http://researchspace.auckland.ac.nz

ResearchSpace@Auckland

Copyright Statement

The digital copy of this thesis is protected by the Copyright Act 1994 (New Zealand).

This thesis may be consulted by you, provided you comply with the provisions of the Act and the following conditions of use:

- Any use you make of these documents or images must be for research or private study purposes only, and you may not make them available to any other person.
- Authors control the copyright of their thesis. You will recognise the author’s right to be identified as the author of this thesis, and due acknowledgement will be made to the author where appropriate.
- You will obtain the author's permission before publishing any material from their thesis.

To request permissions please use the Feedback form on our webpage. http://researchspace.auckland.ac.nz/feedback

General copyright and disclaimer

In addition to the above conditions, authors give their consent for the digital copy of their work to be used subject to the conditions specified on the Library Thesis Consent Form and Deposit Licence.

Note: Masters Theses

The digital copy of a masters thesis is as submitted for examination and contains no corrections. The print copy, usually available in the University Library, may contain corrections made by hand, which have been requested by the supervisor.
Development of a Transdermal Delivery System for Progesterone using Supercritical Carbon Dioxide

James Robert Falconer
BSc, Postgrad Dip, MHealSc, BPharm

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy,
The University of Auckland,
2012
Abstract

BACKGROUND:

Millions of postmenopausal women globally have diminishing bone density, higher rates of ovarian cancers, hot flushes and sweating, insomnia, and postmenopausal depression. The associated costs to the healthcare systems weigh heavily and current treatments prove ineffective or carry significant adverse effects. The use of hormone replacement therapy (HRT) is one of the most popular methods for controlling postmenopausal signs and symptoms. Bio-identical progesterone (PGN) is an endogenous-like steroid that is capable of averting many of the postmenopausal related conditions and has lower rates and severity of adverse effects. Unfortunately, the oral administration of PGN results in very low bioavailability due to PGN’s poor aqueous solubility, physical barriers, and extensive first pass metabolism. Alternatively, transdermal delivery of PGN is a favourable option.

AIM:
The aim of this thesis is to investigate the transdermal delivery potential of PGN in unique dispersion systems prepared using a novel supercritical fluid (SCF) method known as particles from gas saturated suspension (PGSS).

METHODS:
The preformulation phase on PGN included a high performance liquid chromatography (HPLC) method development and validation, x-ray powder diffraction (XRPD), infrared (IR) spectral identification, melting behaviour, saturation solubility, and in vitro dissolution studies. Three excipients were selected as dispersion matrices for PGN primarily based on solubility parameters and saturation solubility, which showed that Gelucire 44/14, a polyethylene glycols (PEG) 400/4000 mixture, and d-α-tocopheryl PEG 1000 succinate (TPGS) were promising. In order to formulate PGN dispersion systems in the selected excipients, a SCF unit was designed, constructed, and developed to perform the PGSS method using supercritical carbon dioxide (SC-CO₂). A factorial design study was used to optimise the PGSS process by identifying significant variables that affected the process yield, in vitro dissolution extent after 20 minutes (E₂₀), and t₁/₂ of PGN release. A non-linear dual first order model was employed to elucidate the mechanisms of PGN release which was found to be dominated by both erosion and diffusion. The dual first order was then compared to conventional release models such as zero and first order, Krosmeyer-Peppas, and Higuchi. The permeability of PGN across nude mouse skin from the dispersion systems prepared by various methods was determined using a Franz diffusion cell. In addition, a SCF-prepared formulation was manufactured with TPGS, an oil (myritol 318), and a penetration enhancer
(transcutol P) which was compared with oil and enhancer free SCF prepared systems, conventionally prepared dispersions, and two controls: an aqueous PGN suspension and a commercial cream.

RESULTS and DISCUSSION:
Improvement in the aqueous dissolution of PGN was observed using the selected excipients, thus Gelucire 44/14, TPGS, and PEG were employed to manufacture PGN dispersion systems using SC-CO$_2$ and PGSS method. The PGSS unit was built and operated successfully to form various dispersion systems, and pilot results using TPGS or Gelucire 44/14 systems produced at 124 bar and 59°C showed promising results in improving the aqueous dissolution of PGN. The factorial experimental design study showed that higher pressure (186 bar), higher temperature (60°C), and longer processing time (30 minutes) all had positive effects on the yield and E$_{20}$ dissolution. On the other hand, the higher pressure and temperature along with a shorter processing time of 10 minutes significantly affected t$_{1/2}$ (p-value < 0.05). The higher loading amount of 9 g and longer sonication time of 10 minutes significantly affected E$_{20}$, while the larger orifice diameter (1/4″) during expansion only affected t$_{1/2}$. The permeability results showed that the SCF formulation in the presence of myritol 318 and transcutol P was significantly different from the controls (p-value < 0.05). Histological examination showed that the enhanced PGN permeability was explained by the dispersion system’s ability to disrupt the SC bilayer and improve the diffusion of PGN into the skin.

CONCLUSION:
Uniform dispersion systems of PGN with Gelucire 44/14, TPGS, and PEG can be formed using the PGSS method. Raman and FTIR spectroscopy, DSC, and XRD were able to successfully characterise the PGN dispersions. The dispersion systems were then optimised using a factorial design study, and the mechanism of PGN release was found to be occurring by the dual processes of erosion and diffusion. TPGS/myritol 318/transcutol P-based systems increased the permeability of PGN across the skin by 2-fold compared to the commercial cream. A transdermal delivery system of PGN has been established using SC-CO$_2$ processing and could be used as a platform to optimise the delivery of other steroids.
Acknowledgements

I would like to express my sincerest gratitude to my supervisor Dr. Jing Yuan-Wen for her advice, encouragement, and support. Dr. Wen was always readily available and provided very helpful guidance. I would also like to thank my former supervisor, Professor Raid Alany, if it had not been for his foresight into my potential as an undergraduate this journey would not have been possible. Both Dr. Wen and Prof. Alany also showed a great willingness to fund necessary project equipment. Due to the developmental nature of planning and constructing the research instrument, quick payment and delivery of relatively expensive parts was very important.

I would like to express my heartfelt gratitude to Dr. Sara Zargar-Shoshtari, my co-supervisor, for providing continuous support throughout the later stages of my research. I acknowledge the ongoing support from Professor Mohammed Farid and Professor John Chen at the Faculty of Engineering, University of Auckland. Their relationship was critical to the success of the collaboration which ensured laboratory space and materials were always available.

Professor John Shaw and the School of Pharmacy have created an excellent postgraduate research environment, and provided much needed support for conference travel. Thank you to Lesley and Anna who help manage us well, even when the school is undergoing renovations and successfully move us around without any major interference. I am grateful to Gamal El Maghraby for his input into the permeability studies. Thanks also to Vivien for helping out from time to time and keeping the HPLC machines working.

I am grateful to Dr. Stephen Tallon and Kristina Fenton of Industrial Research Ltd, in Wellington for their cooperation and use of their laboratory. The encouragement and financial support from Mike Cushman of Pharmaceutical Compounding New Zealand is also greatly valued. I also acknowledge the following University of Auckland members: Peter Buchanan (Department of Chemical & Materials Engineering) for countless times of support, which ensured a successful collaboration, Catherine Hobbis (Faculty of Engineering) for instrument time on the scanning electron microscope, Michel Nieuwootd (Department of Chemistry) for instrument time and help on the Raman and FTIR spectrometers, and John Wilmshurst (Department of Geology) for his time with the powder X-ray diffractometer. For histological work on skin examinations, I am very thankful to Satya Amirapu (School of Medical Science). And finally, thank you to Jacqueline Ross at the Biomedical Imaging Research Unit (School of Medical Science) for excellent help with completing brightfield microscopy.
I also thank the Medical and Health Science Faculty and Staff members at School of Pharmacy for their support throughout my PhD. Without making a comprehensive list, I would also like to name some friends who have accompanied me on similar paths over the last few years – Darren, Sara (again), Ilva, Thilini, Noble, Ali and Alvin.

I am especially thankful to my parents, Fransiska and Dave Falconer who have offered endless emotional support and hours of proof reading. And to my parents-in-law, Foroogh and Shahzad Ghahreman who have made sacrifices to help me get through my PhD. I am indebted for all the support offered by my newest and oldest families.

Last but not least, my greatest thanks goes to Nazanin who has made the last 11 years of my life very meaningful and enjoyable while also keeping me on my toes. My PhD project, work and fatherhood commitments have been all consuming at times but Nazanin helped me find much needed balance. Thank you for tolerating my various preoccupations and for being a long-suffering and always pleasant listener. And a related acknowledgement to our darling Emily who gives life more meaning and makes a difference in my attitude towards life. Although if it wasn’t for her my PhD would have likely finished much sooner. I love both of you very much.
Research outputs from this research

International publications

Falconer, J.R., Wen, JW., Zargar-Shoshtari, S., Chen, J.J., Farid, M., Young, S., Alany, R.G. (2013). Effects of supercritical carbon dioxide processing on optical crystallinity and in vitro release of progesterone and Gelucire 44/14 solid and semi-solid dispersions. IN PRESS. Accepted by Journal of Drug Delivery Science and Technology on 28 February


Peer-reviewed research conference papers and posters


of the 6th Drug Delivery Australia Annual Meeting and Exposition of the Controlled Release Society (CRS-AUS), Melbourne, Australia


**Audio-visual presentations**


Falconer, J.R., Wen, Jingyuan (2012). Supercritical Fluids: A case for delivery of aqueous insoluble drugs. PowerPoint presentation to PharmaTell, School of Pharmacy, FMHS, University of Auckland, Auckland, New Zealand

Falconer, J.R., Alany, R. (2010). Permeability of progesterone delivered from dispersion systems prepared by supercritical fluid processing using porcine skin. PowerPoint presentation to PharmaTell, School of Pharmacy, FMHS, University of Auckland, Auckland, New Zealand

Falconer, J.R., Alany, R. (2009). Formation of solid dispersions from progesterone in various carriers using supercritical carbon dioxide. PowerPoint presentation to PharmaTell, School of Pharmacy, FMHS, University of Auckland, Auckland, New Zealand


Output Summary

5 international peer-reviewed research papers,
9 conference papers and 7 conference posters,
5 oral presentations (Faculty of Medical and Health Sciences)
2 conference podiums (FDB and CRS 2013 - Hawaii)
# Table of Contents

Abstract........................................................................................................................................ii
Acknowledgements..................................................................................................................iv
Research outputs from this research .......................................................................................vi
Table of Contents ......................................................................................................................ix
List of Abbreviations ................................................................................................................xvi
List of Figures ............................................................................................................................xxi
List of Tables ..............................................................................................................................xxiv

## Chapter 1. Introduction ............................................................................................................2

1.1. The postmenopausal problem.................................................................................................2

1.1.1. Osteoporosis ....................................................................................................................4

1.1.2. Pathophysiology .............................................................................................................6

1.1.3. Available treatment options ..........................................................................................8

1.2. The New Zealand Situation ...............................................................................................14

1.2.1. The legal status of PGN ...............................................................................................14

1.3. Progesterone .......................................................................................................................15

1.3.1. Physicochemical properties ..........................................................................................17

1.3.2. Mechanism of action ....................................................................................................17

1.3.3. Pharmacokinetics .........................................................................................................18

1.3.4. Adverse effects .............................................................................................................19

1.3.5. Routes of administration .............................................................................................19

1.3.6. Oral pharmaceuticals and limitations ...........................................................................20

1.4. The Human Skin ................................................................................................................20

1.4.1. The stratum corneum ...................................................................................................22

1.4.2. Drug delivery routes .....................................................................................................25

1.4.3. Factors affecting transdermal drug delivery .................................................................27

1.5. Transdermal strategies .......................................................................................................27

1.5.1. Physical strategies .........................................................................................................28
1.5.2. Pharmaceutical strategies to permeation enhancement ........................................31

1.6. Alternative strategy ................................................................................................45

1.6.1. Solid dispersions ..............................................................................................45

1.7. The main objective ..............................................................................................49

1.8. Aims of the thesis and its structure ......................................................................50

Chapter 2. Preformulation of Progesterone .................................................................53

2.1. Introduction ...........................................................................................................53

2.1.1. Infrared and Raman spectroscopy .................................................................53

2.1.2. Crystaline diffractograms ..............................................................................55

2.1.3. Thermal analysis of PGN .............................................................................55

2.1.4. Solubility parameters .......................................................................................56

2.1.5. Saturation solubility determination ...............................................................58

2.1.6. Optical microscopy – Melting point (HSM)/birefringence .........................58

2.1.7. Chapter aims ...................................................................................................60

2.2. Experimental .......................................................................................................61

2.2.1. Materials ..........................................................................................................61

2.2.2. Excipient selection .........................................................................................61

2.2.3. Current excipients used in the literature .......................................................62

2.2.4. Solubility parameters .......................................................................................69

2.2.5. Saturation solubility .......................................................................................69

2.2.6. Optical microscopy – Melting point (HSM)/birefringence .........................69

2.2.7. Raman spectroscopy .......................................................................................70

2.2.8. Infrared (IR) spectroscopy .............................................................................70

2.2.9. X-ray powder diffraction (XRPD) studies ...................................................71

2.2.10. Differential scanning calorimetry (DSC) .....................................................71

2.2.11. Particle size ....................................................................................................71

2.3. Chromatographic development ............................................................................72

2.3.1. Instruments .....................................................................................................72

2.3.2. HPLC conditions .........................................................................................72

2.3.3. Standard curves .............................................................................................73

2.3.4. Method validation ...........................................................................................73
2.4. Results and discussion.................................................................................75
  2.4.1. Solubility parameters ...........................................................................75
  2.4.2. Saturation solubility ...........................................................................78
  2.4.3. Optical microscopy ..............................................................................80
  2.4.4. Polymorphism of PGN .........................................................................83
  2.4.5. FTIR spectroscopy ...............................................................................86
  2.4.6. XRPD studies .......................................................................................87
  2.4.7. Thermal analysis (DSC) .....................................................................88
  2.4.8. Particle size ..........................................................................................90
  2.4.9. Chromatography and specificity ..........................................................92
  2.5. Conclusion ...............................................................................................97

Chapter 3. Construction of Supercritical Fluid Unit, and Formulation and Characterization of PGN Solid Dispersions .................................................................100
  3.1. Introduction ..............................................................................................100
  3.2. Supercritical fluids ..................................................................................101
    3.2.1. Concept and history ..........................................................................101
    3.2.2. The types of supercritical methods ....................................................105
  3.3. Particles from a gas saturated solution (PGSS) .......................................109
    3.3.1. Concept .............................................................................................109
    3.3.2. Patents ...............................................................................................112
    3.3.3. Future PGSS developments ...............................................................113
    3.3.4. Phase effects on particle formation ....................................................113
  3.4. Equipment setup ......................................................................................117
    3.4.1. Design and construction of the PGSS unit .......................................117
    3.4.2. PGSS setup .......................................................................................117
    3.4.3. The PGSS unit ..................................................................................117
    3.4.4. Particle precipitation chamber ..........................................................121
  3.5. PGSS processing ......................................................................................127
    3.5.1. General outline ..................................................................................127
    3.5.2. Operation procedure .........................................................................127
    3.5.3. Problems and equipment maintenance ..............................................129
3.6. Elementary experiment ............................................................................................................. 130

3.6.1. Materials ................................................................................................................................. 130
3.6.1. Preparation of CS, CM and PM dispersions ............................................................................ 131

3.7. Characterisation analysis ........................................................................................................... 131

3.7.1. Process yield ............................................................................................................................ 131
3.7.2. PGN recovery and uniformity ............................................................................................... 132
3.7.3. Birefringence .......................................................................................................................... 132
3.7.4. Infrared (IR) spectroscopy ...................................................................................................... 133
3.7.5. X-ray powder diffraction (XRPD) studies .............................................................................. 133
3.7.6. Differential scanning calorimetry (DSC) ............................................................................... 133
3.7.7. Scanning electron microscopy (SEM) .................................................................................... 134
3.7.8. HPLC solutions ...................................................................................................................... 134
3.7.9. In vitro dissolution testing ...................................................................................................... 134
3.7.10. Statistical analysis ................................................................................................................ 134

3.8. Results and discussion .............................................................................................................. 135

3.8.1. PGN recovery .......................................................................................................................... 139
3.8.2. Uniformity ................................................................................................................................ 139
3.8.3. Birefringence .......................................................................................................................... 140
3.8.4. FTIR spectroscopy .................................................................................................................. 142
3.8.5. XRPD studies .......................................................................................................................... 144
3.8.6. Thermal analysis ...................................................................................................................... 147
3.8.7. Morphology ............................................................................................................................. 151
3.8.8. In vitro dissolution ................................................................................................................... 153
3.8.9. Discussion ............................................................................................................................... 158

3.9. Conclusion ................................................................................................................................ 162

Chapter 4. Optimisation of SCF processing and In Vitro Evaluation of Progesterone Dispersions ................................................................................................................................. 165

4.1. Introduction .................................................................................................................................. 165

4.1.1. Factorial design experiments ................................................................................................. 165
4.1.2. Dissolution studies .................................................................................................................. 168

4.2. Experimental ............................................................................................................................... 169
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACN</td>
<td>Acetonitrile</td>
</tr>
<tr>
<td>Am</td>
<td>Amenorrhea</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>ANZTPA</td>
<td>Australia New Zealand Therapeutic Products Administration</td>
</tr>
<tr>
<td>ART</td>
<td>Assisted reproductive technology</td>
</tr>
<tr>
<td>ATR</td>
<td>Attenuated total reflection</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
</tr>
<tr>
<td>BIRU</td>
<td>Biomedical Imaging Research Unit</td>
</tr>
<tr>
<td>Δn</td>
<td>Birefringence</td>
</tr>
<tr>
<td>CCD</td>
<td>Charge-coupled device</td>
</tr>
<tr>
<td>CD</td>
<td>Cyclodextrins</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>Maximum concentration</td>
</tr>
<tr>
<td>CMC</td>
<td>Critical micelle concentration</td>
</tr>
<tr>
<td>CS</td>
<td>Cosolvent</td>
</tr>
<tr>
<td>CM</td>
<td>Comelt</td>
</tr>
<tr>
<td>C&lt;sub&gt;t&lt;/sub&gt;</td>
<td>Concentration at time (t)</td>
</tr>
<tr>
<td>d(50)</td>
<td>Average particle size</td>
</tr>
<tr>
<td>d(90)</td>
<td>Upper end particle size</td>
</tr>
<tr>
<td>Cv</td>
<td>Concentration (of drug in the vehicle)</td>
</tr>
<tr>
<td>CED</td>
<td>Cohesive energy density</td>
</tr>
<tr>
<td>COA</td>
<td>Certificate of analysis</td>
</tr>
<tr>
<td>Da</td>
<td>Dalton</td>
</tr>
<tr>
<td>HPE-101</td>
<td>1-[2-(decylthio)ethyl]azacyclopentane-2-one</td>
</tr>
<tr>
<td>DHEA</td>
<td>5-Dehydroepiandrosterone</td>
</tr>
<tr>
<td>DHE</td>
<td>Dihydroergotamine</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl Sulfoxide</td>
</tr>
<tr>
<td>Dm</td>
<td>Diffusion coefficient (membrane)</td>
</tr>
<tr>
<td>DOE</td>
<td>Design of Experiments</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential Scanning Calorimetry</td>
</tr>
<tr>
<td>DRIFT</td>
<td>Diffuse reflectance infrared fourier transform</td>
</tr>
<tr>
<td>DT</td>
<td>Distal tubules</td>
</tr>
<tr>
<td>δ2d</td>
<td>Dispersive interaction</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
</tbody>
</table>
EO  Ethylene oxide
ER  Enhancement ratio
ER  Electrical resistance
EPO  Evening primrose oil
E1  Estrone
E2  Estrodiol
ΔE  Free energy of vaporization
FDA  Food and Drug Administration
FMHS  Faculty of Medical and Health Sciences
FTIR  Fourier transform infrared
g·mol⁻¹  Grams per mole
IUPAC  International Union of Pure and Applied Chemistry
J  Flux
Jss  Steady state flux
GIT  Gastrointestinal tract
GRAS  Generally Recognized as Safe
L  Length (across skin)
HLB  Hydrophilic-lipophilic balance
HPLC  High pressure liquid chromatography
h  Hour
HRT  Hormone replacement therapy
δ2h  Hydrogen bonding interaction
ΔH  Enthalpy of vaporization
ICH  International Conference on Harmonisation
IGF  Insulin-like growth factor
IPM  Isopropyl myristate
IRL  Industrial Research Limited (now Callaghan Innovation)
IR  Infra red
IVF  In vitro fertilization
LMWH  Low molecular weight heparin
LHRH  Luteinizing hormone-releasing hormone
LTP  Localised transport pathways
LOD  Limit of detection
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOQ</td>
<td>Limit of quantification</td>
</tr>
<tr>
<td>logP</td>
<td>Logarithm of octanol/ water partition coefficient</td>
</tr>
<tr>
<td>kDa</td>
<td>Kilo dalton</td>
</tr>
<tr>
<td>K</td>
<td>Partition coefficient</td>
</tr>
<tr>
<td>JTGA</td>
<td>Joint Therapeutic Goods Administration</td>
</tr>
<tr>
<td>min</td>
<td>Minute</td>
</tr>
<tr>
<td>mol·L⁻¹</td>
<td>Moles per Litre</td>
</tr>
<tr>
<td>MP</td>
<td>Melting point</td>
</tr>
<tr>
<td>MR</td>
<td>Mineralocorticoid receptor</td>
</tr>
<tr>
<td>NaDC</td>
<td>Sodium deoxycholate</td>
</tr>
<tr>
<td>NIH</td>
<td>National Institute of Health (US)</td>
</tr>
<tr>
<td>NLC</td>
<td>Nanostructured lipid carriers</td>
</tr>
<tr>
<td>NHANES</td>
<td>National Health and Nutrition Examination Survey</td>
</tr>
<tr>
<td>ne</td>
<td>Refractive Index</td>
</tr>
<tr>
<td>no</td>
<td>Perpendicular light component</td>
</tr>
<tr>
<td>o/w</td>
<td>Oil-in-water</td>
</tr>
<tr>
<td>OECD</td>
<td>Organisation for Economic Cooperation and Development</td>
</tr>
<tr>
<td>PGN</td>
<td>Progesterone</td>
</tr>
<tr>
<td>PGR</td>
<td>PGN receptors</td>
</tr>
<tr>
<td>PGE1</td>
<td>Prostaglandin E1</td>
</tr>
<tr>
<td>PE</td>
<td>Penetration enhancer</td>
</tr>
<tr>
<td>PEG</td>
<td>Polyethylene glycol</td>
</tr>
<tr>
<td>PCNZ</td>
<td>Pharmaceutical compounding New Zealand</td>
</tr>
<tr>
<td>PKC</td>
<td>Protein kinase C</td>
</tr>
<tr>
<td>P-gP</td>
<td>P-glycoprotein</td>
</tr>
<tr>
<td>PLA</td>
<td>Poly lactic acid</td>
</tr>
<tr>
<td>PGSS</td>
<td>Particle from gas saturated solution/suspension</td>
</tr>
<tr>
<td>PM</td>
<td>Physical mixture</td>
</tr>
<tr>
<td>PMA</td>
<td>Polymethacrylate</td>
</tr>
<tr>
<td>P-T</td>
<td>Pressure-Temperature</td>
</tr>
<tr>
<td>PVA</td>
<td>Polyvinylalcohol</td>
</tr>
<tr>
<td>PVP</td>
<td>Polyvinylpyrrolidon</td>
</tr>
<tr>
<td>PSA</td>
<td>Pressure-sensitive adhesive</td>
</tr>
<tr>
<td>δ2p</td>
<td>polar interaction</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomised controlled trials</td>
</tr>
<tr>
<td>R&amp;D</td>
<td>Research and development</td>
</tr>
<tr>
<td>R</td>
<td>Gas constant</td>
</tr>
<tr>
<td>$R^2$</td>
<td>Linear regression</td>
</tr>
<tr>
<td>SAA</td>
<td>Surface active agent (Surfactant)</td>
</tr>
<tr>
<td>SMEDDS</td>
<td>Self-microemulsifying drug delivery systems</td>
</tr>
<tr>
<td>SLN</td>
<td>Solid Lipid nanoparticles</td>
</tr>
<tr>
<td>S-L-V</td>
<td>Solid-Liquid-Vapour (Gas)</td>
</tr>
<tr>
<td>SC</td>
<td>Stratum corneum</td>
</tr>
<tr>
<td>SCF</td>
<td>Supercritical fluid</td>
</tr>
<tr>
<td>SC-CO$_2$</td>
<td>Supercritical carbon dioxide</td>
</tr>
<tr>
<td>Sv</td>
<td>Solubility (of drug in the vehicle)</td>
</tr>
<tr>
<td>Sm</td>
<td>Solubility (of drug in the membrane)</td>
</tr>
<tr>
<td>$\delta$</td>
<td>Solubility parameter</td>
</tr>
<tr>
<td>SSRI</td>
<td>Selective serotonin-reuptake inhibitor</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SE</td>
<td>Standard error</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>T</td>
<td>Temperature</td>
</tr>
<tr>
<td>$T_m$</td>
<td>Melting Point</td>
</tr>
<tr>
<td>TGA</td>
<td>Therapeutic goods administration</td>
</tr>
<tr>
<td>TPGS</td>
<td>D-$\alpha$-tocopheryl polyethylene glycol 1000 succinate (Vitamin E)</td>
</tr>
<tr>
<td>TWL</td>
<td>Transepidermal water loss</td>
</tr>
<tr>
<td>5-FU</td>
<td>5-Fluorouracil</td>
</tr>
<tr>
<td>VDR</td>
<td>Vitamin D receptor</td>
</tr>
<tr>
<td>UK</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet light</td>
</tr>
<tr>
<td>US FDA</td>
<td>United States Food and Drug Administration</td>
</tr>
<tr>
<td>USA</td>
<td>United States of America</td>
</tr>
<tr>
<td>USP</td>
<td>United States Pharmacopeia</td>
</tr>
<tr>
<td>UV</td>
<td>Ultra violet</td>
</tr>
<tr>
<td>Vm</td>
<td>Molar volume</td>
</tr>
<tr>
<td>v/v</td>
<td>Volume by volume</td>
</tr>
<tr>
<td>w/o</td>
<td>Water-in-oil</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>w/w</td>
<td>Weight by weight</td>
</tr>
<tr>
<td>XRPD</td>
<td>X-ray powder diffraction</td>
</tr>
<tr>
<td>$\lambda_{\text{max}}$</td>
<td>Wavelength at maximum absorbance</td>
</tr>
</tbody>
</table>
List of Figures
Figure 1-1. General menopause symptoms, adapted from Cairm.com [3].........................3
Figure 1-2. Illustration of bone matrix changes before (left image) and after (right image) menopause, reproduced and adapted from [20].........................................................5
Figure 1-3. Individual and mean plasma PGN levels throughout the peri-menopausal and post-menopausal years (Am = amenorrhoea). Reproduced with permission from Elsevier [21].....................................................................................................................6
Figure 1-4. Remodeling on the bone surface. Redrawn from [22, 26]...............................8
Figure 1-5. Molecular structure and the IUPAC numbering system for cholesterol............16
Figure 1-6. Molecular structure of (a) PGN and other steroids; ....................................16
Figure 1-7. The basic structure and layers of the human skin. Adapted from [110]. .........16
Figure 1-8. The human SC showing the macroscopic and molecular domains with the suggested routes of drug penetration. Reproduced with permission from John Wiley and Sons [119].......................................................................................................................23
Figure 1-9. An idealized representation of the bilayers within the intercellular lipid regions of the stratum corneum. Reproduced with permission from John Wiley and Sons [119]. .......25
Figure 1-10. The routes of drug penetration through the human stratum corneum (SC); (a) sweat-pores, (b) across the SC, and (c) the hair shafts. Reproduced with permission from John Wiley and Sons [119].......................................................................................................................26
Figure 1-11. Graphic representation of drug permeation across the skin from; ...............32
Figure 1-12. Different strategies for skin permeation enhancement. Reproduced with permission from Elsevier [113, 164].......................................................................................................................33
Figure 1-13. Possible sites for penetration enhancer action in the intercellular lipid matrix of the stratum corneum; .......................................................................................................................33
Figure 1-14. Chemical structures of (a) transcutol P and (b) ethylenediaminetetraacetic acid. .................................................................................................................................34
Figure 1-15. Cross section of various patches; .................................................................43
Figure 1-16. The three modes of drug incorporation into a solid dispersion where;............46
Figure 1-17. The pressure-temperature phase diagram for a pure substance. .................46
Figure 1-18. Factors with possible influence on drug release. .......................................50
Figure 2-1. Incident light rays passing through birefringent material. Adapted from [281]. ..60
Figure 2-2. Gelucire 44/14 contains triglycerides mainly of lauric acid with PEGs by esterification of glycerol with fatty acids .................................................................65
Figure 2-3. General structure of a fatty acid, mono-, di-, and tri-glycerides: ..................65
Figure 2-4. The PEG monomer, n= 400 or 4000. .................................................................67
Figure 2-5. Molecular structure of TPGS – Vitamin E [305]. ..................................................68
Figure 2-6. Saturation solubility test for selected excipients for PGN formulation. ..........79
Figure 2-7. Hot-stage microscope images for pure substances and the excipient mixtures with PGN at their observed points of melting.................................................................81
Figure 2-8. PGN crystal dispersion under polarised light microscopy (100 × magnification). (a): non-ionic cream, (b): PGN dispersed in non-ionic cream, and (c): pure PGN crystals under 400 × magnification.................................................................82
Figure 2-9. Light microscopy (200 × magnification) pictures of PGN; ................................83
Figure 2-10. Raman spectra of solid state PGN before melting (a) and during melting (b). The Raman shift is visible in the overlay of the two spectra. .....................................................84
Figure 2-11. In situ spectra of peak shifting during PGN transformation after melting.......84
Figure 2-12. Raman spectra for PGN crystals before and after SC- CO₂ treatment showing no transformation changes. ..................................................................................85
Figure 2-13. FTIR spectra for PGN crystals and the three excipients. .................................87
Figure 2-14. X-ray diffractograms for PGN, .................................................................88
Figure 2-15. DCS thermograms of PGN and selected excipients; ......................................89
Figure 2-16. Particle size distribution for pure PGN powder .............................................91
Figure 2-17. Chromatograms of various PGN solutions; ..................................................93
Figure 2-18. Chromatogram and UV spectrum (insert) of standard PGN (20 µg·mL⁻¹) solution.......................................................................................................................94
Figure 2-19. The typical calibration curve relating the concentration of PGN (X-axis) to the area under the curve (Y-axis) (n = 3). Error bars are within data points.................95
Figure 3-1. The pressure-temperature phase diagram for a pure substance. ....................102
Figure 3-2. Quaternary phase diagram for CO₂ represented with pressure as a function of temperature. The arrows show the direction of a possible S-L-V projection..............104
Figure 3-3. Effect of density vs. pressure for carbon dioxide. Reproduction (adaption) from international tables of fluid state [423, 424]. .........................................................105
Figure 3-4. A simplified representation of SCF setups for particle formation, adapted from [433]. .................................................................................................................106
Figure 3-5. The basic process of the PGSS method..........................................................110
Figure 3-6. A typical PGSS equipment setup, adapted from [491]. ..................................111
Figure 3-7: Density and viscosity changes for carbon dioxide with pressure at 37°C. Reproduced with permission from Springer [521]. .........................................................114
Figure 3-8: Solubility of PGN in supercritical carbon dioxide

Figure 3-9: Effect of density on the solubility of PGN

Figure 3-10: The PGSS unit located in the Chemical & Materials Engineering Laboratories, UOA, NZ

Figure 3-11: The CO₂ cylinder (left) and the joining assembly to the SCF syringe pump (right). Both the cylinder and pump have separate controlling valves that open and close individually.

Figure 3-12: The SCF syringe pump controller (left) and the connecting tube from the SCF pump and sample cylinder (right)

Figure 3-13: The sample cylinder and pressure gauge fitted together before processing

Figure 3-14: The nozzle assembly before being connected (in place without bolts screwed down) to close the particle precipitation chamber

Figure 3-15: Configuration of the sample cylinder with particle precipitation chamber and nozzle assembly inside the chamber

Figure 3-16: Diagram of the open particle precipitation chamber. Lower Right: the stainless steel micro-filter with supporting sheet and base

Figure 3-17: Water bath and heat pump controller with sample cylinder attached

Figure 3-18: The final PGSS unit setup used in this thesis

Figure 3-19: The three main steps for operating the self-built SCF unit at the University of Auckland

Figure 3-20: Sampling places inside the expansion chamber (SCF unit base). The collection points are marked X, Y and Z

Figure 3-21: The particle precipitation chamber with the cap removed after PGSS processing (view facing down into the chamber)

Figure 3-22: Polarized light microscopic images (200 × magnification) of with and without PGN, prepared from various dispersion methods. The top row images are without PGN

Figure 3-23: FTIR spectra of PGN and PGN dispersions

Figure 3-24: Power X-Ray diffractograms of PGN and PGN dispersions

Figure 3-25: Thermographs of PGN and Gelucire 44/14

Figure 3-26: Thermographs of PGN and TPGS

Figure 3-27: Thermographs for PGN and PEG 400/4000

Figure 3-28: SEM images before and after SCF processing (1:5 ratio samples)

Figure 3-29: Dissolution profiles of dispersions of PGN with Gelucire 44/14 prepared by PGSS and various conventional methods
Figure 3-30. Dissolution profiles for PGN and TPGS dispersions prepared by PGSS and conventional methods.

Figure 3-31. Dissolution profiles of solid dispersions of PGN and PEG 400/4000 processed by PGSS and prepared by different conventional methods.

Figure 4-1. A two factor, two level design used to investigate Factors A and B within certain constraints with the experimental domain.

Figure 4-2. Release profiles of the dispersion systems containing PGN;

Figure 4-3. Spread plots of the responses: (1) processing yield; (2) extent dissolution after 20 minutes; (3) time required to dissolve 50% of PGN.

Figure 4-4. Pareto chart of standardised effects on percentage yield (Alpha = 0.05).

Figure 4-5. Main effects plot for yield percentage of PGN dispersions.

Figure 4-6. Interaction plot for yield percentage of PGN dispersions.

Figure 4-7. Pareto chart of standardised effects on PGN dissolution extent at 20 minutes (Alpha = 0.05).

Figure 4-8. Main effects plot for dissolution extent after 20 minutes of PGN dispersions.

Figure 4-9. Interaction plot for dissolution extent after 20 minutes of PGN dispersions. *Statistically significant interactions are shown as shaded boxes (p-value < 0.05).

Figure 4-10. Pareto chart of standardised effects on time at 50% PGN dissolution (Alpha = 0.05).

Figure 4-11. Main effects plot for t_{1/2} of PGN dispersions.

Figure 4-12. Interaction plot for t_{1/2} of PGN dispersions.

Figure 4-13. Response surfaces for: (R1) processing yield; (R2) extent dissolution after 20 min.; (R3) Time for 50% w/v of PGN to dissolve (t_{1/2}); and (R4) PGN uniformity.

Figure 4-14. Percentage of cumulative PGN amount released from Gelucire 44/14 systems over 60 minutes. Data points represent mean ± SD, n = 3.

Figure 5-1. Schematic of a Franz cell used to evaluate drug permeation. Reproduced and adapted from [593].

Figure 5-2. Microscopy pictures under polarised light (200 × magnification);

Figure 5-3. Extent of PGN permeation with selected penetration enhancers after 24 hours.

Figure 5-4. Skin PGN permeation profile for the SCF formulations.

Figure 5-5. Skin PGN permeation profile for the final SCF and control formulations (water and market cream).

Figure 5-6. Skin PGN permeation profile for the conventional cosolvent (CS) formulations.
Figure 5-7. Skin PGN permeation profile for the conventional comelted (CM) formulations. .......................................................... 224
Figure 5-8. Skin PGN permeation profile for the physically mixed (PM) formulations……. 225
Figure 5-9. Release profile of PGN from water, SCF-TPGS and market cream. 232
Figure 5-10. Comparison of transdermal flux and formulation release rate within 24 hours for the market cream, water control and final SCF dispersion. 234
Figure 5-11. Transverse sections taken from a light microscope of untreated hairless mouse skin: .......................................................... 235
Figure 5-12. Transverse sections taken from a light microscope of water treated (a) and (b), and market cream (c) and (d) treated hairless mouse skin: ........................................... 236
Figure 5-13. Transverse sections taken from a light microscope of nude mouse skin treated with the final SCF – TPGS dispersion: .......................................................... 237
Figure 5-14. Representative transverse sections taken from a light microscope of nude mouse skin treated with selected conventional dispersions: ........................................... 238
Figure 5-15. FTIR spectrum of untreated mouse skin; .......................................................... 239
Figure 5-16. Expanded FTIR spectrum of untreated mouse skin showing amide shifts. 240
Figure 5-17. Expanded FTIR spectra of mouse skin showing the CH stretching regions. 241
Figure 5-18. PGN stability data over 6 months for the final SCF-TPGS formulation. 243
Figure 5-19. Transdermal flux of PGN across the skin (left axis) as a function of solubility saturation (right axis) for excipients at 30% w/w; .......................................................... 245
Figure 5-20. The effect on permeation rate (flux) of PGN across the skin between different methods of preparation. .......................................................... 247
Figure 5-21. Schematic illustration of monomer-micelle equilibrium. 248
Figure 5-22. The structural aspects of a semi-solid cream: .......................................................... 249
Figure 5-23. Major stages in PGN delivery from a transdermal formulation into the skin and into systemic circulation. Micelles sourced from Gelucire 44/14 or TPGS formulations. 250
List of Tables
Table 1-1. Lipid composition of the human stratum corneum [117]. ........................................................................... 24
Table 2-1. Summary of supercritical fluid methods for various steroids and common excipients/cosolvents. .................................................................................................................. 63
Table 2-2. Fatty acid composition of Gelucire 44/14 [310]. ......................................................................................... 65
Table 2-3. Solubility parameter calculations for PGN using the Hoftyzer and van Krevelen method .................................................................................................................................................. 76
Table 2-4. Estimated total solubility parameter for PGN. ............................................................................................. 76
Table 2-5. Solubility parameters ($\delta$) of PGN and various excipients [262, 264, 355, 358-360]. .................................. 77
Table 2-6. Particle size parameters for PGN. ............................................................................................................. 90
Table 2-7. Repeatability of HPLC assay for determination of PGN. ............................................................. 95
Table 2-8. Accuracy and precision of HPLC assay for determination of PGN. ..................................................... 96
Table 2-9. HPLC recovery data for PGN and PGN spiked with blank forms of each excipient and market cream ........................................................................................................................................ 97
Table 3-1. Orders of magnitude of physical properties in three states of matter [405]. ................................. 102
Table 3-2. Critical properties of various solvents [406]. ................................................................................................................ 103
Table 3-3. Operation conditions, PGN: excipient ratio and yield ........................................................................ 136
Table 3-4. Difference in PGN amount loaded and recovered from various methods ............................................. 139
Table 3-5. Percent PGN recovered after SCF processing from excipient at 3 sample locations ............................. 140
Table 3-6. Release extent and rates for the Gelucire 44/14 dispersions ........................................................... 155
Table 3-7. Release extent and rates for the TPGS dispersions ................................................................................ 157
Table 3-8. Release extent and rates for the PEG 400/4000 (50:50) dispersions ...................................................... 158
Table 4-1. Selected parameters of the experimental domain ........................................................................... 172
Table 4-2. Experimental matrix for the $2^{7-3}$ design for PGN dispersions ....................................................... 173
Table 4-3. Experimental matrix for the $2^{7-3}$ design factors and responses for the PGN dispersions. .............................................................................................................................................. 177
Table 4-4. PGN recovery/drug loading after SCF processing for each sample ................................................. 178
Table 4-5. Summary of regression coefficients of the significant factors calculated with the stepwise method (standard error, SE, in parentheses). .................................................................................. 183
Table 4-6. ANOVA table for refined models ...................................................................................................... 184
Table 4-7. Estimated effects and coefficients for processing yield ($R^2 = 99.97$%). ........................................ 184
Table 4-8. Comparison of observed and estimated processing yields from the full (Equation 15) and simplified model (Equation 16). ................................................................. 186
Table 4-9. Estimated effects and coefficients for dissolution after 20 minutes (R² = 99.99%). 189
Table 4-10. Estimated effects and coefficients for t₁/₂ for PGN dispersions (R² = 100%). 192
Table 4-11. The best experimental conditions required for the highest extent of PGN dissolution, processing yield and PGN uniformity ................................................................. 195
Table 4-12. Extent dissolution after 60 minutes and area under the percentage drug released vs time curve (AUC) of PGN and Gelucire 44/14 dispersions. ........................................ 200
Table 4-13. Release parameters for the PGN dispersions obtained from zero and first order models .......................................................................................................................... 201
Table 4-14. Release rate constants for the PGN dispersions obtained from the Higuchi model 201
Table 4-15. Release parameters for the PGN dispersions obtained from Korsmeyer-Peppas model .......................................................................................................................... 202
Table 4-16. Release parameters for the PGN dispersions obtained from dual first order model 203
Table 5-1. Comparison of PGN permeation data across the skin or synthetic membranes from lowest to highest values. .................................................................................................................. 209
Table 5-2. Parameters for the penetration enhancers ............................................................................................................................... 219
Table 5-3. Electrical resistance for mouse skin membranes ............................................................................................................................... 220
Table 5-4. Pharmacokinetic parameters for the controls and PGN dispersion systems ........ 226
Table 5-5. Permeability data for controls and SCF prepared formulations ......................... 228
Table 5-6. Statistical comparison of cumulative extent of PGN permeated over 24 hours using one–way ANOVA .................................................................................................................. 230
Table 5-7. Statistical comparison of PGN flux data using one–way ANOVA ....................... 231
Table 5-8. Release kinetic data for controls and final SCF-TPGS formulations .................. 233
Table 5-9. FTIR summary of amide stretching regions for specific peaks for various formulations and controls .................................................................................................................. 241
Table 5-10. FTIR summary of CH stretching regions for specific peaks for various formulations and controls .................................................................................................................. 242
Chapter 1:

Introduction
Chapter 1. Introduction

Many postmenopausal women can have reduced bone mass, higher rates of ovarian cancers, hot flushes and sweating, insomnia, and depression compared to menopausal women. The healthcare costs from conditions associated with postmenopause add financial stress on individuals, governments, and private insurance companies globally. Many treatment options available today can be either ineffective or carry significant side effects. The use of hormone replacement therapy (HRT) through use of synthetic sex steroids is the most popular way to control postmenopausal conditions. Bioidentical progesterone (PGN) is a hormonal steroid that is able to reduce, even prevent many postmenopausal related conditions. The oral delivery of PGN undergoes metabolism by the liver, thus to get a desired response requires higher doses with increased adverse effects. Various other techniques and delivery routes have been tested including the transdermal route which involves passive diffusion across the skin. The aim of this thesis was to investigate the potential of a transdermal delivery vehicle containing a PGN dispersion using a supercritical fluid (SCF) method known as particles from gas saturated suspension (PGSS).

1.1. The postmenopausal problem

Menopause is defined as the time of a woman's final menstruation. This is when a woman's fertility is considered to end, although the loss of fertility is not an immediate event. Fertility begins to decline gradually as the number of eggs in the ovaries falls [1]. This fall is associated with reduced levels of the reproductive hormones oestrogen and PGN. The onset of menopause is different for every woman but usually menopause occurs between the ages of 45 and 55 [1]. In some women it may occur as early as 40 years, although this is often a hereditary trait. Evidence suggests that menopause is delayed in those women that start to menstruate early [1, 2]. Menopause occurs naturally but may also be induced by surgery (e.g. oophorectomy) or by ovarian damage caused by chemotherapy or radiation. A woman is described as postmenopausal if it has been more than a year since her last menstrual period [2]. The perimenopausal transition
Chapter 1: Introduction

is from the time that symptoms of menopause begin, until 12 months after the last menstrual cycle [1].

From the onset of premenopausal age women suffer a variety of signs and symptoms, to name a few; diminishing bone density (increasing risk of osteopenia and osteoporosis), excessive sweating, ovine cancers, insomnia, and postmenopausal depression. Figure 1-1 gives a list of the many general symptoms women of menopausal age may suffer [3]. The common symptoms of hot flushes and sweating are known as the vasomotor symptoms [4-6].

![Figure 1-1. General menopause symptoms, adapted from Cairm.com [3].](image)

Postmenopausal women utilise a diverse range of pharmaceuticals, including HRT. In 2005, it was estimated that 41% of French women aged between 50 and 69 years were treated by HRT, corresponding to about 2.6 million French women [7]. According to the National Health and Nutrition Examination Survey III (NHANES III), an estimated 14 million American women over the age of 50 years are affected by low bone density at the hip, and 5 million more have bone density that measures 2.5 standard deviations or more, below the mean at the hip [8]. Although actual numbers are difficult to ascertain, it is possible to estimate that there are tens of millions of postmenopausal women in the world today.

Through the use of steroidal HRT the signs and symptoms of postmenopause can be reduced or controlled. The history of HRT for climacteric symptoms started in the late 1920s
Chapter 1: Introduction

with injectable estrogen [9]. Shortly after that oral estrogen was developed and introduced to the market [10]. Over the following twenty years, oestrogen was prescribed alone for postmenopausal women. It was then realized that estrogen only therapy was associated with up to 10-fold increase in the risk of endometrial cancer [11, 12]. Accordingly, it was devised that a progestogen be added with estrogen [13]. At first endogenous PGN was used but oral administration of PGN is subject to pre-systemic metabolism by the liver and gastrointestinal tract (GIT). This means the oral administration of PGN has poor bioavailability and variable blood levels [14]. So, synthetic forms of progestogen (progestins) were developed that improved bioavailability. Unfortunately, progestins have increased rates and severity of adverse effects, to name a few, such as bloating, weight gain, metabolic disorders, Alzheimer’s disease, and depression [4, 15, 16]. They are also associated with the development of breast cancer in women taking combined hormone therapy [17-19]. Hence, there is the need for an alternative drug delivery system for PGN. PGN is an endogenous steroid that is capable of averting, to some extent, many of post-menopausal related conditions and may be associated with lower rates and severity of adverse effects.

1.1.1. Osteoporosis

Osteoporosis is claimed to affect approximately 28 million Americans and cost $14 billion annually (2001 estimate). The bones are so fragile that fractures occur with little physical stress, including normal daily activities [2]. Figure 1-2 shows the loss of bone density in a femur joint in a woman of postmenopausal age. Such changes in the bone structure, if severe, can lead to osteoporosis [20]. Bone weakness can provide the underlying cause for increased bone fractures, as the bone is no longer able to resist concentrated pressure on certain areas, for example hips and joints. This means that falling and fracture risk can be often strongly linked to postmenopausal aged women. The declining levels of PGN and oestrogen are associated with osteoporosis and often compromising quality of life [16].

4
Chapter 1: Introduction

Figure 1-2. Illustration of bone matrix changes before (left image) and after (right image) menopause, reproduced and adapted from [20].

PGN is a naturally occurring hormone, secreted from the corpus luteum (ovaries) during the latter half of the menstrual cycle and is involved with estrogen down-regulation [16]. It is also involved in gestation during pregnancy and promotes uterine endometrial growth for embryogenesis [16]. Ovarian follicular activity and therefore circulating levels of PGN and estradiol declines with age or following hysterectomy [16]. In Figure 1-3, the PGN levels can be seen to subside dramatically from postmenopausal age [21].
1.1.2. Pathophysiology

Osteoporosis has become a major medical problem as the human lifespan has increased. It is characterized by the loss of bone mass that leads to reduced bone strength and fractures [22, 23]. In the early 1800s, Sir Astley Cooper, an English surgeon, first described “the lightness and softness that (bones) acquire in the more advanced stages of life” [22, 23]. He also noted that “this state of bone, favours much the production of fractures” which is possibly the most debilitating and costly consequence of osteoporosis [22].

Estrodiol (E2) and estrone (E1) deficiency are known to be a contributing factor of postmenopausal osteoporosis [24], and PGN are known to play a role in bone metabolism, affecting bone turnover and promoting bone formation by acting on osteoblasts [24, 25]. Both estradiol and PGN receptors, E2R and PGR, respectively, have been found on human osteoblasts [24]. Although the declining levels of hormonal steroids play a role in bone cell function, it is important to note that there are several interlinking pathways and regulatory mechanisms, which are involved in the development of osteoporosis [22]. Furthermore, the heterogeneity of
Chapter 1: Introduction

Osteoporosis may also be due to receptor transformation, nuclear transcription factors, and enzymes, as well as polymorphism of the vitamin D receptor (VDR) [22]. There has been rapid expansion of information over the last decade, both in clinical and laboratory studies; however, there is still conflicting evidence on what is and what is not clinically important. What is undisputed, is that osteoporosis is a complex, polygenic disorder, and more studies are needed to define specific compounds and genes in osteoporosis pathogenesis [22].

In order to understand how reduced bone formation and increased resorption causes skeletal weakness, it is necessary to have some background information on bone remodelling. The bone remodelling process was first outlined by Frost, et al. (1969) [22], as illustrated in Figure 1-4. The activation of stems cells to form osteoclasts, begins the process, and these cells perform what is known as resorption or breakdown of bone tissue. An interaction with inflammatory cells, e.g. T lymphocytes, as well as hematopoietic precursor stem cells and cells of the oestoblast lineage, activate osteoclastic cells. When osteoclasts have formed, the resorption phase is of limited duration and is quickly followed by the reversal phase. Between the resorption and reversal phases the bone surface is covered with mononuclear cells, however bone re-formation does not start at this point [22]. The period between resorption and reversal phases is short compared with the time required for osteoblast to rebuild bone tissue. This means that an increase in the rate of osteoclastic activity will result in a loss of bone mass. Excessive resorption may also cause loss of trabecular structures, not allowing for a template needed for bone formation [22]. Therefore, a slow bone formation phase during remodelling is a critical part of the pathogenesis of osteoporosis.
The balance between bone breakdown and re-building occurs between numerous factors, as described earlier, such as genetic, mechanical, vascular, nutritional, hormonal and local [27]. PGN is a hormone that has an anabolic effect on bone, directly through osteoblast receptors, and through receptor competition with glucocorticoids [27]. As stated, bone remodeling is also regulated by local factors, such as, IGF-I and II (insulin-like growth factor I and II). These are polypeptides similar in structure to insulin and are synthesized by the liver and oestoblasts [27]. IGF synthesis is regulated by hormones. Growth hormone (GH), estrogens and PGN increase their production, while glucocorticoids inhibit it [22, 27]. PGN also helps to mediate the oestoblast/oestoclast interaction that is actively responsible for bone remodelling. IGF-II is most profuse in the bone matrix and is also the most abundant local factor, however its effects on the skeleton are yet not fully understood [27].

1.1.3. Available treatment options

1.1.3.1. Hormonal therapy

HRT aims to restore estrogen and PGN to pre-menopausal levels in order to reduce symptoms and to protect against the long-term effects of reduced estrogen and PGN levels [1]. Some of the long-term effects that HRT can help protect women against include cardiovascular disease and
osteoporosis. The risk of developing these conditions increases as levels of the hormones fall [1, 4]. HRT may be given in various forms and dosages. A combination of estrogen and PGN is usually given, except in women who have had a hysterectomy [1]. HRT preparations contain a similar combination of hormones to those in the contraceptive pill but in lower strength. HRT is available as tablets, skin patches, creams, gels, intravaginal rings and pessaries. Some women may be unable to use HRT, for example, those with a history of blood clots or breast cancer [1].

The use of oral HRT is the main course of action for postmenopausal women in and outside New Zealand. The primary concern is with synthetic steroids that may cause severe adverse effects including cancers, even with medium term use, for example 5 years. While medroxyprogesterone 17-acetate (Provera) and norethisterone (Primolut N) are some of the most effective hormonal treatments available, they also have a poor side effect record, hence forming addition health problems [2]. This raises ethical issues with the use of medium to long term synthetic steroids for post-menopausal women. HRT should be used at the lowest effective dose for the shortest possible time.

Estrogen can be given in the form of a cream, gel, pessary or vaginal ring to women who are suffering from vaginal dryness and consequent discomfort on intercourse [1, 2, 4]. The main hormones given are estradiol (eg, Estring® vaginal ring, Oestrogel®, Sandrena® gel, Vagifem® vaginal tablets) or estriol (eg, Gynest® cream, Ovestin® cream, Ortho-Gynest® pessary) [1]. Tablets and patches usually contain a combination of estrogen and PGN but may contain oestrogen alone if the woman has had a hysterectomy [1]. Sometimes the hormones are given on a cyclical basis, which can produce a monthly bleed, mimicking natural menstruations [1]. While this is acceptable for premenopausal women with symptoms, it may not be the best method for women who have not had a period for several years [1, 4]. Estradiol is often combined with drospirenone, dydrogesterone, levonorgestrel, orehisterone, norgestrel or medoxyprogesterone [4]. The patches are usually replaced every three to four days and the tablets are taken once daily [1]. Tibolone (Livial®) is a synthetic hormone that may be prescribed to prevent symptoms in premenopausal women [1]. It may also be used as second-line therapy for the prevention of osteoporosis in postmenopausal women who are contraindicated for HRT [1, 2]. A hormone called calcitonin (salmon) (Miacalcic®) may be used in the short term to treat postmenopausal osteoporosis [1].
Chapter 1: Introduction

The main semi-solid transdermal preparations for postmenopausal women in New Zealand is PGN dispersed in non-ionic (aqueous) cream such as Progesterone Cream™ by Pharmaceutical Compounding New Zealand (PCNZ), MyGest® by Young Again, and internationally Serenity® by Wellsprings, but there has been controversial evidence regarding its efficiency. It has been claimed that through transdermal delivery of PGN the following can be achieved; the inhibition of endometrial growth, reduction of the incidence of uterine cancer, inhibition of bone loss, preventing of vasomotor symptoms, reduction in fibrocystic breast disease and breast cancer and increasing the sense of well being. These claims have been peer reviewed and the possible benefits of PGN are generally not in question, there are however questions about the ability of delivery systems to provide sufficient amounts of PGN into the blood stream [14, 28, 29]. There are studies that have shown low plasma level of PGN after transdermal delivery [30-33]. Many other studies have even recorded negative clinical effects with only a few studies showing positive effects particularly on bone density or the vasomotor symptoms [14, 28, 29]. The controversy may be due to the poor permeation of PGN across the skin, therefore, it is essential to optimize the transdermal delivery of PGN using a novel formulation.

1.1.3.2. Non-hormonal therapy

Until recently, hormonal therapy was considered the first line option for the management of osteoporosis, but due to complications associated with long-term use, alternative treatments are being considered. As osteoporosis is associated with postmenopausal age, the treatment focus is often around bone density. There are studies that have found efficacy for bisphosphonates, vitamin D, and calcium in the treatment of osteoporosis.

First-line treatments for the prevention of osteoporosis include a group of drugs known as bisphosphonates, for example, alendronate (eg, Fosamax®), etidronate (as Didronel® PMO), ibandronic acid (Bonviva®) and risedronate (Actonel®). These drugs can also be used to treat established osteoporosis.

Alendronate, the most commonly used bisphosphonate, was associated with a 2.3% mean increase in spinal bone mineral density (BMD) compared with a mean loss of 3.2% in the placebo group [34]. The mechanism of action involves the inhibition of osteoclast-mediated bone-resorption, thus reduction of bone density loss. In another study using a double-blind,
placebo-controlled groups, BMD in the tibia showed a significant difference between treatment groups after 24 months (p-value < 0.05) [35]. Although the evidence for alendronate is positive, it is unfortunately not without its side effects, some of which can be severe. The major side effect is ulceration of the esophagus, however the risk of this can be reduced by swallowing while upright for 30 minutes with a full glass of water. The more general side effects include constipation, nausea, skin rash, and muscle pain [34].

Cholecalciferol (vitamin D₃) is the third and most active form of vitamin D that significantly reduces the progressive nature of osteoporosis. In an analysis of postmenopausal women, the relative risk (RR) for vitamin D₃ preventing fall injury was 0.92 and in preventing non-vertebral fractures the RR was 0.81 [36]. Based on the results of this study it was found that there is a reduction in the risk of fall injury among patients treated with vitamin D₃ alone compared with placebo. It has been suggested that vitamin D₃ should have a primary role in effective osteoporosis management [36]. In a systemic review of 167 research articles containing the highest quality of randomised controlled trials (RCTs) from vitamin D₃ efficiency for prevention of fall fractures in older adults [37]. It was concluded that the evidence was fair from the studies showing an association with vitamin D and bone health outcomes, specifically BMD loss and fall injuries.

There are several studies that have found calcium to slow bone degradation in aging women and in some studies also increase bone density. Although calcium is one of the easiest and inexpensive strategies for preventing osteoporotic fractures, calcium supplementation is nevertheless controversial [38]. The conclusion of one study involving calcium supplementation was that calcium alone has a small positive effect on bone density. In 1,806 participants, calcium was more effective than placebo in reducing rates of bone loss after two or more years of treatment. The pooled difference in percentage change from baseline was 2.05% for total body bone density [38]. In another study, BMD was significantly linked with dietary calcium intake (p-value < 0.02) [39]. These data support the hypothesis that calcium intake is a determinant of skeletal health in postmenopausal women [39]. The Food and Drug Administration (FDA) in the US has permitted a bone health claim for calcium-rich foods, and the National Institute of Health (NIH) approved the statement that high calcium intake reduces the risk of osteoporosis [38].

So far, the listed treatments have been presented separately, however in practice a mixture of these treatments is often used. In one study using both alendronate and vitamin D₃
there was a significant increase in calcium absorption of 0.07 ng·mL$^{-1}$, from a baseline reading of 0.31 ng·mL$^{-1}$ [40]. Although there are no other studies showing such a marked response from this combination and the exact mechanism is not fully understood. Another study showed alendronate in combination with vitamin D and calcium increased lumbar spine BMD by 5.2% at 48 weeks compared with an increase of 1.3% in subjects receiving vitamin D and calcium alone [41]. A combination product containing alendronate and vitamin D$_3$ (Fosamax Plus®) is available and is typically used alongside calcium supplements to prevent bone loss [1].

Furthermore, one of the main complaints from postmenopausal women is hot flushes, therefore many non-hormonal treatments have focussed on this symptom. Several recent studies have found efficacy for certain antidepressants and anticonvulsants in the treatment of hot flushes [42-44]. Paroxetine, a selective serotonin-reuptake inhibitor (SSRI) was shown to decrease hot flash scores by 62% (using 12.5 mg·day$^{-1}$) and 65% (with 25.0 mg·day$^{-1}$) compared to the placebo [45]. Venlafaxine, a serotonin-norepinephrine reuptake inhibitor, has also shown efficacy in treating hot flushes [46]. In this study the treatment group showed reduced hot flush scores of up to 60%, while the placebo score was 27% from baseline [46]. It was also shown that venafaxine improved sleep quality and overall mental health [47]. Adverse effects of these antidepressants include nausea, headache, dry mouth, somnolence, decreased appetite, and insomnia [47].

Gabapentin is an anticonvulsant, that has significantly reduced hot flushes in some patients [42]. A controlled study of 59 women found lower hot flush frequency in 45 of the women, compared with 29 in the placebo [42]. Although the mechanism is not fully understood, this gamma-aminobutyric acid derivative is thought to modulate the hypothalamic calcium channels, which in turn controls the thermoregulatory set point [43, 48]. Side effects of this compound include dizziness and peripheral oedema.

Clonidine, an alpha-adrenergic agonist, helps hot flushes by increasing the thermoregulatory sweating threshold. Two placebo-controlled studies found that oral clonidine reduced hot flushes by 46%, while transdermal clonidine reduced it by 80% [49, 50]. There have been two large studies on patients receiving tamoxifen that showed significant reductions in hot flushes for oral and transdermal clonidine compared with placebo [51, 52]. Side effects of clonidine include hypotension, dry mouth, and sedation [52].
Chapter 1: Introduction

1.1.3.3. Non-drug therapy

There are numerous alternatives to medication, many of which have little or no evidence base, but are rational and may help to some degree. Although not necessarily related to the treatment of osteoporosis, these approaches are generally useful to postmenopausal women. The most obvious approaches are to exercise, avoid caffeine and nicotine, attend relaxation classes such as yoga, tai chi, or meditation [53, 54]. Evidence from RCTs conclude that aerobic exercise can improve vasomotor and other menopausal symptoms as well as improving psychological health and quality of life [53]. Other non-RCT studies have also found significant amelioration of vasomotor symptoms using relaxation procedures [55, 56].

Various natural remedies have been considered for reducing hot flushes including vitamin E, Menoquil, Remifemin, and ginseng [57-60]. Unfortunately, there are mixed results from many of these alternatives with some showing large beneficial changes, while others show no improvement [61]. Generally the natural remedies show low frequency of adverse effects such as ginseng and wild yams, while others such as oxitriptan have common adverse effects such as dry mouth and nausea and vomiting, and may cause blood abnormalities [61]. Some studies have also been incomplete due to significant adverse effects, where over 20% of participates have been reported as withdrawing from studies. Another general disadvantage of natural remedies is the lack of information regarding possible drug interactions, rendering them of limited use for those women on polypharmacy, especially while taking warfarin or digoxin.

Isoflavones have estrogenic properties and are found in many soy products and red clover [62]. Black cohosh is another plant-derived substance used to treat hot flushes. However, a moderately sized “review of 22 controlled studies, 12 on soy and 10 on other botanical compounds, found no consistent improvement of hot flushes relative to placebo” [62].

Other possible considerations are to drink less or even avoid alcohol, spicy foods, and dress lightly and in layers [63]. Furthermore, it is in general, regardless of age, a long-term advantage to eat a low-fat diet, monitor and control, if required, blood pressure, moderate exposure to sunlight (vitamin D₃ relationship), and finally moderate cholesterol intake [63]. In one study, BMD was significantly associated with body weight (p-value < 0.01) [39]. Therefore, generally watching dietary intake and ensuring regular exercise are important for women of postmenopausal age.
1.2. The New Zealand Situation

In New Zealand (NZ), there is an aging population of which there are approximately 578,500 men and women aged 65 years, which has grown from 12% of the population to 13% one decade later (ending December 2010) [64]. Women in this age group slightly outnumber men but this difference is at 5 to 2 in those 85 years or older. The elderly proportion of the NZ population is estimated to be more than 25.5% by the year 2051, and is predicted to be upwards of 627,000 women aged 65 years or older [65].

There are numerous empirical reports in NZ on menopause and bone density loss. There were an estimated 84,000 osteoporotic fractures in 2007, with more than 60% of these occurring in women and the total cost of osteoporosis is estimated to be over $1.15 billion per year [66]. Not all of these figures can be attributed to menopause, however it is reasonable to conclude that menopause is a significant contributing factor in bone density loss and fractures.

1.2.1. The legal status of PGN

From a legal perspective, endogenous PGN has an interesting position in New Zealand. Under the Medicines Act 1981, a 'medicine' is defined in Section 3 of the Act as: ‘any substance or article, other than a medical device, that is manufactured, imported, sold, or supplied wholly or principally - For administering to one or more human beings for a therapeutic purpose; …’ [67].

PGN is not marketed or registered in New Zealand for a therapeutic purpose related to any post-menopausal symptoms. The only formulation registered as a medicine containing bioidentical PGN is Crinone 8% which is a vaginal gel used for in vitro fertilization (IVF) and assisted reproductive technology (ART). The PGN injection (Gestone) is indicated for the treatment of dysfunctional uterine bleeding and various treatments of infertility [68]. The PGN capsule (Utrogestan) 100 mg (by Pharmaco NZ Ltd) has provisional consent for adjunctive use with oestrogen replacement therapy where there is an increased risk of endometrial cancer [68]. There are no other PGN containing brands or formulations listed as registered medicines for HRT with MedSafe [67]. An alternative way to register PGN and its various formulations would be to list it as a 'herbal remedy' as defined in Section 2 of the Act. However, PGN is wholly extracted from plants into a fine powder, and therefore cannot be a herb, which typically consists of several if not hundreds of other various molecules. This means creams or ointments
containing PGN for HRT purposes come under the provisions called ‘Section 25’ and ‘Section 29’ of the Act, which pertain to the prescribing and dispensing practices, respectively, of an unregistered substance. Under these provisions PGN is classified as a prescription medicine except in medicines containing 1 mg or less per litre or per kg of PGN [68].

Furthermore, this ambiguous classification for unmodified PGN may be clarified under unified legislation between Australia and New Zealand. It was proposed in 2003 that the regulation of complementary and alternative medicines in Australia and New Zealand would be managed under a Joint Therapeutic Goods Administration (JTGA) or Australia New Zealand Therapeutic Products Administration (ANZTPA). This would have drawn a line in terms of placing endogenous PGN into a registered drug class, however too many differences in regulations hampered this goal and changing governments has resulted in a halt in progress. In August 2007, Minister Annette King announced that NZ would no longer proceed with any joint legislation at this stage and postponed any work on it until a sensible and acceptable compromise can be put in place by all parties [69]. In Australia the Therapeutic Goods Administration (TGA) has currently listed natural PGN in the same drug class (S4) as artificial PGNs.

1.3. Progesterone

PGN, also known as pregn-4-ene-3,20-dione, is a C-21 steroid hormone involved in the female menstrual cycle, pregnancy (supports gestation), and embryogenesis of humans. In this thesis, PGN has been used to provide a possible platform for other steroids. PGN belongs to the steroid family that all contain the cyclopentanoperhydrophenanthrene nucleus system and predominately synthesised from cholesterol derivatives originally present in the liver [70]. It is important to note that all hormonal steroids are formed from cholesterol at various sites throughout the human body. Figure 1-5 shows the numbering system for cholesterol according to the International Union of Pure and Applied Chemistry (IUPAC) system, which is the same numbering system used for all steroids including PGN [71].
Figure 1-5. Molecular structure and the IUPAC numbering system for cholesterol. The same numbering is used for all steroids including PGN [71]. The letters (a), (b), (c), and (d) represent the four cycloalkane rings.

Figure 1-6(a), shows the molecular structure of PGN, together with several other steroids showing the relative similarities and differences of each structure [70-72]. PGN contains two ketones, an oxygenated functional group on carbons 3 and 20, two methyl branches between rings A and B, and C and D, on carbons 10 and 13, respectively [71]. PGN is a naturally occurring hormone, as described earlier, and is secreted from the corpus luteum (ovaries) during the latter half of the menstrual cycle [16]. A major source of PGN outside of the human female comes from diosgenin, extracted from Mexican yam root. It is identical in chemical structure to endogenous PGN of the human ovary and placenta [16].

Figure 1-6. Molecular structure of (a) PGN and other steroids;
Chapter 1: Introduction

(b) estradiol, (c) medroxyprogesterone acetate, (d) betamethasone-17-benzoate, (e) testosterone, and (f) 5-Dehydroepiandrosterone (DHEA). Adapted from various sources [70, 71].

1.3.1. Physicochemical properties

PGN has a relatively small molecular size (314.5 g·mol⁻¹) [73], excellent solubility in octanol with ≥ 90% v/v [74], a logP of 3.5 [75], and poor aqueous solubility (≤ 7 µg·mL⁻¹) [76]. The melting point of PGNs polymorph Form I is approximately 131°C, while Form II is approximately 120°C [77]. Form I of PGN is not moisture or light sensitive and is very stable. PGN has no ionisable functional groups and is one of the least aqueous soluble endogenous steroids.

1.3.2. Mechanism of action

PGN exerts its primary action through the intracellular PGN receptor, although a distinct membrane bound PGN receptor has also been postulated [78, 79]. In addition, PGN is a highly potent antagonist of the mineralocorticoid receptor (MR), the receptor for aldosterone and other mineralocorticosteroids [26]. It prevents MR activation by binding to this receptor with an affinity exceeding even those of aldosterone and other corticosteroids such as cortisol and corticosterone [26, 80].

PGN has a number of physiological effects that are amplified in the presence of estrogen [26, 81]. Estrogen through estrogen receptors upregulates the expression of PGN receptors [82]. Also, elevated levels of PGN potently reduce the sodium-retaining activity of aldosterone, resulting in natriuresis and a reduction in extracellular fluid volume [26]. PGN withdrawal, however, is associated with a transient increase in sodium (Na²⁺) retention (reduced natriuresis, with an increase in extracellular fluid volume) due to the compensatory increase in aldosterone levels, this in turn helps contend with the mineralocorticoid receptor blockade by the previously higher levels of PGN [26, 83]. PGN exerts an antiovulatory effect when administered during days 5 to 25 of the normal menstrual cycle [84].

The kidney has also been investigated as a site of PGN receptors, which has hormone influence on electrolyte physiology, in particular calcium (Ca²⁺) reabsorption [85]. In one study, the distal tubules (DT) treated with PGN enhanced calcium uptake from 0.60 to 0.84 pmol·µg⁻¹ per 10 seconds (p-value < 0.05) [85]. This has implications for osteoporosis, as described earlier in section 1.1.1.
Chapter 1: Introduction

1.3.3. Pharmacokinetics

Depending on the route of administration, oral or parenteral (vaginal, intramuscular, transdermal), PGN can manifest different effects that are due to differences in metabolism [86]. Many of the metabolites are formed in the liver and are excreted via the urine. PGN is metabolised in the liver by cytochrome P450 (or CYPs) 2C9, 2C19, and 3A4 causing oxidation. PGN can also be metabolised in the gastrointestinal tract (GIT) tract via 3-beta-hydroxysteroid dehydrogenase, and in the skin via 5-alpha reductase [87-89]. Allopregnanolone and pregnanolone are major metabolites of PGN and act through binding to the GABA<sub>A</sub> receptor in the brain where they contribute to reducing neurotransmission [90]. One study that evaluated the pharmacokinetics of oral PGN, indicated that a high proportion of PGN was rapidly broken down to allopregnanolone via hepatic metabolism [90]. High concentrations of allopregnanolone and pregnanolone have been reported to have anxiolytic, sedative, anaesthetic and antiepileptic effects when administered to animals and humans [90]. In women with premenstrual dysphoric disorder, the deterioration of physical and psychological symptoms is related to an increase in PGN and allopregnanolone concentrations during the late luteal phase of ovulatory cycles [90, 91]. GABA<sub>A</sub> receptor sensitivity to neurosteroids decreases with extended or repeated exposure. Therefore, pregnanolone and allopregnanolone may have more pronounced effects in postmenopausal women than in women with ovulatory cycles.

The half life of PGN can vary greatly, depending of the ROA, but a common range is between 34.8 and 55.1 hours [91]. In one study, after the initial administration of 50 mg or 100 mg in a vaginal tablet form, a maximum serum concentration (C<sub>max</sub>) of 20.43 nmol·L<sup>-1</sup> and 31.61 nmol·L<sup>-1</sup> (p-value < 0.05) was reached after time taken to reach C<sub>max</sub> (t<sub>max</sub>) of 6.1 and 6.4 hours, respectively [92]. The terminal half-life was 13.1 and 13.7 hours, respectively [92]. In another study measuring percutaneous absorption, the application of a PGN (30 mg/day) cream to the skin over 4 weeks gave variable results. Serum PGN concentrations among the women participants gave mean concentrations ranging from 1.6 to 3.3 ng·mL<sup>-1</sup>, while the terminal half-life in circulation was measured as 19 minutes [31]. The variability can also be explained by the effects of biphasic elimination. It has been demonstrated that substantial amounts of PGN appears to be present in human adipose tissue [85]. The first phase of elimination can occur rapidly over a few hours, which may represent the unbound PGN free in circulation, while the
second phase occurs much later perhaps has much as 60 hours, which represents the PGN protein bound and stored in adipose/fatty tissues.

There are also differences in binding of PGN to steroid binding proteins in systemic circulation [86]. Protein binding of PGN can be as high as 96 – 99%, but for specific proteins; such as sex hormone-binding globulin (SHGB) there is no binding while dihydrotestosterone and cortisol bind approximately 100% and corticosteroid-binding globulin (CBG) has approximately 36% PGN binding [86].

1.3.4. Adverse effects

PGN, whether it is the endogenous form or a synthetic progestin, taken orally, at relatively high doses can have various adverse health effects. For example, at doses of 400 mg PGN can cause increased fluid retention, migraine, asthmatic attack, cardiac and renal dysfunction. Blood clots that can result in strokes and heart attacks, leading to death or long-term disability; pulmonary embolus or breast cancer can also develop as a result of high doses or long-term progestogen therapy [4, 93]. Progestogens are associated with an increased risk of thrombotic disorders such as thrombophlebitis, cerebrovascular disorders, pulmonary embolism, and retinal thrombosis [1, 6]. Common adverse effects of progestogens include cramps, abdominal pain, headache, constipation, diarrhea, nausea, vomiting, breast enlargement, joint pain, flatulence, hot flushes, decreased libido, thirst, increased appetite, nervousness, drowsiness, excessive urination at night [1, 4, 6]. Psychiatric effects include depression, mood swings, abnormal crying, insomnia, forgetfulness, sleep disorders [1, 6, 93].

1.3.5. Routes of administration

The most common route of synthetic PGN administration is orally and absorption occurs in the small intestine [94]. From the intestinal tract, PGN goes into the splanchnic circulation before flowing into the liver [95, 96]. The use of progestins are not popular due to their undesirable effects and variable efficacy [94]. Numerous studies have found PGN can be readily absorbed through several other routes, including transdermally, subdermally, nasally, intramuscularly, sublingually, vaginally or rectally [94, 97, 98].
Chapter 1: Introduction

1.3.6. Oral pharmaceuticals and limitations

Oral PGN is subject to pre-systemic metabolism in the gastrointestinal tract and liver, along with poor aqueous solubility, which limits the amount of PGN absorption. This all means the oral administration of PGN has poor bioavailability and variable blood levels [14]. Hence there is the need for an alternative drug delivery system for PGN. Delivery of drugs through the skin offers several advantages over the conventional routes of administration (ROA) such as the oral and injection routes. These advantages include bypassing metabolism by the liver and GIT, avoiding the invasiveness and increased risk of infection from injections, improved regimen adherence and less frequent and severe side effects [99]. A number of drugs have been delivered through the skin, such as scopolamine [100, 101], nicotine [102-104], PGN [16, 93], estradiol [105, 106], and testosterone [107].

The use of steroidal hormones such as PGN for the control of climacteric symptoms has pharmaceutical implications [108]. It has been proposed that delivery through the skin is the more suitable ROA for PGN. As with other drugs, the delivery of steroidal hormones into the human body requires physical and chemical knowledge of the target drug and ROA. Before the available transdermal strategies are overviewed, it is important to have an understanding of the skin’s structure and function.

1.4. The Human Skin

The skin covers approximately 10% of the total body mass, making the skin the largest organ of the human body. As shown in Figure 1-7, the skin consists of three layers: epidermis (containing the stratum corneum - SC), dermis, and subcutaneous tissues [109]. There are also several associated appendages: hair follicles, sweat ducts, apocrine glands and nails. The skin functions can be generally classified as protective, homeostatic, and sensing. This organ is in a state of constant regeneration and repair [109]. The skin is also a means by which medication can be applied to deliver local (known as topical delivery) and/or systemic effects (known as transdermal delivery).
There are eccrine and apocrine sweat glands that exist as skin appendages on humans. The eccrine glands (2 – 5 million) secrete sweat (pH 4.0 – 6.8) which may contain proteins, antibodies/antigens and drugs [97]. The principle job is to help control heat and sweat induced from emotional stress (the clammy palm syndrome) [111]. The apocrine sweat glands form with pilosebaceous follicles through puberty into adulthood in the armpit (axilla), breast areola, and perianal area [97]. The oily sweat contains proteins, lipids, lipoproteins, and saccharides. Surface bacteria are responsible for metabolizing this odourless sweat into personal body odour [112].

Hair follicles form all over skin except the lips, palms, soles, and some parts of sex organs. Sebaceous glands are found with hair follicles just above the muscle tissue. The hair follicle is limited as a route for drug delivery because there are too few (absorption area is about 0.1% of total surface area) [97, 113].

In summary, the structure of the skin consists of several layers and this can restrict passage of drug penetration [111]. The external layer, or SC, contains further layers with mainly hydrophobic and some hydrophilic areas providing a relatively successful barrier to drug molecules and micro-organisms. The SC layer is generally the most defensive region and will now be discussed in more detail in the following section.
Chapter 1: Introduction

1.4.1. *The stratum corneum*

The SC to hypodermis (at 0.4 - 5 mm thick) consists of a protein matrix (collagen, elastin, reticulin, and fat) networked in an amorphous substance known as mucopolysaccharide [97]. The SC is the outer most layer of the epidermis and is the major barrier for drug absorption. It consists of between 15 to 20 layers of flat, anucleated, polyhedral-shaped dead cells, known as corneocytes [114]. These corneocytes are arranged in the so called bricks and mortar arrangement (see Figure 1-8), where the keratin rich corneocytes (bricks) are embedded in the intercellular lipid-rich matrix (mortar). The water content of the SC is at a minimum of 30% and maximum content of 50% [115].

The corneocytes regions (or bricks) are considered to be dead (the nonviable epidermis as it has no blood supply), with very little lipid content. Their major structural components are aggregates of keratins arranged as bundles of individual keratin filaments [116]. The most important chemical aspect is the sulphur-containing diamino acid cystine. Keratins are a family of alpha-helical polypeptides ranging from 40,000 to 70,000 Daltons [117]. The major keratin existing in SC is alpha-keratin [118]. These components make this layer of corneocytes dense and relatively impervious to external compounds.
The intercellular lipid matrix (or mortar) is different to other biological membranes. The lipids do not contain the usual phospholipids, rather are mostly composed of ceramides, cholesterol, cholesteryl esters, fatty acids, and a small fraction of cholesterol sulphate [117]. The lipid breakdown of the SC is shown in Table 1-1.
Table 1-1. Lipid composition of the human stratum corneum [117].

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Amount in SC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesteryl esters</td>
<td>10</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>-</td>
</tr>
<tr>
<td>Fatty acids</td>
<td>9.1</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>26.9</td>
</tr>
<tr>
<td>Ceramide 1</td>
<td>3.2</td>
</tr>
<tr>
<td>Ceramide 2</td>
<td>8.9</td>
</tr>
<tr>
<td>Ceramide 3</td>
<td>4.9</td>
</tr>
<tr>
<td>Ceramide 4</td>
<td>6.1</td>
</tr>
<tr>
<td>Ceramide 5</td>
<td>5.7</td>
</tr>
<tr>
<td>Ceramide 6</td>
<td>12.3</td>
</tr>
<tr>
<td>Glucosylceramides</td>
<td>-</td>
</tr>
<tr>
<td>Cholesteryl sulfate</td>
<td>1.9</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>-</td>
</tr>
<tr>
<td>Others</td>
<td>11.1</td>
</tr>
</tbody>
</table>

The ceramides are the major group of lipids, which may help reduce the epidermal water loss [120]. Furthermore, the ceramides are important for the adhesion properties of the corneocytes [121]. Ceramides (cera = Latin for wax) consist of a fatty acid and C_{18} amino alcohol, known as a sphingosine. Many of the lipid species that form the bilayer structure of the SC differ in type and chain length, as shown in Figure 1-9.

Early research suggested that lipids surrounded keratin filaments [122, 123]. It has been reported that the extracellular lipids between the corneocytes are arranged in multiple lamellar structures that form continuous lipid phases occupying approximately 20% w/w of the total SC [124]. This may be due to the polar groups of ceramides which are capable of forming hydrogen bonding [114]. The cholesteryl sulphate can further help stabilize the lamellar structure found in the SC [125].
Chapter 1: Introduction

Figure 1-9. An idealized representation of the bilayers within the intercellular lipid regions of the stratum corneum. Reproduced with permission from John Wiley and Sons [119].

The lamellae regions are oriented parallel to the surface of the SC [126, 127]. Research provided by small angle X-ray diffraction shows that the intercellular lipids in the SC form two lamellar phases that are 6 and 13 nm apart [128-131]. Wide angle X-ray diffraction studies have disclosed that human SC is mostly made-up of crystalline lipids which have orthorhombic lateral alkyl chain structure. SC is also described as a gel-like phase that has a loose hexagonal arrangement [127, 132].

1.4.2. Drug delivery routes

There are several main routes of drug penetration (1) transepidermal, which is across the epidermis and can be further broken into sub-groups: 1(a) transcellular – through epidermal cells and 1(b) intercellular – between cells across the lipid domain, and (2) transappendagenal via the sweat ducts/pores and through hair follicles with their associated sebaceous glands [97]. Figure
Chapter 1: Introduction

1-10, gives a diagram of the skin structure and main routes of penetration. The follicle route may be important for ions and large molecules that cross the SC with difficulty [109].

The SC and dermis are the most predominant routes of drug absorption. Blood vessels, nerves and lymph nodes intersect the dermis matrix. The blood supply comes within 0.2 mm of the skin’s surface and the large blood volume acts as a ‘sink’ for diffusing drug molecules [97]. Usually the deeper dermal layers do not affect drug absorption, although some drugs, for example the non-steroidal anti-inflammatory family may reach as far as the muscle [133]. The dermis may also bind other molecules, such as testosterone, decreasing systemic absorption [97]. If a drug is very lipophilic in nature, then as it meets the dermis, which is more aqueous than the SC region, the partitioning can be dramatically reduced, affecting permeation into systemic circulation. As the drug passes from the SC to viable layers the concentration gradient falls, thus affecting flux across the skin [111]. The rate determining step in absorption can then become dermis clearance, rather than barrier penetration [97].

Figure 1-10. The routes of drug penetration through the human stratum corneum (SC); (a) sweat-pores, (b) across the SC, and (c) the hair shafts. Reproduced with permission from John Wiley and Sons [119].

The thin, tough, and generally impermeable nature of the SC makes this layer a major rate-limiting barrier in transdermal drug delivery [97, 134]. The entire epidermal layer actually
Chapter 1: Introduction

provides diffusional resistance and does not allow drugs to pass easily. Low molecular weight agents (e.g. < 500 g·mol⁻¹) have a better ability to pass through the skin with little resistance. The lipid bilayers of the intercellular pathway offer the main route of absorption [97, 113]. Diffusion is passive, controlled by physicochemical properties of the drug. Once passed through the dermis, drugs pass through into systemic circulation.

1.4.3. Factors affecting transdermal drug delivery

Although the skin is advantageous over the oral route for the delivery of drugs such as PGN, it is not without its problems, due to the defensive structure and function of the skin as well as physical-chemical properties of a drug. The level of drug absorption across the skin can be affected by several factors:

a. the physicochemical nature of the drug, such as:
   i. molecular size, particle size and distribution, and MP,
   ii. solubility, ionisation (pKa), and crystalline form,
   iii. partition coefficient (lipophilicity/hydrophilicity);

b. the skin condition (i.e. age, hydration, temperature, blood flow, and pH);

c. the formulation (e.g. gel, patch, cream or roll-on);

d. and the ability of various penetration enhancers to temporarily disrupt the SC.

Various functions of the skin, such as secretion of oils and enzyme activity, adds to the skin’s arsenal against foreign material, which must be overcome in order for drug administration to take place [135, 136]. Many drug molecules are hydrophobic in nature, such as PGN, hence display a limited solubility in an aqueous based transdermal formulation, but this same drug property allows them to pass easily through most of the skins layers, which can be the rate limiting step in the path of a drug into the human body. Following is an outline of the possible strategies used to overcome the limitations of transdermal drug delivery.

1.5. Transdermal strategies

Various strategies for transdermal drug delivery have been developed including the use of chemical penetration enhancers [137, 138], preparation of supersaturated drug delivery systems [139], electrically driving molecules into or through the tissue using iontophoresis [140] or
Chapter 1: Introduction

physically disrupting the skin structure, for example by electroporation or sonophoresis [141, 142]. Vesicular drug delivery systems may provide another possible alternative [143]. The strategies involving the formulation of a drug, to name a few, such as using solvents and co-solvents, liposomes, or reducing particle size, have been described as pharmaceutical, while using electrical or sonophoresis methods have been listed as physical strategies. The pharmaceutical strategies are described later in Section 1.8, and the physical strategies are outlined in more detail below. Finally the use of solid dispersions as a possible method to formulate a transdermal delivery system for PGN will be introduced.

1.5.1. Physical strategies

1.5.1.1. Iontophoresis

The electrical driving of charged molecules into tissue is known as iontophoresis. This strategy has been used for ionic drugs, proteins and peptides [144, 145]. Iontophoresis is an effective way to physically facilitate the transport of solutes across skin for both local and systemic effects [145, 146]. The general practise is to pass a low current (approximately 0.5 mA·cm⁻²) through a drug solution containing an electrode directly above it, which is intact with the skin [97]. The electrode placed above the drug solution carries the charge. Another electrode is placed somewhere else on the body to complete the circuit, this is known as grounding. The driving mechanism is by electrical repulsion of the charged molecules from the drug loaded electrode. The electromigration has been shown to depend on molecular weight, where small ions (e.g. Sodium) being transported primarily by repulsion. Larger molecules (over 1,000 Da) undergo migration via electro-osmosis where the movement across the skin involves convective solvent flow that is current induced [97]. The main pathways of transport by iontophoresis are the transappendageal and paracellular routes [147]. A problem with this method is that the current concentrates via the low resistance area such as hair follicles [97]. This may be enough to damage growing hair and cause irreversible conformational changes in the skin peptides [142, 148, 149]. However, improved technologies and the combination of other enhancement techniques may result in the need for less intense levels of current, thus reducing the side effect problems [150, 151].
Chapter 1: Introduction

1.5.1.2. Electroporation

Transdermal delivery of drugs using electroporation is another way to help permeation through the skin. The use of electroporation for drug delivery is relatively new and is being actively researched [142]. It is similar to iontophoresis in that it uses a current. The technology involves the formation of aqueous pores in the lipid bilayers by applying rapid (micro- and milli-second) electrical pulses (100 – 1,000 V·cm⁻¹) [97]. The opening/closing of most protein channels is known to depend in some way on the transmembrane electric potential, and the gating potentials of these channels are around the 50-mV [152]. By applying a large voltage to the skin, these channels allow passage of ions and water, forming reversible aqueous cavities [153]. These pores expand in the 100 µs time frame to reseal (incompletely) within milliseconds. This time frame is shown to be sufficient for drugs to be transported through the skin [152]. The main mechanisms by which drug transport is reached are electrorepulsion, electro-osmosis, pressure and diffusion [154, 155]. Flux has been recorded as being increased up to 10,000 fold for charged molecules [97]. Electroporation has been used for a wide range of molecules including the permeation of peptides such as vasopressin, LHRH, and calcitonin [97].

1.5.1.3. Sonophoresis

The first published report on low frequency (typically up to 100 kHz) sonophoresis for transdermal drug delivery dates back to the 1950s [156]. Sonophoresis, also known as ultrasound, has been used for anaesthetics, proteins and peptides, including insulin and low molecular weight heparin (LMWH). This technique involves application of a formulation over the skin followed by ultrasonic energy. This method is gaining more traction over the last 25 years but is primarily only used in physiotherapy and sports medicine [97]. Significant attention has been dedicated to understand the mechanisms of sonophoresis [156]. Adding the ultrasonic source to the skin disrupts the lipid packing in the intracellular spaces of the SC. This forms cavities called localised transport pathways (LTP) which is how drugs can be transported across the skin [157].

General agreement has been reached that acoustic cavitation, the formation and collapse of gaseous cavities, is responsible for low-frequency sonophoresis [158]. The radius of the cavitation bubbles is related to the frequency and acoustic pressure amplitude [156, 158]. The maximum bubble radius is estimated to be between 10 and 100 µm [158]. Two types of
cavitation, stable or inertial, have been evaluated for their role in sonophoresis. Stable cavitation relates to the periodic growth and oscillations of bubbles, while inertial cavitation is the violent growth and collapse of cavitation bubbles [158].

In the clinical setting there are two main ultrasound procedures used. The first is simultaneous sonophoresis where both the formulation and ultrasound are applied to the skin at the same time [156]. The permeation rate is correlated to the ultrasound intensity and frequency. The second procedure is known as pretreatment sonophoresis where the ultrasound is used prior to the application of the formulation. The permeability of the drug occurs over 1 – 3 hours continuously without the need for further ultrasound [156]. The main problem with sonophoresis is the need for an ultrasonic probe and equipment [97]. Furthermore, use of the ultrasound requires technical skill such as learning to focus the probe correctly in the SC.

1.5.1.4. Microneedle array

The SC barrier can be bypassed by micro-injection. This strategy employs titanium or solid silicon sheets with numerous needle like projections (up to 400 microneedles) on the surface of a patch or similar device [159]. The needles are simply used to penetrate the skins surface [97]. Forming microscopic pores allows the passage of macromolecules including proteins and DNA vaccines [160]. The ‘poke with patch’ approach allows the transport of drugs via diffusion or possibly iontophoresis if an electric field is applied [160]. Another approach is ‘coat and poke’ where the needles are first coated with a drug and then inserted into the skin. The skins surface has no drug reservoir and all the drug to be delivered is on the microneedle [160]. A variation on the ‘coat and poke’ approach is the ‘dip and scrape’ technique, where microneedles are first dipped into a drug solution and then scraped across the skin surface [160]. This action leaves behind drug within microabrasions created by the tips of the microneedles. Flux is claimed to increase almost 100,000 times [97]. Proper needle design can assure insertion into the skin preventing needle fracture or pain [160]. Using solid microneedles, Martanto, et al. (2003) delivered insulin to diabetic hairless rats, with arrays of 105 needles measuring 1,000 µm in length forming a sharp microscopic tip [160, 161]. Over a 4 hour period, blood glucose levels steadily decreased by as much as 80% [160, 161]. Microneedles containing a hollow core, compared to the solid needles discussed above, offer the possibility of transporting drugs by diffusion or, for more rapid rates of delivery, by pressure driven flow [160].
Chapter 1: Introduction

1.5.1.5. High-velocity particles

The PowderJect system shoots particles through the SC into the dermis using helium gas at supersonic speeds (approximately Mach 2-3) [97]. This is a painless way to deliver pharmaceuticals through the skin and in some cases, requires less than 1% of drugs delivered by traditional means. Injection requires that drugs or vaccines be formulated as microscopic solid particles, which are sent through a gas jet inside the hand-held PowderJect device [162]. Various molecules including; peptides, proteins or genes, can be used according to their particle parameters relating to size, mass, and density [163]. Unfortunately there are problems with bruising and uncontrollable loss of particles due to bouncing off the skin surface [97]. Similar devices such as Intraject have been used to deliver vaccines using a liquid rather than particles.

1.5.2. Pharmaceutical strategies to permeation enhancement

Where there is a membrane present for a drug to pass through, a mathematical representation can be drawn. Once the passage of a drug has reached steady-state, the rate of drug permeation across the membrane can be calculated. The rate in which a drug passes through the skin (known as flux, \( J \)) can be described as Equation 1 and is derived from Fick’s first law [113]:

\[
J = \frac{dm}{dt} = \frac{D \cdot C_0 \cdot K}{h} \tag{1}
\]

where, \( \frac{dm}{dt} \), represents the amount of permeant movement across the skin as a function of time, \( C_0 \) is the concentration of the diffusing material just within the membrane surface (usually taken as the concentration in the vehicle), \( K \) is the skin/vehicle partition coefficient, \( D \) is the diffusion coefficient of the drug in the membrane, and \( h \) is the diffusional path length across the skin (skin thickness) [113]. The three main permeation enhancement strategies that have been based in Fick’s first law are: a) increase the \( D \), b) increase drug partitioning into the membrane (\( K \)), and c) increase the degree of saturation of the drug in the vehicle (\( C_0 \)) [113]. In Figure 1-11, some of the varying approaches are shown to give the expected changes in skin permeation of a drug.
Figure 1-11. Graphic representation of drug permeation across the skin from;
(a) standard vehicle, (b) the increase in diffusion coefficient by four-fold using a penetration enhancer, (c) increasing solubility of a drug in the skin, and (d) changing the saturation of a drug in a vehicle. Both (c) and (d) have an effect on the partition coefficient into the skin. Reproduced with permission from Elsevier [113].

Changing the saturation of a drug in a vehicle is based on the interaction between the drug and the vehicle; the other two strategies are about the effect of the vehicle on the barrier function of the SC. For example, using a penetration enhancer to disorder the SC intercellular lipids or by solvating lipid components [113]. A flow diagram of these strategies has been represented in Figure 1-12. This figure considers the influence of penetration enhancement on the diffusion coefficient, solubility of a drug in the SC, and the use of a supersaturated vehicle to increase diffusive forces for drug permeability across the skin.
1.5.2.1. Improving diffusion coefficient

The diffusion coefficient can be changed by disordering the SC lipid bilayer. Using penetration enhancers (PEs) such as fatty acids is a common tool used to increase skin permeation [113]. Enhancing penetration is achieved by the incorporation of the enhancer molecule at or near the polar head groups of the lipid bilayer, or between the hydrophobic regions of the SC lipids as illustrated in Figure 1-13 [164].

(a) at or near the polar head groups and/or (b) between the hydrophobic tails of the bilayer. This also illustrates the change from relative order (as a normal physical state of the intercellular
lipids responsible for the barrier function) to a more disordered state due to the action of the enhancers. Reproduced with permission from Elsevier [164].

Oleic acid is an example of a fatty acid known to cause phase separation in the SC lipids, thus changing its barrier properties [113]. Azone and diethylene glycol monoethyl ether (transcutol P), like oleic acid, are composed of a polar head group and an alkyl chain. The molecular structure of transcutol P is shown in Figure 1-14(a). A formulation of finasteride containing transcutol P, a monoethyl ether of diethylene glycol, in a concentration of 0.25% increased skin absorption nearly 3.6 times in the first 15 minutes compared to the enhancer-free control [165]. The highest enhancement ratio (ER) was gained using 1% transcutol P (ER = 5.98) [165]. In this study, among the various transdermal formulations, transcutol P 1% w/w in combination with water, propylene glycol and 2-propanol (30, 10, and 60) showed the highest enhancement ratio [165].

![Chemical structures of (a) transcutol P and (b) ethylenediaminetetraacetic acid.](image)

Figure 1-14. Chemical structures of (a) transcutol P and (b) ethylenediaminetetraacetic acid.

Ethylenediaminetetraacetic acid (EDTA), shown in Figure 1-14(b), at 20 mM showed a marked increase in the release of lactate dehydrogenase [166]. EDTA also induced a selective increase in the paracellular flux, indicating that EDTA enhanced the transport of lactate dehydrogenase across the skin. Yamashita, et al. (1987) [167] using a voltage-clamp design method reported that EDTA at 10 mM selectively enhanced the transport of sulphanilic acid via a
paracellular pathway [166, 167]. It is claimed that EDTA depletes calcium in the extracellular space including the tight junctional region and activates protein kinase C (PKC), resulting in expansion of the paracellular route [168]

Urea can also improve transdermal permeation of a drug. Improvement in permeability is achieved through hydration of the skin surface and through the formation of hydrophilic diffusion channels within the SC [169]. Cyclic urea forms are biodegradable and non-toxic consisting of a polar parent moiety and a long chain alkyl ester group [169]. This indicates that enhancement mechanism may be a consequence of both hydrophilic solubility and lipid disruption mechanism [169].

There are many other PEs available, such as dimethyl sulfoxide (DMSO) and the terpene family. All the aforementioned enhancers have shown varying degrees of success at improving either/or both skin permeation and diffusivity [170]. The use of specific PEs is discussed later in Chapter five.

1.5.2.2. Improving drug solubility

Increasing the amount of drug solubility is an important factor for simple formulations, especially alongside formulations that use chemical penetration enhancers [170]. Solubility can be optimized by increasing the drug concentration or changing the drug solubility in the vehicle. Both methods increase the thermodynamic activity of drug in the vehicle, therefore improved skin permeation [170].

The bioavailability of betamethasone-17-benzoate from ointments has been investigated based on the formulations composition [171]. As the ointments become more saturated with betamethasone-17-benzoate, its permeability increased according to Fick’s law of diffusion. When more drug was added to form suspension systems, the penetration rate was independent of solubility. The observed effects for the solution and suspension bases were similar though the amount of drug required for the maximal effect was different, reflecting the different drug solubility's in each vehicle [170]. The results showed that this steroid’s thermodynamic activity in the vehicle is critical, and it is not the overall drug amount, but the amount dissolved.

The solubility method is in itself a very simple approach and has the potential to remarkably increase skin permeation. However, a small increase in the degree of saturation may lead to the supersaturation of the formulation. Although this may be ideal for some drugs,
Chapter 1: Introduction

supersaturated systems are generally thermodynamically unstable, and over time undergo drug crystallization [170]. The stability of such formulations can be improved by using polymers [170, 172-175], although long term storage has stability complications that are difficult to avoid. Alternatively a supersaturated formulation can be manufactured to improve stability problems through evaporation of volatile components once applied to the skin, increasing water uptake from the skin, and pre-mixing cosolvents prior to application such as polymers that prevent crystallisation [170]. Furthermore, the addition of a penetration enhancer can alter the thermodynamic activity of formulation components. The solubility of the drug may change, which can increase or decrease the degree of saturation. The enhancer itself may also undergo changes in saturation which may be positive or negative towards the drug penetration. If the dissolution is higher for the PE, then the PE will better penetrate the skin and increase drug permeation. However, improving enhancer delivery to the skin is not always ideal and limited by the degree of skin irritation in vivo. Therefore, some in vitro data that show promise may not be marketable due to side effects [170].

1.5.2.3. Improving the partition coefficient

Another critical parameter is the octanol water partition coefficient (P) and has implications for drug permeability through the skin. The P between an oil/octanol and water is a measure of a drug’s lipophilicity. Given a drug has a low molecular weight, the ideal lipophilicity (logP) value for drug permeation through the skin is between 1 – 3 [97, 119]. Therefore, drugs with logP < 1 and, to some extent > 3, will have difficulty moving across the skin.

A way to increase permeation across the skin is to increase drug partitioning into the skin; that is increase the skin-formulation partition coefficient. This is another important factor that can affect the permeation rate across the skin. The skin-formulation partition coefficient can be increased by using agents that can improve the drug solubility in the skin (specifically the SC layer) such as propylene glycol or disrupting the SC lipid bilayer through penetration enhancers. Examples of penetration agents that can achieve this include PEG, transcutol P, ethanol, and N-methyl pyrrolidone [176].

A study examining the ability of saturated PEG to help permeate ibuprofen showed improvement with increasing concentrations of PEG [177]. The results suggest that the vehicle may be modifying the partition properties of the membrane [177]. In another study, PEG was
found to improve skin permeation of metronidazole [178]. The mechanism of PEG is explained by the solvent drag effect and altering the skin's solubility parameter. The solubility parameter of the SC is approximately 9.8 and in the presence of PEG (solubility parameter 14-19 depending on length) it is shifted to 13.8 [170].

Transcutol P is a penetration enhancer, as described earlier, but can also be used to increase the solubility of drugs in the skin [170]. The permeation enhancement through terpenes, other than through their disruptive nature, has been shown with increased drug solubility in isooctane [170]. This suggests improved permeation by means of increased solubility in the skin [179].

Ethanol has been used to improve the flux of estradiol through skin without changing the diffusion coefficient [170]. In this study the improvement was found in the apparent partition coefficient and concluded that it was due to the solubility of the drug in the membrane. Similarly, the increased permeation of levonorgestrel through the skin has been claimed to occur as a function of increased diffusion [180]. However, at high concentrations (up to 60%) of ethanol, it has been reported that the main mechanism to penetrate the skin is via reduced barrier function of the SC lipids [181, 182].

1.5.2.4. Combination method to improving permeation

It makes sense that synergistic effects can be observed by using multiple methods, as described in the preceding sections, to improving drug permeation. It was shown that PEs, according to formulation restrictions and ideal concentrations, can function on both drug solubility and diffusion coefficient [170]. Combining PEs could also improve permeation, depending on their required concentration and on the drug substance. There are several examples where combination methods have been employed to increase skin permeation. For example, the use of azone and PEG, which raise the diffusion coefficient and increases the drug solubility in the SC, leading to improved significantly skin permeation for a range of drugs such as metronidazole [178], 5-FU [183], and estradiol [170, 183].

1.5.2.5. Nanoparticles

Nanoparticles are colloidal systems with particles less than 1 µm in diameter suspended in a liquid. Nanoparticle technology has proven particularly useful for large molecules, such as
proteins. There are several forms of these nanoparticles, such as solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) [184, 185]. Solid lipid nanoparticles (SLN, Nanopearls®) are made of lipids that are solid at room temperature, where the surface being covered by a surfactant shell stabilises the dispersion. Nanostructured lipid carriers (NLC) are mixtures of solid and fluid lipids, the fluid lipid phase is reported to be embedded into the solid lipid matrix [186] or to be localised at the surface of solid platelets and the surfactant layer [187]. Application of nanoparticle dispersions or lipid particle loaded cream or gel to the skin surface causes structural changes. Water evaporation results in a transition of the lipid matrix to a more highly ordered structure leading to drug discharge [188]. Through the formation of an occlusive lid over a skin surface the transepidermal water loss (TWL) can be slowed. It is thought that improved hydration (via preventing TWL) at least temporarily, opens the compact structure of the horny layer, thus increasing the permeability across the barrier [185, 189, 190]. Generally nanoparticles and their formulation components in current use are regarded as biocompatible and show minimal toxicological results [185].

1.5.2.6. Liposomes

Forty-seven years have passed since liposomes were first discovered [191]. Since then liposomes have evolved into model drug carriers with clinical effectiveness [192, 193]. “Due to their versatile nature, liposomes can be used for diverse applications, which impose quite different requirements on the carrier” [192].

Liposomes have one or more hydrophobic spherical bilayers with a hydrophilic core and hydrophilic outer layer [192, 194]. Liposomes can be uni- and multi-lamellar and range in diameter from approximately 50 - 150 nm [192]. A range of similar lipid based systems are available, such as niosomes, proliposome, and ethosomes [195]. These can be large or small multi-lamellar systems and will not be discussed further.

One of the main rationales for liposomal use is that they may serve as carrier for lipid soluble and aqueous soluble drugs [196]. While liposomes have been investigated for many types of drugs, they have been particularly useful in the delivery of anticancer, antibiotic, and antifungal agents, as well as proteins and peptides [196]. Three other important rationales for liposomal use include; (1) sustained release depots for dermally active agents such as antibiotics, corticosteroids, and retinoic acid, (2) penetration enhancing effects of phospholipids or non-ionic
ether surfactants on the lipid layers of the SC and epidermis, and (3) rate limiting systems for systemic absorption, viz controlled release transdermal delivery systems [196]. All the lipid based systems used today are considered to be non-toxic and biodegradable [197].

The formulation process involves a wide variety of assessments such as drug retention, stability, particle integrity, processing, partitioning behaviour, and scale-up [195]. The processing step basically consists of hydrating the lipids, leading to liposome formation; the liposomal size, and drug loading steps then follow and may occur one after the other or at the same time [198].

Among the major problems with lipid based systems is the storage instability. “Shelf life of liposomal drug systems may be limited by insufficient chemical stability of both the active ingredient and bilayer-forming lipids or by insufficient physical stability of the liposomes” [198]. Hydrolysis of ester bonds and oxidation of unsaturated fatty acids contained in the lipid bilayer(s) are the leading causes of liposomal degradation [197, 198]. The use of surfactants as a replacement to phospholipids is one way this problem is being resolved, forming noisomes. Physical stability is another problem that may affect particle size as well as drug content within the liposome, known as leakage. Another problem occurs with aggregation where sedimentation or floatation of the liposome systems (except niosomes – non-inoic based liposome systems) causing vesicle grouping. Using a charge to induce electrostatic repulsion or modifying the particle size so Brownian motion can keep the liposome suspended are ways to control aggregation [198].

Although liposomal based systems offer substantial benefits demonstrated with some drugs, the technical demands have often led to delays in commercialisation [198]. For such reasons, many of the bigger pharmaceutical industries have been terminating their R&D work on these lipid based systems [198]. There is however a clear need for novel technologies, and liposomes offer an opportunity that someday may be more viable financially and practically.

1.5.2.7. Micro- and nano-emulsions

Microemulsions are generally thermodynamically stable, transparent, isotropic systems consisting of an oil, water, and surface active agent (surfactant or SAA) [199]. The system contains two immiscible liquid phases, one of which is a fine droplet dispersed in the other continuous phase. The system is stabilised by using the surfactant. Two main types are used in
the pharmaceutical industry; oil-in-water (o/w) and water-in-oil (w/o) microemulsions. They are differentiated by whether the continuous phase is aqueous or oily [97]. More complicated systems exist with multiple oil or water phases and are used in delayed drug action delivery systems. The droplet size determines the category of the emulsion, such as micro- or nano-systems. For comparison, larger colloidal systems have droplet sizes between 0.1 and 100 µm, and are relatively unstable [97]. The droplet size in microemulsions can be between 10 and 200 nm in diameter. Nanoemulsions or submicron emulsions have a droplet size of greater than 50 nm. In some cases, the use of a single surfactant is not sufficient to reduce the interfacial tension, so cosurfactants are added. The cosurfactants also have the ability to reduce the film rigidity and improve the interfacial curvature [200]. Another important factor that distinguishes micro- and nanoemulsions is that microemulsions form spontaneously, while nanoemulsions require energy input to form. The advantages of microemulsions include low viscosity, practical preparation, wide ranging drug solubility, surfactant based penetration enhancement, and avoiding enzyme breakdown [201]. The mechanisms for improved drug delivery across the skin are focused around the penetration effects on the SC layer and enzymatic protection. PGN delivery through the skin has been investigated using microemulsions. In one study the use of isopropyl myristate (IPM)-based microemulsion systems was found to increase the solubility values of PGN up to 3300-fold, compared to those in water [202]. In another study the transdermal flux of PGN delivered across porcine ear skin was increased 4-fold or higher (p-value < 0.05) compared to the PGN solution in oil [203].

Another advantage of some of the micro- and nano-sized emulsions is their improved storage stability. For example, the self-microemulsifying drug delivery systems (SMEDDDS) may contain little or no water while under storage conditions, rendering them a suitable medium for drugs susceptible to hydrolysis [204]. Although micro- and nano-emulsions have many advantages, they require the use of proportionally high amounts of surfactants compared with standard emulsions which restricts the formulation to only a few acceptable options [97].

1.5.2.8. Cyclodextrins

For more than 30 years, cyclodextrins (CDs) have attracted research interest because of their possible use in a wide range of fields including pharmaceutics [205]. They were first discovered in 1891 by A. Villiers [206]. CDs are cyclic oligosaccharide chains consisting of six α-
cyclodextrin, seven β-cyclodextrin, eight γ-cyclodextrin, or more glucopyranose units linked by α-(1,4) bonds [207]. CDs may serve as organic host molecules with their internal cavity able to accommodate one or two guest molecules [208]. “The binding of guest molecules within the CD is not fixed or permanent but rather is a dynamic equilibrium” [207]. The binding strength depends on the ‘host-guest’ complex and how well this fits together, and on specific local interactions between surface atoms [207]. The potential guest list for molecular encapsulation in CDs is quite diversified and includes chained aliphatics, aldehydes, ketones, alcohols, organic acids, fatty acids, aromatics, gases, and polar compounds such as halogens, oxyacids and amines [209].

The CD molecules are relatively large, with molecular weights ranging from 1000 to over 1500 [207, 209]. Due to their size and the hydrated outer surface, CD molecules will not easily permeate biological membranes [207]. However they can stabilise active compounds, reduce the volatility of drug molecules, and protect the drug from degradation [207]. CDs have been used in transdermal drug delivery. Their generally low irritancy is an added advantage of these molecules. In one study, the CD based ointment was found to significantly increase the transfer of HPE-101 into the skin of hairless mice, compared to the ointment only base, leading to higher drug permeation [210]. Although most penetration enhancers have an effect on the lipid SC layer of the skin, CDs are known to increase drug availability at the surface of the biological barrier [207]. Despite significant improvement of drug bioavailability using CDs, their use can be limited due to irritation effects, ironically even though reduction in drug irritancy is their one main advantage [211]. For example, in one study the β-CD extracted all major classes of lipids from an isolated SC of hairless rats, thereby permanently reducing the barrier function of the skin [212]. This caused significant skin irritation and this degree of skin irritation is not acceptable for transdermal delivery systems.

1.5.2.9. Transdermal patches

One of the innovative breakthroughs in transdermal delivery science has been the development of transdermal patches [97]. Transdermal patches, also known as transdermal drug delivery systems (TDDS), are designed to control the release rate of a drug into the skin. The first commercial patch, Transderm-Scop®, a delivery system for scopolamine, was developed by Alza Corporation (Palo Alto, CA, USA) in 1980 for motion sickness [213]. Since then numerous
drugs have been marketed using a patch system, including fentanyl, nicotine, oxybutynin, rivastigmine, nitroglycerin, and testosterone.

Patches are more convenient and effective than parenteral, and even many oral therapies [97, 214]. The release rate is well below the maximum the skin tissue can receive, thus the patch, not the SC, controls the diffusion rate. A patch typically contains an adhesive layer, a release layer, and an impermeable outer layer. The manufacturing of TDDS can be classified into five main categories; (a) monolith/matrix system; (b) membrane-controlled; (c) adhesive-controlled; (d) microreservoir; and (e) matrix dispersion [214-216], as shown in Figure 1-15.
All transdermal patches adhere to the skin using a pressure-sensitive adhesive (PSA) [214, 215]. The simplest form of patch is the matrix kind, where the drug matrix is the adhesive.
Chapter 1: Introduction

For these patches, the release rate of the drug usually follows the Higuchi square root of time. One such example is the Nitro-DurRII (Key Pharmaceuticals, Inc.) which is used for angina pectoris [214]. The membrane controlled patches are a little more complicated where the drug is enclosed in a compartment with an impermeable backing and semipermeable polymer membrane facing the skin. Estraderm (Ciba Pharmaceutical Co.) and CatapresR-TTS (Boehringer-Ingelheim) are such patches using this technology [214]. In a similar design, adhesive-controlled patches use the drug reservoir concept, without an additional rate controlling membrane. The micro-reservoir systems also have a drug reservoir, but in this case the reservoir is formed by suspending an aqueous solution in a water soluble polymer [214, 217]. The final patch system is the polymer matrix, where a drug is dispersed throughout an inert polymer matrix. The drug is physically mixed into a hydrophilic or lipophilic polymer, pressed into a disc shape, then glued to an occlusive plastic backing. The medicated disc has a defined surface area and thickness to predetermine the release rate and quantity [214]. The polymers used for transdermal patches must be biodegradable and non-toxic [218].

1.5.2.10. Prodrug

The SC poses one of the main barriers to effective passage of drugs, so when a drug does not possess the necessary properties, the drug can be modified towards an ideal partition and/or diffusion coefficient and solubility [97]. Another way is to use a prodrug. A prodrug is an active substance with an inactive moiety. After diffusion to the skins surface and absorption, the prodrugs fully convert to the parent drug through enzymatic cleavage [219]. “Considerable efforts have been made to deliver prodrugs utilizing various dosage forms and delivery routes, including injectable oil, biodegradable implant, and injectable microsphere” [219].

In a study of nalbuphine, the passive permeation results demonstrated that the ester prodrugs had higher permeation rates than the parent drug on its own [219]. In another study with the same drug, release from PLA-based matrices were examined [220]. The prodrug release data was consistent with the Higuchi model, which suggests the prodrug release occurred through a diffusion mechanism [220]. The main advantage of using a prodrug is the increased penetration rate through a chemical attachment, which may be easier than having to re-formulate a delivery system [221].
Chapter 1: Introduction

1.6. Alternative strategy

1.6.1. Solid dispersions

Many types of skin preparations, ranging from powders, through to semi-solids to liquids have been used as dermatological vehicles. In pharmaceutics, the physicochemical properties are important, as is understanding how each property influences bioavailability [97]. A single phased system (e.g. solution) may be manufactured relatively easily without excessive knowledge, but limit the formulation possibilities and is unlikely to achieve a significant improvement in bioavailability [97]. Complex multiphased systems are often necessary, but the formulation can lead to complications such as drug and/or formulation instability and mixing problems. Patients also tend to favour certain types of product textures such as creams rather than gels or ointments [97]. This section outlines the basis of solid-state vehicles as a formulation strategy that will be investigated in this research.

Solid and semi-solid dispersions have been used as a way to improve aqueous solubility of many drugs for various delivery routes. The formulation of solid and molecular dispersions, amorphous dispersions, and metastable polymorph dispersions are some examples of how solubility has been increased [222]. Improving the aqueous solubility of a compound is a major challenge and has implications for bioavailability [223].

Solid dispersions are made up of molecules that are held in close proximity to each other by intermolecular forces [97]. The solid state material may consist of at least two components, generally a hydrophilic excipient and a hydrophobic drug [222]. The drug can be dispersed in crystalline form, in amorphous form or molecularly. Crystalline forms contain molecules that are arranged in a specific order, which repeats over and over again within a particle [97, 222]. When molecules in a solid state are not packed in an orderly manner, they are said to be amorphous [97]. In Figure 1-16, a schematic representation has been drawn showing the modes of drug incorporation in a solid dispersion.
Chapter 1: Introduction

Figure 1-16. The three modes of drug incorporation into a solid dispersion where; (a) shows a crystalline particle, (b) amorphous particle, and (c) molecularly dispersed.

There are various preparation methods of solid dispersions, and the more common or conventional methods have been reported in the literature. Conventional dispersion preparation methods include comelting (or fusion), solubilisation of a drug in solvent, and possibly cosolvents followed by evaporation, and physical mixing such as by mortar and pestle. These methods have been used with varying degrees of success, however they are not without their limitations [222]. For instance, melting and physical mixing may lead to an undesirable drug transformation, or use of organic solvents or water that leaves products with unwanted residue(s) or hydrates that slow dissolution [97, 222]. However, even with these limitations in mind, solid dispersion formulation remains a flexible method allowing for a variety of processing and excipient options, when forming a delivery system for poorly aqueous soluble drugs [222].

The pharmaceutical industry has also adopted a range of solid-state engineering methods to reduce drug particle size and modify the crystalline structure to improve solubility. Commonly used techniques include spray drying, emulsion–solvent extraction, and particle comminution based on high shear, cavitation, or impaction processes, such as ball milling, media milling, jet milling and fluidization [224-226]. These operations offer a significant improvement in drug particle formation and are implemented for most current dosage productions. However, technical drawbacks associated with the conventional processes have become significant. For example, typical comminution processes often lead to wide or uneven particle size distributions, heat-sensitive drugs can be degraded, and conversion into unwanted or uncontrollable polymorphism can occur [227] [97]. The spray drying processes may also cause the precipitated particles to collide and agglomerate within the hot gas media [228]. In addition, inefficient energy consumption and overuse of organic co-solvents, especially for the antisolvent precipitation or surfactants in these operations, may pose real or perceived public health and environmental
safety issues [229]. Furthermore, these techniques may be insufficient for poorly water soluble drugs [230]. Moreover precise control of the drug particle properties including their size, shape, surface properties, and crystalline purity/density is also required [230]. These characteristics are important for control of the pharmacokinetic properties of a drug, such as absorption, thus bioavailability.

Supercritical fluid (SCF) processing has been used as a relatively new pharmaceutical method to engineer the solid-state of drugs, including improve solubility and manufacture solid-state formulations. This alternative method has been used for various purposes that included forming drugs with a smaller particle size, reducing or eliminating residual organic solvents, improving flowability, and enhancing drug dispersion systems [222]. Due to the exclusive thermodynamic properties of SCFs, mass transfer (drug-SCF dissolution) is highly effective. The properties of SCF are briefly outlined in section 1.7.1.1 and thoroughly in Chapter 3. Generally the dissolution or dispersion capacity of SCF can be significantly higher than that of conventional methods that use solvents and comelting and other mixing techniques [226, 231]. It has been proposed that SCF processing can be used as a way to improve PGN dissolution, thus enhancing its skin permeation. To date SCF methods have not been investigated for the development of a transdermal PGN formulation. In fact, to date, there are only SCF extration methods that have been used in food and herbal products such as carotenoid separation from fruits and vegetables by Norac Technologies in Canada. A single pharmaceutical product, Levadex® for migraines (made by MAP Pharmaceuticals, now owned by Allergan), is currently under review with the FDA (USA), and is expected to be accepted sometime in 2013. It is SCF processed to reduce the particle size of dihydroergotamine (DHE) for inhalation and has a markedly improved bioavailability profile over sprays and oral formulations.

1.6.1.1. Supercritical fluid technology

SCF technology has been used extensively in the food industry for more than 50 years and to some extent in pharmaceutical industry since the 1980s, and not exclusively used for solid-state formulation. In this thesis the focus is wholly on the solid-state aspects of SCFs. A supercritical fluid is defined as a state of matter above its critical temperature (Tc) and pressure (Pc) where distinct liquid and gas phases no longer exist. [230, 232, 233]. Figure 1-17 shows a pressure-
Chapter 1: Introduction

temperature phase diagram for a pure substance. The lowest point at which the two variables meet on a phase diagram is known as the critical point (Cp).

![Phase Diagram](image)

**Figure 1-17.** The pressure-temperature phase diagram for a pure substance.

Cp = critical point.

The supercritical state is neither gas nor liquid, but in a mesophase. It can have the density of a liquid, while at the same time have the diffusivity and viscosity of a gas. The main advantage of this mesophase is the increased dissolving capacity compared to a liquid or gas alone [234, 235].

SCF methods often use the SCF as either a solvent or anti-solvent for a drug and excipient [222]. These methods typically have, in addition to the SCF, a solvent, a matrix and a drug that are dissolved and sprayed through a nozzle into a precipitation chamber, with atmospheric pressure forming particles [222]. There are many different SCF methods but the mains ones include: particles from a gas saturated solution (PGSS), rapid expansion of supercritical solution (RESS) for carbon dioxide (CO$_2$)-soluble drugs, and gas anti-solvent (GAS) for CO$_2$-insoluble drugs [236-239]. The PGSS process is the SCF method used in this research.

Some SCF methods do not use organic solvents and hence may be referred to as ‘solvent free’. Organic solvents can be used to deliver a dissolved drug into a SCF, but are removed later
during the expansion phase. Moneghini, et al. (2001) [240] described the use of acetone to
dissolve PEG and carbamazepine while claiming to use a solvent free method. The use of CO₂ as
a SCF is also considered a solvent free technique because it is relatively more environmentally
friendly [222]. Supercritical carbon dioxide (SC-CO₂) treatment of pharmaceuticals has also
recently received increased attention because CO₂ is clean, safe and inexpensive [241, 242].
Furthermore, it is claimed the energy consumption associated with the general SCF techniques is
lower than that of most traditional processing methods [243]. A more extensive outline on SCF
technologies and methods is given in Chapter 3.

1.7. The main objective

The transdermal delivery of endogenous PGN is considered as one of the prime ways to control
postmenopausal signs and symptoms. Currently there are numerous companies manufacturing
transdermal PGN delivery systems. These products provide varying results that have
questionable, even controversial effectiveness. The delivery systems employ, by most
definitions, ‘conventional’ preparation methods that have failed to produce a single product with
improved effectiveness.

PGSS using supercritical carbon dioxide (SC-CO₂) is a unique method for preparing solid
drug dispersions. It is capable of forming stable crystalline and molecular semi-solid dispersions
with suitable drug loading and uniformity. Although this technology has been used for decades,
it has had little exposure in the pharmaceutical industry. Furthermore, studies on the effects of
processing and formulation parameters on the properties of PGSS are limited.

It was hypothesised that process parameters (such as pressure, temperature, and nozzle
orifice size) and excipient properties (e.g. melting point and solubility) affect the characteristics
of PGSS dispersions, and these can be optimized to form PGSS semi-solid dispersions with
desired quality, such as: improved aqueous dissolution and bioavailability. The following flow
diagram shows the various factors involved that have potential to affect drug release, therefore
permeation and ultimately bioavailability, see Figure 1-18.
Figure 1-18. Factors with possible influence on drug release.

SC-CO₂ technology and PGSS methods are safe and potentially efficient way to prepare transdermal delivery systems of PGN. Due to the high pressure of SC-CO₂ processing and greater mixing efficiency, it is anticipated that the PGSS method will perform as well, and possibly outperform other selected methods currently available such as comelting and using cosolvents.

Based on this information and the hypothesis, studies were carried out with the following main objective: To investigate the potential of SC-CO₂ processing as a method to form semi-solid PGN dispersion systems for transdermal delivery.

1.8. Aims of the thesis and its structure

Although there is extensive research investigating the biochemical and physical barriers to absorption of PGN using various transdermal formulations (e.g. creams, gels, patches, microemulsions), to date no such studies have reported on semi-solids prepared using SCFs. Given the novel aspects of SCF processing and the lack of research on the formulation of PGN via SCF processing for transdermal delivery, the aims of this thesis were:

(1) to provide background knowledge and introduce the research information about PGN and transdermal delivery (Chapter 1);
Chapter 1: Introduction

(2) to select suitable excipients in order to formulate a semi-solid dispersing system, i.e. preformulation (Chapter 2);

(3) to develop and validate an HPLC method for PGN (Chapter 2);

(4) to develop, build, and operate a novel SC-CO2 unit for preparation of PGN dispersion systems (Chapter 3);

(5) to determine the solubility and \textit{in vitro} dissolution profile of PGN and characterise the dispersion systems using XRPD, FTIR spectroscopy, and DSC (Chapter 3);

(6) to optimise the PGN dispersion systems using a factorial experimental design (Chapter 4); and

(7) to investigate the strategies to improve the permeability of PGN across mouse skin using dispersion systems in the absence or presence of a penetration enhancer.
Chapter 2:

Preformulation of Progesterone
Chapter 2. Preformulation of Progesterone

2.1. Introduction

Preformulation is the stage of development during which, amongst others, the stability, solubility, dissolution, ionization, states of matter, and partitioning properties of drug substance are determined to aid in the design of an appropriate delivery system. In a basic definition it is the stepwise plan to characterise the physicochemical properties of a drug, "to learn about its tendencies" [244]. The goal of preformulation in dosage form development is to address the "basic and applied principles important to the characterization of drugs, excipients, and products” [244]. Information is also gathered regarding the biological activity and in situ stability of the drug which are essential to successful formulation design. It is widely accepted that a drug’s poor water solubility and low permeability across the skin are major factors that can contribute to poor bioavailability. The greatest limitation of PGN is that it is a very poorly water soluble drug which contributes to its poor bioavailability.

In this chapter preformulation studies will be carried out including the development and validation of an HPLC method for PGN. There is numerous data on the physicochemical of PGN throughout the literature. Several preformulation studies have been repeated and confirmed such as FTIR and XRPD but there is few data on SCF and PGN. Therefore, this chapter aims to investigate some fundamental physical aspects of PGN including spectral characterisation using FTIR and XRPD and melting point determination under light microscopy. Results from hotstage melting will also examine the crystalline structure of PGN using Raman spectroscopy and birefringence. Furthermore, the rational behind the selection of excipients used in SCF formulations will be discussed with the appropriateness for PGN in mind. Chapter 3 will continue with the investigation of PGN with the selected excipients under different SCF conditions.

2.1.1. Infrared and Raman spectroscopy

Vibrational spectroscopy was first recognised as infrared (IR) spectra observed by Sir William Herschel (1800) [245]. Much later Abney and Festing (1881) had photographed absorption bands
for 53 compounds [246]. IR spectra were observed through transmittance of infrared light focused through a sample. All molecules vibrate and these vibrations can be excited by absorbing electromagnetic radiation. The frequency where the absorption occurs provides information about the identity atoms within a molecule [247]. Observing the energy difference between the excited state and ground vibration state typically occurs within the wavenumber region of 4000 – 10 cm\(^{-1}\). Polymorphs of a drug have different intermolecular order leading to specific vibration spectrum.

In Raman spectroscopy, the vibration energy is measured from the inelastic light scattering of a sample from a laser in the visible, near IR, and UV wavelengths. Raman spectroscopy is named after the physicist Sir Chandrasekhara Raman (1928) from India who established that an original beam of light emerged from a sample at right angles and with different wavelength (Raman scattering) [248]. Normally, molecules absorb light and are excited into a partial quantum mode (i.e. higher vibrational mode) and emit wavelengths of equal frequency as the incoming wavelengths, so that there is no net change in energy detected [248]. Such observations are known as elastic effects in a process known as Rayleigh scattering. It was then found that a very small amount of energy, approximately 0.0001\% of the original energy, accounts for inelastic effects [187]. The most common are those in which a molecule releases a wavelength of lesser energy and these events are referred to as Stokes shifts [248]. The opposite effect may also occur, referred to as anti-Stokes shifts, in which a molecule releases a wavelength of higher energy than the wavelength it absorbs [248]. When a functional group undergoes excited vibrations, the scattered inelastic light with show Raman scattering providing a fingerprint to accurately identify a molecule.

IR and Raman spectroscopy are often used together and are considered to be complimentary to each other. The IR and Raman spectra were obtained to identify and characterise PGN in this chapter. There are several publications that were used to confirm the spectra obtained for both techniques [249-251].
Chapter 2. Preformulation

2.1.2. *Crystaline diffractograms*

It was Johannes Kepler (1611) first described the hexagon symmetry of snowflakes, as the packing of solid water (ice) particles (crystals) [252]. By 1784, through experiments conducted by René Haüy, the idea was born that crystals of a substance are the regular three-dimensional arrangement of molecules.

X-rays were first discovered by Wilhelm Röntgen (1895) [253]. X-rays are a high energy form of electromagnetic radiation ranging from 200 eV to 1 MeV, although there are no sharp boundaries in the electromagnetic spectrum [254]. It was not until 1912 when crystals were considered able to be used as diffraction gratings for X-rays, and experiments by the Bragg’s (father and son) were able to make the connection that the observed scatterings were from the crystal lattice [255].

X-ray powder diffraction (XRPD) has since provided the most precise data about the existence of different crystal structures [254]. Diffractograms of drugs yield numerous peaks that can be correlated to the spacing of atoms in a crystal lattice [256]. In XRPD, a monochromatic X-ray is aimed at a powder sample and the scattered radiation angle is detected. Using Bragg’s law\(^1\) the distance between the planes of crystal (d-spacing) can be measured [257]. As XRPD provides information about the crystal structure, it can be used to characterise drugs and its polymorphs.

2.1.3. *Thermal analysis of PGN*

Thermal analysis of a sample involves the physical transformation such as phase transitions, causing more or less heat to flow to it than the reference to maintain both at the same temperature [258]. In DSC, which was developed by Watson and O’Neill (1972), energy is directly measured allowing for precise values of heat capacity [259]. An endothermic (melting) or exothermic event (recrystallization) is recorded as a change in baseline temperature. This change is revealed as a peak, and the area under the curve correlates with its associated enthalpy. Different molecules and different polymorphs give different thermodynamic

\(^{1}\) Bragg’s law: \(2d \sin \Theta = n\lambda\), where \(d\) is the spacing between diffracting planes, \(\Theta\) is the incident angle, \(n\) is any integer, and \(\lambda\) is the wavelength of the beam. These specific directions appear as spots on the diffraction pattern called *reflections* [254]. Dorset, D.L., X-ray Diffraction: A Practical Approach. *Microscopy and Microanalysis*, 1998. 4(05): p. 513-515, [255]. Bragg, W.L., The Specular Reflexion of X-rays. *Nature* 90, 1912. 90(2250): p. 410.
parameters such as MP, heat capacity, and heat of fusion [260]. Therefore, different molecules and polymorphic changes can be characterized by DSC.

2.1.4. Solubility parameters

Pharmaceutical formulations use excipients to improve the delivery of a drug molecule by increasing solubility and/or efficacy of drug loading. The design of pharmaceutical formulations is produced from the knowledge of: (a) the physico-chemical properties of the drug and excipients, (b) the interactions between each component and (c) the potential changes to each component under different environmental conditions [261, 262]. It is well known that the characteristics of the drug-excipient relationship determine the properties and performance of the formulation [261]. Furthermore, the most significant factor for drug loading and retention of a formulation is the solubility of the drug in the excipient [263].

Currently, although less so in industry, the development of pharmaceutical formulations occurs by trial and error without a rational procedure to find the most suitable excipients or carriers [261]. This means the selection of an appropriate excipient can become time consuming and an expensive event [261]. Solubility parameters provide a useful and inexpensive guide to predicting the solubility of drugs in different excipients.

Solubility parameters help to characterize several important features of a drug or excipient, including miscibility, compatibility and adsorption [264]. The application of solubility parameters provides predictive information about the magnitude of the interactions between the different components of a formulation and to some extent the stability of a final product [264]. The use of solubility parameters in pharmaceutical dosage form design has been presented throughout the literature [261, 262, 264, 265].

The solubility parameter is a function of the energy of vaporization as indicated by the cohesive energy density (CED), see Equation 2.

\[
\delta = (CED)^{1/2} = \left[ \frac{\Delta H - RT}{Vm} \right]^{1/2} = \left[ \frac{\Delta E}{Vm} \right]^{1/2}
\]  

Equation 2
where δ is the solubility parameter; ΔH the enthalpy of vaporization (J·mol\(^{-1}\)); R is the gas constant, 8.314 (J·mol\(^{-1}\)·K\(^{-1}\)); T equals temperature; Vm the molar volume (mL·mol\(^{-1}\)); and ΔE the free energy of vaporization.

The solubility parameter, as defined in Equation 2 has also been referred to as the *Hildebrand parameter* after Joel H. Hildebrand and was proposed by him in 1936 [264, 266, 267]. A development from the Hildebrand parameter is the Hansen parameter which considers the cohesive energy as a sum of contribution from dispersive (δ²\(d\)), polar (δ²\(p\)) and hydrogen bonding (δ²\(h\)) interactions, respectively, as denoted in Equation 3 [268].

\[
\frac{\delta^2}{T} = \frac{\delta^2}{d} + \frac{\delta^2}{p} + \frac{\delta^2}{h}
\]

**Equation 3**

In 1976, according to the van Krevelen and Hoftyzer group method, the cohesive energy of a substance is additive and can be divided into three group contributions, see Equation 4 [269]. All solubility parameters were estimated using the van Krevelen and Hoftyzer group contribution method, which is also a three dimensional (3D) component method [270]. Van Krevelen and Hoftyzer expressed each group contribution in the following form:

\[
\delta_d = \frac{\sum F_d}{V}, \delta_p = \sqrt{\frac{\sum F_p^2}{V}}, \delta_h = \sqrt{\frac{\sum E_h}{V}}
\]

**Equation 4**

where \(F_d\), \(F_p\), and \(E_h\) are factional group contributions for dispersion, polar, and hydrogen bonding components, respectively; and \(V\) is molar volume [271].

The final equation used to group each contribution was proposed by Hoy (1970) [272]. According to this equation, the values of each component are individually evaluated, and the combined (total) forces can be calculated using Equation 5 [272].

\[
\delta_t = \sqrt{\delta_d^2 + \delta_p^2 + \delta_h^2}
\]

**Equation 5**
Chapter 2. Preformulation

The solubility parameters and its components (Hoofzyer-Van Krevelen and Hoy) have been estimated for PGN using algorithmic methods and confirmed from the literature. The result calculated in this equation was found to be similar to the values provided in the literature. In order to predict from all the estimated values, the mean was taken from the estimated calculation and literature values.

For good solubility between a drug and excipient, the difference in solubility parameters between drug and excipient (or \( \Delta\delta \)) must be less than or equal to 2, for moderate solubility the difference must be between 5 and 9, while poorly soluble can be defined as a difference solubility parameters greater than or equal to 10 [268, 270].

2.1.5. Saturation solubility determination

The saturation solubility point occurs when the solution of a substance can no longer dissolve more of that substance [273]. The saturation point is the point of maximum concentration for a solute and depends mostly on the temperature and pressure [263]. Several different methods can be used for solubility determination.

The shake-flask method involves forming a saturated solution at equilibrium, as observed by excess undissolved drug [274]. After filtration the concentration is determined by HPLC analysis. Although time consuming, this method provides accurate results and equipment is generally available in most laboratories [273]. Turbidimetry is a high through-put method where a drug is dissolved in an organic solvent, typically 10 mM dimethyl sulfoxide (DMSO). When light scattering first occurs, further aliquots of solution are added and each addition plotted against turbidity so that solubility can be back- extrapolated to the point of first drug precipitation [274]. The potentiometric method is based on an acid-base characterisation shift in a titration curve associated with drug precipitation [274]. However, this method is only useful for ionisable drugs and can be difficult to quantify poorly soluble drugs. Equipment and software has been developed to automate the process and improve solubility determination [275, 276].

2.1.6. Optical microscopy – Melting point (HSM)/birefringence

Observation using the visual aid of a light microscope was one method used to test melting point and birefringence. The melting point step was conducted using a hot stage microscope (HSM). The main components of a light microscope are the light source, two filters; one above
(analysing filter) and the other below the sample stage (polarising filter), magnifying lens, and ocular lens (eyepiece). HSM in pharmaceutical research is used in a variety of ways to confirm transitions observed using other techniques [277]. HSM may be used for the solid-state characterization of bulk drugs, evaluation of crystal forms and hydrates, and other physio-chemical properties [277, 278]. In pharmaceutics the illumination techniques are also commonly used on birefringent samples.

Birefringence, also known as optical anisotropy and double refraction, is defined as $\Delta n = n_e - n_o$, where $n_e$ is the refractive index of light polarized parallel (extraordinary) to the optical axis and $n_o$ is the polarizations perpendicular (ordinary) to the optical axis [279]. The refractive index is a function of the molecular structure, orientation and packing within a substance [279]. The incident light interacts with the substance and so generating contrast with the background which is visible through the ocular lens. In Figure 2-1, incident light which contains parallel and perpendicular components is split into light waves travelling at different directions and speeds [280]. Anisotropic substances separate light into these two waves, where the perpendicular light waves are detected by the analysing filter, confirming the presence of crystals form the substance. Isotropic substances appear dark as the incident light passes unchanged through the sample. Light microscopy also provided information of the particle size of PGN.
Chapter 2. Preformulation

![Diagram](image)

Figure 2-1. Incident light rays passing through birefringent material. Adapted from [281].

The ability of a drug to re-scatter light gives a unique opportunity to observe the degree of dissolved drug in a formulation, either to confirm solid solution or the boundary of saturation in a given excipient(s). One of the important goals in this research was to improve the degree of dissolved PGN in the selected excipients, thus improve the amount of free PGN available for permeation. The improved solubility can be ensured by the reduced absence of birefringence under cross-polarized light. In order to characterise PGN and its birefringence, samples were examined for crystallised PGN. The excipients are amorphous and do not show any birefringence.

2.1.7. Chapter aims

The aims of this chapter are to:

- select commonly used excipients in SCF formulations from the literature,
- identify PGN and selected excipients with IR and Raman spectroscopy,
- determine the effects of SC-CO$_2$ on PGN using Raman spectra,
- characterise PGN and selected excipients using XRPD,
- determine the melting point of PGN using DSC,
Chapter 2. Preformulation

- investigate the solubility of PGN in selected excipients,
- examine the birefringence of PGN mixed with the selected excipients, and
- determine the HPLC conditions for PGN analysis

2.2. Experimental

2.2.1. Materials

Industrial grade PGN was purchased from Pharmaceutical Compounding New Zealand (PCNZ) (Auckland, NZ) which was sourced from Pharmaca & UpJohn-Pfizer (New York, USA). Progesterone Cream™ was purchased from PCNZ (Auckland, NZ). PEG 400 and 4000 was brought from BDH, VMR International Ltd. Poole, England. Vitamin E (as TPGS) was obtained from Zhejiang Shiner Chemical Company Ltd. (Zhejiang, China). Gelicure 44/14 was donated from Gattefossé Corporation (New Jersey, USA). Methanol and acetonitrile were HPLC grade obtained from Sigma-Aldrich (Auckland, NZ). Liquid CO₂ (purity 98%) was purchased from BOC Gas (Auckland, NZ). All other reagents and chemicals were of analytical grade. Triple-distilled MilliQ water was obtained in-house by reverse osmosis (Millipore, USA). All samples were used without any further purification steps.

2.2.2. Excipient selection

The next stage of the research involved the selection of various excipients to be used for the delivery of PGN via the transdermal route. The use of suitable excipients/carriers is essential in the development of a novel formulation for PGN, because PGN is a poorly aqueous soluble endogenous steroid. The novel formulation will involve SCF processing using CO₂. Literature searching, solubility parameters, saturation solubility and birefringence tests were performed to decide on appropriate carriers for PGN that would improve aqueous solubility. SCF processing has generally been used for oral formulations, thus the common excipients used in SCF processing are usually for oral delivery formulations. However the excipients were selected with a transdermal formulation in mind, therefore each excipient must also have been used in transdermal formulations.
Chapter 2. Preformulation

2.2.3. *Current excipients used in the literature*

Breifly, there are three main SCF methods, rapid expansion from supercritical solution (RESS), gas anti-solvent (GAS), and particles from gas saturated solution (PGSS). These methods will be further explained in section 3.2.2. There are several papers that employ the different SCF methods that use various lipid and/or surfactant based excipients. Table 2-1 below lists the literature using several common excipients and the SCF method used with each excipient.
Chapter 2. Preformulation

Table 2-1. Summary of supercritical fluid methods for various steroids and common excipients/cosolvents.

<table>
<thead>
<tr>
<th>Method</th>
<th>Excipient(s)/cosolvent(s)</th>
<th>Supercritical fluid</th>
<th>Drug/solute</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RESS</td>
<td>n/a</td>
<td>CO₂</td>
<td>α-tocopherol</td>
<td>[282, 283]</td>
</tr>
<tr>
<td>RESS</td>
<td>n/a</td>
<td>CO₂</td>
<td>PGN</td>
<td>[284-286]</td>
</tr>
<tr>
<td>RESS</td>
<td>n/a</td>
<td>CO₂</td>
<td>testosterone</td>
<td>[284]</td>
</tr>
<tr>
<td>GAS</td>
<td>Gelucire 44/14, TPGS, PEG 8000, PVP K30</td>
<td>CO₂</td>
<td>carbamazepine</td>
<td>[225, 243]</td>
</tr>
<tr>
<td>GAS</td>
<td>Poly(50/50-DL-lactide-co-glycolide)</td>
<td>CO₂ + N₂</td>
<td>hydrocortisone</td>
<td>[287]</td>
</tr>
<tr>
<td>PGSS</td>
<td>n/a</td>
<td>CO₂</td>
<td>glycerides</td>
<td>[288]</td>
</tr>
<tr>
<td>RESS/PGSS</td>
<td>Gelucire 44/14, PEG 1000, PEG 1450, PEG 8000</td>
<td>CO₂</td>
<td>various poorly soluble drugs e.g. HIV protease inhibitor</td>
<td>[289-292]</td>
</tr>
<tr>
<td>Derivative GAS/SAS</td>
<td>n/a</td>
<td>CO₂, propane, n-butane</td>
<td>PGN</td>
<td>[293]</td>
</tr>
<tr>
<td>GAS</td>
<td>PEG 4000</td>
<td>CO₂</td>
<td>carbamazepine</td>
<td>[240]</td>
</tr>
<tr>
<td>RESS</td>
<td>PVA, PEG, PVP</td>
<td>CO₂</td>
<td>ibuprofen</td>
<td>[294]</td>
</tr>
<tr>
<td>GAS</td>
<td>Gelucire 44/14, TPGS, PEG 8000</td>
<td>CO₂</td>
<td>carbamazepine</td>
<td>[230]</td>
</tr>
<tr>
<td>RESS</td>
<td>n/a</td>
<td>CO₂</td>
<td>PEG 1500</td>
<td>[295]</td>
</tr>
<tr>
<td>GAS</td>
<td>PEG and PLA</td>
<td>CO₂</td>
<td>insulin</td>
<td>[296]</td>
</tr>
<tr>
<td>GAS</td>
<td>water, methanol, and water/methanol mixtures</td>
<td>CO₂</td>
<td>lobenzarit</td>
<td>[297]</td>
</tr>
<tr>
<td>RESS</td>
<td>ethanol and ethyl acetate</td>
<td>CO₂</td>
<td>cellulose triacetate</td>
<td>[298]</td>
</tr>
<tr>
<td>PGSS</td>
<td>hydrogenated palm oil</td>
<td>CO₂</td>
<td>theophylline</td>
<td>[299]</td>
</tr>
<tr>
<td>PGSS</td>
<td>n/a</td>
<td>CO₂, DME, alcohols</td>
<td>polyether imide</td>
<td>[300]</td>
</tr>
</tbody>
</table>
It was found from the literature that common excipients used in SCF methods are varied, including having lipid and aqueous solubility properties. Also from the literature, a range of excipients were checked for use in transdermal formulations. Using the literature, three excipients where selected to conduct experiments for this study: Gelucire 44/14 [301-303], Vitamin E (TPGS) [304-306], and a PEG combination of 400 and 4000 (ratio 50:50) [178, 307, 308]. It was expected that these excipients would improve the aqueous solubility of PGN. To test their ability, each excipient was investigated using solubility parameters (see section 2.3.2), saturation solubility (see section 2.3.3), and birefringence (in section 2.3.4). There were no publications at the time of writing with these excipients and PGN using the PGSS method for use in transdermal delivery. Following is an outline on each of the selected excipients.

2.2.3.1. Gelucire 44/14

The lipid-based amphiphilic carrier, Gelucire 44/14, was selected to determine its effect on PGN dissolution [230]. Gelucire 44/14 can be obtained by the polyglycolysis of hydrogenated palm kernel oil and PEG 1500 [309]. Manufacturing of Gelucire 44/14 must meet the requirements of the European Pharmacopoeia 4th Edition (2002) under the ‘lauroyl macrogolglyceride’ monograph [310]. The molecular structure of Gelucire 44/14 is shown in Figure 2-2, it is a saturated polyglycolized glyceride made-up of a defined combination of approximately 20% w/w mono-, di-, and triglycerides, approximately 72% w/w mono- and di-fatty acid esters of PEG 1500, and approximately 8% of free PEG, with molecular weight ranging between 30 to 1500 [230, 311]. The numbers in its name represent the MP at 44°C and the HLB value of 14. Gelucire 44/14 is commonly used as an oral bioavailability enhancer, associated with strong inhibition of the enterocytic efflux transporter (known as P-gP inhibition) and strong inhibition of the enterocytic drug metabolizing enzyme CYP3A4 [312, 313]. Gelucire 44/14 also acts as a non-ionic water dispersible surfactant and is an excellent excipient that can solubilize poorly soluble drugs [314]. Figure 2-3 gives the general molecular structures of the various components of Gelucire 44/14.
Figure 2-2. Gelucire 44/14 contains triglycerides mainly of lauric acid with PEGs by esterification of glycerol with fatty acids.

(a) fatty acid, (b) monoglyceride, (c) diglyceride, and (d) triglyceride.

Table 2-2 provides the list of fatty acids found in Gelucire 44/14 and the possible percentages of each [310, 315]. Gelucire 44/14 has been used in self-emulsifying drug delivery systems (SEDDS) for both oral and transdermal formulations [310].
Chapter 2. Preformulation

Gelucire 44/14 contains PEG esters and pure PEG that form lamellar crystals [316]. The PEG chains in these crystals form lamellae determined by the length of a PEG chain in a helical configuration [317]. Furthermore, there may be trilaurin crystals, amorphous regions, which are less than 17% of the total, and some liquid glycerol, which may be approximately 3% [316].

The hydration of Gelucire 44/14 through humid air shows typical results for a compound that contains crystals and amorphous regions. The glycerol is the most hydrophilic component of Gelucire 44/14, which absorbs water as the other components remain crystalline and do not swell and some water may be absorbed in the amorphous regions [316]. Water loading is approximately 1% over much of the relative humidity (RH) range (30 – 60%), which may be encountered during storage of the excipient [316]. Then, at RH 70% the water uptake increases rapidly, which may be related to the dissolution of the another hydrophilic component, which is PEG 33 [316]. PEG 33 dissolves when the RH reaches above 75% and mixtures of PEG may dissolve at lower humidity [316]. The hygroscopic ability of Gelucire 44/14 then depends on the solution regions containing glycerol and PEG 33 [317]. The other hygroscopic components of PEG esters and glycerides do not swell or dissolve until the RH is greater than 80% [316]. At RH above 90%, Gelucire 44/14 contains 40 % w/w water, and it has turned into a more viscous dispersion compared to that at RH 50%, thus viscosity increases with rising humidity [318, 319]. The viscosity of Gelucire 44/14 at 25°C is ≥ 250,000 mPa·s, while in molten form viscosity is approximately 100 mPa·s, and at temperatures above 60°C and loaded with 50% water the viscosity increases to approximately 7,000 mPa·s [316, 319, 320].

2.2.3.2. PEG 400 and 4000

PEG is the abbreviation for polyethylene glycol or properly, poly (ethylene glycol), which is a compound containing repeating units of ethylene glycol. PEGs are polymers of ethylene oxide. PEGs are commonly used as versatile functional fluids and chemical intermediates primarily through reacting with fatty acids to make esters [321]. As PEG molecular weight increases, viscosity and freezing point increases, and solubility in water decreases. However, even at the highest molecular weights, PEGs are highly water-soluble. Low molecular weight PEGs (< 600) are clear liquids at 25°C, while higher molecular weight PEGs (> 2000) are in solid state form [322]. The low molecular size of PEG favours the formation of solutions or suspensions, while the high molecular size favours solid dispersions [323]. Some PEG
products have been blended to produce materials with a specific viscosity, texture, or melting point, and immunogenicity [324]. Thus, a mixture of PEG 400 and 4000 would favour the formation of a semi-solid dispersion.

PEG is commercially available as mixtures of different oligomer lengths of defined molecular weight (MW). For example, PEG 4000 is made up of oligomers having a mean MW of 4,000 g·mol⁻¹. The repeating monomer of PEGs is shown in Figure 2-4. PEG has been used as a carrier polymer for the attachment, via end groups, of drugs such as penicillin V, aspirin,amphetamine, quinidine and atropine [325].

![Figure 2-4. The PEG monomer, n= 400 or 4000.](image)

PEGs are FDA approved and are generally considered non-toxic, hydrophilic, highly flexible, and uncharged, although there are some studies that have shown that the low MW glycols such as PEG 300 can form metabolites with known toxicity [307]. Only PEG 400 and 4000 were investigated in this research and careful consideration would be required for animal and human trials.

In relation to SCF processing, the solubility of CO₂ in PEGs depends only on the pressure (between 20-250 bar) and temperature (between 20-250°C), and not on the molar mass of PEG [326]. In one study the solubility of CO₂ in PEG was found to decrease with increasing temperature, and increase with increasing pressure [326]. Generally, the solubility of CO₂ is highest at 43°C and greater than 100 bar [326]. Similar observations have been made on PEGs with molar mass ranging from 400 to 35,000 g·mol⁻¹ [327, 328].

2.2.3.3. Tocopherol PEG 1000 succinate (TPGS)

Vitamin E has been used for more than 55 years in experimental and clinical dermatology [306]. Vitamin E refers to a group of eight fat-soluble compounds that include both tocopherols and tocotrienols [329]. The most commonly digested form of vitamin E is γ-Tocopherol which can be found in corn oil, soybean oil, margarine and dressings [330]. Alpha-tocopherol is the most biologically active form of vitamin E, and is the second most common form of vitamin E. This variant of vitamin E can be found most abundantly in wheat germ oil, sunflower, and safflower oils [331]. It is a potent antioxidant that limits the
production of reactive oxygen species produced during fat oxidation [332]. The form of vitamin E used in this research is D-α-Tocopheryl polyethylene glycol 1000 succinate (TPGS) [305]. The molecular structure of TPGS is shown in Figure 2-5. Vitamin E TPGS is a water-soluble derivative of natural vitamin E, which is formed by esterification of vitamin E succinate with polyethylene glycol 1000 (Eastman Chemical Company, USA) [333]. The viscosity of TPGS varies depending on temperature, at 25°C it is approximately 120,000 mPa·s, while the melton form is approximately 660 mPa·s at 40°C, and approximately 298 mPa·s at 60°C [334, 335].

![Molecular structure of TPGS – Vitamin E](image)

Figure 2-5. Molecular structure of TPGS – Vitamin E [305].

Vitamin E is an essential nutrient that is receiving growing attention in the skin care industry because of its antioxidant properties [306]. While some antioxidants such as glutathione or ubiquinol-10 can be synthesized by humans, levels of cutaneous vitamin E depend on its oral intake or transdermal delivery [306]. A study analyzing dietary data from 10,000 participants found that the majority of men and women in the United States do not meet the current recommendations for vitamin E intake [336]. From the literature TPGS has been used for various delivery systems including oral and skin vehicles, the added advantage is that it may also provide antioxidant benefits to the formulation and perhaps the surface skin to which it is applied.
Chapter 2. Preformulation

2.2.4. Solubility parameters

The Hoptyzer/Van Krevelan (and partial Hansen method) and Hoy methods for the estimation of solubility parameters for PGN in comparison with Gelucire 44/14, TPGS and PEG 400/4000 were conducted. Two methods are examined to improve the accuracy of predictions, in conjunction with a calculated solubility parameter for PGN. The solubility parameter values, from the literature, permit a relatively reliable selection of compatible excipient with PGN. The calculated value for PGN was performed using the basic molecular structure of PGN and the reference valves (including dispersion coefficient $d_d$, polarity $d_p$, and hydrogen bonding $d_h$) from the literature [269, 337]. It was predicted that Gelucire 44/14, TPGS, and PEG mixture, would be the ideal carriers of PGN because all their parameters value differences were less than 2. Gelucire 44/14 and TPGS were estimated to show the highest solubility in theory, while PEG 400/4000 was estimated to show less biocompatibility in terms of their solubility paramters.

2.2.5. Saturation solubility

Solubility of PGN in the selected excipients was measured according to methods outlined in the literature [230, 338]. An excess amount of PGN was added to a solution containing 0 – 30% w/v of TPGS, Gelucire 44/14 or PEG mixture. The samples were sonicated for 30 minutes at 25°C and then shaken at 30°C for 24 hours in a water bath. Following that, the suspensions were centrifuged at 10,000 rpm for 10 minutes and the supernatant removed through a filtered of 0.45 µm nylon (Millipore, USA). The supernatant was collected and analysed by an HPLC method previously outlined in the literature [339-343].

2.2.6. Optical microscopy – Melting point (HSM)/birefringence

The procedure carried out on different samples was as follows: approximately 1 mg of each excipient; Gelucire 44/14, TPGS and PEG 400/ 4000, and PGN in solid state were separately placed over glass slides and heated to 100°C rapidly, then up to 155°C at 10 °C·minute$^{-1}$ using furnace equipment (Mettler Toledo FP84HT-TA, Switzerland) and microscopy cell. During thermal treatment the melting behaviour was observed using a Leica DMR microscope (Wetzer GmbH, 020-525.024, Germany) with polarised light (12V, China) and confirmed by light transmission changes using a central processor (Mettler Toledo FP90, Switzerland). Pictures were taken with a Nikon Coolpix 4500 camera through the light polarizing microscope ocular lens attachment.
Preparation for crystalline PGN birefringence involved weighing 1 g samples and spreading on a clean glass slide. Observations were performed using a brightfield Leica DMR polarized light microscope (10x and 20x objectives) (Leica, GmbH, Germany). Pictures were taken using a Nikon coolpix 4500 camera.

2.2.7. Raman spectroscopy

The polymorphic transformations of PGN were investigated with a Renishaw System 1000 Raman Micro-Spectrometer (Gloucestershire, UK). The laser excitation was a 785 nm solid state diode laser attenuated to 0.26 mW at the sample and focused to a spot size of 1 µm using a 50 x objective. The detector was a thermoelectrically cooled (TEC) charge-coupled device (CCD), the spectral resolution was 6 cm\(^{-1}\), entrance slit width 50 µm and accumulation time was 10 second. The Raman spectra were recorded in back scattered mode of a few granules of powder samples in their native state without the need for sample preparation. All the spectra recorded were reproducible to ±0.5 cm\(^{-1}\) shift.

Spectra were acquired with the Windows Raman environment (WiRE) software designed by the manufacturer. Display of the spectral trace (Raman Shift in cm\(^{-1}\) versus intensity in arbitrary units) and arithmetical calculations (peak fitting, parameter estimation, base line corrections and other analysis) were done with the GRAMS (Galactic) software. Acquisition of spectra was done in the extended scanning mode in the range of 3200 – 200 cm\(^{-1}\) shift from the Laser line.

The heating stage was a Linkam THMS600 (Linkam Scientific Instruments Ltd, Surrey GU4 8RU, UK) with a Linkam TMS94 temperature controller. The temperature was increased at a rate of 10°C per minute. The chamber was purged with nitrogen (N\(_2\)) gas to avoid condensation of water on the glass cover of the stage. Several readings were made at regular intervals from 25 to 150°C while the temperature rise was stalled.

2.2.8. Infrared (IR) spectroscopy

FTIR was employed to examine the spectrum of PGN without excipient and before exposure to SC-CO\(_2\). The IR spectra of samples were recorded on a Bruker Tensor 37 (Bruker Optik, GmbH, Germany) spectrometer using an ATR attachment. For the characterization of solid forms, the samples were placed on the diamond crystal surface and were gently compressed using a pressure clamp. Scanning was performed in the 400 – 4000 cm\(^{-1}\) region with a resolution 4 cm\(^{-1}\), from 24 - 32 parallel scans. Data was analyzed using OPUS software.
Chapter 2. Preformulation

(Bruker Optik GmbH, Germany) version 6.5. Data was shown as the reciprocal of wavelength (i.e. wavenumbers or cm\(^{-1}\)), and will be given as cm\(^{-1}\) hereafter.

2.2.9. X-ray powder diffraction (XRPD) studies

XRPD analysis for PGN and excipient samples was performed with a powder diffractometer with a copper (Cu) K\(\alpha\) radiation (\(\lambda = 1.5406\) Å) (Eindhoven, Netherlands). The scanning angle \(2\theta\) ranged from 2° to 40°, and the scan steps were conducted at 0.020° with a counting time of 1 second-step\(^{-1}\). The current and voltage used during operation were 40 mA and 40 kV, respectively. Results were analyzed using Diffrac (plus) EVA (Bruker AXS Inc., USA) software.

2.2.10. Differential scanning calorimetry (DSC)

In this research, the transdermal route of delivery was targeted for PGN. The melting behaviour of a drug is an important factor in the flux across the skin [344]. The melting point of a selected drug for transdermal delivery is recommended to be less than 200°C [112]. The melting point of a drug also provides information on its polymorphic transformation in solid state. Temperature is known to be a major contributing factor to polymorphism, therefore melting point (MP) of a drug and the heating level during the formulation process must be considered. There are five known polymorphs of PGN identified, of which Forms I and II are the most predominate and relatively easily interchangeable [250, 345, 346].

The melting point of PGN and excipients was determined by DSC, using a Q1000 TzeroTM module (Thermal Analysis – TA Instruments, USA). Heat flow calibration of the instrument was done using indium as a reference material. Powder samples (5-10 mg) were taken in crimped aluminium pans and heated at 10 °C·minute\(^{-1}\) above the MP of the drugs. Data was analyzed using Universal Analysis software (Thermal Analysis – TA Instruments, USA) version 4.1D. All the measurements were performed in duplicate. MP is reported as the onset temperature.

2.2.11. Particle size

Particle size analysis of the pure PGN powder was performed by laser diffraction (LD), using a Mastersizer 2000 (Malvern Instruments, UK) instrument. For measurement, each sample was added drop-wise to the dispersion unit. The laser obscuration range was maintained between 10 and 20%. A refractive index value of 1.3 was used to analyze the particle size.
Chapter 2. Preformulation

The analysis was performed in triplicate and the mean values of distribution were documented. Mean particle size was expressed as $d(0.5)$ (50% of the particle volume below a certain size) and $d(0.9)$ values (90% of particle volume below a particular size). Volume distribution of the microparticles was obtained using software supplied by the manufacturer (Malvern Instruments, UK) and the average particle size was expressed as volume mean diameter ($\text{Vol. [4, 3]}$) in micrometers as described in the literature [347-349]. Span was used to describe polydispersity and calculated as $[d(0.9) - d(0.1)] / d(0.5)$.

2.3. Chromatographic development

One of the important steps in preformulation is the development of an analytical method to qualify and quantify the drug candidate. HPLC is often the standard, providing data with excellent sensitivity, specificity, and low maintenance costs. There are several publications for qualifying and quantifying PGN [350-353] and other steroids, and their metabolites [339, 340, 342, 343] using HPLC methods. In this section, the HPLC method for PGN was developed and validated according to International Conference on Harmonisation (ICH) guidelines [354]. The accepted test requirements are for accuracy, precision, specificity, detection limit (LOD), quantitation limit (LOQ), linearity and range [354].

2.3.1. Instruments

HPLC analysis was performed using an Agilent 1200 HPLC system (manufactured by Agilent Corporation, Germany), comprising a quaternary pump, an autosampler, vacuum solvent microdegasser, and photodiode array UV detector (G1315B). Acquired data was observed on ChemStation® software (Agilent Corporation, Germany) using a Pentium computer. The UV spectrum of PGN was initially tested using UV-VIS spectroscopy (Libra S32, Biochrom Ltd., England) between 200 and 400 nm, which was confirmed using the peak purity function on Chemstation® running five overlapping wavelengths.

2.3.2. HPLC conditions

Analysis of PGN was carried out with a Gemini C18 HPLC analytical column (Jupiter 5u C18 300R, 250 mm × 4.6 mm, particle size 5 µm, Phenomenex, USA) fitted with a C18 guard column (10 × 3.0 mm). The ACN and fresh Milli-Q water (collected at 18.2 MΩ-cm) were filtered through a 0.45 µm nylon filter (Millipore, USA) and sonicated for 15 minutes before use. The sample was eluted isocratically with the mobile phase, which was HPLC
grade acetonitrile (ACN) and water, 75 and 25% v/v, respectively. Constant flow rate of 1 mL·minute⁻¹ was used. The eluding time for PGN was 9.1 minutes, and the column temperature was maintained at 37°C. The volume of samples injected was 20 µL and analysis was carried out at a wavelength of 244 nm. Every second spectrum was recorded for peak purity analysis. The purity test is outlined later in section 2.8.4.1.

2.3.3. Standard curves

A stock solution was prepared by accurately weighing 10 mg of PGN into a volumetric flask to which methanol (70 mL) was added followed by sonication (Bandelin sonorex, Germany) for 10 minutes. Methanol was added to volume (100 mL) to give a final stock solution concentration of 0.1 mg·mL⁻¹ PGN. Standard curves for PGN were measured over a range of PGN from 1, 10, 25, 50, 75, 100, 150, and 200 µg·mL⁻¹ by transferring stock solution into separate graduate tubes and diluting with mobile phase. Standard curves were only accepted for a high degree of linear regression (R² > 0.99). Standard solutions of PGN were stored at 4°C for no more than 2 hours before being discarded. The HPLC auto sampler tray was also set and maintained at 4°C.

2.3.4. Method validation

2.3.4.1. Specificity

The specificity was demonstrated by comparing the peak area of standard PGN, PGN spiked with Gelucire 44/14, PGN spiked TPGS, and PGN spiked PEG 400/4000. The HPLC chromatograms were visually inspected for interfering peaks with each other. Each excipient was prepared from four conditions before spiking the PGN solution; a) melted, b) dissolution in methanol, c) attrition, and d) SC-CO₂ exposure. Briefly, for (a), excipient (5 g) was melted on a hot stage while stirring using a glass rod. The heating element was then switched off and the melted solution was allowed to cool to room temperature (25°C) during which the dispersion recrystalized. The solid form of each recrystalised excipient was stored at 4°C in a refrigerator, until required for testing.

In the solvent evaporation, for (b), each excipient (1 g) was individually dissolved with a minimum volume of methanol in a conical flask at room temperature (25°C). The solvent was removed from the excipient by evaporation at a temperature (3 to 10°C below the excipient melting point) over 72 hours while shaken at 50 cycles per minute. The solid product was collected and stored at 4°C in a refrigerator.
Chapter 2. Preformulation

In the attrition method, for (c), the excipient was weighted (2 g) and placed in a mortar and pestle. Attrition was conducted for 2 minutes at room temperature (25°C). The solid was removed from the mortar using a spatula and stored at 4°C in a refrigerator.

In the SC-CO₂ method, for (d), each excipient (1 g) was exposed to CO₂ under supercritical conditions for 10 minutes, then precipitated out of the SC-CO₂ during expansion from the sample cylinder. Supercritical conditions were maintained at 40 ± 2°C and 88 ± 4 bar for each excipient run. The recrystallised solid was collected and stored at 4°C in a refrigerator.

A specificity test run was also conducted with non-ionic cream. The cream was not processed or exposed to any chemicals or enhancement. The various components were cetostearyl alcohol, chlorocresol, liquid paraffin, paraffin white soft, and water purified. A market cream prepared with non-ionic cream was used throughout the thesis as a control.

Prior to HPLC determination, excipient solutions were passed through a syringe with 0.45 µm nylon filters (Millipore, USA). The obtained solution was then diluted 4 fold with mobile phase. A final concentration of PGN in the spiked samples was 100 µg·mL⁻¹.

In addition, the purity of PGN peak obtained in the chromatogram was assessed, using Agilent Chemstation® software. UV spectra were obtained at five points across the peak; two points before the peak apex (leading front), one point at the apex and two points after the apex (tailing front). The peak purity was assessed by examining the similarity of the UV spectra obtained at five points. When an impurity or degradation product coelutes with the PGN peak the five UV spectra obtained across the peak are not similar. The peak purity analysis was always carried out for all the samples throughout the study.

2.3.4.2. Linearity
A standard curve was obtained by plotting the area under the curve (AUC) against eight concentrations of the standard PGN solution (n = 3) in the range 1 - 200 µg·mL⁻¹. The slope, y-intercept and the line of best fit of the curve were evaluated by linear regression analysis. Regression analysis was performed using collected data on a spreadsheet in Excel 2007 (Microsoft® Corporation, CA, USA) on an Intel Core™ 2 Dell computer.

2.3.4.3. Sensitivity
The sensitivity was determined by testing the limit of detection (LOD) and limit of quantification (LOQ). Each test was measured by repeatedly diluting and analysing the tested
concentrations of PGN. The lowest concentration of the drug that was detected (LOD) had to provide a peak height three times the baseline noise which was referenced against a blank solution. The lowest quantifiable concentration (LOQ) was set as ten times the signal to noise level, also referenced by injecting a blank.

2.3.4.4. Repeatability

The repeatability was performed by calculating the percentage of relative standard deviation (%RSD) for results of three repeated solutions of PGN at the lower, middle and upper range. These PGN concentrations were set at 25, 100 and 175 µg·mL⁻¹ (n = 3).

2.3.4.5. Recovery

Recovery of PGN was calculated by comparing the results for the directly-injected PGN standards (50, 100 and 150 µg·mL⁻¹) with the concentration of PGN in the spiked samples (50, 100 and 150 µg·mL⁻¹). The percentage recovery was measured from the slope and the intercept of the calibration curve.

2.3.4.6. Accuracy and precision

This step was conducted at two levels: accuracy and precision, according to ICH guidelines. All PGN solutions were made in triplicates for accuracy tests. The intra-day accuracy was tested at three concentrations of 50, 100 and 150 µg·mL⁻¹ on the same day. Inter-day accuracy were determined by analysis of the same three standard solutions on three consecutive days. Percentage of accuracy was calculated by dividing the means of the measured concentrations to their expected concentrations. The precision was determined using set concentrations over three consecutive days.

2.4. Results and discussion

2.4.1. Solubility parameters

The solubility parameter of progestrone has been calculated based on molecular structure using the group-contribution method [262, 265, 355] and from the literature [356]. The results are listed in Table 2-3 and Table 2-4.
Table 2-3. Solubility parameter calculations for PGN using the Hoftyzer and van Krevelen method.

<table>
<thead>
<tr>
<th>Group</th>
<th>F_d</th>
<th>F_p^2</th>
<th>E_h</th>
<th>ΣzV/cm^3·mol(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>(3) CH₃</td>
<td>1870</td>
<td>0</td>
<td>0</td>
<td>80.5</td>
</tr>
<tr>
<td>(7) CH₂ (rings)</td>
<td>2390</td>
<td>0</td>
<td>0</td>
<td>327.7</td>
</tr>
<tr>
<td>(4) CH</td>
<td>350</td>
<td>0</td>
<td>0</td>
<td>-3</td>
</tr>
<tr>
<td>(1) C=</td>
<td>70</td>
<td>0</td>
<td>0</td>
<td>-5.5</td>
</tr>
<tr>
<td>(2) C=O</td>
<td>580</td>
<td>2372000</td>
<td>10500</td>
<td>21.6</td>
</tr>
<tr>
<td>(2) C (4 bonds)</td>
<td>-140</td>
<td>0</td>
<td>0</td>
<td>-38</td>
</tr>
<tr>
<td>(4) ring (5 - 6 atoms)</td>
<td>2560</td>
<td>0</td>
<td>0</td>
<td>64</td>
</tr>
<tr>
<td>(1) conjugated double bond</td>
<td>-100</td>
<td>0</td>
<td>0</td>
<td>-2.2</td>
</tr>
<tr>
<td>Σ</td>
<td>7580</td>
<td>2372000</td>
<td>10500</td>
<td>445.1</td>
</tr>
</tbody>
</table>

Table 2-4. Estimated total solubility parameter for PGN.

<table>
<thead>
<tr>
<th>Solubility Parameter</th>
<th>Estimated value (MPa)(^{1/2})</th>
</tr>
</thead>
<tbody>
<tr>
<td>δ_d</td>
<td>17.03</td>
</tr>
<tr>
<td>δ_p</td>
<td>3.46</td>
</tr>
<tr>
<td>δ_h</td>
<td>4.86</td>
</tr>
<tr>
<td>δ_t</td>
<td>18.04</td>
</tr>
</tbody>
</table>

The estimated total solubility parameter (δ_t) calculated from the square root of the various components (dispersion forces δ_d, hydrogen bonding δ_h and polar interactions δ_p) was 18.04. This was relatively similar to the reported value for PGN using the Hoftyzer and van Krevelen method of 19.46 [258, 270]. This calculated difference is likely to be a result of sourcing different group contribution values from different literature.

It is important to note that the selection has been made based on excipient phase (e.g. liquid or solid) at room temperature and compatibility (or closeness) of the solubility parameter. In Table 2-5, from Tween 80 to PEG 400, these excipients are in liquid state at room temperature and are the most likely to be miscible with PGN (Group 1), while the remaining excipients are either solid or have a solubility parameter difference greater than 2. These results were similar to those found in the literature, although there are studies showing poor predictability using solubility parameters and observed solubility [357]. It is claimed the departure of the observed and calculated solubilities is evidence of solvent-solute interactions [357].
Table 2-5. Solubility parameters (δ) of PGN and various excipients [262, 264, 355, 358-360].

<table>
<thead>
<tr>
<th>Compound</th>
<th>Method 1 (MPa)$^{1/2}$</th>
<th>Method 2 (MPa)$^{1/2}$</th>
<th>Mean</th>
<th>Difference in (δ)</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroids (General)</td>
<td>25.30</td>
<td>17.20</td>
<td>21.3</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>25.30</td>
<td>23.70</td>
<td>24.5</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>PGN (estimated 18.04)*</td>
<td>19.46</td>
<td>-</td>
<td>18.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tween 80</td>
<td>19.48</td>
<td>-</td>
<td>19.5</td>
<td>0.7</td>
<td>1</td>
</tr>
<tr>
<td>Cetiol B</td>
<td>17.77</td>
<td>-</td>
<td>17.8</td>
<td>1.0</td>
<td>1</td>
</tr>
<tr>
<td>Glycofurol</td>
<td>-</td>
<td>18.2</td>
<td>18.2</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Labrasol</td>
<td>18.83</td>
<td>-</td>
<td>18.8</td>
<td>0.05</td>
<td>1</td>
</tr>
<tr>
<td>Labrafil M 1944 CS</td>
<td>18.37</td>
<td>-</td>
<td>18.4</td>
<td>0.4</td>
<td>1</td>
</tr>
<tr>
<td>Miglyol 812</td>
<td>17.36</td>
<td>-</td>
<td>17.4</td>
<td>1.4</td>
<td>1</td>
</tr>
<tr>
<td>Lauroglycol FFC</td>
<td>18.05</td>
<td>-</td>
<td>18.1</td>
<td>0.7</td>
<td>1</td>
</tr>
<tr>
<td>TPGS</td>
<td>20.08</td>
<td>17.81</td>
<td>18.9</td>
<td>0.1</td>
<td>1</td>
</tr>
<tr>
<td>Gelucire 44/14</td>
<td>21.09</td>
<td>19.00</td>
<td>20.0</td>
<td>1.2</td>
<td>1</td>
</tr>
<tr>
<td>PEG 400</td>
<td>19.82</td>
<td>20.20</td>
<td>20.0</td>
<td>1.2</td>
<td>1</td>
</tr>
<tr>
<td>PEG 2000</td>
<td>19.51</td>
<td>-</td>
<td>19.5</td>
<td>0.7</td>
<td>1</td>
</tr>
<tr>
<td>PEG 4000</td>
<td>22.50</td>
<td>21.10</td>
<td>21.8</td>
<td>3.0</td>
<td>1&amp;2</td>
</tr>
<tr>
<td>PEG 10000</td>
<td>20.80</td>
<td>-</td>
<td>20.8</td>
<td>2.0</td>
<td>1&amp;2</td>
</tr>
<tr>
<td>Triethyl citrat</td>
<td>17.13</td>
<td>-</td>
<td>17.1</td>
<td>1.6</td>
<td>1</td>
</tr>
<tr>
<td>Dimethicon Abil 350</td>
<td>-</td>
<td>15.05</td>
<td>15.1</td>
<td>3.7</td>
<td>1&amp;2</td>
</tr>
<tr>
<td>PEG 35000</td>
<td>23.41</td>
<td>-</td>
<td>23.4</td>
<td>4.7</td>
<td>1&amp;2</td>
</tr>
<tr>
<td>Pluronic F68</td>
<td>24.84</td>
<td>-</td>
<td>24.8</td>
<td>6.1</td>
<td>2</td>
</tr>
<tr>
<td>PVP 30</td>
<td>22.40</td>
<td>20.70</td>
<td>21.6</td>
<td>2.9</td>
<td>1&amp;2</td>
</tr>
<tr>
<td>Citric Acid</td>
<td>29.40</td>
<td>25.90</td>
<td>27.7</td>
<td>9.0</td>
<td>2</td>
</tr>
<tr>
<td>PVA</td>
<td>31.70</td>
<td>30.30</td>
<td>31.0</td>
<td>12.3</td>
<td>3</td>
</tr>
<tr>
<td>Mannitol</td>
<td>39.10</td>
<td>38.70</td>
<td>38.9</td>
<td>20.2</td>
<td>3</td>
</tr>
<tr>
<td>Lactose</td>
<td>35.70</td>
<td>33.00</td>
<td>34.4</td>
<td>15.7</td>
<td>3</td>
</tr>
<tr>
<td>Sucrose</td>
<td>36.00</td>
<td>33.50</td>
<td>34.8</td>
<td>16.1</td>
<td>3</td>
</tr>
<tr>
<td>Water</td>
<td>42.17</td>
<td>42.32</td>
<td>42.3</td>
<td>23.5</td>
<td>3</td>
</tr>
</tbody>
</table>

*Calculated previously in this research. Highlighted rows represent the selected excipients.

Method 1: Hoftyzer and van Krevelan method
Method 2: Hoy method

The Hoftyzer and Van Krevelan method is a partial Hansen measure of solubility parameter. The group is determined by the degree of difference in the solubility parameter,
for example, a difference of 2 or less is considered to have miscible compounds (assigned Group 1), a difference of 5 to 9 is practically miscible (Group 2) and a difference of 10 or more between two compounds is considered immiscible (Group 3). The condition of solubility parameter difference being less than 5 is assumed to be the limit of solubility [358]. In this case the difference has been determined by comparing PGN with each excipient. PGN has been estimated to have a mean solubility parameter of 18.8, taken from the literature, as outlined above, and by mathematical calculation which was referenced from the literature.

Research has shown that inverse gas chromatography can be applied for the estimation of solubility parameter for PEGs and theoretical solubility parameter [359]. It is important to note the values of determined solubility parameter depend on the structure of examined PEGs and the temperature of experiment [359]. A very comprehensive study by Özdemir, et al. (2007) worked through several methods to define the predicted solubility of a range of PEGs [360]. According to results of calculations, the total solubility parameters determined with regard to other approaches, apart from one study, have been found as 20 ± 2 (J·cm⁻³)¹/₂ [360]. This result was in excellent agreement with those previously calculated and experimentally determined values.

2.4.2. Saturation solubility

As shown previously, solubility is said to be maximal when the solubility parameter of the solvent is similar to that of the solute. Theory predicts that solvents with a solubility parameter approximate to that of the solute will act as a strong solvent [357]. To confirm the solubility parameter studies, the selected excipients underwent a saturation solubility test. As predicted all three excipients were able to dissolve PGN, although not to the same extent. It was found that Gelucire 44/14 and TPGS have the highest solubility, which is consistent with the predicted solubility parameters, see Figure 2-6. Saturation solubility studies indicated the selected excipients at 30% w/v had dramatically improved the aqueous solubility of PGN from 7.1 µg·mL⁻¹ to 67.5 µg·mL⁻¹ in PEG 400/4000, to 410.3 µg·mL⁻¹ in TPGS, and 2129.9 µg·mL⁻¹ in Gelucire 44/14. At 5% w/v of Gelucire 44/14 in water showed a 78.5 fold increase of solubility for PGN and at 30% w/v a 313 fold increase of PGN solubility. The 5% w/v loading of TPGS in water showed a 10.6 fold increase of PGN solubility, while at 30% w/v there was a 58.6 fold increase. The PEG 400/4000 (50:50 mixture) at 5% w/v and 30% w/v in water there was only a 6 and 9.6 fold increase in PGN solubility, respectively. The saturation
solubility results provided evidence that the selected excipients were promising candidates for the PGSS method and preparation of PGN dispersion systems.

Figure 2-6. Saturation solubility test for selected excipients for PGN formulation.

--- Mean aqueous solubility of pure PGN (~ 7.1 µg·mL⁻¹).

*Significantly different PGN solubility to that of the aqueous PGN suspension (p-value < 0.01).

Unfortunately to date, there has been no literature reporting on the saturation solubility of the selected excipients with PGN specificity, although there are several studies showing the use of these excipients to improve aqueous solubility for a range of delivery routes. For example, TPGS forms micelles at concentrations ≥ 0.2 mg·mL⁻¹ in water and improves the aqueous solubility of amprenavir 20-fold [309]. Other studies have shown that improved aqueous solubility with TPGS also lead to improved permeation [308, 361].

Gelucire 44/14 has been used to solubilize 200 mg of the water-insoluble cholesterol-lowering agent fenofibrate in Cil (Germany) and Lipirex (France) [309]. A 1% concentration of Gelucire 44/14 has been demonstrated to produce a 280-fold increase in solubility for some drugs [362]. The increase of solubility in the presence of Gelucire 44/14 has been explained by increased wettability and micellar solubilisation [362]. This is described as the surfactant effect, for Gelucire 44/14, where a decrease in the interfacial
Chapter 2. Preformulation

tension occurs between the drug and dissolving solution. The gelucire based excipients solubilise many drugs and have been used frequently with poorly water soluble drugs. Polyethylene glycol 300 (PEG 300) and polyethylene glycol 400 (PEG 400) are commonly used due to their solubilizing capabilities and are generally considered to be among the safest organic cosolvents [363, 364].

Furthermore, the preparation method may influence the solubility outcome. For example, one study showed that aqueous solutions of a PEG did not increase the solubility of UC-781, while mixing PEG powder directly with the drug then into pure water showed a reasonable increase [362]. It was argued that the direct mixing in solid state lead to higher concentration of the excipient at the surface of the drug, acting as a driving force for the drug to get into solution [362]. It was also stated that the cohesion of the drug particles was reduced.

2.4.3. Optical microscopy

2.4.3.1. Melting point (HSM)

Physical mixtures were prepared to test the melting point dynamics using hot-stage microscopy [365]. Figure 2-7 shows the images for pure substances and the binary mixtures with PGN and excipients at their corresponding points of melting. It was noted that the melting temperatures for the individual substances were in accordance with their reported values, while the melting temperatures of the physical mixtures occurred relatively sooner. Light transmittance was used to confirm the visual observations, a representative graph is shown in Appendix I. For all three excipients, any undissolved PGN crystals began to dissolve into the excipient as soon as the excipient went into liquid state. The PGN particles would disappear well before the melting point of the pure drug was reached, which is consistent with similar studies [366]. It is also of note that the excipients themselves had lower onset melting points in the presence of PGN. It is also expected, as it is well known that other constituents and impurities can lower the MPs of substances, although the reasons for these effects remain poorly understood [366].
Figure 2-7. Hot-stage microscope images for pure substances and the excipient mixtures with PGN at their observed points of melting.
(a) untreated PGN before melting, (b) PGN crystal melting observable at 129°C, (c) PEG 400/4000 before melting, (d) PEG 400/4000 melting at 54°C, (e) pure TPGS before melting, (f) TPGS at melting point 36°C (1:5), (g) Gelucire 44/14 before melting, (h) Gelucire 44/14 melting at 44°C, (i) binary mixture of PGN/PEG (5:1) before melting, (j) PGN/PEG (5:1) melting at 49°C, (k) PGN/TPGS (5:1), (l) PGN/TPGS (5:1) melting onset lower at 30°C, (m) PGN/Gelucire 44/14 (5:1) before melting, (n) PGN/Gelucire 44/14 (5:1) melting at 42°C,
Chapter 2. Preformulation

Figure 2-7 continued. and (o) PGN/Gelucire 44/14 (5:1) completely melted at 44°C, where only the PGN particles remain visible under the microscope (200 × magnification). Circles indicate a suggested region to observe before melting occurred and arrows point to obvious melting onset in the suggested region.

For the PEG-based mixture, similar results were observed for a range of drugs, including ciprofloxacin and PEG 4000 prepared by physical mixing [367]. Similar results have been found using triamterene with PEG or Gelucire 44/14 prepared from physical mixtures [368]. It was claimed in one study that, while dispersions in PEGs or Gelucire 44/14 improve the in vitro release rates of the drugs, the magnitude of the increase depends on the molecular weight of the PEG, the drug:carrier ratio, and the phase interaction between the drug and the carrier [368]. This knowledge was given consideration during the optimisation phase of the formulation in Chapter 4, using a factorial design experiment.

2.4.3.2. Birefringence

Crystalline PGN re-scatters (or double refracts) polarized light while dispersed in non-ionic cream or water. Figure 2-8 shows a representative near uniform dispersion of PGN crystals throughout excipients from a non-ionic cream. The excipients are amorphous and do not show any birefringence, see Figure 2-8:A.

![Figure 2-8. PGN crystal dispersion under polarised light microscopy (100 × magnification). (a): non-ionic cream, (b): PGN dispersed in non-ionic cream, and (c): pure PGN crystals under 400 × magnification.](image)

The ability of a drug to re-scatter light gives a unique opportunity to observe the degree of dissolved drug in a formulation, either to confirm solid solution or the boundary of saturation in a given excipient(s). One of the important goals in this research was to improve the degree of dissolved PGN in the selected excipients, thus improve the amount of free PGN available for permeation. The improved solubility can be ensured by the reduced absence of birefringence under cross-polarized light. This technique is quick way to check the solubility of a drug in test formulations without having to first complete a dissolution test [369].
Chapter 2. Preformulation

2.4.4. Polymorphism of PGN

A normal light microscope was coupled with the Raman spectroscope allowing visual observations to be made. Figure 2-9 shows the microscopy pictures taken at four different time points during the melting of PGN powder from room temperature. There were quite obvious appearance changes, with the pre-melting powder appearing as white particles on the silicon sample tray, the melted product was transparent, while the cooled PGN product appeared more fused and gave the impression to be adhesive in nature.

![Microscopy pictures of PGN at different time points](image)

Figure 2-9. Light microscopy (200 × magnification) pictures of PGN; (a) powder samples at room temperature, (b) just before melting at 128°C, (c) melted at 136°C, (d) and after cooling at 22°C.

Polymorphism is important because it changes the physical properties of a drug, e.g. solubility/melting. Often the less stable form of a drug is the more aqueous soluble and has a lower melting point. PGN is known to have at least two polymorphic forms: prisms (alpha-) and needles (beta-) PGN, both of which have an orthorhombic crystalline system [250, 346]. Raman is one way to detect any polymorphic changes. Work completed by Wang, *et al.* (2000) has investigated the alpha- and beta- forms of PGN using Raman spectroscopy coupled with a hot stage [250].

According to the literature, Raman is able to detect a single band shift (from 1662 to 1667 cm⁻¹), representing alpha- or Form I and beta- or Form II forms, respectively [250]. This transformation occurred when PGN was melted above 155°C. As confirmed in this research,
see Figure 2-10, PGN can be transformed due to melting [250]. Since all the experimental research in this thesis never involved temperatures near the melting point of PGN, transformation effects due to temperatures above MP for PGN are not relevant. Figure 2-11 shows the data collected in situ of these crystallizations, the two end spectra at 1667 and 1662 cm\(^{-1}\), are for Form II and Form I, respectively.

Figure 2-10. Raman spectra of solid state PGN before melting (a) and during melting (b). The Raman shift is visible in the overlay of the two spectra.

Figure 2-11. In situ spectra of peak shifting during PGN transformation after melting.
Chapter 2. Preformulation

The polymorphic transformation from Form II to Form I was monitored in situ at different temperatures ranging from 25 to 155°C. The transformation rates were found to change as the temperature increases and decreases, as shown in Figure 2-10. This trend suggests that this polymorphic system is monotropic and Form I is the thermodynamically stable form, while Form II is the metastable form [250]. It has been demonstrated that Raman spectroscopy can distinguish between Form I and Form II PGN crystals and this is consistent with published research.

As part of the preformulation phase the solid state PGN powder was treated with supercritical carbon dioxide (SC-CO₂) to determine if such exposure would cause transformational changes in polymorphs as described above. From the literature there is no evidence of polymorphism induction from supercritical fluids involving CO₂, and PGN transformation is mediated only by organic solvents and temperature phenomenon [221]. The use of supercritical fluids is described in the following chapter. Figure 2-12 shows the Raman spectra for PGN when untreated and treated with SC-CO₂. The results show no evidence of polymorphism due to SC-CO₂ treatment.

![Raman spectra for PGN crystals before and after SC-CO₂ treatment showing no transformation changes.](image)

Figure 2-12. Raman spectra for PGN crystals before and after SC-CO₂ treatment showing no transformation changes.
Chapter 2. Preformulation

2.4.5. FTIR spectroscopy

In order to study the possible interactions between PGN and the excipients in their solid states, the characteristic fingerprints were gathered using FTIR. Figure 2-13 shows the spectra for PGN in solid state, the PEG mixture of 4000 and 400, TPGS and Gelucire 44/14. The spectra provide quite distinctive peaks both in the same and different regions. PGN has the two peaks where the C=C bands are observed between 1600 – 1660 cm\(^{-1}\). More specifically, peaks at 1614 and 1658 cm\(^{-1}\) were observed which can only be attributable to PGN. In the case of TPGS and Gelucire 44/14 the ester carbonyl stretching was observed at 1734 cm\(^{-1}\), while no such band was seen for PEG. Over-lapping of the CH stretching band of PGN at 2945 cm\(^{-1}\) and that of each excipient at 2880 cm\(^{-1}\) was observed with varying intensities.

For each excipient, definitive bands were also detected at higher and lower wavenumbers [362, 370, 371]. The absorption band at 3400 – 3650 cm\(^{-1}\) is from the terminal hydroxyl group which is obvious for TPGS and somewhat observable for Gelucire 44/14 [371]. The bands between 1050 – 1250 cm\(^{-1}\) are due to the C-O stretching which is present in all the excipients but not PGN [371].
This background information provided important knowledge about each substance, and was used to help determine the possibility of interactions with PGN.

2.4.6. XRPD studies

Figure 2-14 shows the X-ray diffractograms for PGN, TPGS, Gelucire 44/14 and PEG that were investigated. The diffraction spectrum for pure PGN showed that the drug was crystalline in nature as observable with the numerous distinctive peaks. The characteristic peaks and intensities detected at \(2\theta\): 10.7°, 12.8° and 16.96° respectively, corresponds well with the reported \(\alpha\)-form of PGN [250, 346]. The corresponding peaks for (b) Gelucire 44/14, (c) TPGS and (d) polyethylene glycol (PEG), at \(2\theta = 19.3°\) and 23.9° were also revealed. All three excipients contain varying amounts of PEG from 1000 to 4000 monomer units.
To investigate the possible solid solution formation between drug and excipient, the XRPD was completed and used at various times during the research. The primary purpose was for determining crystallinity and polymorphic forms of each substance.

2.4.7. Thermal analysis (DSC)

The thermograms for PGN, PEG 4000/400, Gelucire 44/14 and TPGS are shown in Figure 2-15. The thermograms of each pure substance have given a single exothermic peak corresponding to the melting point of PGN at 129.81°C, PEG mixture of 4000 and 400 (50:50 ratio) at 55.2°C, Gelucire 44/14 at 42.9°C and TPGS at 32.1°C. The sample with PEG displayed a melting exotherm with wavy shoulders, indicating the presence of more than one pure crystal which is consistent with the literature [362, 372].
Figure 2-15. DCS thermograms of PGN and selected excipients;
(a) pure PGN, (b) PEG 400/4000, (c) Gelucire 44/14, and (d) TPGS. Exo-therm: up.
2.4.8. Particle size

Figure 2-16 shows the particle size and distribution range of PGN powder that has not undergone preparation or formulation processing. A representative report of the full computed data is given in Appendix IIa. Table 2-6 lists the particle size parameters for PGN in solid state. The main observation is the already small particle size of the preformulation PGN with a surface weighted mean of 77.3 ± 0.5 µm. Also of note are the obvious two distributions, one at approximately 10 µm, and the other at approximately 100 µm, also due to polydispersity of the PGN crystals.

Table 2-6. Particle size parameters for PGN.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean diameter (µm) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>d(0.5)</td>
<td>110.62 ± 0.50</td>
</tr>
<tr>
<td>d(0.9)</td>
<td>193.60 ± 0.59</td>
</tr>
<tr>
<td>Vol. (4,3)</td>
<td>117.35 ± 0.48</td>
</tr>
<tr>
<td>Surface weighted mean</td>
<td>77.31 ± 0.50</td>
</tr>
<tr>
<td>Span</td>
<td>1.25 ± 0.00</td>
</tr>
<tr>
<td>Uniformity</td>
<td>0.39 ± 0.00</td>
</tr>
</tbody>
</table>

Results are provided as mean ± SD, n = 3
According to the Noyes-Whitney equation, see Equation 6 below, the dissolution of a drug depends on, amongst others, the surface area of the drug particles. In order for the maximum amount of drug to be readily available for absorption it must first be completely dissolved. The certificate of analysis (COA) from Pharmacia & UpJohn company states that the PGN particle size is 100% between 5 and 20 µm. The difference observed could be explained from the use of different methods used. It was noted that no surfactant was used in the water, so smaller particles could have agglomerated giving larger particle sizes, while the use of a surfactant may have induced dissolution leading to smaller particle sizes. There is no method described in the COA for PGN.

\[
\frac{dm}{dt} = \frac{kA(Cs - C)}{h}
\]

\text{Equation 6}

where \( \frac{dm}{dt} \) is the dissolution rate, \( k \) the dissolution solid rate constant, \( A \) the surface area of drug particles, \( Cs - C \) is the concentration gradient in dissolution, and \( h \) is the diffusion layer.
Chapter 2. Preformulation

thickness [97]. Once the drug is dissolved it is free to be absorbed at the molecular level. For transdermal delivery the molecular mass must be low, ideally less than 600 Da, which can provide a high diffusion coefficient [97]. The molecular weight of PGN is approximately 315 g·mol$^{-1}$, therefore ensuring the particles of PGN are dissolved in the formulation is a critical factor.

2.4.9. Chromatography and specificity

Specificity was assessed by comparing the chromatograms of the standard PGN with corresponding spiked samples. Representative chromatograms have been shown for each sample in Figure 2-17. The standard PGN exhibited a sharp and symmetric peak at 8.90 minutes, well separated from any impurities (i.e. no impurities observed in the time frame tested). The spiked PGN samples (with excipients) maintained the peak corresponding to PGN at the expected retention time, the range was 8.78 to 8.98 minutes for the given samples. Under these chromatographic conditions, interferences from the matrix components on the retention time of standard PGN were not observed.
Figure 2-17. Chromatograms of various PGN solutions; 
(a) the standard PGN (100 µg·mL⁻¹) solution, (b) the PGN samples which were spiked with Gelucire 44/14 after melting, (c) the PGN samples which were spiked with TPGS after solvent recrystalisation, (d) the PGN samples which were spiked with PEG after attrition, (e) the PGN samples which were spiked with TPGS after exposure to SC-CO₂ (continued next page).
2.4.9.1. Peak purity

Peak purity was done in addition to specificity to confirm the complete separation of PGN from other substances without dependence on visual detection of small inferences. The chromatogram and UV spectrum of PGN are shown in Figure 2-18. The UV spectrum reveals a peak wavelength at 244 nm, therefore this wavelength was used for the peak purity test of the HPLC assay. The chromatogram of PGN shows a sharp single peak at 8.7 - 9.1 minutes. The peak purity spectrum showed excellent resolution of greater than 2, indicating PGN had sufficient separation, and that no other substance was coeluting with PGN near this time retention interval.

Figure 2-18. Chromatogram and UV spectrum (insert) of standard PGN (20 µg·mL⁻¹) solution.
Chapter 2. Preformulation

2.4.9.2. Linearity, sensitivity and repeatability

The assay exhibited linearity between the area under the curve (y) and the corresponding concentration of PGN (x), over the 1 – 200 µg·mL\(^{-1}\) range tested. Figure 2-19 shows a representative standard curve produced for PGN concentrations 1 to 200 µg·mL\(^{-1}\). A typical equation was \(y = 53.37 \times + 109.48\) (n = 3). The results of linear regression analysis showed that the correlation coefficient (R2) of the standard curve was 0.9992. The other linear regression data included the slope (56.09 ± 0.53) and intercept (34.81 ± 18.61). The detection limit (LOD) and quantification limit (LOQ) was 0.225 µg·mL\(^{-1}\) and 0.450 µg·mL\(^{-1}\), respectively. The repeatability was obtained from the results from replicate measurements (n = 3) of one sample and it was expressed as %RSD. The HPLC method was reproducible with relative standard deviation (RSD) < 2.1%. The results for repeatability are shown in Table 2-7.

![Figure 2-19. The typical calibration curve relating the concentration of PGN (X-axis) to the area under the curve (Y-axis) (n = 3). Error bars are within data points.](image)

<table>
<thead>
<tr>
<th>Concentration (µg·mL(^{-1}))</th>
<th>Mean AUC</th>
<th>Variation (% RSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>1432.81 ± 29.8</td>
<td>2.08</td>
</tr>
<tr>
<td>100</td>
<td>5562.61 ± 37.3</td>
<td>0.67</td>
</tr>
<tr>
<td>175</td>
<td>9532.70 ± 93.4</td>
<td>0.90</td>
</tr>
<tr>
<td>Mean</td>
<td>1.22</td>
<td></td>
</tr>
</tbody>
</table>

Results are provided as mean ± SD, n = 3 at each concentration.
Chapter 2. Preformulation

2.4.9.3. Recovery, accuracy and precision

The intra-day and inter-day accuracy and precision of the HPLC assay were determined by three replicate measurements of each sample at low, medium and high concentration. Accuracy for the HPLC method was tested between 20, 100 and 180 amounts of the test concentration, which was defined as 100 µg·mL\(^{-1}\) of PGN in methanol. Precision, as described earlier, was determined by repeated injections of the standard solution to test repeatability and inter-day assay precision. The between day assays were conducted over three consecutive days. Results were summarised in Table 2-8. In the assay, the mean intra-day and inter-day accuracy were 101.2% and 100.3%, respectively, which indicate that the measured test values match well with the actual values.

<table>
<thead>
<tr>
<th>Concentration (µg·mL(^{-1}))</th>
<th>Mean calculated AUC</th>
<th>Accuracy (%)</th>
<th>Precision (% RSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intra-day:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>20.56 ± 0.40</td>
<td>102.8</td>
<td>1.95</td>
</tr>
<tr>
<td>100</td>
<td>101.20 ± 1.83</td>
<td>101.2</td>
<td>1.81</td>
</tr>
<tr>
<td>180</td>
<td>179.50 ± 2.89</td>
<td>99.7</td>
<td>1.61</td>
</tr>
<tr>
<td>Mean:</td>
<td>101.2</td>
<td>1.79</td>
<td></td>
</tr>
<tr>
<td><strong>Inter-day:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>19.92 ± 0.31</td>
<td>99.6</td>
<td>1.55</td>
</tr>
<tr>
<td>100</td>
<td>101.14 ± 0.38</td>
<td>101.1</td>
<td>0.38</td>
</tr>
<tr>
<td>180</td>
<td>180.54 ± 1.98</td>
<td>100.3</td>
<td>1.09</td>
</tr>
<tr>
<td>Mean:</td>
<td>100.3</td>
<td>1.01</td>
<td></td>
</tr>
</tbody>
</table>

Results are provided as mean ± SD, n = 3 at each concentration.

The recovery data of PGN at three concentrations are shown in Table 2-9. Recovery for the HPLC method was tested between 50, 100 and 150 amounts of the test concentration, which was defined as 100 µg·mL\(^{-1}\) of PGN in methanol. The ICH guideline states that the recovery of the drug must be between 98% and 102% of the defined test range [354]. In all cases, the mean recovery of PGN was > 98% and < 102%. Based on the data from this section, the HPLC assay for PGN determination was reliable, accurate, and precise.
Chapter 2. Preformulation

Table 2-9. HPLC recovery data for PGN and PGN spiked with blank forms of each excipient and market cream.

<table>
<thead>
<tr>
<th></th>
<th>Defined concentration (µg·mL⁻¹)</th>
<th>Actual concentration</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PGN alone:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>49.2 ± 0.21</td>
<td>98.40</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>99.4 ± 0.34</td>
<td>99.40</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>150.8 ± 0.24</td>
<td>100.53</td>
<td></td>
</tr>
<tr>
<td><strong>Spiked with Gelucire 44/14 (melted):</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>50.5 ± 0.19</td>
<td>101.0</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>99.8 ± 0.44</td>
<td>99.8</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>150.9 ± 0.24</td>
<td>100.6</td>
<td></td>
</tr>
<tr>
<td><strong>Spiked with TPGS (solvent evaporation):</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>50.4 ± 0.20</td>
<td>100.8</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>100.3 ± 0.33</td>
<td>100.3</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>151.0 ± 0.41</td>
<td>100.7</td>
<td></td>
</tr>
<tr>
<td><strong>Spiked with PEG 400/4000 (milled):</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>50.4 ± 0.23</td>
<td>100.8</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>100.6 ± 0.37</td>
<td>100.6</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>149.9 ± 0.17</td>
<td>99.8</td>
<td></td>
</tr>
<tr>
<td><strong>Spiked with TPGS (SC-CO₂ processed):</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>49.3 ± 0.32</td>
<td>98.6</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>98.3 ± 0.41</td>
<td>98.3</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>151.6 ± 0.39</td>
<td>101.1</td>
<td></td>
</tr>
<tr>
<td><strong>Spiked with non-ionic cream:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>50.8 ± 0.45</td>
<td>101.6</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>101.2 ± 0.57</td>
<td>101.2</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>152.3 ± 0.32</td>
<td>101.5</td>
<td></td>
</tr>
</tbody>
</table>

Results are provided as mean ± SD, n = 3 at each concentration.

In summary, the developed C₁₈-HPLC method was a rapid, sensitive, reproducible, and specific assay for determination of PGN. PGN maintained a steady elution whether it was spiked with the selected excipients or non-ionic cream. It is likely to be able to provide a quantitative analysis of PGN during degradation. Although PGN is susceptible to enzyme degradation in the skin, it was not examined, and PGN combined with the selected excipients for transdermal delivery need to be further studied in following investigations.

2.5. Conclusion

In this chapter, we have established the background knowledge required to conduct formulation preparation and characterisation. Preformulation research is essential work to help understand the physicochemical properties of drugs and excipients. Solubility is a critical parameter for transdermal drug delivery. Through a selection process involving a
literature search, solubility parameters, saturation solubility, and optical microscopy, there were three chosen excipients, namely, Gelcure 44/14, TPGS, and PEG 400/4000.

The effects of SC-CO$_2$ on PGN were determined using Raman spectroscopy which found no obvious polymorphism. The characterisation and identify of PGN and selected excipients was conducted with IR spectroscopy, DSC, and XRPD. And finally the HPLC conditions for PGN analysis were determined.

From the preformulation studies conducted in this chapter, it is concluded that PGN has a high MP that would be ideal for a SCF process using CO$_2$, a molecular weight in the ideal range for transdermal delivery, an adequate lipophilicity, and likely to have improved aqueous solubility with the selected excipients. Enhancement of aqueous solubility was an important factor in the preformulation phases and was to be equally important in the formulation phases where the SCF process was to be implemented. As drug delivery across the skin occurs predominately via passive diffusion, increasing the solubility can increase the amount of free drug at the surface of the skin, increasing the concentration gradient from the surface to the dermis, thus improving the permeation rate. Using the preformulation studies of PGN, a formulation was designed using semi-solid lipid and aqueous based excipients that were mixed with a supercritical fluid. In the following chapters, a novel delivery system has been investigated for transdermal delivery of PGN.
Chapter 3:

Construction of Supercritical Fluid Unit, and Formulation and Characterisation of PGN Solid Dispersions
Chapter 3. Construction of Supercritical Fluid Unit, and Formulation and Characterization of PGN Solid Dispersions

3.1. Introduction

The purpose of this chapter was to design, build, operate, and develop a unit for a novel particles from a gas-saturated suspension (PGSS) method using supercritical carbon dioxide (SC-CO\textsubscript{2}). Solid and semi-solid dispersions of endogenous PGN (PGN) were prepared using supercritical fluid (SCF) and compared with three conventional methods; comelting (CM), cosolvent (CS) and physical mixing (PM). Resulting dispersions were characterized by X-ray powder diffraction (XRPD), \textit{in vitro} dissolution, fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), and high performance liquid chromatography (HPLC). The SCF-based particle formation technology is presented as an alternative to possibly overcome the common limitations of conventional methods with respect to mixing, morphology control, polymorphic purity, batch consistency, and regulatory compliance according to good manufacturing practice (GMP)\textsuperscript{2} [373].

The pharmaceutical industry has adopted a range of methods to improve drug dissolution, reduce drug particle size, and modify the crystalline structure; these are widely known as the conventional methods. Commonly used techniques include spray drying, emulsion–solvent extraction, and particle comminution based on high shear, cavitation, or impaction processes such as ball milling, media milling, jet milling and fluidization [224-226, 374]. These operations offer significant abilities in drug particle formation and are implemented for most current dosage productions [375]. However, technical drawbacks associated with the conventional processes can become troublesome. For example, the typical comminution processes often lead to wide or uneven particle size distributions, heat-sensitive

\textsuperscript{2} A set of production testing procedures to help ensure a high quality standard of manufactured pharmaceuticals. All medicines manufactured today must have clearly defined and controlled production lines. An essential component of GMP is the documentation of all the steps taken in production and testing, which also relates to validation and proves compliance with specifications. Medsafe in NZ has a set of guidelines and codes, see website at: \url{http://www.medsafe.govt.nz/regulatory/Guideline/code.asp}
drugs can be degraded, and conversion can occur into unwanted or uncontrollable polymorphs [97, 227]. Spray drying processes may cause the precipitated particles to collide and agglomerate within the hot gas media [228]. Furthermore, inefficient energy consumption and overuse of organic co-solvents, especially for antisolvent precipitation or surfactants in these operations, may pose real or perceived public health and environmental safety issues [229].

In addition, conventional techniques may be unable to resolve many of the newer drugs which are said to be poorly water soluble [230]. More precise control of the drug particle properties including their size, shape, surface properties, and crystalline purity/density are required [230, 376]. These characteristics are important for control of the pharmacokinetic properties of a drug, mostly absorption, i.e. there are consequences for bioavailability.

3.2. Supercritical fluids

3.2.1. Concept and history

In 1822, a French physicist Baron Charles de la Tour describes the critical point of a substance in cannon barrel experiments. He noticed at a certain temperature and pressure a distinct single supercritical fluid phase forms, and was able to determine (although not accurately) the critical point of water. It then was not until the 1950s that the food industry developed efficient extraction techniques using SCFs and the 1980s when SCFs were used for analytical techniques (e.g. HPLC). Over the last two decades or so, the use of SCF in the pharmaceutical industry is being explored, however the use of SCF technologies is not exclusively employed for drug formulation [377]. For example, SCF has been used recently to thin coat ceramic tiles [378], for sensing applications [379], production of metal complexes [380], formation of particles for explosives [381-383], aerosol science and manufacturing [384], and to improve the properties of milk [385]. Due to the exclusive thermodynamic properties of SCFs, dissolution is substantially improved and dissolution capacity is greatly increased, compared to conventional spraying or extraction techniques [226, 231, 386, 387]. In fact, SCF technology is being used for improved spraying and coating processes [388-391], to improve extraction methods [392-395], and even used as part of other techniques such as in a fluidized bed [396, 397]. SCF technology has also been used to form various types of formulations, including micelles [398, 399], microencapsulation [400, 401], formation of microcapsules [402], and even synthesis of pharmaceuticals [403]. Regulatory
bodies also are shining the light on the importance of particle properties, and harmonization guidelines emphasise the characterization of crystallinity and polymorphism [230, 404].

A SCF, as defined earlier, is as a state of matter above its critical temperature (Tc) and pressure (Pc) where distinct liquid and gas phases cease to exist, see Figure 3-1 [230, 232, 233].

![Pressure-temperature phase diagram for a pure substance.](image)

Figure 3-1. The pressure-temperature phase diagram for a pure substance.

The supercritical state is neither gas nor liquid, but a mesophase. It has the density of a liquid, yet the diffusivity and viscosity of a gas, hence the main advantage is the increased dissolving capacity (viz mass transfer) compared to a liquid [234, 235]. The relative values that can be seen with a supercritical fluid verses a liquid and gas of the same substance are shown in Table 3-1 [405].

<table>
<thead>
<tr>
<th>Property</th>
<th>State of matter</th>
<th>Gas</th>
<th>Supercritical Fluid</th>
<th>Liquid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density (kg·m⁻³)</td>
<td></td>
<td>1</td>
<td>200 – 1000</td>
<td>600 -1600</td>
</tr>
<tr>
<td>Diffusivity (106 m²·s⁻¹)</td>
<td></td>
<td>10</td>
<td>1</td>
<td>10⁻³</td>
</tr>
<tr>
<td>Viscosity (103 kg·m·s⁻¹)</td>
<td></td>
<td>10⁻²</td>
<td>10⁻²</td>
<td>1</td>
</tr>
</tbody>
</table>

There are many different liquids that can be used as a supercritical fluid, refer to Table 3-2.
Carbon dioxide is a commonly used SCF because it has a relatively low critical temperature (approximately 31.1°C), and pressure (approximately 72.8 bar) \([407]\). SC-CO\(_2\) treatment of pharmaceuticals has recently received great attention because CO\(_2\) is a clean, inexpensive, easily available, and non-hazardous in these quantities relative to hydrocarbons, and especially the low critical temperature makes it attractive for processing heat-sensitive drugs \([241, 242, 323]\). The energy consumption associated with CO\(_2\) use is also lower than that of traditional particle processing methods \([243]\). In comparison to other solvents, CO\(_2\) is also attractive as it is considered to be chemically inert, non-toxic, odourless, tasteless, and GRAS (generally regarded as safe) \([230]\). Carbon dioxide is also suitable as a solvent for a range of polar substances that are often water insoluble \([408, 409]\). SC-CO\(_2\) has also been used successfully to dissolve a range of polymers \([410-412]\), including PEG \([326, 413]\), poly(1,1,2,2-tetrahydroperfluorodecyl acrylate) \([414]\), poly-lactic acids \([415]\), and benzoic acid \([416]\). Carbon dioxide has also been used in combination with other solvents such as, acetone and ethanol \([417]\), methanol \([418]\), and even other SCFs such as trifluoromethane (CHF\(_3\)) \([419]\).

### Table 3-2. Critical properties of various solvents \([406]\).

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Molecular weight g·mol(^{-1})</th>
<th>Critical temperature °C</th>
<th>Critical pressure bar (psi)*</th>
<th>Density g·cm(^{-3})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon dioxide (CO(_2))</td>
<td>44.01</td>
<td>31.1</td>
<td>72.8 (1056)</td>
<td>0.469</td>
</tr>
<tr>
<td>Water (H(_2)O)</td>
<td>18.02</td>
<td>374.3</td>
<td>218.3 (3166)</td>
<td>0.348</td>
</tr>
<tr>
<td>Methane (CH(_4))</td>
<td>16.04</td>
<td>-82.6</td>
<td>45.4 (658.5)</td>
<td>0.162</td>
</tr>
<tr>
<td>Ethane (C(_2)H(_6))</td>
<td>30.07</td>
<td>32.3</td>
<td>48.1 (697.6)</td>
<td>0.203</td>
</tr>
<tr>
<td>Propane (C(_3)H(_8))</td>
<td>44.09</td>
<td>96.8</td>
<td>41.9 (607.7)</td>
<td>0.217</td>
</tr>
<tr>
<td>Ethylene (C(_2)H(_4))</td>
<td>28.05</td>
<td>9.39</td>
<td>49.7 (720.9)</td>
<td>0.215</td>
</tr>
<tr>
<td>Propylene (C(_3)H(_6))</td>
<td>42.08</td>
<td>91.9</td>
<td>45.4 (658.5)</td>
<td>0.232</td>
</tr>
<tr>
<td>Methanol (CH(_3)OH)</td>
<td>32.04</td>
<td>239.6</td>
<td>79.8 (1157.4)</td>
<td>0.272</td>
</tr>
<tr>
<td>Ethanol (C(_2)H(_5)OH)</td>
<td>46.07</td>
<td>240.9</td>
<td>60.6 (878.9)</td>
<td>0.276</td>
</tr>
<tr>
<td>Acetone (C(_3)H(_6)O)</td>
<td>58.08</td>
<td>235.1</td>
<td>46.4 (672.9)</td>
<td>0.278</td>
</tr>
</tbody>
</table>

Pressure conversion: 1 MPa = 10 atm or bar = 145 psi = 2059 kg·cm\(^{-2}\). Pressure will be presented in bar hereafter.
Figure 3-2. Quaternary phase diagram for CO$_2$ represented with pressure as a function of temperature. The arrows show the direction of a possible S-L-V projection.

Temperature and pressure are an important influence of the state of CO$_2$, especially for creating its SCF. Increasing pressure without changing temperature at 17°C does not change the state of matter for carbon dioxide into a SCF. But temperature changes from 32°C can induce SCF status for CO$_2$ at relatively low pressure, approximately 73 bar [420, 421].

The effect on density caused by changing pressure for CO$_2$ in the supercritical phase is shown in Figure 3-3 [422]. It can be seen that pressure above 70 bar does not increase density, while changing temperature can change density with high pressure.
Figure 3-3. Effect of density vs. pressure for carbon dioxide. Reproduction (adaptation) from international tables of fluid state [423, 424].

3.2.2. The types of supercritical methods

SCF particle formation technology has evolved in many different forms during the last 20 years. The three most widely used methods are: particles from a gas saturated solution (PGSS) – (as utilised in this research), rapid expansion of supercritical solution (RESS) for CO₂-soluble drugs, and gas anti-solvent (GAS) for CO₂-insoluble drugs [236-239]. These SCF methods can be further broken up into various forms but are more or less based on the three above categories. Other possible supercritical fluid methods (to date) include: precipitation with compressed antisolvent (PCA), supercritical antisolvent (SAS), aerosol solvent extraction system (ASES), and solution enhanced dispersion by supercritical (SEDS) processing [230, 425]. Numerous review articles on a large range of SCF methods are available in the literature [234, 405, 426-432]. Although there are several interpretations of what constitutes a typical arrangement, and high degrees of overlapping parameters within each setup, a simple schematic of just four of these methods is shown in Figure 3-4 [433]. The schematic has been drawn in relation to whether the SCF is the solvent or anti-solvent (left or right sides) and continuous or dispersed (top or bottom), but even this is not to be taken in an absolute sense. A brief overview of a few selected SCF methods follows.
3.2.2.1. RESS

The rapid expansion of supercritical solution (RESS) method has been described in the literature from as early as 1984 [433]. In the RESS process, the solute is dissolved in SCF, then expanded through a nozzle to break-up the expanding solution into solid particles [434, 435]. The solute can also be dissolved in an organic solvent, then injected into SCF followed by volumetric expansion to form particles and removal of solvent [436]. The choice of solvent is very important as it must dissolve drugs of a polar nature, thus enhance solubility in the SCF [298, 436]. Also it is argued that the nozzle dimensions are critical to the formation of fine powder in RESS [437-440], although other studies have shown it is possible to form smaller particle sizes without sophisticated nozzle technology [441]. It may be that the materials used determine whether or not the nozzle is required.
Chapter 3. Formulation

The RESS process is divided into three major parts; the pre-extraction chamber, an extraction chamber, and the expansion chamber [422, 436]. RESS is simpler and cheaper compared to SAS, but solubility of polar drugs in supercritical CO$_2$ is poor, which almost makes RESS an impractical process. Since carbon dioxide, as a nonpolar molecule, exhibits weak Van der Waals forces, this means it is a weak solvent for most poorly aqueous-soluble pharmaceuticals that have strong intermolecular forces [226, 231].

For pharmaceuticals the particle size is important since it can limit the bioavailability of poorly soluble active drugs [286, 442]. In RESS the main purpose so far, for drugs in solid state, has been to reduce particle size [443]. Various studies have obtained data where the concentration of the drug in the blood were taken, from healthy volunteers at given intervals after dosing, as a function of particle surface area [444]. The quantity absorbed for a particle size of 2.7 µm is 2-fold more than that obtained with a particle size of 10 µm [444]. For further information, refer to the literature where the RESS method has been extensively reviewed [445-461].

Specifically for PGN, its solubility in CO$_2$ [234, 285, 462] is sufficient for the RESS process, however the starting particle size range was 10 – 100 µm, which was considered small enough for the formulation development of this research. Also, RESS used on smaller particles (50 µm or less) can cause agglomeration to occur due to adhesion forces leading to larger sized particles [284, 436, 463]. Appendix IIb provides the representative results of the RESS procedure on PGN powder. While the mean particle size dropped to 44.3 ± 1.5 µm from 77.4 µm (before RESS), the particle distribution became less monodisperse which could have been due to agglomeration. The span after RESS increased to 2.25 from 1.25 before RESS, indicating a larger particle size range. Furthermore, the RESS method is ideal for poorly water-soluble compounds, and can generate particle sizes in the 1-5 µm range which can be ideal for dosing by inhalation of drugs. However, the RESS method and unit (with 300 µm nozzle assembly) tested did not produce particle size distributions in the 5 micron or submicron regions. This may have been due to the low solubility of PGN in SC-CO$_2$, although soluble, it could be inadequate to produce particle sizes under 5 microns. Changing the SCF could have been one way to alter the method. For example, acetaminophen produced by the RESS process using near-critical dimethyl ether (DME) as the solvent, increased the solubility up to 15 g·kg$^{-1}$ in DME by several orders of magnitude higher than in CO$_2$ [433]. However, changing the SCF type was not considered due to complications such as logistics, and for fluids such as DME, safety and toxicity issues that would require regulatory approval, and additional appropriate lab facilities such as floor detectors, alarms, and improved
ventilation systems. The RESS process was therefore considered, but deemed not useful, nor practical for this research, so the SAS and GAS process were then considered.

3.2.2.2. SAS and GAS

Because of the low solubility of many compounds in CO$_2$, it is an effective anti-solvent for many substances, as in the supercritical anti-solvent (SAS) and gas anti-solvent (GAS) processes [297, 433, 464]. In the SAS process, a solution is mixed with the SCF where the solvent is highly soluble in the supercritical fluid but the solute is not [433]. The solvent expands into the SCF until the solute is no longer soluble and a solid precipitate forms [224, 433]. The key advantages are the low temperatures used, the rapid mass transfer (viz dissolution), and fine particle nucleation, for a wide range of drugs compared to the RESS process, and the higher solubilities that can be achieved by choosing an appropriate solvent:SCF mix [224, 465, 466].

Some examples where the SAS methods have been used include: insulin, hydrocortisone, lysozyme, albumin; also plant and dairy products have been processed [433, 467-469]. In the GAS process, which is similar to the SAS process, the solution forms the continuous phase and the supercritical anti-solvent is introduced, slowly decreasing the solvent capacity of the fluid until particles precipitate out [433]. The supercritical fluid can remove the majority of the solvent from the system with a continuous SCF line feeding into the system [470]. This has been used for many substances, including proteins [471-473], but it has limited control over the particle size [297, 474].

The major disadvantage of the antisolvent processes is the presence of residual solvent in the product [433]. Suitable solvents for use with carbon dioxide include organic alcohols. Solvents that are toxic, and hence less desirable, such as dichloromethane (DCM) and dimethyl sulfoxide (DMSO), have often been used [433] in both SAS and GAS methods [475, 476]. Other possible SCF are C2H6 and NH3 where substances can be directly precipitated from aqueous solution [477].

Variations of the anti-solvent SCF processes have been made to form more complicated setups, including the use of surfactants and sonication to aid phase dispersion. Surfactants have been added to limit re-agglomeration or deterioration of particles that are formed [433]. More comprehensive reviews of all the possible SCF methods and their applications have been published elsewhere [296, 478-486].
Chapter 3. Formulation

Several SAS runs were completed at IRL laboratories in Wellington, NZ. However, PGN was confirmed to be too soluble in SC-CO2, and did not behave as an anti-solvent with the SCF, leading to no product recovery. A confirmation test of PGN solubility in SC-CO2 was not conducted because it would require the construction of a separate unit built specifically for solubility measurements. Several studies have outlined the requirements to investigate substances under SCF conditions [487-490]. The PGN was washed through the expansion chamber along with the methanol and recovered in the waste bucket in solution. The SAS process was abandoned at this stage, which left only the PGSS method to pursue, which has the main attraction of simplicity for mixing purposes.

3.3. Particles from a gas saturated solution (PGSS)

3.3.1. Concept

A less conventional method, as used in this thesis, involves the use of SCF to produce PGSS. The process involves the dissolution of a melted or liquid suspended material(s) in a supercritical fluid, producing a gas-saturated solution/suspension that is later expanded through a nozzle to form solid particles or droplets [300, 491, 492].

The main principle of PGSS is the mixing process as described by Jung, Wissinger, and Liau (1985) [491, 493, 494]. This process is grounded on the fact that the solubilities of gases in liquids and solids are usually high, and even higher solubilities can occur if such liquids and solids are together in a compressed gas phase [491]. Although a large variety of substances can form particles using PGSS, it is not necessarily a requirement for that substance to be soluble in the supercritical fluid. For example, it is known that some polymers that can draw up high amounts of supercritical carbon dioxide (10 – 40%) do not actually dissolve in the SCF because the polymer may either swell or melt at a temperature well below (~10 to 50°C) its melting/glass transition temperature [491, 493-495].

The gas saturated solutions/suspensions are formed by dissolving a compressed gas in the bulk of a liquid [496]. The solution is then rapidly expanded into a precipitation vessel, resulting in the evaporation of the gas, and solidification of the liquid. During expansion the liquid cools below its solidification temperature, because of the Joule-Thompson effect\(^3\) [497], evaporation and/or volume-expansion of the gas, producing solid or semi-solid

---

Chapter 3. Formulation

particles [498]. Unlike other SCF techniques, PGSS does not necessarily require materials to be soluble in carbon dioxide [496]. The pivotal parameter is often the melting profile of a material in the presence of carbon dioxide, thus knowledge of the P-T trace and S-L-V equilibrium is required to give the pressure needed to melt and form a liquid at a given temperature, refer to Figure 3-2 [491, 496, 498]. The PGSS procedure can be drawn as a basic flow diagram, see Figure 3-5.

![Diagram of PGSS process]

**Figure 3-5. The basic process of the PGSS method.**

As illustrated in the flow diagram in Figure 3-5, the PGSS process consists of four steps:

- **Step 1.** Preparing the drug and excipient in a sample cylinder before pre-mixing conditions.
- **Step 2.** Forming the supercritical suspension/solution to mix the drug and excipient together (that is the SCF acts as the solvent for both solutes).
- **Step 3.** Rapid expansion of the SCF mixture to non-SC conditions.
- **Step 4.** Recovery of the final product (at this point the SCF has become supersaturated and particles precipitate out of SCF).

As will be described later, the density of a SCF can be changed by manipulating the operational conditions. For PGN the higher density state improves the solvent capacity of the SCF. For most SCFs, the density can be increased with increasing pressure and lower temperature [226, 284, 499]. Hence the pre-mixing phase of PGSS can be achieved by compressing the SCF to a higher pressure, given it is already at a sufficient temperature (e.g. for carbon dioxide a minimum of 31.1°C) [226, 231]. As the solvent capacity of the SCF increases, a greater amount of the solutes (drug and excipient) will dissolve in the SCF and a
Chapter 3. Formulation

saturated solution and/or suspension forms [500-502]. During the expansion phase, the SCF depressurises to atmospheric conditions (viz non-critical conditions). As the pressure decreases rapidly due to expansion, the temperature also decreases and changes the equilibrium between the SCF and solutes, resulting in a supersaturated state [231]. As the SCF continues to leave the solutes, the once dissolved solutes precipitate to form mixed particles of the drug and excipient. In work by de Sousa, et al. (2006) it was found that for Gelucire 43/01 (a similar molecular structure to Gelucire 44/14) the melting point came down (at 12°C lower than at atmospheric pressure), and the solubility of carbon dioxide increased with pressure (up to approximately 0.5 g/g of Gelucire 43/01) [496]. The temperature effect was the opposite (under constant pressure), i.e. solubility decreases with increasing temperature [496].

The time that the SCF undergoes expansion and precipitation of a substance has been estimated to be within the $10^{-7}$ to $10^{-5}$ seconds range [503, 504]. This rapid depressurisation and precipitation helps create uniform mixtures due to nucleation and facilitates small particle size formation in a narrow size distribution [441, 505]. As no other solvents are used, the PGSS process produces a final product that is free of organic solvents, because a SCF (such as carbon dioxide or water) is used instead as the solvent. This is advantageous in the food and pharmaceutical industries [226, 506]. A typical setup is represented below in Figure 3-6.

![Figure 3-6. A typical PGSS equipment setup, adapted from [491].](image)

The drug and excipient to be mixed are placed inside a sample cylinder, and will later be dissolved or dispersed in SCF. In this diagram, there is a stirring rod with paddles shown inside the sample cylinder and is used to increase the dissolution/dispersion rate. For our research adding a stirring apparatus was cost prohibitive and sonication was explored as a
way to help dissolution and dispersion to occur. Other examples to help mixing include using magnetism and canister cylinders [507]. More recently, a number of studies have found that ultrasonification of a dispersion in a PGSS process can result in the formation of more uniformly mixed products [508-510].

Also of note is the delivery of carbon dioxide into the sample cylinder from a non-supercritical gas cylinder holding up to 20 L of liquid gas, shown on the left of this figure (CO$_2$ arrow). Usually the delivered CO$_2$ is 98-99.99% pure and that is claimed to be sufficient to form a SCF with desired properties [511, 512]. However, some research suggests that more pure (SCF grade) CO$_2$ is required, possibly as high as 99.99% [224]. One study found that a linear dependence of critical temperature with critical pressure over a range of concentrations for the solvents and drug components that may be influenced by the CO$_2$ purity [513]. However there are no studies that have completed the same set of experiments using different CO$_2$ purities, so the evidence for CO$_2$ purity is inconclusive.

The PGSS process has been applied in various ways with different products being formed. For example, Shine and Gelb [514] showed that it is possible to form microspheres when a suspension is saturated with SC-CO$_2$ prior to atomisation. Their patent was for a process called polymer liquefaction using supercritical salvation (PLUSS). What is interesting is that the excipient/polymer and the drug are not necessarily soluble in the SCF. The mixture of the drug and encapsulating polymer, under SCF conditions, is capable of swelling the polymer under a temperature and pressure sufficient to maintain the fluid in a SCF state. The pressure is then rapidly released resulting in solidification of the polymer around the drug to form microcapsules [491, 514].

3.3.2. Patents

There are many patents filed for processes that use the PGSS concept. Some patents describe applications related to paint spraying and coating technologies [491, 515]. A patented PGSS process, by Weidner, et al. (1995) uses a SCF dissolved into a melted substance or suspension to form a gas-saturated solution, this mixture is then expanded through a nozzle and solid particles are formed due to the sharp temperature drop which is caused by fluid expansion [491, 516].

Another patent, by Sievers, et al. (1995) describes a new CO$_2$ based aerosolization process that is compatible with water-soluble compounds. A supercritical emulsion is formed and forced through a restrictor and decompressed to make an aerosol [517]. The
aerosolization parameters include temperature, pressure, flow rates and restrictor diameter. It is claimed the dispersion results from a mechanical effect during the short contact time in the ‘mixing tee’ and/or inside the tubing, and the high velocity expansion of the two-phase mixture [517]. According to Sievers, CO$_2$ solubility is so high in aqueous media that even within the short contact time, some dissolution occurs, improving the resulting dispersion [491, 517]. There are several versions of this process developed and still being developed.

In 1997, Karasawa patented a method of pulverising solid particles using SCF. The solid particles were suspended in a fluid in a SCF state [491]. The suspension was then expanded through a jet to form particles, avoiding agglomeration of the processed particles, which can be a major problem with PGSS techniques [491, 518].

3.3.3. Future PGSS developments

Particle design using PGSS is widely used in large scale, compared with other SCF methods under development. The simplicity of the concept, leading to low costs, and wide range of applications, provides a platform for PGSS development [491]. Many patents have been granted, since the 1995 filing of the basic PGSS process in Europe and the USA. A common area of development surrounds the expansion phase of PGSS where the nozzle assembly plays an important role [519, 520]. There may be continued growth in this area regarding the ‘mixing tee’, capillary flow, restrictor size and type, and so forth. Another area may include the method of mixing in the SCF, for example use of built-in stirring bars or rods, resins, and ultra-sonication.

Furthermore, patents are also successfully being granted for aerosol drug delivery [517] with different process variations, apparatus setups, and portable devices for static nebulisation, and forming micro-emulsions using RESS or PGSS concepts are being developed [491].

3.3.4. Phase effects on particle formation

A reasonable level of understanding is important when manipulating the conditions of the experiment to produce changes in crystallisation in a controlled manner, and obtain a product with targeted physicochemical properties [521]. For optimisation of the technique using supercritical fluids, the solubility of the model drug must be known as a function of pressure and temperature [286]. In this section some of the thermodynamic principles of fluid phases under critical conditions are reviewed is relation to the possible effects on crystallisation.
3.3.4.1. Single component systems

A pure substance can exist in three different states of matter – solid, liquid, and gas. Pressure and temperature are two independent factors which determine the state of a substance [521]. A system containing only one substance is said to be a single component system.\(^4\)

Equilibrium between different phases is represented by the phase boundary curves and all three states of matter coexist at the triple point [523]. The phase transition between a liquid and gas occurs along the vaporisation curve and ends at the critical point where the maximum pressure and temperature exists for a given substance. At temperatures and pressures above this point it is called the supercritical region, see Figure 3-1. Physical properties vary between liquids and gases, while physical variation can occur for a substance in its supercritical state. The variation in density and viscosity of carbon dioxide in relation to pressure at a constant temperature is shown in Figure 3-7.

Figure 3-7: Density and viscosity changes for carbon dioxide with pressure at 37°C. Reproduced with permission from Springer [521].

---

\(^4\) Gibbs' phase rule, was proposed by Josiah Willard Gibbs in the 1870s using the formula; \( F = C - P + 2 \), where \( P \) is the number of phases in thermodynamic equilibrium with each other and \( C \) is the number of components [522]. Atkins, P.W., de Paula, J., Physical chemistry. 8th ed. 2006, Oxford: Oxford University Press. A phase is a form of matter that is homogeneous in chemical composition and physical state. Typical phases are solid, liquid and gas. The number of components (C) is the number of chemically independent constituents of the system. The number of degrees of freedom (F) in this context is the number of intensive variables which are independent of each other.
Chapter 3. Formulation

For carbon dioxide both the density and viscosity increases dramatically from approximately 150 – 170 bar at a relatively low temperature of 37°C. This means that while the CO₂ is the only phase present, there can be varying degrees of density and viscosity that may have influences on solubility even within the supercritical state of CO₂. Depending on whether SC-CO₂ is acting as solvent or anti-solvent could be as simple as changing the pressure of the system during processing.

3.3.4.2. Two component systems

For binary mixtures there are two chemically independent components, and in addition to temperature and pressure, other variables help make up the composition (e.g. density, viscosity) of each phase.

Supercritical fluids show significantly increased solvent power that can be altered over a wide range through changing pressure and/or temperature, especially for high molecular weight and low vapour pressure solids [521]. For example, solubility increases with increasing pressure (at temperatures between 40°C and 60°C) for PGN in supercritical carbon dioxide (see Figure 3-8).

![Figure 3-8: Solubility of PGN in supercritical carbon dioxide.](image)

Chapter 3. Formulation

The effect of temperature is usually different, due to the vapour pressure changes of the solute [286, 521]. For PGN, the solubility is lower with higher temperature up to a certain pressure (approximately 235 ± 15 bar), then temperature seem to have less influence on solubility in SC-CO$_2$ [286].

The effect of density on the solubility is shown in Figure 3-9 for PGN. The line provided is obtained from the correlation between the logarithm of the solubility and the logarithm of the SC-CO$_2$ density [422].

![Figure 3-9: Effect of density on the solubility of PGN.](image)


It can be seen that the higher the density of CO$_2$, the higher the solubility of PGN in SC-CO$_2$. This is important when considering operating conditions for the PGSS method and formulation. In the following sections, a PGSS processing method, using carbon dioxide (CO$_2$) and various excipients, has been developed to provide improved aqueous solubility of PGN. Several PGSS operating conditions were identified as being able to alter the resulting dispersions, which could modify the aqueous dissolution of PGN.
Chapter 3. Formulation

3.4. Equipment setup

3.4.1. Design and construction of the PGSS unit

A major goal of this research was to develop a SCF unit at Auckland University that could be used for processing PGN into a delivery system. The unit was modified, according to reasons outlined earlier, into an operational form to perform the PGSS method and this unit would be used as an alternative mixing technique to more conventional methods such as using fusion or cosolvents. The PGN products would be examined for polymorphic changes before and after PGSS treatment.

3.4.2. PGSS setup

This section describes the four main components for the PGSS unit: the whole unit, the sample cylinder, the nozzle assembly and the particle precipitation chamber.

3.4.3. The PGSS unit

The PGSS unit used in this research is shown in the Figure 3-10. A complete list of materials and equipment used for this project setup are in Appendix III.
In Figure 3-11, liquid CO$_2$ was received by the high pressure (SCF) syringe pump from the CO$_2$ cylinder and delivers liquid CO$_2$ in the PGSS system. Appendix IV gives the details for the CO$_2$ connection package required, including an in-line filter. Both 98% and 99.9% (SCF grade) CO$_2$ were utilised originally, however no apparent difference in the products was noted from the different CO$_2$ purities. Therefore, the CO$_2$ liquid of 98% purity was used for all the experiments.
Figure 3-11. The CO$_2$ cylinder (left) and the joining assembly to the SCF syringe pump (right). Both the cylinder and pump have separate controlling valves that open and close individually.

The syringe pump (Teledyne Isco Series 260D with controller and pump module, Lincoln, USA) was purchased from AI Scientific (Auckland, NZ) and is capable of delivering liquid CO$_2$ and has a maximum delivery pressure of 1,000 bar.

Figure 3-12 shows the controller module used to set the system pressure, and the connecting tube from the SCF pump and sample cylinder. The type of pump is not important, as many studies use various types including piston, HPLC and syringe pumps [510]. The important considerations are to ensure the capacity of the pump can provide sufficient pressure for the SCF of interest. The key ability of the pump in this research is to able to deliver liquid CO$_2$ to pressures well above the critical point. Often a heating unit is fitted just prior to the pump because at sub-critical levels the liquid may expand, as it drops pressure, thus cooling and blocking the tubing and pump itself. This was dependent on the distance between the CO$_2$ source and pump and insulation of the pump(s) and connecting pipes. As in this research, and in several previous studies the CO$_2$ was able to be delivered directly into the pump without heating [524-526].
Figure 3-12. The SCF syringe pump controller (left) and the connecting tube from the SCF pump and sample cylinder (right).

The sample cylinder, from Swagelok (Solon, USA), was made of stainless steel with double ended DOT-Compliant thread, volume 300 cm$^3$ and maximum loading pressure of 213 bar. Figure 3-13 shows the sample cylinder. Liquid CO$_2$ was delivered to the top end of the sample cylinder and controlled with a needle valve. Once the needle valve was closed, the pressure of the sample cylinder was monitored with a gauge attached between the needle valve and sample cylinder. Of note is the presence of a relief valve installed adjacent to the outlet needle valve, which was necessary if the system became over pressurised. Also, there was a rupture disc adjacent to the sample needle valve that would break before the system experienced pressures exceeding the recommended maximum pressure, at approximately 203 ± 6.3 bar. There are many types of sample cylinders, however the main consideration was that the cylinder must be able to withstand high pressures within the required boundaries of the research pressure parameters. Appendix V provides details of the sample cylinder selected. Most of the literature studies employ relatively small cylinders, similar to the size used in this research [527-529].
3.4.4. Particle precipitation chamber

The precipitation chamber also acts as the expansion chamber, these terms will be interchanged hereafter. This chamber was made from 316 stainless steel capable of withstanding pressure up to 1379 bar. This is almost 7 times stronger than the maximum pressure allowable in the sample cylinder. The chamber setup and connection with the sample cylinder is shown in Figure 3-14. The chamber had a working space that measures 34.5 mm in diameter and 65 mm in height.
Figure 3-14. The nozzle assembly before being connected (in place without bolts screwed down) to close the particle precipitation chamber.

Specifications of precipitation chambers used in the literature are not readily available. Several studies have described the chamber in limited detail, either in the text or use of a schematic diagram [226, 530-532]. The lack of information could be partly due to the patents that have been accepted for various SCF processes. For example, several US patents have described methods for coating where the SCF unit structure is critical [533-535], European patents on the SCF unit construction [536, 537], and German patents have also undisclosed information [538]. The most important aspects are that the volume is sufficient for the experimental design, capable of withstanding high pressure dependent on the SCF being used and can allow rapid expansion, therefore ensuring precipitation of PGN. It was also important that it does not leak gas uncontrollably or leak water inside the chamber. Most chambers in the literature used a chamber similar to the one used in this research [539-541].
Figure 3-15. Configuration of the sample cylinder with particle precipitation chamber and nozzle assembly inside the chamber.

In Figure 3-16, the laser cut filter is shown, which has been made from stainless steel which is set inside the chamber to retain the PGN/excipient dispersions formed. The filter was purchased from Pall Industries Process Ltd, Auckland, NZ and has a pore size of 0.5 μm. A larger stainless steel filter (1.4 mm thick with 1.09 mm pores) was placed under the filter sheet as support for the smaller filter sheet. A copper O-ring was placed between the lid and base to ensure the system was tightly sealed. The reducing connector (1/8” to ¼”) is shown projecting out from the base of the cap, which was used as a form of nozzle, or entry point, into the precipitation chamber. There are numerous forms of nozzle use and definitions in the literature. As discussed earlier, it may or may not be important in PGSS processing. There are
several studies that use an open ended tube system for entry of the in-process materials into the precipitation chamber [503, 542, 543].

Figure 3-16. Diagram of the open particle precipitation chamber. Lower Right: the stainless steel micro-filter with supporting sheet and base.

The system pressure was measured in two places, at the syringe pump and at the sample cylinder, and data was recorded using PicoLog data logging software on a desktop computer. The locations of pressure measurements are important, for instance, the major pressure area is inside the sample cylinder, thus installation of a reliable pressure gauge or transducer to measure the cylinder pressure continuously during processing was paramount. All SCF units, regardless of method, use pressure monitoring technology.
Figure 3-17. Water bath and heat pump controller with sample cylinder attached.

As liquid CO$_2$ was added to the sample cylinder, the sample cylinder was detached from the precipitation chamber and placed diagonally in the heated water bath. This ensured the sample cylinder was mostly submerged in water. During expansion the water bath could be used to control temperature around the base of the reattached sample cylinder and particle precipitation chamber. The temperature was controlled using a coiled heat pump and controller (Grant, USA). See Figure 3-17 showing the relative positions of the water bath and heat pump with the sample cylinder attached in position.

As liquid CO$_2$ was delivered into the sample cylinder pressure, the system pressure at this point was controlled solely with the syringe pump, as a set pressure on the controller interface would be pre-determined and adjusted accordingly when required. The valves on the syringe pump and sample cylinder could be used in an emergency (e.g. system blockage, or leak) that would stop CO$_2$ fluid from entering the sample cylinder. A ball valve was
located between the sample cylinder and precipitation chamber, refer to Figure 3-15, which was fully opened when depressurising. A micro-needle valve was placed beneath the precipitation chamber (at the base of the water bath) which could be adjusted to control the release rate of the SCF mixture from the sample cylinder into the precipitation chamber. Also the male/ female connector below the ball value could be opened instantly to completely depressurise the chamber if required. All the connecting tubes, reducing connectors, washers and nuts were made from high pressure 316 stainless steel. The final PGSS system used in this research is shown schematically in Figure 3-18 with specific locations and types of valves.

![Figure 3-18. The final PGSS unit setup used in this thesis;](image)

(a) Liquid CO₂ cylinder, (b) syringe pump, (c) pressure gauge, (d) sample cylinder, (e) precipitation chamber, (f) heat pump, (g) water line, (h) temperature transmitter, (i) computer with PicoLog software, (j) pressure transducer, (k) CO₂ vapour (outlet), in line arrows indicate flow of CO₂, (l) isolation valve, (m) ball valve, (n) needle valve, (o) needle valve with rupture disc, (p) relief valve, and (q) water bath.
This PGSS unit setup is similar in design to various units used in the literature [288, 299, 300, 516].

3.5. PGSS processing

3.5.1. General outline

As described in some detail earlier, the PGSS process consists of four steps: preparing the drug and excipient, forming the supercritical mixture, rapid expansion to non-SCF conditions and recovery of the final product. This can be further broken down into three simpler steps that describe the procedure: preparation, processing and recovery. Each step was important and had the potential to influence the final product. The selection of each excipient will be discussed in the following chapter.

3.5.2. Operation procedure

3.5.2.1. Preparation step

The experiment started when a sample comprising of PGN/excipient of a desired weight and ratio was fed into the top of the sample cylinder (or pre-expansion vessel) with the ball valve attached at the bottom. The liquid CO₂ cylinder valve was opened, and the syringe pump calibrated to zero pressure, followed closely with the syringe pump priming to fill the syringe to maximum volume of approximately 265 mL which took up to about 5 minutes. As the syringe pump fills, the precipitation chamber is prepared with the filter in place and closed with the lid using a copper O-ring and 9 screws. The closed cylinder containing the sample (PGN and excipient) was then connected to the CO₂ syringe pump and placed inside the water bath unattached, at this stage, to the precipitation vessel. The system was then ready for processing with SC-CO₂.

3.5.2.2. Processing step

The cylinder needle valve was opened and high pressure liquid CO₂ was preliminarily fed into the system at a constant flow rate (83 to 85 mL-minute⁻¹) to increase the system pressure, using the syringe pump. At the same time, the water bath heater was operated to obtain the desired system temperature.
Chapter 3. Formulation

After the system conditions reached the desired pressure and temperature, the syringe pump was switched off and the needle value closed. The sample cylinder was then disconnected from the CO₂ supply. The pressurised sample cylinder was then taken to the sonicating water bath for 10 minutes to allow for improved mixing (bath temperature maintained at either above or below the melting point of the excipient being examined – e.g. Gelucire 44/14 had a water bath temperature of 40 ± 2°C). The set temperature for each sample was initially random and was later decided on using a systematic approach to determine the optimal level of heat. Each processing step ranged from 10 to 60 minutes, this step also underwent review to determine the ultimate processing time for each sample.

3.5.2.3. Recovery step

After sonnication the pressurised sample cylinder was connected to the precipitation chamber for release of CO₂ and separation from the PGN and excipient dispersions. The ball valve (fixed between the sample cylinder and expansion chamber) and the outlet valves were fully opened quickly to depressurize the system to atmospheric conditions. The syringe pump delivering liquid CO₂ was already stopped at this point, so as to not have continuous CO₂ flow into the unit, and conserve CO₂ for subsequent runs.

After the system pressure decreased to ambient conditions, the precipitation chamber was opened and the dispersions were collected from the filter sheet for analysis. The products were stored in closed glass vials. The sample cylinder and precipitation chamber was then washed using methanol.

The three steps; (1) preparation, (2) processing and (3) recovery can be seen in a flow diagram, refer to Figure 3-19. Via this procedure, the initial dispersions were formed using different pressures, temperatures, PGN/excipient ratios and varying amounts of CO₂ that were tested and analysed for yield only.
3.5.3. Problems and equipment maintenance

Given the high pressure that each run would subject on the system, it was not surprising that regular maintenance was required to ensure the system functioned well. For example, the ball valve (between the sample cylinder and precipitation chamber) has three Ethyl propylene
(EP) O-rings that would crack after 6 to 10 runs, so would need to be checked before each run and replaced when required. Nitro-based O-rings were considered because they would have expanded and contracted less with exposure to CO₂ and temperatures over 50°C, however they were cost prohibitive.

Due to the stainless steel needle and ball valves being removed, reattached and again removed from the sample cylinder for each run, Teflon tape was required. This helped ensure that no fusing of the steel parts occurred which could damage the threads used to screw each part together and also helped to seal the cylinder.

Furthermore, the opening and closing of the precipitation chamber resulted in the copper O-ring being easily worn, potentially causing a leak and rendering a failed run. Therefore, the copper O-ring was heated regularly to help reshape it and ensure that no leakages occurred and the system operated safely. Finally, the stainless steel filter sheet was deformed after the release of SC-CO₂ from the sample cylinder, which therefore required a coarse stainless steel filter to be placed underneath the collecting micro-filter for every run as a support, and the filter sheet was checked frequently for any damage.

Although all the problems were fixed on an ad hoc basis, each problem as it arose would hold up progress until new or replacement equipment had been ordered and delivered. At one point, all experimental work was halted at the research lab including where the SCF unit was located due to fires (from other experiments), requiring new safety checks of everyone’s equipment. On safety checks of my unit, it was noted that the unit did not have a way to relieve over pressure, and the existing valves were all manual. Hence, a rupture disc was ordered and installed, along with a relief valve. Also the precipitation chamber did not have a certified maximum pressure value, which required some theoretical and mathematical calculations to determine it was within safety margins before work was allowed to continue.

3.6. Elementary experiment

3.6.1. Materials

Industrial grade PGN and non-ionic cream (NIC) was purchased from PCNZ (Auckland, New Zealand) which was sourced from Pharmaca & UpJohn-Pfizer (New York, USA). PEG 400 and 4000 was purchased from BDH, VMR International Ltd. (Poole, England). Vitamin E TPGS was obtained from Zhejiang Shiner Chemical Company Ltd. (Zhejiang, China). Gelicure 44/14 was received by kind donation from Gattefossé Corporation, (New Jersey, USA). Sample cylinder, stainless steel tubing, male/female unions, valves, gauges, and CO₂
Chapter 3. Formulation

filters were purchased from Swagelok (Auckland, NZ). The syringe pump (Teledyne Isco Series 260D) with controller and pump module (Lincoln, USA) was purchased from AI Scientific (Auckland, NZ). Methanol and acetonitrile were HPLC grade obtained from Sigma-Aldrich (Auckland, NZ). Liquid CO₂ (purity 98%) was purchased from BOC Gas (Auckland, NZ). Triple-distilled MilliQ water was obtained in-house by reverse osmosis (Millipore, USA). All samples were used without any further purification steps. Various other materials and equipment are listed in Appendix III.

3.6.1. Preparation of CS, CM and PM dispersions

In the solvent (cosolvent – CS) evaporation method, PGN and excipient were dissolved in a minimum volume of methanol in a conical flask at room temperature (25°C). The solvent was removed by evaporation at room temperature (25°C) over 72 hours while shaken at 50 cycles per minute. The resulting semi-solid product was removed and dried in an oven at 30°C (below the melting points of all three excipients) for 5 hours. The solid dispersions were then tested for dissolution immediately after being removed from the conical flask.

The melting (comelt – CM) recrystallization method involved stirring with a glass rod the PGN and excipient on a hot stage in a beaker and brought to temperature (135°C), slightly above the melting point of PGN (approximately 131°C). The heating element was then switched off and the melted solution was allowed to cool to room temperature (25°C). The solid dispersions of each excipient were then analysed accordingly.

In the physical mixing (PM) method, PGN and excipient were weighed in a 1 to 1 ratio and placed in a mortar and pestle. Mixing was conducted for 2 minutes, which was sufficient time to allow the PGN and excipient to mix with each other. The mixing occurred at room temperature (25°C) and the product was removed for analysis from the mortar using a spatula.

3.7. Characterisation analysis

The procedures previously described in Chapter 2 were again used in this chapter. The characterisation studies included HPLC as described in section 2.8.

3.7.1. Process yield

The process yield of sample was measured by weighing the sample cylinder loaded with selected ingredients before processing and after processing.
3.7.2. PGN recovery and uniformity

The measurements for uniformity were conducted for samples loaded with PGN between the positions X, Y and Z inside the precipitation chamber, see Figure 3-20. This was also a form of validation, as samples tested were taken from intra- and inter-day batches.

![Figure 3-20. Sampling places inside the expansion chamber (SCF unit base). The collection points are marked X, Y and Z.](image)

The amount of drug contained in each dispersion was determined upon dissolving in methanol. Individual samples of the produced dispersions were dissolved in 10 mL of methanol and placed in a water bath (30°C), with ultrasonic agitation for 15 minutes. The resulting solutions were stored at 4°C in a refrigerator, until required for analysis under HPLC.

3.7.3. Birefringence

Crystalline PGN re-scatters (or double refracts) polarized light while dispersed in non-ionic cream or water. The excipients are amorphous and do not show any birefringence.

The ability of a drug to re-scatter light gives a unique opportunity to observe the degree of dissolved drug in a formulation, either to confirm solid solution or the boundary of saturation in a given excipient(s). One of the important goals in this research was to improve the degree of dissolved PGN in the selected excipients, thus improve the amount of free PGN available for permeation. The improved solubility can be ensured by the reduced absence of birefringence under cross-polarized light.

For each sample, 1 g was placed on a clean glass slide and covered carefully with a coverslip. Observations were performed using a brightfield Leica DMR polarized light microscope (10x, 20x or 100x oil immersion objectives) (GmbH, Germany). Pictures were taken using a Nikon coolpix 4500 camera.
3.7.4. Infrared (IR) spectroscopy

FTIR was employed to investigate the composition for PGN with excipient after exposure to SC-CO₂. The IR spectra of samples were recorded on a Bruker Tensor 37 (Bruker Optik, GmbH, Germany) spectrometer using an ATR attachment, as described in section 2.3. IR spectra of the samples were recorded following high pressure conditions at 83 and 124 bar. Spectra were also recorded for dispersions after PGSS processing of the samples at different temperatures. For PEG-based dispersions the temperatures were 40°C and 56.9°C, for Gelucire 44/14 dispersions there were 39°C and 56.9°C observations and TPGS dispersions were observed at 41°C and 56.5°C. The spectra reported in this chapter were obtained by measuring the spectrum of the pure powder of PGN, the excipients without the drug, and compared with dispersions with expipient and PGN. Spectra are shown with dispersions of either ratios of 1:1 or 1:5 parts, PGN to excipient, respectively. Data was analyzed using OPUS software (Bruker Optik GmbH, Germany) version 6.5.

3.7.5. X-ray powder diffraction (XRPD) studies

XRPD analysis for PGN dispersions was performed with a powder diffractometer with a copper (Cu) Kα radiation (λ= 1.5406 Å) (N.V. Philip’s, Gloeilampenfabriken – Eindhoven, Netherlands). The scanning angle 2θ ranged from 2° to 40°, as described in section 2.4. Spectra were recorded for dispersions after PGSS processing of the samples at different temperatures (39, 40, 41, 56.9 and 56.5°C). Spectra are shown with dispersions of either ratios of 1:1 or 1:5 parts, PGN to excipient, respectively.

3.7.6. Differential scanning calorimetry (DSC)

The melting point of PGN with excipient was determined by DSC using a Q1000 TzeroTM module (TA Instruments, USA). Heat flow calibration of the instrument was done using indium as a reference material, as described in section 2.2.10. Powder samples (5-10 mg) were taken in crimped aluminium pans and heated at 10 °C·minute⁻¹ above the MP of the disperions. Data was analyzed using Universal Analysis software (TA Instruments, USA) version 4.1D. All the measurements were performed in duplicate. MP is reported as the onset temperature.
Chapter 3. Formulation

3.7.7. **Scanning electron microscopy (SEM)**

The surface characteristics of PGN dispersions were observed using a SEM (Philips XL30S FEG: Eindhoven, Netherlands) The samples were prepared for SEM by sputter coating with platinum for 180 seconds at 1.1 kV (Polaron SC 7640 Sputter Coater: Hertfordshire, UK) before viewing under the microscope. The samples had a platinum coating thickness of approximately 75 nm and images were taken with 200 times magnification.

3.7.8. **HPLC solutions**

PGN samples at room temperature were centrifugated at 10,000 rpm for 10 minutes and the supernatant collected for analysis. The drawn sample was then filtered using 0.45 µm nylon filters (Millipore, USA). The amount of PGN contained in each sample was determined upon dissolving in mobile phase by a dilution factor of no more than 4. When the solutions were diluted they were placed in the HPLC storage tray at 2 – 8°C ready for autoinjection or stored in freezing conditions (-20°C) for no longer than 4 weeks. After every third sample solution, the column was washed with methanol with a total run time of 10 minutes per wash.

3.7.9. **In vitro dissolution testing**

*In vitro* dissolution tests were performed according to the rotating paddle method (USP 24) and the dissolution apparatus used was model SR8PLUS, Hanson Research (California, USA). The dissolution medium used was 900 mL of fresh Milli-Q water. Samples of PGSS-CO₂ treated PGN/excipient and untreated PGN (18 ± 0.05 mg) were weighed and applied to the pre-equilibrated dissolution medium (temperature = 32 ± 0.2°C) and the paddle was operated at a stirring rate of 100 ± 0.7 rpm. Dissolution samples of 4 mL were withdrawn every 60 seconds over the first 10 minutes, then every 10 minutes for the following 50 minutes, and replaced with 4 mL of fresh dissolution medium after each withdrawal. Each test sample was filtered using a 0.45 µM filter unit (Millipore, USA) and the absorbance was immediately measured at 254 nm in a Agilent series 1200 HPLC (Agilent Corporation, Germany). All samples were tested in triplicate.

3.7.10. **Statistical analysis**

Statistical analysis was performed with Minitab® Release 15.0 software (Minitab Inc. State College, PA, USA). The data was tested for significance using analysis of variance
Chapter 3. Formulation

(ANOVA) with a 95% confidence interval (p-value < 0.05). Raw data was collected in Excel 2007 (Microsoft® Corporation, CA, USA) on an Intel Core™ 2 Dell computer.

3.8. Results and discussion

The elementary PGSS experiment focused on the effect of the excipient ratio (PGN to Excipient by weight), temperature and pressure on the yield and dissolution of the produced dispersions. The effects of pressure and temperature on production percentage yield are outlined in Table 3-3. Very good percentage yield (> 80%) could be achieved at temperatures both below and above the melting point of the selected excipients. Lower yields were observed at temperatures below MP and when the pressure (thus CO₂ density) was low (< 85 bar).

A typical post-SCF processing observation of the precipitation vessel after opening is shown in Figure 3-21. One of the critical conditions for PGSS carbon dioxide processing is the melting behaviour of the target compound [496]. In this case, PGN is not altered as it’s melting point is above 130°C, while the melting points of the used excipients is 60°C and below.

![Figure 3-21. The particle precipitation chamber with the cap removed after PGSS processing (view facing down into the chamber).](image)

The higher yields may be explained by a combination of higher pressure and larger amounts of CO₂, which is directly related to higher pressure. By raising the pressure of the cylinder at temperatures near 60°C, this would increase the CO₂ loading and the proportion of PGN and excipient was reduced. This made the CO₂ environment more dominant, and as a
result, the suspended PGN particles and excipients became better diffused and possibly more dissolved, also allowing for improved flow into the precipitation chamber. Furthermore, higher pressure facilitates removal of the suspended mixture from the sample cylinder, hence increasing yield. Lower operation temperatures (less than the melting point of excipients) did not significantly influence the process yield of the PEG-based dispersion. Whist lower temperatures affected the Gelucire 44/14 dispersions with a lower processing yield; the higher temperatures gave a high and low yield for the TPGS dispersions. However, the yield was probably also influenced by the PGN to excipient ratio leading to PGN crystal agglomeration and blocking the tubing between the sample cylinder and precipitation chamber. The tested SCF conditions and yield values are listed in Table 3-3. It was found that binary dispersions with a drug to excipient ratio of 1 to 5, nominal SC-CO$_2$ pressure of 124 bar, and system temperature of ranging between 56 – 59°C were favorable processing conditions to produce promising yields (> 86%). There are few studies on PGSS that specify the process yield obtained, however one study stated that an increase in the expansion pressure yielded lower degrees of aggregation [299]. In this study a dispersion of theophylline and hydrogenated palm oil was formed, but the research was focused on the different types of morphological structures produced by PGSS, rather than process yield. In a recent study using PGSS to formulate β-carotene the process yield was between 5 to 44% for SC-CO$_2$ conditions at 80 – 100 bar and temperature of 45°C [544]. This study had at least two differences in the PGSS method including the use of a mechanical stirred built inside the sample cylinder and utilized a 600 µm spray nozzle. In another study using PGSS technology to produce particles of PEG filled with lavandin essential oil, yields of up to 66% were achieved [545]. Due to the use of individual designs and constructions of SCF units and non-standardized SCF procedures of operation, any comparisons must be taken with limited value.

<table>
<thead>
<tr>
<th>Excipient</th>
<th>System temperature ± SD (°C)</th>
<th>Cylinder pressure ± SD (bar)</th>
<th>CO$_2$ amount in cylinder (g) *</th>
<th>Nominal CO$_2$ density kg·m$^{-3}$</th>
<th>PGN/Excipient ratio</th>
<th>Product yield**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelucire 44/14</td>
<td>56.9± 1.0</td>
<td>124 ± 2.5</td>
<td>152</td>
<td>507</td>
<td>1 to 5</td>
<td>93.3</td>
</tr>
<tr>
<td>TPGS</td>
<td>56.5± 1.2</td>
<td>124 ± 1.9</td>
<td>152</td>
<td>507</td>
<td>1 to 5</td>
<td>95.7</td>
</tr>
<tr>
<td>PEG 400/4000</td>
<td>58.9± 1.0</td>
<td>124 ± 2.1</td>
<td>152</td>
<td>507</td>
<td>1 to 5</td>
<td>86.3</td>
</tr>
<tr>
<td>PEG 400/4000</td>
<td>40.0± 0.9</td>
<td>83 ± 2.4</td>
<td>112</td>
<td>372</td>
<td>1 to 1</td>
<td>80.1</td>
</tr>
</tbody>
</table>

Table 3-3. Operation conditions, PGN: excipient ratio and yield.
An important factor in the formation of dispersion is the drug solubility in the excipient/carrier such as solvent evaporation methods; however in the case of the PGSS method it could be less important. In this study, the lower yields observed in the dispersions with a higher drug to excipient ratio (1 to 1) had lower amounts of PGN dissolved in SC-CO$_2$. For example, as the initial total weight for all the SCF dispersions was 3 g, the 1 to 1 ratio of drug to excipient had 1.5 g PGN compared to that of 0.5 g for the 1 to 5 ratio dispersions. Furthermore, the lower pressure (83 bar), which is directly proportional to SC-CO$_2$ density, would lead to lower PGN amounts being dissolved in SC-CO$_2$. Thus, a suspension likely formed when a drug to excipient ratio of 1 to 1 was used. Various studies have shown that PGN solubility in SC-CO$_2$ could be as low as $1.64 \times 10^{-4}$ (mole fraction) at 115 bar and 40°C [286, 293, 546], however it is possible to drastically improve the PGN solubility in SC-CO$_2$ by increasing the CO$_2$ density [230, 547]. However, there is a threshold at which SC-CO$_2$ density is not associated with improved PGN solubility and this occurs at approximately 205 bar for temperatures between 40°C to 60°C (where the solubility mole fractions are $7.20 \times 10^{-4}$ and $7.37 \times 10^{-4}$, respectively) [286, 548]. In this study, the maximum pressure utilized was 124 bar at 59°C, so PGN solubility in SC-CO$_2$ was below ideal conditions for maximum solubility (approximately $1.78 \times 10^{-4}$). This means the higher drug to excipient loading of 1:1 ratios had a mole fraction of approximately $170 \times 10^{-4}$, compared to that of the 1:5 ratio dispersions where the mole fraction of PGN to SC-CO$_2$ was approximately $32 \times 10^{-4}$. It is possible that the high pressure condition of SC-CO$_2$ treatment forced more PGN to interact with the excipient per unit area [295]. Interestingly, the SCF-based PGN dispersions formed under higher pressure (124 bar) did outperform the SCF products formed from the lower pressure (86 bar) based on dissolution testing. Furthermore, the SC-CO$_2$ may induce other excipient changes like swelling, foaming, crystallization, and viscosity or density reduction [295, 428, 549, 550]. Further research investigating the solid-liquid-vapor (S-L-V) curves and solubility of SC-CO$_2$ with drug and excipients are required to understand the effects of density and relationships with PGN dissolution rate.

The rate of expansion can be important, where rapid cooling on expansion into the precipitation vessel can improve dispersion uniformity because there is less time for
recrystallisation and agglomeration [428]. During processing the ball valve was opened in the same way each time, however once the valve was fully opened the rate of expansion was not necessarily the same. It was noted that the PGN-based dispersions had a drastically slower time period where liquid CO$_2$ was evaporated. For example, the Gelucire 44/14 and TPGS expansion times for approximately 10 to 20 seconds, while the PEG expansion time typically was 60 to 90 seconds. In this study, all the PEG-based PGN dispersions underperformed in dissolution testing including the SCF-prepared dispersions relative to the Gelucire 44/14 and TPGS dispersions prepared by the PGSS method, although there was improvement compared to that of the control (untreated PGN). This result is similar to some PEG-based formulation studies where dissolution rates were significantly better compared with controls [230, 551-554]. Moreover, other studies have shown that the larger molecular weight PEG-based formulations tend to produce slower dissolution results unless prepared in combination with cyclodextrins [555, 556].

It has also been demonstrated that CO$_2$ can be used as a transferring medium to bring a solute (or drug) from fluid phase into the polymer or carriers internal structure [373]. The CO$_2$ changes the physical structure of a polymer so it becomes more flexible and accepting of guest molecules even under moderate pressure and temperature [373]. Recent studies have shown that the drug-polymer encapsulation is possible for CO$_2$-soluble [557-559] as well as for CO$_2$-insoluble solutes [373, 560]. For CO$_2$ soluble drugs, the loading efficiency depends on the solubility of a drug in CO$_2$, which can be controlled by pressure [373]. Supercritical fluids can be utilised through their solvent power abilities to control the degree of encapsulation. Furthermore, the advantages SCFs over “the conventional methods include not only the simplicity of process that needs only one-step operation but also the capability of complete removal of the processing medium from end-product, which is useful for the organic solvent-sensitive applications” [373].

Based on the combined experimental results (those shown in Table 3-3), it was decided that the binary dispersions (ratio 1:5), nominal SC-CO$_2$ pressure of 124 bar and system temperature of approximately 57°C were favorable processing conditions from a yield viewpoint (> 86% yields). The higher drug loading formulations of (1:1) caused larger PGN crystals to form and showed lower yields (63 – 80%). Therefore, samples processed under higher supercritical conditions with 5 times more excipient than PGN were thought to be the best PGN dispersions. Both PGSS processed dispersions for each excipient were characterized and compared with conventional methods. Namely, untreated PGN and CO$_2$
treated PGN with the excipients Gelucire 44/14, TPGS and PEG 400/4000 were investigated for changes in dissolution of PGN under various supercritical conditions, morphology and crystalline structure within each excipient.

### 3.8.1. PGN recovery

Chromatographic analyses confirmed the maintenance of consistent drug loading and recovered PGN with each excipient after exposure to SC-CO$_2$, and from the conventionally prepared methods. A full summary of test runs together with operating parameters and total yields are listed in Appendix VI. Given the known ratios, such as 1 part PGN with 1 part excipient or 5 parts excipient, the drug loading into each excipient could be measured after PGSS processing. It was found that the binary dispersion of PEG 400/4000 with PGN was within 1.96% of the expected PGN loading, while TPGS and Gelucire 44/14 were within 3.22% and 1.96%, respectively, shown in Table 3-4. All the conventional dispersions showed little or no measured difference, and any difference was probably due to experimental error from handling, e.g. weighing and dilution for HPLC preparation.

<table>
<thead>
<tr>
<th>Dispersion</th>
<th>PEG 400/4000</th>
<th>TPGS</th>
<th>Gelucire 44/14</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCF (1:5)</td>
<td>1.96%</td>
<td>3.22%</td>
<td>1.69%</td>
</tr>
<tr>
<td>SCF (1:1)</td>
<td>2.15%</td>
<td>0.58%</td>
<td>-0.44%</td>
</tr>
<tr>
<td>CM</td>
<td>0.54%</td>
<td>-0.25%</td>
<td>0.87%</td>
</tr>
<tr>
<td>CS</td>
<td>-0.19%</td>
<td>0.33%</td>
<td>0.15%</td>
</tr>
<tr>
<td>PM</td>
<td>1.54%</td>
<td>0.29%</td>
<td>nil</td>
</tr>
</tbody>
</table>

Results are provided as mean ± SD, n = 3 at each concentration.

### 3.8.2. Uniformity

The dispersion was sampled at three places on the filter in the expansion chamber, after completion of the SCF processing. The expected amount was the initial amount of PGN weighed during preparation, and the results indicated variability of uniformity for the SCF process. The recovery of PGN from all the samples was 95.3% to 100.6% for the 1:5 dispersions and 94.6% to 103.8% for the 1:1 dispersions of the expected amounts. Generally, the samples with a 1:5 ratio of PGN to excipient showed better uniformity values, as did the Y area of the sample recovery area studied. Overall, the recovery of PGN samples between
the positions from X, Y and Z of each run was relatively similar (see Table 3-5), suggesting that control over PGN recovery is possible under the conditions tested.

Table 3-5. Percent PGN recovered after SCF processing from excipient at 3 sample locations.

<table>
<thead>
<tr>
<th>Sample position</th>
<th>Constituent Ratio</th>
<th>PEG 4000</th>
<th>TPGS</th>
<th>Gelucire 44/14</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>SCF (1:5)</td>
<td>97.04±1.4%</td>
<td>96.54±2.0%</td>
<td>95.31±3.1%</td>
</tr>
<tr>
<td></td>
<td>SCF (1:1)</td>
<td>95.88±1.2%</td>
<td>96.78±1.2%</td>
<td>101.04±1.5%</td>
</tr>
<tr>
<td>Y</td>
<td>SCF (1:5)</td>
<td>98.07±1.3%</td>
<td>100.58±1.4%</td>
<td>98.31±1.7%</td>
</tr>
<tr>
<td></td>
<td>SCF (1:1)</td>
<td>97.65±1.5%</td>
<td>96.78±1.9%</td>
<td>100.44±2.2%</td>
</tr>
<tr>
<td>Z</td>
<td>SCF (1:5)</td>
<td>95.85±2.0%</td>
<td>95.79±1.7%</td>
<td>98.25±2.3%</td>
</tr>
<tr>
<td></td>
<td>SCF (1:1)</td>
<td>103.02±1.9%</td>
<td>103.78±2.4%</td>
<td>94.56±3.7%</td>
</tr>
</tbody>
</table>

Results are provided as mean ± SD, n = 3 at each concentration.

3.8.3. Birefringence

Visual inspection of samples shows that the PGN was generally well dispersed through each excipient, shown in Figure 3-22. Given the variability in the ability to spread each sample consistently, it was also observed that both Gelucire 44/14 and TPGS samples showed the most concentrated dispersions of fine particles (50-100 µm), while for the market cream and PEG samples there are less well dispersed particles. For PEG there appears to be agglomeration/aggregation of particles not seen in the other samples. As mentioned previously, it is necessary to consider the possible differences in the image analysis may be due to experimental application rather than unique characteristics of the samples.
Figure 3-22. Polarized light microscopic images (200 × magnification) of with and without PGN, prepared from various dispersion methods. The top row images are without PGN.

3.8.4. FTIR spectroscopy

Characteristic bands of PGN are seen in all three dispersions with different excipients processed from different methods, as shown in the FTIR results, Figure 3-23 [249]. Figure 3-23(a) represents untreated PGN alone with the C=C bands observed between 1600 – 1680 cm\(^{-1}\), which can only be attributable to PGN. For each excipient definitive bands were also detected at higher and lower wavenumbers [362, 370, 371]. In the case of TPGS and Gelucire 44/14 the ester carbonyl stretching was observed at 1734 cm\(^{-1}\), while no such band was seen for PEG 400/4000 (Figure 3-23d). Over-lapping of the CH stretching band of PGN at 2945 cm\(^{-1}\) and that of each excipient at 2880 cm\(^{-1}\) was observed. In the dispersions, the CH stretching band of each excipient was decreased. The absorption band at 3400 – 3650 cm\(^{-1}\) is from the terminal hydroxyl group which is obvious for Gelucire 44/14 [spectra: (e), (h) and (n) and somewhat observable for TPGS (spectra: (c) and (l)] [371]. The bands between 1050 – 1250 cm\(^{-1}\) are due to the C-O stretching which is present in all the excipients but not PGN [371]. A very broad band was also visible at 3430 cm\(^{-1}\) which accounted for the presence of water confirmed by the generally broad endotherms detected in the DSC experiments (see section 3.8.6).
Figure 3-23. FTIR spectra of PGN and PGN dispersions;
(a) untreated PGN, (b) Gelucire 44/14, (c) TPGS, (d) PEG 400/4000, (e) SCF-PGN/ Gelucire 44/14 (1:5), (f) SCF-PGN/TPGS (1:5), (g) SCF-PGN/PEG 400/4000 (1:5), (h) solvent-PGN/Gelucire 44/14 (1:1), (i) solvent-PGN/TPGS (1:1), (j) solvent-PGN/PEG 400/4000 (1:1), (k) mixing-PGN/Gelucire 44/14 (1:1), (l) mixing-PGN/TPGS (1:1), (m) mixing-PGN/ PEG 400/4000 (1:1), (n) SCF-PGN/PEG 400/4000 (1:1), (o) SCF-PGN/PEG 400/4000 (1:1), (p) SCF-
PGN/PEG 400/4000 (1:1), (q) comelted PGN/Gelucire 44/14 (1:1), (r) comelted PGN/TPGS (1:1) and (s) comelted PGN/PEG 400/4000 (1:1).
Generally, the FTIR spectra of the dispersions were only a summation of drug and excipient spectra. This would suggest that there was minimal or no interactions between the drug and excipients and PGN was able to maintain its crystallinity. If the drug and excipient interacted, then the functional groups in the spectra would show band shifting and/or broadening in comparison to the untreated PGN. The incorporation of PGN into Gelucire 44/14, TPGS and PEG 400/4000 did not obviously modify their bands, their pure FTIR spectra are shown in Figure 3-23(b), (c) and (d), respectively. Each moiety of Gelucire 44/14 has C=O groups that can potentially form hydrogen bonds with the drug. But steric hindrance may reduce the capacity for intermolecular interactions. Given there are no nitrogen atoms available, thus making the carbonyl group the only possible source of hydrogen bonding. The bands at 1050 cm\(^{-1}\) and 1250 cm\(^{-1}\) represent the symmetrical and asymmetrical C-O stretching of the Gelucire 44/14, which were replaced by broader bands suggesting possible –CH group hydrogen bonding with PGN. Similar interactions were observed with the TPGS and PEG dispersions containing PGN. Thus, a bonding interaction and reduced mobility of PGN within each dispersion may indicate that a stable amorphous form of PGN was produced inside the excipients. Further investigations are required such as XRPD to observe crystalline form changes, although he Raman spectra indicated no significant polymorphic or amorphous transformations occurred when pure PGN was tested under SC-CO\(_2\) conditions.

3.8.5. XRPD studies

Figure 3-24(a), contains the X-ray powder diffractogram (XRPD) pattern of untreated PGN. It shows a relatively high magnitude of peak intensities and the three highest peaks, in the characteristic diffraction peaks, were detected at 2\(\theta\) of 10.7\(^{\circ}\), 12.8\(^{\circ}\) and 16.96\(^{\circ}\) respectively, which corresponds well with the reported α-form of PGN [250, 346]. The SC-CO\(_2\) treated PGN within each excipient did not exhibit any different peaks [diffratograms (e) to (g) for ratios 1:5 and (n) to (p) for ratios 1:1].

Figure 3-24 also reveals corresponding peaks for (b) Gelucire 44/14, (c) TPGS and (d) PEG 400/4000, at 2\(\theta\) = 19.3\(^{\circ}\) and 23.9\(^{\circ}\). All three excipients contain varying amounts of PEG from 1000 to 4000 units, which exist in crystalline form. None of the excipients formed low broad peaks, suggesting that none of the excipients were amorphous before or after preparation. No changes in the crystal morphology were seen based on the diffractograms for the differently prepared formulations, except for the comelted product with TPGS. Melting is
well known to be one way to transform PGN into β-form, which matched the known extra peak at 13.9° and loss of the peak at 10.7°, as shown in Figure 3-24(r) [250, 346]. This change in crystal form did not result in a higher dissolution rate compared to comelted Gelucire 44/14 product. Both the comelted Gelucire 44/14 and TPGS-based products had similar dissolution (approximately 40% after 60 minutes), compared with the PEG 400/4000-based product, approximately 25% after 60 minutes. This means the difference in dissolution cannot be attributable to the crystalline transformation observed from the XRPD. No other polymorphic transitions occurred including in the SCF processed products, which is also consistent with the Raman result for the SCF processed PGN (Figure 2-12). The cosolvent prepared products are shown in Figure 3-24 for (h) Gelucire 44/14, (i) TPGS and (j) PEG 400/4000 and PGN (ratios 1:1). The physical mixing products with PGN are represented by diffractograms for (k) Gelucire 44/14, (l) TPGS and (m) PEG 400/4000 (ratios 1:1), and the comelted products for (q) Gelucire 44/14, (r) TPGS and (s) PEG 400/4000 (ratios 1:1). All the observed lines in the XRPD of the dispersions were attributable to either the PGN or excipient. The overall magnitude of peak intensities were decreased when both PGN and excipient were present, indicating a dilution effect, rather than change in degree of crystallinity or amorphism.
Figure 3-24. Power X-Ray diffractograms of PGN and PGN dispersions;
(a) untreated PGN, (b) Gelucire 44/14, (c) TPGS, (d) PEG 400/4000, (e) SCF-PGN/Gelucire 44/14 (1:5), (f) SCF-PGN/TPGS (1:5), (g) SCF-PGN/PEG 400/4000 (1:5), (h) solvent-PGN/Gelucire 44/14 (1:1), (i) solvent-PGN/TPGS (1:1), (j) solvent-PGN/PEG 400/4000 (1:1), (k) mixing-PGN/Gelucire 44/14 (1:1), (l) mixing-PGN/TPGS (1:1), (m) mixing-PGN/PEG 400/4000 (1:1), (n) SCF-PGN/PEG 400/4000 (1:1), (o) SCF-PGN/PEG 400/4000 (1:1),
(p) SCF-PGN/PEG 400/4000 (1:1), (q) comelted PGN/Gelucire 44/14 (1:1), (r) comelted PGN/TPGS (1:1), and (s) comelted PGN/PEG 400/4000 (1:1).
3.8.6. Thermal analysis

The thermograms for PGN, PEG 400/4000, Gelucire 44/14, TPGS and various mixtures are shown in Figure 3-25, 26 and 27. The thermograms of each pure substance have given a single exothermic peak corresponding to the melting point (MP) of PGN at 129.81°C, PEG mixture of 400 and 4000 (50:50 ratio) at 55.2°C, Gelucire 44/14 at 42.9°C and TPGS at 32.1°C.

As Gelucire 44/14 content increased, the PGN peak moved to a lower temperature. The exotherms for TPGS are shown in Figure 3-25. The MP of PGN changed from 129.8°C, to 128.9°C with 1:5 ratio of Gelucire 44/14, and then 101.9°C when the ratio increased to 1:1 with Gelucire 44/14. Also the Gelucire 44/14 MP changed a lower temperature, from 42.9°C, to 41.9°C and finally 33°C for the higher Gelucire 44/14 ratio of 1:1. This is consistent with mixing different ingredients, and indicates a degree of dispersion has occurred.
Figure 3-25. Thermographs of PGN and Gelucire 44/14;
(a) PGN, (b) Gelucire 44/14, (c) SCF prepared dispersion of PGN with Gelucire 44/14 1:5, and (d) PGN with Gelucire 44/14 1:1. Exotherm: up.

For the TPGS-based dispersions there were obvious changes in the melting behaviours for both PEG and PGN. The PGN with TPGS exotherms dropped from 131.9°C to 104.5°C and 118.3°C for the 1:5 and 1:1 dispersions, respectively. The exotherms for TPGS are shown in Figure 3-26.
Figure 3-26. Thermographs of PGN and TPGS;
(a) PGN and (b) TPGS. The dispersions of PGN and TPGS (1:5) are shown in (c) and the dispersion of the PGN and TPGS (1:1) are shown in (d). Exotherm: up.

For the PEG-based dispersions there were also obvious changes in the melting behaviours for both PEG and PGN. The PGN exotherms dropped from 131.9°C to 129.3°C.
while the PEG content increased from 1:5 to 1:1. The exotherm for the PEG dispersions are shown in Figure 3-27.

Figure 3-27. Thermographs for PGN and PEG 400/4000; (a) PGN and (b) PEG. The mixtures are shown in (c) PGN with PEG (1:5) and (d) PGN with PEG (1:1). Exotherm: up.
3.8.7. Morphology

Figure 3-28 shows the SEMs of untreated PGN and SCF processed PGN with each excipient. The untreated PGN particles had a sphere like habit with smooth surfaces and were clustered. The particles were within the size range of about 1 to 5 μm and the clusters up to about 50 μm. On the other hand, micrographs of untreated excipients showed a continuous surface consistent with the unaided visual appearance (Figure 3-28;b, d and f). From the SEM results, the dispersions processed with SCF generally showed that the PGN and excipient had mixed well, as PGN particles were not visible. This is consistent with reported literature that drug crystals were entrapped inside excipient pores and/or molecularly dispersed [230]. It is not unusual for drug particles to undergo a change in morphology due to SCF conditions and possibly the nozzle geometry [234], however PGN is unaffected by SC-CO$_2$ and this is indirectly confirmed with the SEM because there were no obvious prisms or needle like structures present in any of the images.
Figure 3-28. SEM images before and after SCF processing (1:5 ratio samples);
(a) PGN alone, (b) Gelucire 44/14, (c) PGN with Gelucire 44/14, (d) PEG 4000, (e) PGN with PEG 400/4000, (f) TPGS, and (g) PGN with TPGS (1500 × magnification).
3.8.8. In vitro dissolution

The in vitro dissolution profiles for both untreated and PGN dispersions are shown in Figure 3-29 to Figure 3-31. As expected a substantial increase in both the rate and extent of PGN dissolution was observed with SC-CO$_2$ prepared PGN dispersions compared to the untreated sample over 60 minutes. The untreated PGN dissolution rate is so slow that even after 3 hours the extent of dissolution was under 71% PGN released, an extended time period of 3 hours for untreated PGN has been provided in Appendix VII. The rate of PGN dissolution for the selected excipient dispersions prepared by conventional and SCF processing outperformed the untreated PGN powder, especially the SCF-based PGN dispersions. For instance, after 10 minutes, 87.4% of SC-CO$_2$ processed PGN with Gelucire 44/14 (Figure 3-29), under pressure (124 bar) and above melting point (57°C), was dissolved compared to only 7.0% dissolution of the untreated PGN at the same time point. After 60 minutes the Gelucire 44/14-based PGN (1:5) dispersion prepared by PGSS showed the highest dissolution extent that was 3.2-fold greater than pure PGN, followed by the Gelucire 44/14-based PGN dispersion prepared by PM (1:1) and CS (1:1) that were both approximately 2.5 times greater than pure PGN (p-value < 0.05).
Figure 3-29. Dissolution profiles of dispersions of PGN with Gelucire 44/14 prepared by PGSS and various conventional methods.

SCF = supercritical fluid, PM = physical mixing, CS = cosolvent (methanol) and CM = comelted. Results represent means values ± SD, n = 3.

Table 3-6 lists the dissolution parameters for extent after 20 and 60 minutes for Gelucire 44/14 based dispersions prepared under various methods. PGN prepared by a SCF method showed increased dissolution extent over 60 minutes: the 1:5 dispersion being the best followed by PM and CS dispersions. The SCF 1:1 showed dissolution extent and rate on par with the CM semi-solid dispersions (46.93 ± 2.65% in 60 minutes). The poor dissolution of the untreated micronized commercial PGN was attributed to poor wetting due to the very low aqueous solubility. The Gelucire 44/14 (1:5) prepared by SCF processing showed the best dissolution (> 90% in 20 minutes), which is consistent with other Gelucire 44/14 dispersions formulated from SCF processing [230, 547]. However, the higher drug load of the 1:1 dispersions had dissolution rates that were not significantly higher than PGN dispersions prepared by conventional methods. In one study the Gelucire 44/14-based dispersions showed the least increase in dissolution rate compared to other dispersions containing PVP and/or
Chapter 3. Formulation

TPGS [323]. Based on the ratio of the drug to excipient, a lower PGN to Gelucire 44/14 ratio was thought to be optimal for dissolution of these dispersions. Thus, the dissolution rate was sensitive to the ratio of PGN to Gelucire 44/14 utilized and the method of preparation. Because all the dispersions of PGN showed higher dissolution rates higher than 0.57 µg·minute\(^{-1}\), it was expected that they would show a better bioavailability than pure PGN.

Table 3-6. Release extent and rates for the Gelucire 44/14 dispersions.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Extent 20 Minutes (%)</th>
<th>Extent 60 Minutes (%)</th>
<th>Rate Release (%·minute(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCF 1:1</td>
<td>12.69 ± 1.34</td>
<td>43.65 ± 1.56</td>
<td>1.20 ± 0.16</td>
</tr>
<tr>
<td>SCF 1:5</td>
<td>92.68 ± 4.76</td>
<td>96.25 ± 4.07</td>
<td>8.20 ± 0.34</td>
</tr>
<tr>
<td>CS</td>
<td>45.49 ± 2.08</td>
<td>71.84 ± 1.97</td>
<td>4.29 ± 0.12</td>
</tr>
<tr>
<td>PM</td>
<td>61.14 ± 2.22</td>
<td>76.67 ± 2.89</td>
<td>6.37 ± 0.41</td>
</tr>
<tr>
<td>CM</td>
<td>26.67 ± 1.54</td>
<td>46.93 ± 2.65</td>
<td>1.96 ± 0.25</td>
</tr>
<tr>
<td>Untreated PGN</td>
<td>10.51 ± 1.07</td>
<td>29.86 ± 1.94</td>
<td>0.57 ± 0.07</td>
</tr>
</tbody>
</table>

Data are mean ± SD, n =3.

For TPGS and PEG 400/4000 at 10 minutes, dissolution percentages were 44% and 62%, respectively (Figure 3-30 and Figure 3-31) under the same conditions as the Gelucire 44/14 formulations were processed. This improved extent of dissolution over conventional methods, is most probably due to the improved dispersion of PGN particles and molecules throughout each excipient, hence increasing the amount of PNG available for release. This is also seen when comparing the SC-CO\(_2\) processed dispersions (under maximum pressure, 124 bar) with the conventional methods. It is possible that the high pressure condition of CO\(_2\) treatment forced more PGN to interact with the excipient per unit area [295]. Also the rapid cooling on expansion into the precipitation vessel can improve dispersion because there is less time for recrystallisation and agglomeration [428]. Another important aspect is the melting point depression of the excipient when dissolved in supercritical carbon dioxide [295]. Although CO\(_2\) is barely able to dissolve polar molecules, it is able to plasticize many polymers owing to its capability to solubilize into the polymer [295, 428]. Furthermore, the CO\(_2\) may induce other polymer changes like swelling, foaming, crystallization and viscosity or density reduction [295, 428, 549, 550].
Figure 3-30. Dissolution profiles for PGN and TPGS dispersions prepared by PGSS and conventional methods.

SCF = supercritical fluid, PM = physical mixing, CS = cosolvent (methanol), and CM = comelted. Results represent means values ± SD, n = 3.

Table 3-7 lists the dissolution parameters for extent after 20 and 60 minutes for TPGS based dispersions prepared under various methods. Remarkably, the PGSS method of preparation for both TPGS-based PGN dispersions with different drug to TPGS ratios proved significant giving approximately a 10.5 times better dissolution rate compared to its conventional counterparts. It was noted that the TPGS systems containing both 1:1 and 1:5 parts PGN to TPGS gave similar release rates, 6.5 and 5.2 %·minute⁻¹, respectively. Using a Student T-test of the mean values and SD, the difference was not significant (p-value > 0.05). It seemed the co-solvent effect of the TPGS improved PGN dissolution to a similar extent. This may have also been a phenomenon between the SC-CO₂ and excipients (espically TPGS), such as swelling and/or re-crystallisation extent, which is discussed in more detail in section 3.8.9.
### Table 3-7. Release extent and rates for the TPGS dispersions.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Extent 20 Minutes (%)</th>
<th>Extent 60 Minutes (%)</th>
<th>Rate Released (%·minute⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCF 1:1</td>
<td>56.43 ± 1.56</td>
<td>95.67 ± 3.21</td>
<td>6.48 ± 0.12</td>
</tr>
<tr>
<td>SCF 1:5</td>
<td>68.25 ± 1.76</td>
<td>99.44 ± 3.56</td>
<td>5.20 ± 0.55</td>
</tr>
<tr>
<td>CS</td>
<td>36.35 ± 1.33</td>
<td>60.91 ± 2.78</td>
<td>2.13 ± 0.19</td>
</tr>
<tr>
<td>PM</td>
<td>21.59 ± 1.09</td>
<td>53.14 ± 1.99</td>
<td>1.21 ± 0.10</td>
</tr>
<tr>
<td>CM</td>
<td>13.41 ± 1.21</td>
<td>47.11 ± 1.34</td>
<td>0.76 ± 0.11</td>
</tr>
<tr>
<td>Untreated PGN</td>
<td>10.51 ± 1.07</td>
<td>29.86 ± 1.94</td>
<td>0.57 ± 0.07</td>
</tr>
</tbody>
</table>

Data are mean ± SD, n =3

The preparations with PEG 400/4000 (50:50) dispersion resulted in the slower release of PGN compared with untreated PGN, with only 4.9 % released after 10 minutes from the co-solvent method and 10.1 % after 10 minutes for the physical mixing (Figure 3-31).

Figure 3-31. Dissolution profiles of solid dispersions of PGN and PEG 400/4000 processed by PGSS and prepared by different conventional methods.
SCF is supercritical fluid, PM is physical mixing, CS is cosolvent (methanol), and CM is comelted. Results represent means values ± SD, n = 3.

Table 3-8 lists the dissolution parameters for extent after 20 and 60 minutes for PEG-based dispersions prepared under various methods. For the different co-precipitates obtained by the SCF method, the 1:5 PEG-based formulation was the best and was not closely followed by any of the other formulations (Figure 3-31). This result was comparable with those from the corresponding SCF dispersions; however this was not true for the corresponding conventional solid dispersions. All the conventional dispersions showed a much slower dissolution rate than the SCF processed dispersions with 1:5 ratio, although the PEG-based formulations appear to have generally underperformed compared to the TPGS and Glucire 44/14 dispersions. This result is different to other PEG-based formulations where dissolution was significantly better [230, 551-554]. Other studies have shown that the larger PEG-based formulations tend to produce slower dissolution results [556] unless prepared in combination with cyclodextrins [555].

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Extent 20 Minutes (%)</th>
<th>Extent 60 Minutes (%)</th>
<th>Rate Released (%·minute⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCF 1:1</td>
<td>20.72 ± 1.22</td>
<td>31.17 ± 1.78</td>
<td>1.10 ± 0.16</td>
</tr>
<tr>
<td>SCF 1:5</td>
<td>69.00 ± 2.25</td>
<td>81.83 ± 3.41</td>
<td>4.88 ± 0.27</td>
</tr>
<tr>
<td>CS</td>
<td>8.04 ± 0.89</td>
<td>17.71 ± 1.66</td>
<td>0.84 ± 0.13</td>
</tr>
<tr>
<td>PM</td>
<td>16.69 ± 1.09</td>
<td>35.28 ± 2.31</td>
<td>0.82 ± 0.23</td>
</tr>
<tr>
<td>CM</td>
<td>15.74 ± 0.92</td>
<td>27.75 ± 1.59</td>
<td>0.62 ± 0.19</td>
</tr>
<tr>
<td>Untreated PGN</td>
<td>10.51 ± 1.07</td>
<td>29.86 ± 1.94</td>
<td>0.57 ± 0.07</td>
</tr>
</tbody>
</table>

Data are mean ± SD, n = 3.

3.8.9. General Discussion

Over the last few decades, several SCF approaches have been utilized for enhancing the dissolution rate of poorly aqueous soluble drugs, including the use of organic solvents to form favorable delivery properties (e.g reduced particle size). However, the PGSS method can be conducted without the need for an organic solvent, in a time efficient process, and at relatively low temperatures (< 32°C) which is ideal for heat sensitive drugs.

The chapter aim to develop and operate a novel PGSS process to form PGN dispersions using SC-CO₂ was achieved. Also the formation of the novel PGN dispersions prepared under SCF conditions was able to show significantly improved PGN dissolution
rates. It was found that binary dispersions with a drug to excipient ratio of 1 to 5, nominal SC-CO$_2$ pressure of 124 bar, and system temperature of ranging between 56 – 59°C were favorable processing conditions to produce promising yields (> 86%). There are few studies on PGSS that specify the process yield obtained, however one study stated that an increase in the expansion pressure yielded lower degrees of aggregation [299]. In this study a dispersion of theophylline and hydrogenated palm oil was formed, but the research was focused on the different types of morphological structures produced by PGSS, rather than process yield. In a recent study using PGSS to formulate β-carotene the process yield was between 5 to 44% for SC-CO$_2$ conditions at 80 – 100 bar and temperature of 45°C [544]. This study had at least two differences in the PGSS method including the use of a mechanical stirred built inside the sample cylinder and utilized a 600 µm spray nozzle. In another study using PGSS technology to produce particles of PEG filled with lavandin essential oil, yields of up to 66% were achieved [545]. Due to the use of individual designs and constructions of SCF units and non-standardized SCF procedures of operation, any comparisons must be taken with limited value.

An important factor in the formation of a dispersion is the drug solubility in the excipient/carrier such as solvent evaporation methods, however in the case of the PGSS method it could be less important. In this study, the lower yields observed in the dispersions with a higher drug to excipient ratio (1 to 1) had lower amounts of PGN dissolved in SC-CO$_2$. For example, as the initial total weight for all the SCF dispersions was 3 g, the 1 to 1 ratio of drug to excipient had 1.5 g PGN compared to that of 0.5 g for the 1 to 5 ratio dispersions. Furthermore, the lower pressure (83 bar), which is directly proportional to SC-CO$_2$ density, would lead to lower PGN amounts being dissolved in SC-CO$_2$. Thus, a suspension likely formed when a drug to excipient ratio of 1 to 1 was used. Various studies have shown that PGN solubility in SC-CO$_2$ could be as low as 1.64 × 10$^{-4}$ (mole fraction) at 115 bar and 40°C [286, 293, 546], however it is possible to drastically improve the PGN solubility in SC-CO$_2$ by increasing the CO$_2$ density [230, 547]. However, there is a threshold at which SC-CO$_2$ density is not associated with improved PGN solubility and this occurs at approximately 205 bar for temperatures between 40°C to 60°C (where the solubility mole fractions are 7.20 × 10$^{-4}$ and 7.37 × 10$^{-4}$, respectively [286, 548]. In this study, the maximum pressure utilized was 124 bar at 59°C, so PGN solubility in SC-CO$_2$ was below ideal conditions for maximum solubility (approximately 1.78 × 10$^{-4}$). This means the higher drug to excipient loading of 1:1 ratios had a mole fraction of approximately 170 × 10$^{-4}$, compared to that of the 1:5 ratio dispersions where the mole fraction of PGN to SC-CO$_2$ was approximately 32 × 10$^{-4}$. It is
possible that the high pressure condition of SC-CO$_2$ treatment forced more PGN to interact with the excipient per unit area [295]. Interestingly, the SCF-based PGN dispersions formed under higher pressure (124 bar) did outperform the SCF products formed from the lower pressure (86 bar) based on dissolution testing. Furthermore, the SC-CO$_2$ may induce other excipient changes like swelling, foaming, crystallization, and viscosity or density reduction [295, 428, 549, 550]. Further research investigating the solid-liquid-vapor (S-L-V) curves and solubility of SC-CO$_2$ with drug and excipients are required to understand the effects of density and relationships with PGN dissolution rate.

The rate of expansion can be important, where rapid cooling on expansion into the precipitation vessel can improve dispersion uniformity because there is less time for recrystallisation and agglomeration [428]. During processing the ball valve was opened in the same way each time, however once the valve was fully opened the rate of expansion was not necessarily the same. It was noted that the PGN-based dispersions had a drastically slower time period where liquid CO$_2$ was evaporated. For example, the Gelucire 44/14 and TPGS expansion times for approximately 10 to 20 seconds, while the PEG expansion time typically was 60 to 90 seconds. In this study, all the PEG-based PGN dispersions underperformed in dissolution testing including the SCF-prepared dispersions relative to the Gelucire 44/14 and TPGS dispersions prepared by the PGSS method, although there was improvement compared to that of the control (untreated PGN). This result is similar to some PEG-based formulation studies where dissolution rates were significantly better compared with controls [230, 551-554]. Moreover, other studies have shown that the larger molecular weight PEG-based formulations tend to produce slower dissolution results unless prepared in combination with cyclodextrins [555, 556].

The Gelucire 44/14 (1:5) prepared by SCF processing using higher pressure (186 bar) showed immediate dissolution (> 90% within 20 minutes), which is consistent with other studies investigating Gelucire 44/14 dispersions formulated from SCF processing [230, 547]. However, the higher drug loading of the 1:1 ratio and processing with lower SCF pressure (83 bar) formed dispersions that had dissolution rates significantly lower than PGN dispersions prepared by higher pressure and lower PGN loading and was similar to the dissolution rates observed for the dispersions prepared by conventional methods. In one study the Gelucire 44/14-based dispersions with a lower Gelucire 44/14 content also showed the least increase in dissolution rate compared to other dispersions containing PVP and/or TPGS [323]. Generally, the dissolution rate was sensitive to the ratio of PGN to excipient utilized
and the method of preparation. In addition, because all the dispersions of PGN showed higher dissolution rates than 0.57 %·minute$^{-1}$, it was expected that they would show a better bioavailability than pure PGN.

Another critical condition for PGSS processing is the melting behavior of the target drug and excipients [496, 556]. This may partly explain why the TPGS-based PGN dispersions of either drug to excipient ratio produced similar dissolution profiles. Of the three excipients selected, TPGS has the lowest melting point, which may have enhanced SC-CO$_2$ diffusion and solubility, thus forming a molecular dispersion during processing and improved drug entrapment. Also the dissolution testing was conducted at 37°C, which is above the melting point of TPGS, leading to a lower viscosity, thus higher disintegration/diffusion rate. However, TPGS can form micelles at relatively low concentrations, which would build an entangled network with a high macroscopic viscosity [561]. Further research is needed to investigate the micelle formation, viscosity, and melting point behavior of TPGS.

Overall, the PGN/Gelucire 44/14 (1:5) dispersion obtained by SCF processing under 124 bar at 57°C produced the most improved in vitro dissolution followed by the TPGS-based and PEG-based products under the same SCF conditions. The relative ability of Gelucire 44/14 based formulations to produce a rapid release profile is consistent with other studies that utilized Gelucire 44/14 [302, 556, 562]. The SCF dissolution profiles for the lower SCF conditions (84 bar, 40 – 45°C) were comparable with their corresponding conventional dispersion, except for the TPGS-based dispersions that had been processed under both SCF conditions which showed improved dissolution.

Conventional methods in the pharmaceutical industry have an overwhelming presence and transformation into the use of SCF processes into commercial manufacturing requires scale-up into large batch sizes [323]. In order to progress towards the wider use of SCF methods in industry, the SCF processing units must be simple, cost sensitive, and show significantly improved formulation of drug delivery systems. The SCF process used in this study, was contained within a closed system, without moving parts, and maintained at low cost with high-grade stainless steel and CO$_2$. More research is required to evaluate the possible factors involved in SCF processing possibly using a factorial design experiment, and assess whether or not SCF processing has significant advantages over conventional methods. Furthermore, by developing a platform into which a range of steroids and other drugs from different therapeutic classes, with varying physicochemical parameters, can be evaluated, the versatility of the PGSS method would be established.
3.9. Conclusion

Novel solid dispersion systems of PGN with selected excipients and PGSS method using SC-CO$_2$ gave higher dissolution rates compared to conventionally prepared dispersion systems. The operating conditions of the SC-CO$_2$ processing affected the yield and *in vitro* dissolution of PGN. Operating conditions necessary for a high production yield (> 86 %) and rapid dissolution rate of PGN were identified as high pressure (124 bar), a temperature either of 40 or 59°C, and PGN:excipient ratio of 1 to 5. The dispersions prepared by conventional methods and PGSS method, incorporated PGN in crystalline form suggesting a stable dispersion of PGN was formed. The light microscopy images of the dispersion systems formed from various methods showed the structural variations of these dispersion systems. Interestingly the introduction of more Gelucire 44/14 from 50% to almost 91% prepared under the same SCF conditions did not completely dissolve the PGN crystals. Nevertheless, the lower amount of PGN incorporated into Gelucire 44/14 did not negatively impact on the observed particle size, rather showed a reduced particle size dispersed uniformly. The micrographs may also reveal a correlation between crystalline extent of PGN and the release of PGN over time from the Gelucire 44/14 dispersion systems. The 1:10 SCF prepared systems produced the least and smallest PGN crystals and they produced the highest release rates than that of the 1:5 and 1:1 dispersion systems. The use of the PGSS method also plays a significant role in drug release as shown by the more effective uniform mixing when compared to that of the conventionally prepared dispersion systems.

Earlier research from Raman analysis also revealed that there was no change in the crystalline structure of PGN after exposure to SC-CO$_2$. Although the lipid based excipients are often plagued by stability issues and are difficult to prepare by conventional methods, this study showed that with the constituent amounts used greater stability for the drug was possible and the relatively simple SCF-based process has its own merits. Improvements in dissolution rate, time efficient processing, and stability of PGN within the dispersions prepared by SCF processing provide significant advantages to this alternative to conventional methods. The results showed that PGN is able to be dispersed, possibly uniformly, throughout each excipient (PEG 4000 and TPGS and Gelucire 44/14) as revealed by XRPD and FTIR, regardless of the method used. The SC-CO$_2$ based-PGSS method appeared to be able to incorporate more PGN crystals/molecules, possibly more uniformly throughout the excipients, which may explain the dissolution results. The CO$_2$-treated PGN with various excipients showed a significantly enhanced dissolution, compared to the untreated PGN.
particles and binary dispersions produced by the conventional cosolvent, comelting, and physical mixing methods. The SCF-based particle formation technology is presented as an alternative to the conventionally prepared dispersion systems. However, this technology is not without its own problems. While the versatile operating conditions that are possible with SCF and their mixtures may be advantageous, it is difficult to prepare products with ideal properties without having to perform many experiments. Improving dissolution, for example, using a wide range of processing parameters (to name a few; temperature, pressure, release rate, and processing time) could take over hundred experiments to investigate. This would increase to several hundred experiments if all known factors were fully tested, as it is unknown which factors could affect the final product’s ability to dissolve PGN in an aqueous environment. Using the elementary experiments, the model Gelucire 44/14 formulation was optimised using numerous parameters with SC-CO$_2$. Gelucire 44/14 was selected for the following experiments because it at this stage it showed the most promising results based on in vitro dissolution. In the following chapter, a delivery system has been optimised for PGN processing using PGSS and evaluated by in vitro dissolution.
Chapter 4:

Optimisation of SCF processing and *In Vitro* Evaluation of Progesterone Dispersions
Chapter 4. Optimisation of SCF processing and In Vitro Evaluation of Progesterone Dispersions

4.1. Introduction

A limitation of SCF processing is that, although it provides high speed results, it is difficult to know if it has been used efficiently, and numerous experimental conditions can be utilized as there is no standardised reference to follow. This could be due to the fact that it is a relatively new technique in pharmaceutical sciences. The use of a screening experiment could greatly improve the production efficiency by signalling important parameters for optimisation of a formulation. In doing so, with regards to this research, the mixing of the drug within an excipient can be advantageously enhanced using a PGSS method [496].

The main aim of this study was firstly to investigate the effect of several experimental parameters on the supercritical fluid processing of PGN with the model excipient Gelucire 44/14, and secondly to find the experimental conditions to give the best PGN dispersions. The study outlined in this chapter employed a symmetrical fractional factorial design at two levels, i.e. ‘high’ and ‘low’ (or ‘+1’ and ‘-1’) that investigate factors, or independent variables, identified in the PGN dispersion process. This is expressed as $2^{k-p}$, where $k$ is the number of variables at ‘2’ levels and $p$, or generators, are the number of columns in the experimental domain constructed from a full factorial design $2^\beta$ ($\beta = k - p$) [563, 564]. This means that the number of experiments increases exponentially as the number of variables increase. Thus, full factorial designs are usually more practical when only four or less variables are investigated [565]. For example, if a full-factorial design requires $nk$ runs, and there were three levels tested with five possible variables, a total of 125 runs would be required. Each of these runs can often have replicates, further increasing the experimental costs and efforts.

4.1.1. Factorial design experiments

Procedures that require multiple steps, and use of large amounts of organic solvents, are often very expensive and time consuming [566]. For investigations involving large numbers of variables, a fractional factorial study is an effective means to identifying principal variables
with a fraction of the experiments required in a full design, which also means minimal resources can be used. Condensed experimental designs are particularly useful as screening studies, especially where costs are limited and resources are scarce [567]. There are numerous methods to conduct a less than full factorial design; (a) fractional factorial design, D-optimum designs, Ray experimental design, Plackett-Burman design, and central composite designs (such as Doehlert or Box–Behnke approaches) [568]. These and other such approaches are generally referred to as a design of experiments (DOE) or experimental design. The fractional factorial design is known as symmetrical when the experimental matrix is investigated at a set level, whereas a matrix of variables with different set levels (e.g. ‘high’ and ‘low’ for some; and ‘extended’ and ‘medium’ for others) is typical of an asymmetrical factorial design. The D-optimum and Ray designs are often used in examining a constant mixing ratio of chemicals in various doses that result in varying levels of biological effect [569]. These designs are ideal for experiments where interactions can occur with any number of chemicals, but requires the individual chemical dose-response curves to be available. Plackett-Burman designs (Plackett and Burman 1946) are multivariate experiments that allow the simultaneous study of several variables and are faster to implement than univariate methods. The limitation, as with most condensed designs, is that the main effects or responses may be confounded by two way interactions [570]. These designs are very useful for detecting large main effects, and requires the interactions to be relatively negligible compared with the main effect(s).

Up until now, the designs outlined assume the relationship between the variables and responses are linear (these designs are often referred to as ‘first-order’ designs), however, where a non-linear (or quadratic) effect can occur, more complicated experimental designs can be used such as central composite designs, and are referred to as ‘second-order’ designs. The Doehlert design (Doehlert 1970) is employed to generate response surfaces with a good estimation of the variable parameters of a quadratic mathematical model. These designs allow for the study of three independent variables at a different number of levels [571]. The experimental domain is explored with a minimum number of experiments around its centre point, and by the variations of each variable around the centre. In the Box–Behnke (Box and Behnke 1960) approach each independent variable is placed at one of three equally spaced positions in an experimental matrix [230]. The main drawback of this design is that at least three levels are required to sufficiently fit a curved response. Factorial experiments are analyzed using ANOVA or regression analysis.
One of the pivotal points of a fractional factorial design is the resolution which defines the extent to which the main effects and interactions are confounded or aliased. The technical definition of resolution for a fractional factorial design is “equal to the smallest number of characters in any word appearing in the defining relation” [563]. Designs with resolutions less than II are considered to have effects and interactions that are too interlinked to produce conclusive results. A resolution III design can provide the fundamental basis to which only the main effects are tested. This means that there is a level of confounding within such studies but that may be acceptable. Resolution IV and V designs are able to provide conclusive main effects with increased information about the interaction effects between the variables [572].

The centre points of the design are the controls and are included in the experimental domain. The centre points serve a bi-fold function; firstly, the provide a check for curvature. If the dependent variable mean at the centre point is significantly different from the overall mean at all other points of the design, then it can be assumed that the factors are not linearly related to the dependent variable. Secondly, centre points act as a guide for stability. If the individual means of centre points are significantly different to each other then it is assumed the study design is unstable and not reproducible. It is important to note that while experimental runs of the matrix are generated in random order, the centre points are added at set values at regular intervals that are not in random arrangement.

Unfortunately, a typical SCF experiment tends to be heuristic in nature, where selected variables appear to be randomly controlled. There are currently no multivariate studies applied to the optimization of SCF processing of PGN under different conditions with Gelucire 44/14. This study has provided essential information about the behaviour of the ternary system (SCF:PGN:excipient) by studying the interactions among the variables tested. In addition, with a relatively small number of extra runs, modelling of multifactorial response surfaces was also completed, giving yet another perspective on the relationship between PGSS processing conditions and PGN dispersions.

The SCF method utilized in this study is known as the PGSS which has been previously outlined section 3.5. The seven variables measured were temperature, pressure, sample loading, processing time, sonication time, PGN:excipient ratio (1:1, 1:5 and 1:10), and orifice diameter. This design was used to determine the effects of the seven variable parameters thought to have an effect on the performance of the SCF based method. The responses measured were in vitro dissolution rate, yield and and t_{1/2} of the dispersions formed.
from SCF processing with carbon dioxide. For this chapter, the use of a fractional factorial design, when compared to a full factorial design, reduces the number of experimental runs from 128 to 16.

4.1.2. Dissolution studies

In solid-state dissolution, factors such as wetting, particle size/surface area, disintegration, and agglomeration/clumping can complicate analysis [323]. By using a disc the surface area remains constant, avoiding assumptions associated with these complications. In sink conditions, the dissolution of a drug can be obtained from a constant hydrostatic system using Equation 7 [573].

\[ C = \left( \frac{S}{V} \right) k \times C_s \times t \]  

Equation 7

where \( C \) is drug concentration at time \( t \), \( S \) is the surface area of the disk, \( V \) is the volume of the test solution, \( k \) is the intrinsic dissolution rate constant, and \( C_s \) is solubility [573]. Therefore, by rearrangement the dissolution rate (\( DR \)) from a unit surface area can be calculated from Equation 8:

\[ DR = \left( \frac{C}{t} \right) \left( \frac{V}{S} \right) = k \times C_s \]  

Equation 8

apparent \( DR \) of each dispersion can then be determined and compared to that of pure PGN. Studies have shown that drugs with a \( DR \) below 0.1 mg·minute\(^{-1}\)·cm\(^{-2}\) typically have limited absorption dependent on dissolution rate [574]. The \( DR \) of PGN is expected to fall within this category, although no literature has determined this value. It is also expected that the \( DR \) will increase for the various formulations prepared by both conventional and SCF processing. There are several studies that have conducted dissolution testing according to surface area for Gelucire 44/14 and it was shown that the \( DR \) was sensitive to the ratio of drug to excipient used and preparation method [575, 576].

Another aim of this chapter was to develop a simple mathematical equation of fitted drug release from semi-solid dispersions of PGN. \textit{In vitro} dissolution has become an important element in drug development. Drug dissolution from solid dosage forms has been described by various kinetic models in which the dissolved amount of drug (\( Q \)) is a function
Chapter 4. Optimisation

of the time, \( t \) or \( Q = f(t) \) [577]. Some of these analytical models include, zero-order, first-order, Higuchi, and Korsmeyer-Peppas methods. Briefly, a zero-order, or linear model is where drug dissolution from a pharmaceutical dosage form is independent of drug amount present (i.e. the dosage form does not disaggregate and releases the drug slowly but does erode). In a first-order model (Gibaldi and Feldman 1967) the drug release phenomena is proportional to the amount of drug remaining in a dosage form that will diminish over time [578]. These profiles give the appearance of a curved response against time. Higuchi (1961) first described drug release as a diffusion process based on Fick’s law, and is dependent on the square root of time [577]. This relation was first proposed by Higuchi to describe the dissolution of drugs in suspension from ointment matrixes and assumes the drug is uniformly dispersed. The Korsmeyer-Peppas model describes a constant that incorporates the structural and geometric characteristics of the drug dosage form, and is indicative of the drug release mechanism [289, 577]. It measures different phases of dissolution occurring between disintegration and diffusion, which may or may not occur simultaneously. These models are described in more detail later in the chapter.

Furthermore, the optimised Gelucire 44/14 formulation processed using SC-CO\(_2\) and conventional dispersions prepared using co-solvent, co-melted, and physical mixing methods were evaluated using the different in vitro dissolution models, and the dual-first order approach. This was done to elucidate the release mechanism of PGN from the manufactured dispersions.

4.2. Experimental

4.2.1. Materials

Industrial grade PGN was purchased from Pharmaca & Upjohn-Pfizer Company (New York, USA). Gelucire 44/14 was donated by Gattefossé Corporation (New Jersey, USA). Methanol and acetonitrile was high-performance liquid chromatography (HPLC) grade, which was purchased from Sigma-Aldrich (Auckland, NZ). Liquid CO\(_2\) (purity 98%) was purchased from BOC Gas (Auckland, NZ). Triple-distilled MilliQ water was obtained in-house by reverse osmosis (Millipore, USA). All samples were used without any further purification steps.
Chapter 4. Optimisation

4.2.2. SCF processing and experimental domain

The dispersions were formed using an in-house particles from gas saturated suspension (PGSS) method, as described in section 3.5. Briefly, liquid carbon dioxide was pumped into a Swagelok sample cylinder (Solon, USA) containing unmixed materials (PGN and Gelucire 44/14). The materials were then dispersed under the designed conditions predetermined in the experimental domain. After each condition was meet, the SCF dispersion was released into the attached expansion chamber at atmospheric pressure and room temperature. At the end of the dispersion process, the dry semi-solid mass was removed and placed in glass vials. The products were stored at 4°C in a refrigerator, until required for testing.

Formation of PGN dispersions using SCF are mainly governed by the density of the fluid and therefore, pressure (bar) and temperature (°C) were expected to be very important factors. Seven variables were identified in the dispersion processed, shown in Table 4-1. The selected factors and their corresponding ranges (+1, upper and -1, lower levels) were determined after preliminary screening experiments. Two centre point runs (0) were also included, set at a medium level between the upper and lower levels. Figure 4-1 shows a simplified diagram of a two factor design with two levels. It helps to visualise the concept of experimental design methods, and the shown diagram is simplified because it is for just two factors. Many experiments often investigate many more factors with several levels to be tested, which cannot be drawn with a diagram. In this research, there are seven factors investigated at two levels.

All pressures are in the supercritical region for carbon dioxide (i.e. no sub-critical experimental runs were done). The temperature range was decided to be above 60°C, below 20°C and similar to (approximately 40°C) the melting point of Gelucire 44/14.
Figure 4-1. A two factor, two level design used to investigate Factors A and B within certain constraints with the experimental domain.

The hollow circles represent predictive levels outside the restricted design for selected parameters. Redrawn and adapted from [579] and [580].
Table 4-1. Selected parameters of the experimental domain.

<table>
<thead>
<tr>
<th>Factor*</th>
<th>Low level (-1)</th>
<th>Centre point (0)</th>
<th>High level (+1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Pressure (bar)</td>
<td>90</td>
<td>135</td>
<td>186</td>
</tr>
<tr>
<td>B Temperature (°C)</td>
<td>20</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>C Sample load (g)</td>
<td>3</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>D Processing time</td>
<td>15</td>
<td>22</td>
<td>30</td>
</tr>
<tr>
<td>E Sonication (minutes)</td>
<td>0</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>F Drug:excipient ratio</td>
<td>1:1</td>
<td>1:5</td>
<td>1:10</td>
</tr>
<tr>
<td>G Orifice diameter (inch)</td>
<td>small (1/16)</td>
<td>medium (1/8)</td>
<td>large (1/4)</td>
</tr>
</tbody>
</table>

* Factor selection and parameter levels are based on typical research variables and equipment constraints.

The duplication of centre point was used to estimate the experimental error. All experiments were randomly performed without replication. The measured responses were defined as the process yield (%), in vitro dissolution (extent) after 20 minutes, and $t_{1/2}$.

4.2.3. Experimental matrix

The factorial design of $2^{7-3}$ was used to give 16 experimental runs plus two centre points (totalling 18 runs), instead of the 128 runs required for a full $2^7$ factorial design. The experimental matrix is provided in Table 4-2, together with the corresponding factors for A, B, C, D, E, F, and G. A recurring result between the centre points (runs 3 and 11) for every response would imply the SCF processing was reproducible.
Table 4-2. Experimental matrix for the $2^{7-3}$ design for PGN dispersions.

<table>
<thead>
<tr>
<th>Run</th>
<th>Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>X1</td>
<td>1</td>
</tr>
<tr>
<td>X2</td>
<td>1</td>
</tr>
<tr>
<td>X3</td>
<td>0</td>
</tr>
<tr>
<td>X4</td>
<td>-1</td>
</tr>
<tr>
<td>X5</td>
<td>-1</td>
</tr>
<tr>
<td>X6</td>
<td>1</td>
</tr>
<tr>
<td>X7</td>
<td>-1</td>
</tr>
<tr>
<td>X8</td>
<td>-1</td>
</tr>
<tr>
<td>X9</td>
<td>1</td>
</tr>
<tr>
<td>X10</td>
<td>-1</td>
</tr>
<tr>
<td>X11</td>
<td>0</td>
</tr>
<tr>
<td>X12</td>
<td>1</td>
</tr>
<tr>
<td>X13</td>
<td>1</td>
</tr>
<tr>
<td>X14</td>
<td>1</td>
</tr>
<tr>
<td>X15</td>
<td>1</td>
</tr>
<tr>
<td>X16</td>
<td>-1</td>
</tr>
<tr>
<td>X17</td>
<td>-1</td>
</tr>
<tr>
<td>X18</td>
<td>-1</td>
</tr>
</tbody>
</table>

Legends: A = Pressure, B = temperature, C = sample load, D = contact time with CO$_2$, E = sonication, F = Drug: excipient ratio and G = orifice diameter. Highlighted rows are the centre points.

4.2.4. Resolution

The factors A to D on the experimental matrix were from a full $2^4$ factorial design. Factors E, F and G are the generators which were formed by multiplying the previous four columns. That is E = A•B•C, F = B•C•D, and G = A•C•D, and the generating relations can be expressed as:

\[ I = ABCE, I = BCDF, I = ACDG \]
Chapter 4. Optimisation

The shortest word in the generated design was four units, providing a resolution IV experimental design. The complete description of the fractional factorial design in this chapter is written as $2^{7-3}(IV)$.

4.2.5. Regression modeling

Multiple regression gives a mathematical relationship between responses and independent variables [580]. A fractional factorial design provides sufficient data to fit a linear regression, such as given below for seven factors (see Equation 9):

$$Y = b_0 + b_1A + b_2B + b_3C + \ldots + b_{14}BD + b_{15}ABD + \varepsilon$$  \hspace{1cm} \text{Equation 9}

where $Y$ represents the response, $b_0$ the intercept, $b_i$ the parametric coefficients of the model obtained by regression, the $A$, $B$, $C$ and $D$ are the independent experimental factors (coded variables) and $\varepsilon$ is the error term derived from the centre points.

4.2.6. Statistical analysis of experimental design

Data analysis was carried out with Minitab® Release 15.0 software 2007 (Minitab Inc. State College, PA, USA). The data was tested for significance by analysis of variance (ANOVA) with a level of significance of 5% ($p$-value $\leq 0.05$).

4.2.7. PGN recovery

The different products manufactured from PGN, Gelucire 44/14, and SC-CO$_2$ mixing were formed using the PGSS process outlined previously in section 3.5. The PGN recovery was determined by testing each experimental run from the factorial design, replicated three times. The amount of PGN was quantified using the HPLC method detailed in section 2.4.

4.2.8. Preparation of CS, CM and PM dispersions

Preparation of controls were prepared by three conventional methods: (1) the solvent (cosolvent – CS) evaporation method, PGN and excipient were dissolved in a minimum volume of methanol in a conical flask and heated, (2) the melting (comelt – CM) recrystallization method involved stirring PGN and excipient in a beaker on a hot stage at temperature (135°C), and (3) the physical mixing (PM) method, involving the mixing of PGN and excipient to the required ratio (e.g. 1 to 1), in a mortar and pestle. The conventional
formulations were prepared using the methods outlined in more detail previously in section 3.6.1.

4.2.9. In vitro dissolution

Samples removed from the filter located at the base of the expansion chamber (PGSSS unit) were used for the dissolution testing. The recovered samples were weighted to 4 mg +/-0.2 mg and used in the dissolution studies. The dissolution rates of the PGN dispersions were measured by using a USP apparatus 5 (paddle over disk), Hanson Research, SR8PLUS dissolution apparatus, California, USA; at 100 rpm in 900 mL 37 +/-1°C. No pH buffer was used due to small fraction of sample used and the fact that PGN is non-ionisable at pH levels found in the human body. The pH ranged from 4.5 to 4.8 over the duration of the experiments and did not change on addition of loaded sample disks. It is well known that atmospheric carbon dioxide freely moves between water and air and the equilibrium is approximately pH 4.5 [581]. Each dissolution was carried out in triplicate.

4.2.10. Response surface curves

Data obtained by performing experiments of the factorial design (outline shown in Table 4-1) were used to analyse the response surface and visualize the best possible processing conditions inside the experimental domain. The three dimensional plots of the response surfaces for the extent of dissolution after 20 minutes, process yield and PGN uniformity after SC-CO$_2$ processing; in these plots only the variables pressure (A) and temperature (B) are examined, while the remaining variables were fixed at optimum levels previously determined in this research.

4.2.11. Analysis of release profiles

The four models chosen to describe the release profiles were based on known physical geometry of the PGN particles and the fact that the systems examined do not disintegrate. For analysis the following models were used [338, 582];

Zero-order release: \[ Y = k_0 \cdot t \]  
First-order release: \[ \ln Y = k_1 \cdot t \]  
Higuchi release: \[ Y = k_H \cdot t^{1/2} \]  
Korsmeyer-Peppas release: \[ Y = k_{K-P} \cdot t^n \]
In these equations, Y is the cumulative % drug release at time (t): \( k_0, k_1, k_H \) and \( k_{K-P} \) are release rate constants and \( n \) is the exponent that describes the release mechanism of the formulation.

In an attempt to understand the release mechanism from the dispersion systems under investigation, a dual first order (Equation 14) model was developed to estimate the drug release rates attributable to diffusion and erosion;

Dual first-order release:

\[
y = (H_1 \cdot [1 - \exp(-B \cdot x)]) + K_1 \cdot x
\]

Equation 14

where \( H_1 \) represents % PGN released by diffusion (before erosion occurs), \( B \) is the rate constant (first order release) for the diffusion process, \( K_1 \) is the erosion rate (%·minute\(^{-1}\)), and \( x \) is the cumulative % amount PGN released by \textit{in vitro} dissolution. It was assumed that the linear release rate was practically equal to the rate of erosion and it is known that the excipient (Gelucire 44/14) is more hydrophilic than PGN. The dual first order model was optimized in QtiPlot software Version 0.9.7.12 Copyright © 2004 - 2011 originally produced by Ion Vasilief and the models were iterated using the Levenberg-Marquardt method. Kinetic parameters were analyzed using a one-way analysis of variance (ANOVA) by Tukey’s pairwise comparison at 95% confidence interval using Minitab® Release 15.0 software (Pennsylvania, USA).

4.2.12. Modelling study statistics

After dissolution, the estimated amount of PGN released from the dispersions were compared to each other using equations 1 to 4. The dual first-order equation was modelled using QtiPlot software, Version 0.9.7.12, Copyright © 2004 - 2011 originally produced by Ion Vasilief. Statistical analyses were performed by using the Minitab® Release 15.0 software (Minitab Inc. State College, PA, USA).

4.3. Results and Discussion

4.3.1. Experimental matrix

The factorial design of \( 2^{7-3} \) was used to give 18 experimental runs including the centre points. The experimental matrix is provided in Table 4-3 together with the corresponding responses for \( Y_1, Y_2 \) and \( Y_3 \). The recurring result between the centre points (runs 3 and 11) for every response implies the SCF processing was relatively reproducible. The extent of PGN
dissolution after 20 minutes for centre point runs 3 and 11 gave the largest variation result, 55.8 and 60.3 minutes, respectively.

<table>
<thead>
<tr>
<th>Run</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>Yield %</th>
<th>Extent 20 minutes</th>
<th>t_{1/2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>X1</td>
<td>1</td>
<td>-1</td>
<td>1</td>
<td>1</td>
<td>-1</td>
<td>-1</td>
<td>1</td>
<td>67.70</td>
<td>85.55</td>
<td>6.81</td>
</tr>
<tr>
<td>X2</td>
<td>1</td>
<td>1</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
<td>1</td>
<td>1</td>
<td>78.70</td>
<td>58.48</td>
<td>17.65</td>
</tr>
<tr>
<td>X3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>62.70</td>
<td>55.83</td>
<td>4.64</td>
</tr>
<tr>
<td>X4</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
<td>2.40</td>
<td>40.65</td>
<td>2.80</td>
</tr>
<tr>
<td>X5</td>
<td>-1</td>
<td>1</td>
<td>-1</td>
<td>1</td>
<td>1</td>
<td>-1</td>
<td>1</td>
<td>77.80</td>
<td>54.12</td>
<td>4.19</td>
</tr>
<tr>
<td>X6</td>
<td>1</td>
<td>-1</td>
<td>-1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>82.00</td>
<td>62.03</td>
<td>4.97</td>
</tr>
<tr>
<td>X7</td>
<td>-1</td>
<td>-1</td>
<td>1</td>
<td>-1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>50.20</td>
<td>64.60</td>
<td>5.34</td>
</tr>
<tr>
<td>X8</td>
<td>-1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>-1</td>
<td>1</td>
<td>-1</td>
<td>91.00</td>
<td>75.35</td>
<td>5.20</td>
</tr>
<tr>
<td>X9</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>-1</td>
<td>1</td>
<td>-1</td>
<td>-1</td>
<td>65.30</td>
<td>71.81</td>
<td>16.00</td>
</tr>
<tr>
<td>X10</td>
<td>-1</td>
<td>1</td>
<td>-1</td>
<td>-1</td>
<td>1</td>
<td>1</td>
<td>-1</td>
<td>63.30</td>
<td>77.40</td>
<td>5.94</td>
</tr>
<tr>
<td>X11</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>60.30</td>
<td>60.28</td>
<td>4.80</td>
</tr>
<tr>
<td>X12</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>94.70</td>
<td>71.68</td>
<td>10.28</td>
</tr>
<tr>
<td>X13</td>
<td>1</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
<td>1</td>
<td>-1</td>
<td>1</td>
<td>57.00</td>
<td>63.96</td>
<td>14.67</td>
</tr>
<tr>
<td>X14</td>
<td>1</td>
<td>1</td>
<td>-1</td>
<td>1</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
<td>88.70</td>
<td>62.99</td>
<td>6.64</td>
</tr>
<tr>
<td>X15</td>
<td>1</td>
<td>-1</td>
<td>1</td>
<td>-1</td>
<td>-1</td>
<td>1</td>
<td>-1</td>
<td>57.30</td>
<td>57.61</td>
<td>9.54</td>
</tr>
<tr>
<td>X16</td>
<td>-1</td>
<td>-1</td>
<td>1</td>
<td>1</td>
<td>-1</td>
<td>1</td>
<td>-1</td>
<td>21.70</td>
<td>73.47</td>
<td>5.52</td>
</tr>
<tr>
<td>X17</td>
<td>-1</td>
<td>1</td>
<td>1</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
<td>31.70</td>
<td>71.29</td>
<td>7.43</td>
</tr>
<tr>
<td>X18</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
<td>1</td>
<td>-1</td>
<td>1</td>
<td>1</td>
<td>61.10</td>
<td>61.50</td>
<td>5.69</td>
</tr>
</tbody>
</table>

Legends: A = Pressure, B = temperature, C = sample load, D = contact time with CO\textsubscript{2}, E = sonication, F = Drug: excipient ratio and G = orifice diameter. Highlighted rows are the centre points.

4.3.2. *PGN* recovery

The dispersions produced from SCF processing were taken from the filter plate without any further preparation and weighed into sample disks for drug release evaluation.

The recovery of PGN from the samples was between 77.04% and 106.95%, indicating large variability of uniformity for the SCF process. The recovery of PGN was therefore, generally uncontrollable for the set of conditions tested, shown in Table 4-4. Photos of the samples recovered are given in Appendix VIII.
Table 4-4. PGN recovery/drug loading after SCF processing for each sample.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean PGN Recovery (%) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>X1</td>
<td>115.00 ± 2.24</td>
</tr>
<tr>
<td>X2</td>
<td>105.77 ± 0.65</td>
</tr>
<tr>
<td>X3</td>
<td>100.19 ± 0.037</td>
</tr>
<tr>
<td>X4</td>
<td>77.04 ± 3.56</td>
</tr>
<tr>
<td>X5</td>
<td>106.95 ± 1.54</td>
</tr>
<tr>
<td>X6</td>
<td>106.40 ± 1.09</td>
</tr>
<tr>
<td>X7</td>
<td>99.04 ± 0.15</td>
</tr>
<tr>
<td>X8</td>
<td>103.90 ± 2.04</td>
</tr>
<tr>
<td>X9</td>
<td>102.77 ± 1.94</td>
</tr>
<tr>
<td>X10</td>
<td>98.75 ± 0.56</td>
</tr>
<tr>
<td>X11</td>
<td>98.28 ± 0.62</td>
</tr>
<tr>
<td>X12</td>
<td>99.00 ± 0.49</td>
</tr>
<tr>
<td>X13</td>
<td>103.09 ± 1.86</td>
</tr>
<tr>
<td>X14</td>
<td>102.03 ± 1.23</td>
</tr>
<tr>
<td>X15</td>
<td>104.08 ± 1.29</td>
</tr>
<tr>
<td>X16</td>
<td>99.56 ± 0.097</td>
</tr>
<tr>
<td>X17</td>
<td>101.53 ± 1.34</td>
</tr>
<tr>
<td>X18</td>
<td>103.04 ± 1.78</td>
</tr>
</tbody>
</table>

Highlighted rows are the centre points.

4.3.3. Dissolution results

Figure 4-2 shows the release profiles of PGN from Gelucire 44/14 of different ratios; identification of the analyte extracted from the matrix was performed by injecting standard solutions into HPLC with UV-Vis detection (DAD). As various parameters potentially affect the PGSS process, the optimization of the experimental conditions represents a critical step in the development of a SCF method [566]. It is known, for example, solubility of the analyte [521, 566] can be controlled by the composition, density and temperature of the SCF, moreover the recovered product is not only dependent on the operating conditions but also on the sample characteristics, e.g. water content, matrix type, particle size, viscosity, etc., making selection of optimum conditions difficult, especially with subsequent reliable quantification [229, 285, 286, 422, 496, 566]. Furthermore, on the basis of preliminary experiments and the literature, some experimental parameters were not varied or examined, e.g. continuous CO2 flow [230, 243], different collection filters [491], multiple SC fluids
Chapter 4. Optimisation

[507], use of a nozzle (e.g. 300 µm) [428, 498, 507], the use of a modifier (e.g. ethanol) [491, 566], and the release rate into the precipitation chamber [491].
Figure 4-2. Release profiles of the dispersion systems containing PGN;
(a) PGN release from Gelucire 44/14 prepared 1:1 drug:excipient ratio (high pressure, 186 bar), (b) PGN release from Gelucire 44/14 prepared 1:1 drug:excipient ratio (low pressure, 90 bar), (c) PGN release from Gelucire 44/14 prepared 1:10 drug:excipient ratio (high pressure, 186 bar), (d) PGN release from Gelucire 44/14 prepared 1:10 drug:excipient ratio (low pressure, 90 bar), and (e) PGN release from Gelucire 44/14 prepared 1:5 drug:excipient ratio (medium pressure, 135 bar).
Chapter 4. Optimisation

4.3.4. Responses from the experimental design

Data obtained by performing experiments of the factorial design (Table 4-3) were graphed according to each response (Figure 4-3). As it can be seen from these plots, the main effects considered have not shown any obvious correlations, which may mean there were some variables not examined that had an effect, or too many variables were examined for the given response. The main effects of the variables sample load (C), sonication time (E), drug: excipient ratio (F) and orifice diameter (G) were not significant for all the responses, whereas main effects of the variables pressure (A), temperature (B) and processing time (D) resulted in significant difference (p-value < 0.05). In addition, interaction effects of the variables (A) and (F) were significant for all the responses except that for t1/2. On the basis of the results of the factorial design obtained for t1/2, the variable (F) could have been excluded from the experiment, because it was not significant either as main or interaction effect.
Figure 4-3. Spread plots of the responses: (1) processing yield; (2) extent dissolution after 20 minutes; (3) time required to dissolve 50% of PGN.

A summary of the significant results for the $Y_1$, $Y_2$, and $Y_3$ studies are shown in Table 4-5. The results were analysed for the standard error of the coefficients, t-values, p-values, and regression of coefficients. The results show that for all the responses, pressure (A), temperature (B), and processing time (D) were significant factors.
Table 4-5. Summary of regression coefficients of the significant factors calculated with the stepwise method (standard error, SE, in parentheses).

<table>
<thead>
<tr>
<th></th>
<th>Coefficient (SE)</th>
<th>t-value</th>
<th>p-value</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Process % yield</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>61.9 (0.4)</td>
<td>145.68</td>
<td>0.004</td>
<td>99.97%</td>
</tr>
<tr>
<td>A</td>
<td>12.0 (0.4)</td>
<td>28.26</td>
<td>0.023</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>11.9 (0.4)</td>
<td>28.21</td>
<td>0.023</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>11.2 (0.4)</td>
<td>26.29</td>
<td>0.024</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>10.4 (0.4)</td>
<td>24.41</td>
<td>0.026</td>
<td></td>
</tr>
<tr>
<td>AF</td>
<td>-6.1 (0.4)</td>
<td>-14.41</td>
<td>0.044</td>
<td></td>
</tr>
<tr>
<td><strong>Dissolution extent</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(after 20 minutes)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>67.0 (0.09)</td>
<td>749.91</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>2.2 (0.09)</td>
<td>24.9</td>
<td>0.025</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>3.3 (0.09)</td>
<td>37.59</td>
<td>0.017</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>6.9 (0.09)</td>
<td>77.07</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>1.3 (0.09)</td>
<td>14.62</td>
<td>0.043</td>
<td>99.99%</td>
</tr>
<tr>
<td>E</td>
<td>2.9 (0.09)</td>
<td>31.91</td>
<td>0.020</td>
<td></td>
</tr>
<tr>
<td>AB</td>
<td>-1.4 (0.09)</td>
<td>-15.46</td>
<td>0.041</td>
<td></td>
</tr>
<tr>
<td>AF</td>
<td>-5.9 (0.09)</td>
<td>-65.62</td>
<td>0.010</td>
<td></td>
</tr>
<tr>
<td>AG</td>
<td>1.3 (0.09)</td>
<td>14.39</td>
<td>0.044</td>
<td></td>
</tr>
<tr>
<td>BD</td>
<td>-5.7 (0.09)</td>
<td>-63.33</td>
<td>0.010</td>
<td></td>
</tr>
<tr>
<td><strong>t 1/2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>8.0 (0.03)</td>
<td>261.45</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>2.7 (0.03)</td>
<td>90.33</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>1.1 (0.03)</td>
<td>36.56</td>
<td>0.017</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>-1.8 (0.03)</td>
<td>-61.08</td>
<td>0.010</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>1.0 (0.03)</td>
<td>31.41</td>
<td>0.020</td>
<td>100%</td>
</tr>
<tr>
<td>AB</td>
<td>0.7 (0.03)</td>
<td>22.72</td>
<td>0.028</td>
<td></td>
</tr>
<tr>
<td>AD</td>
<td>-1.8 (0.03)</td>
<td>-57.40</td>
<td>0.011</td>
<td></td>
</tr>
<tr>
<td>AG</td>
<td>0.6 (0.03)</td>
<td>18.44</td>
<td>0.034</td>
<td></td>
</tr>
<tr>
<td>BD</td>
<td>-0.7 (0.03)</td>
<td>-23.05</td>
<td>0.028</td>
<td></td>
</tr>
</tbody>
</table>

Generally, all seven factors were significant for one or more of the responses investigated, but not all seven factors mattered for each response. The dissolution after 20 minutes was the most complex response where five factors were appeared important with four possible interactions, while process yield and t 1/2 each had four significant factors with only one and four interactions, respectively. A one-way analysis of variance (ANOVA) was carried out on the data obtained by SCF processing (PGSS) and various conventional methods; ANOVA results did show evidence of significant differences between sets of data. Table 4-6 gives the results for the refined models from ANOVA. A more detailed analysis of each response is examined in the following sections.
Chapter 4. Optimisation

Table 4-6. ANOVA table for refined models

<table>
<thead>
<tr>
<th></th>
<th>Sum of squares</th>
<th>Dof</th>
<th>Mean squares</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Process yield</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regression</td>
<td>$8.6 \times 10^3$</td>
<td>7</td>
<td>$1.20 \times 10^3$</td>
<td>9.58</td>
<td>0.001</td>
</tr>
<tr>
<td>Residual error</td>
<td>$1.3 \times 10^3$</td>
<td>10</td>
<td>$0.13 \times 10^3$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>$9.9 \times 10^3$</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Dissolution extent</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(20 minutes)</td>
<td>$1.2 \times 10^3$</td>
<td>7</td>
<td>$0.17 \times 10^3$</td>
<td>1.49</td>
<td>0.021</td>
</tr>
<tr>
<td>Regression</td>
<td>$1.1 \times 10^3$</td>
<td>10</td>
<td>$0.12 \times 10^3$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual error</td>
<td>$2.3 \times 10^3$</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>$2.3 \times 10^3$</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$t_{1/2}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regression</td>
<td>$0.22 \times 10^3$</td>
<td>7</td>
<td>$0.031 \times 10^3$</td>
<td>3.23</td>
<td>0.046</td>
</tr>
<tr>
<td>Residual error</td>
<td>$0.10 \times 10^3$</td>
<td>10</td>
<td>$0.009 \times 10^3$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>$0.32 \times 10^3$</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Dof = degrees of freedom, F = F-test which has an F-distribution under the null hypothesis.

4.3.4.1. Response $Y_1$ – Processing yield of PGN dispersions

Results for the $Y_1$ study were analysed for effect, coefficients, standard error of the coefficients, t-values, and p-values and are shown in Table 4-7.

Table 4-7. Estimated effects and coefficients for processing yield ($R^2 = 99.97\%$).

<table>
<thead>
<tr>
<th>Term</th>
<th>Effect</th>
<th>Coefficient</th>
<th>SE</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-</td>
<td>61.913</td>
<td>0.4250</td>
<td>145.68</td>
<td>0.004</td>
</tr>
<tr>
<td>A</td>
<td>24.025</td>
<td>12.012</td>
<td>0.4250</td>
<td>28.26</td>
<td>0.023*</td>
</tr>
<tr>
<td>B</td>
<td>23.975</td>
<td>11.987</td>
<td>0.4250</td>
<td>28.21</td>
<td>0.023*</td>
</tr>
<tr>
<td>C</td>
<td>-3.925</td>
<td>-1.962</td>
<td>0.4250</td>
<td>-4.62</td>
<td>0.136</td>
</tr>
<tr>
<td>D</td>
<td>22.350</td>
<td>11.175</td>
<td>0.4250</td>
<td>26.29</td>
<td>0.024*</td>
</tr>
<tr>
<td>E</td>
<td>4.175</td>
<td>2.088</td>
<td>0.4250</td>
<td>4.91</td>
<td>0.128</td>
</tr>
<tr>
<td>F</td>
<td>20.750</td>
<td>10.375</td>
<td>0.4250</td>
<td>24.41</td>
<td>0.026*</td>
</tr>
<tr>
<td>G</td>
<td>5.900</td>
<td>2.950</td>
<td>0.4250</td>
<td>6.94</td>
<td>0.091</td>
</tr>
<tr>
<td>AB</td>
<td>-8.125</td>
<td>-4.063</td>
<td>0.4250</td>
<td>-9.56</td>
<td>0.066</td>
</tr>
<tr>
<td>AC</td>
<td>-1.425</td>
<td>-0.713</td>
<td>0.4250</td>
<td>-1.68</td>
<td>0.342</td>
</tr>
<tr>
<td>AD</td>
<td>-3.650</td>
<td>-1.825</td>
<td>0.4250</td>
<td>-4.29</td>
<td>0.146</td>
</tr>
<tr>
<td>AE</td>
<td>-2.525</td>
<td>-1.263</td>
<td>0.4250</td>
<td>-2.97</td>
<td>0.207</td>
</tr>
<tr>
<td>AF</td>
<td>-12.250</td>
<td>-6.125</td>
<td>0.4250</td>
<td>-14.41</td>
<td>0.044*</td>
</tr>
<tr>
<td>AG</td>
<td>-4.700</td>
<td>-2.350</td>
<td>0.4250</td>
<td>-5.53</td>
<td>0.114</td>
</tr>
<tr>
<td>BD</td>
<td>5.950</td>
<td>2.975</td>
<td>0.4250</td>
<td>7.00</td>
<td>0.090</td>
</tr>
<tr>
<td>Centre Points</td>
<td>-</td>
<td>-0.412</td>
<td>1.2750</td>
<td>-0.32</td>
<td>0.801</td>
</tr>
</tbody>
</table>

Legends: A = Pressure, B = temperature, C = sample load, D = contact time with CO$_2$, E = sonication, F = Drug: excipient ratio and G = orifice diameter. *Statistically significant variables (ANOVA, p-value ≤ 0.05).
Chapter 4. Optimisation

By substituting the regression coefficients in Table 4-7 into Equation 15, a mathematical model can be obtained to estimate the percentage yield of PGN processing with SC-CO₂:

\[
Y_1 = 61.913 + 12.012A + 11.987B - 1.962C + 11.175D + 2.088E + 10.375F + 2.950G - 4.063AB - 0.713AC - 1.825AD - 1.263AE - 6.125AF - 2.350AG + 2.975BD - 0.412
\]

**Equation 15**

The effects are statistically significant when the p-value, or the minimum level of significance where the null hypothesis would be rejected, is less than 0.05. Figure 4-4 shows a Pareto chart that was plotted illustrating the level of significance for individual effects and interactions. Using a student’s T-test it was possible to determine the statistical significance of the effects on the percentage yield with 95% confidence. Briefly, a student’s T-test is a two sample test of the null hypothesis that the means of two normally distributed populations are equal. The T-value can be calculated by dividing the coefficient by its standard error.

![Pareto chart of standardized effects on percentage yield (Alpha = 0.05).](image)

*The red line represents the level of significance from 95% of the confidence interval (MINITAB 15.0).*
Chapter 4. Optimisation

The Pareto chart showed that the pressure (A), temperature (B), processing time (D), and PGN:excipient ratio (F) were significant main effects influencing the yield of PGN dispersions manufactured using SC-CO$_2$ processing ranging between 2.4% and 94.7% yield. Using the student’s T-test results and the values in Table 4-7, a simplified model was formed as expressed by Equation 16:

$$Y_1 = 61.913 + 12.012A + 11.987B + 11.175D + 10.375F - 6.125AF$$  \hspace{1cm} \text{Equation 16}

Table 4-8 shows what the estimated yield were compared between the two models and the observed values in the factorial study. The use of the full mathematical model (Equation 15) and the simplified model (Equation 16) produced relatively accurate estimations of the observed processing yield with mean standard errors of 1.7 and 3.1, respectively. This model was able to show that for processing, yield was independent of the insignificant factors; which were the sample load (C), sonication (E), and orifice diameter (G).

Table 4-8. Comparison of observed and estimated processing yields from the full (Equation 15) and simplified model (Equation 16).

<table>
<thead>
<tr>
<th>Run</th>
<th>Observed yield %</th>
<th>Estimated yield % (Full model)</th>
<th>SE</th>
<th>Estimated yield % (Simplified model)</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>67.701</td>
<td>67.5583</td>
<td>1.6941</td>
<td>71.1958</td>
<td>3.0653</td>
</tr>
<tr>
<td>2</td>
<td>78.706</td>
<td>78.5583</td>
<td>1.6941</td>
<td>81.3208</td>
<td>3.0653</td>
</tr>
<tr>
<td>3</td>
<td>62.709</td>
<td>63.8333</td>
<td>1.2671</td>
<td>63.8333</td>
<td>3.8897</td>
</tr>
<tr>
<td>4</td>
<td>2.415</td>
<td>2.2583</td>
<td>1.6941</td>
<td>2.5708</td>
<td>3.0653</td>
</tr>
<tr>
<td>5</td>
<td>77.867</td>
<td>77.6583</td>
<td>1.6941</td>
<td>68.8958</td>
<td>3.0653</td>
</tr>
<tr>
<td>6</td>
<td>82.034</td>
<td>81.8583</td>
<td>1.6941</td>
<td>79.6958</td>
<td>3.0653</td>
</tr>
<tr>
<td>7</td>
<td>50.278</td>
<td>50.0583</td>
<td>1.6941</td>
<td>45.5708</td>
<td>3.0653</td>
</tr>
<tr>
<td>8</td>
<td>91.056</td>
<td>90.8583</td>
<td>1.6941</td>
<td>91.8958</td>
<td>3.0653</td>
</tr>
<tr>
<td>9</td>
<td>65.345</td>
<td>65.1583</td>
<td>1.6941</td>
<td>72.8208</td>
<td>3.0653</td>
</tr>
<tr>
<td>10</td>
<td>63.334</td>
<td>63.4417</td>
<td>1.6941</td>
<td>64.8792</td>
<td>3.0653</td>
</tr>
<tr>
<td>11</td>
<td>60.301</td>
<td>59.1667</td>
<td>1.2671</td>
<td>59.1667</td>
<td>3.8897</td>
</tr>
<tr>
<td>12</td>
<td>94.796</td>
<td>94.8417</td>
<td>1.6941</td>
<td>99.0042</td>
<td>3.0653</td>
</tr>
<tr>
<td>13</td>
<td>57.017</td>
<td>57.1417</td>
<td>1.6941</td>
<td>44.1792</td>
<td>3.0653</td>
</tr>
<tr>
<td>14</td>
<td>88.70</td>
<td>88.8417</td>
<td>1.6941</td>
<td>90.5042</td>
<td>3.0653</td>
</tr>
<tr>
<td>15</td>
<td>57.300</td>
<td>57.4417</td>
<td>1.6941</td>
<td>52.6792</td>
<td>3.0653</td>
</tr>
<tr>
<td>17</td>
<td>31.739</td>
<td>31.8417</td>
<td>1.6941</td>
<td>30.2542</td>
<td>3.0653</td>
</tr>
<tr>
<td>18</td>
<td>61.127</td>
<td>61.2417</td>
<td>1.6941</td>
<td>63.2542</td>
<td>3.0653</td>
</tr>
</tbody>
</table>

Figure 4-5 shows the main effects of the investigated SC-CO$_2$ processing variables on the percentage yield of PGN dispersions. The plot shows that the use of higher pressure (A) of 186 bar, higher temperature (B) of 60°C, a longer processing time (D) of 30 minutes, and
lower PGN ratio to excipient (Gelucire 44/14) (F) of 1:10 were all important features to the increasing yield of PGN dispersions using the PGSS method.

As described earlier, multiple interactions in this factorial design study do not provide any valuable information due to confounding. This means it is uncertain whether the response was a result of the main factor effect or joint effects from the confounding interactions. The interactions plot in Figure 4-6 illustrates the effects of two-factor interactions on the processing yield of PGN dispersions. The only combination that was able to be compared without be confounded was pressure (A) and PGN:excipient ratio (F). The interaction result shows that a significant increase in yield occurs when a high pressure (186 bar) was used in combination with lower PGN amount to Gelucire 44/14 (1:10) (p-value = 0.044). A higher temperature (60°C) was also deemed to be important (p-value = 0.023), along with the use of a longer processing time (p-value = 0.024), as well as a lower PGN ratio to excipient (p-value = 0.026) to produce the maximum yield of PGN dispersions from the PGSS unit using SC-CO₂.

Figure 4-5. Main effects plot for yield percentage of PGN dispersions.
*Statistically significant factors (p-value < 0.05).
4.3.4.2. Response Y₂ – Dissolution extent after 20 minutes

The statistical parameters for the extent of PGN dissolution after 20 minutes are shown in Table 4-9. Table 4-3 showed that the significant factors gave results ranging between 40.7% and 85.6% PGN dissolved after only 20 minutes. Data is given graphically in a Pareto chart (Figure 4-7).

All of the investigated variables in the SC-CO₂ processing of PGN dispersions, except for PGN:excipient ratio (F) and orifice diameter (G), had a significant influence on the dissolution of PGN over 20 minutes. Of all the variables, the sample loading (C) had the greatest effect on PGN dissolution over 20 minutes (response Y₃), followed by temperature (B), then pressure (A), and lastly processing time (D). The PGN dissolution over the initial 20 minutes for PGN dispersions manufactured using a low sample load (40.7 – 77.4%) was lower than the range of extent dissolved using a high sample load (57.6 – 85.6%).
Table 4-9. Estimated effects and coefficients for dissolution after 20 minutes ($R^2 = 99.99\%$).

<table>
<thead>
<tr>
<th>Term</th>
<th>Effect</th>
<th>Coefficient</th>
<th>SE Coefficient</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-</td>
<td>67.030</td>
<td>0.08938</td>
<td>749.91</td>
<td>0.001</td>
</tr>
<tr>
<td>A</td>
<td>4.465</td>
<td>2.232</td>
<td>0.08938</td>
<td>24.98</td>
<td>0.025*</td>
</tr>
<tr>
<td>B</td>
<td>6.720</td>
<td>3.360</td>
<td>0.08938</td>
<td>37.59</td>
<td>0.017*</td>
</tr>
<tr>
<td>C</td>
<td>13.777</td>
<td>6.889</td>
<td>0.08938</td>
<td>77.07</td>
<td>0.008*</td>
</tr>
<tr>
<td>D</td>
<td>2.613</td>
<td>1.306</td>
<td>0.08938</td>
<td>14.62</td>
<td>0.043*</td>
</tr>
<tr>
<td>E</td>
<td>5.705</td>
<td>2.853</td>
<td>0.08938</td>
<td>31.91</td>
<td>0.020*</td>
</tr>
<tr>
<td>F</td>
<td>-1.898</td>
<td>-0.949</td>
<td>0.08938</td>
<td>-10.62</td>
<td>0.060</td>
</tr>
<tr>
<td>G</td>
<td>-1.266</td>
<td>-0.633</td>
<td>0.08938</td>
<td>-7.08</td>
<td>0.089</td>
</tr>
<tr>
<td>AB</td>
<td>-2.764</td>
<td>-1.382</td>
<td>0.08938</td>
<td>-15.46</td>
<td>0.041*</td>
</tr>
<tr>
<td>AC</td>
<td>1.017</td>
<td>0.509</td>
<td>0.08938</td>
<td>5.69</td>
<td>0.111</td>
</tr>
<tr>
<td>AD</td>
<td>-0.014</td>
<td>-0.007</td>
<td>0.08938</td>
<td>-0.08</td>
<td>0.950</td>
</tr>
<tr>
<td>AE</td>
<td>0.506</td>
<td>0.253</td>
<td>0.08938</td>
<td>2.83</td>
<td>0.216</td>
</tr>
<tr>
<td>AF</td>
<td>-11.730</td>
<td>-5.865</td>
<td>0.08938</td>
<td>-65.62</td>
<td>0.010*</td>
</tr>
<tr>
<td>AG</td>
<td>2.573</td>
<td>1.286</td>
<td>0.08938</td>
<td>14.39</td>
<td>0.044*</td>
</tr>
<tr>
<td>BD</td>
<td>-11.321</td>
<td>-5.660</td>
<td>0.08938</td>
<td>-63.33</td>
<td>0.010*</td>
</tr>
<tr>
<td>Centre Points</td>
<td>-</td>
<td>-3.475</td>
<td>0.26815</td>
<td>-12.96</td>
<td>0.049</td>
</tr>
</tbody>
</table>

Legends: A = Pressure, B = temperature, C = sample load, D = contact time with CO$_2$, E = sonication, F = Drug: excipient ratio and G = orifice diameter. *Statistically significant variables (ANOVA, p-value ≤ 0.05).

A full regression model relating the dissolution extent of PGN after 20 minutes to the SC-CO$_2$ processing conditions was generated from the factorial study and is shown in Equation 17:

$$Y_2 = 67.030 + 2.232A + 3.360B + 6.889C + 1.306D + 2.853E - 0.949F - 0.633G - 1.382AB$$
$$- 0.509AC - 0.007AD + 0.253AE - 5.865AF + 1.286AG - 5.660BD - 3.475$$

Equation 17

The main effects plot in Figure 4-8 shows that a high pressure (A) of 186 bar, high temperature (B) of 60°C, large sample load (C) of 9 g, and more SC-CO$_2$ processing time (D) of 30 minutes all have positive effects on PGN dissolution over the first 20 minutes. Furthermore, using a higher pressure (186 bar) and a smaller orifice diameter (1/16”) also had an apparent effects on PGN dissolution over 20 minutes. The synergetic effect of these two variables is reflected in the interaction plot in Figure 4-9. Interestingly, the amount of PGN dissolution from the dispersions formed from a lower pressure (90 bar) and temperature (20°C) was much lower than from dispersions formed from the opposite conditions. High PGN dissolution over 20 minutes was also expected when the pressure was low in combination with a higher excipient amount. Finally when a longer processing time is used, a higher temperature must also be used in order to increase the PGN dissolution after just 20 minutes.
Chapter 4. Optimisation

Figure 4-7. Pareto chart of standardised effects on PGN dissolution extent at 20 minutes (Alpha = 0.05).

Figure 4-8. Main effects plot for dissolution extent after 20 minutes of PGN dispersions.

*Statistically significant factors (p-value < 0.05).
Figure 4-9. Interaction plot for dissolution extent after 20 minutes of PGN dispersions. *Statistically significant interactions are shown as shaded boxes (p-value < 0.05).

4.3.4.3. Response Y₃ – PGN dissolution when t₁/₂ reached

In the factorial design, the time when 50% of the SC-CO₂ processed PGN dispersions had been dissolved (t₁/₂) ranged between 2.8 and 17.7 minutes, as shown in Table 4-3. Of the seven factors tested, four of the SCF processing variables had a significant effect on the t₁/₂. Table 4-10 shows that pressure (A), temperature (B), processing time (D), and orifice diameter (G) all had effects on PGN dissolution at the 50% time point.

Results have been presented in a Pareto chart (Figure 4-10). Once again, both pressure (A) and temperature (B) were significant factors in the time it took to reach 50% PGN dissolution (response Y₃), just as they were for responses Y₁ and Y₂. The other factors that influenced the time to reach t₁/₂ included the processing time (D) and the orifice diameter (G). The amount of sample loading (C), sonication (E), and PGN:excipient ratio (F) did not significantly affect the time it took to reach t₁/₂.
Chapter 4. Optimisation

Table 4-10. Estimated effects and coefficients for $t_{1/2}$ for PGN dispersions ($R^2 = 100\%$).

<table>
<thead>
<tr>
<th>Term</th>
<th>Effect</th>
<th>Coefficient</th>
<th>SE Coefficient</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-</td>
<td>8.041</td>
<td>0.03076</td>
<td>261.45</td>
<td>0.002</td>
</tr>
<tr>
<td>A</td>
<td>5.557</td>
<td>2.778</td>
<td>0.03076</td>
<td>90.33</td>
<td>0.007*</td>
</tr>
<tr>
<td>B</td>
<td>2.249</td>
<td>1.125</td>
<td>0.03076</td>
<td>36.56</td>
<td>0.017*</td>
</tr>
<tr>
<td>C</td>
<td>0.447</td>
<td>0.223</td>
<td>0.03076</td>
<td>7.26</td>
<td>0.087</td>
</tr>
<tr>
<td>D</td>
<td>-3.757</td>
<td>-1.878</td>
<td>0.03076</td>
<td>-61.08</td>
<td>0.010*</td>
</tr>
<tr>
<td>E</td>
<td>0.643</td>
<td>0.321</td>
<td>0.03076</td>
<td>10.45</td>
<td>0.061</td>
</tr>
<tr>
<td>F</td>
<td>0.066</td>
<td>0.033</td>
<td>0.03076</td>
<td>1.08</td>
<td>0.476</td>
</tr>
<tr>
<td>G</td>
<td>1.932</td>
<td>0.966</td>
<td>0.03076</td>
<td>31.41</td>
<td>0.020*</td>
</tr>
<tr>
<td>AB</td>
<td>1.397</td>
<td>0.699</td>
<td>0.03076</td>
<td>22.72</td>
<td>0.028*</td>
</tr>
<tr>
<td>AC</td>
<td>-0.771</td>
<td>-0.386</td>
<td>0.03076</td>
<td>-12.53</td>
<td>0.051</td>
</tr>
<tr>
<td>AD</td>
<td>-3.531</td>
<td>-1.765</td>
<td>0.03076</td>
<td>-57.40</td>
<td>0.011*</td>
</tr>
<tr>
<td>AE</td>
<td>0.676</td>
<td>0.338</td>
<td>0.03076</td>
<td>10.99</td>
<td>0.058</td>
</tr>
<tr>
<td>AF</td>
<td>-0.491</td>
<td>-0.245</td>
<td>0.03076</td>
<td>-7.98</td>
<td>0.079</td>
</tr>
<tr>
<td>AG</td>
<td>1.134</td>
<td>0.567</td>
<td>0.03076</td>
<td>18.44</td>
<td>0.034*</td>
</tr>
<tr>
<td>BD</td>
<td>-1.418</td>
<td>-0.709</td>
<td>0.03076</td>
<td>-23.05</td>
<td>0.028*</td>
</tr>
<tr>
<td>Centre Points</td>
<td>-</td>
<td>-3.321</td>
<td>0.09227</td>
<td>-35.99</td>
<td>0.018</td>
</tr>
</tbody>
</table>

Legends: A = Pressure, B = temperature, C = sample load, D = contact time with CO$_2$, E = sonication, F = Drug: excipient ratio and G = orifice diameter. *Statistically significant variables (ANOVA, p-value $\leq 0.05$).

The full regression equation derived from the factorial study relating to $t_{1/2}$ for PGN dispersions is shown in Equation 18:

$$Y_3 = 8.041 + 2.778A + 1.125B + 0.223C - 1.878D + 0.321E + 0.033F + 0.966G + 699AB - 0.386AC - 1.765AD + 0.338AE - 0.245AF + 0.567AG - 0.709BS - 3.321$$

Equation 18
The main effect plot in Figure 4-11 shows that SC-CO₂ processing with a high pressure (A) of 186 bar, high temperature (B) of 60°C, short processing time (D) of 10 minutes, and large orifice diameter (1/4”) all have positive effects on reducing time to reach 50% PGN dissolution. This partly consistent with the previous sets of responses, where both pressure and temperature were also high, but the short processing time is the opposite to that found relating to PGN dissolution after 20 minutes. For the orifice diameter (G), this is the first time it was significant enough to be seen in the main effect to a response (Y₃), that is the orifice diameter was an insignificant influence on Y₁ and Y₂.

The interaction plot is presented in Figure 4-12 showing that four major interactions occurred. It was found that using a higher temperature (60°C) and more processing time (30 minutes) had evident effect on the time taken to reach 50% PGN dissolution. It was also clear that the higher the pressure, in combination with higher temperature and larger orifice size were required to improve the t₁/₂ period.
Chapter 4. Optimisation

Figure 4-11. Main effects plot for \( t_{1/2} \) of PGN dispersions.
*Statistically significant factors (p-value < 0.05).

Figure 4-12. Interaction plot for \( t_{1/2} \) of PGN dispersions.
*Statistically significant interactions are shown as shaded boxes (p-value < 0.05).
4.3.5. Response surface curves

By analysing the response surfaces and by mathematical calculations of the highest values of each variable inside the experimental domain, the best possible processing conditions were obtained, shown in Table 2-11. By completing several further sets of runs at the centre points for pressure (A) and temperature (B) while fixing the other factors, the responses can be analysed using MINITAB for any non-linear (quadratic) relationships. Such non-linear models provide more insight into the variables investigated by showing optimised levels within the DOE. Figure 4-13 shows the three dimensional plots of the response surfaces for the process yield (R1), extent of dissolution after 20 minutes (R2), time required to dissolve 50% of PGN (R3), and PGN uniformity after SC-CO₂ processing (R4); in these plots the variables pressure (A) and temperature (B) are examined. Interestingly only the PGN dissolution extent after 20 minutes gave a drastically non-linear curve. This acts as an indirect confirmation that for responses (Y₁ and Y₃) the linear based factorial design was ideal. The result for R2 was also inconsistent with the results from Y₂, where both high pressure and temperature were observed to improve PGN dissolution after 20 minutes, however for the surface response, a high pressure combination with low temperature (and too some extent visa versa) was seen to positively influence dissolution. Further studies need to be conducted related to the response (Y₂) and the seven variables investigated.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Extent after 20 minutes</th>
<th>Processing yield</th>
<th>t₁/₂</th>
<th>Uniformity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure (A)</td>
<td>medium</td>
<td>high</td>
<td>high</td>
<td>high</td>
</tr>
<tr>
<td>Temperature (B)</td>
<td>medium</td>
<td>high</td>
<td>high</td>
<td>high</td>
</tr>
</tbody>
</table>
Figure 4-13. Response surfaces for: (R1) processing yield; (R2) extent dissolution after 20 min.; (R3) Time for 50% w/v of PGN to dissolve ($t_{1/2}$); and (R4) PGN uniformity.

4.3.6. Effect of pressure ($A$) and temperature ($B$)

As has been noted previously the solubility of a drug in a SCF depends on a critical balance between CO$_2$ fluid density and the drug vapour pressure, both are controlled by temperature and pressure of the solvent fluid [566]. In all three main responses, the temperature and pressure were found to be significant main factors. Temperature had no significant interactions for the process yield, but did have an interaction effect for both the dissolution after 20 minutes and dissolution time for half of PGN. This is also confirmed in surface response curves where a higher pressure and temperature would be expected to produce a high yield, faster $t_{1/2}$, and near optimal PGN dispersion uniformity.

A temperature increase may cause an increase in solvating ability of CO$_2$ due to increasing the solute vapour pressure, even while reducing the fluid density [566]. For the dissolution effects, the relationships between temperature and pressure, and the other factors were more complicated than for the process yield.
From the results, it can be inferred that the pressure of the SCF plays an important role in the formation of PGN dispersions using PGSS, in fact a high pressure could be considered the most important factor for all the responses. This means that process yield and dissolution are enhanced as pressure increases. As pressure increases, the fluid density increases and this could have two effects; an increase in the solvating ability of the SC-CO$_2$ and a reduced interaction between the fluid and excipient as a consequence of the lower diffusion at higher density [225, 243, 566]. No square terms appear in the polynomial functions for any of the responses investigated, indicating that the main effects and interactions have occurred due to the influence of the factors examined and not any independent variables.

4.3.7. Effect of sample loading (C)

In order to achieve high process yields, it was considered necessary to examine the amount of sample loaded into the sample cylinder. The maximum pressure of the sample cylinder is approximately 206.8 bar which could be reached with approx. 294.8 g of CO$_2$. PGN solubility below the SCF point of CO$_2$ is less than 0.18 mole fraction ($\times 10^{-4}$), this provided over capacity where the highest amount of PGN was 4.5 g or only a mole fraction of 0.0174 $\times 10^{-4}$, 10 times less than the solubility at the SC level. Given the density of CO$_2$ at its critical temperature and pressure is 0.496 g·cm$^{-3}$, the maximum sample loading (excipient and PGN) weight of 9 g was chosen in this study.

Ironically only the dissolution response measuring PGN extent dissolved after 20 minutes appeared to hold significant value. This indicates that either the loading range considered was ideal or that the parameters used didn’t test the physical boundaries to detect influence. It is possible that varying ratios of PGN to excipient (F) and orifice diameter (G) may have directly affected this variable, see section 4.3.4.2. This is a limiting aspect of fixing set values in a surface response design, when factors are known to have some influence, however it also means that more significant factors, such as pressure in this case, can be better examined.

4.3.8. Effect of time (D) and sonication (E)

The essential advantage in the use of CO$_2$ in this study was its low polarity, making some degree of solubility of both PGN and excipient possible. In order to produce sufficient dissolution via improved dispersion, the process was conducted under static conditions (i.e. closed system); this allowed for a better penetration of the fluid in the excipient matrix than
in a dynamic method where CO₂ is continuously added and released throughout the system. In the model experiment the main effect of time (D) was found to be significant for all three main responses. As described earlier, the CO₂ contact time did not show significant interactions for the process yield, but there was a similar interaction between time (D) and temperature (B) for both dissolution responses. Again this was shown in the surface curves, where temperature can be seen as having a dramatic influence on all the responses tested. By fixing the other variables the relative influence of temperature (B) can be observed for PGN dissolution after 20 minutes, where both a low and high temperature (B) where able to give a slow and fast dissolution response after 20 minutes.

4.3.9. Effect of drug ratio (F) and orifice diameter (G)

The excipient fraction of a formulation can be central in the dissolution of a drug, hence varying ratios of excipient to PGN was investigated in the 2^p-k experiment. For only the process yield the main effect of the variable drug ratio (F) was significant with a positive coefficient, viz the increase in the excipient fraction to PGN led to an increase in process yield. A single two-factor interaction with pressure (A) and drug ratio (F) was also observed to be statistically significant. The interaction result shows a significant increase in yield when a high SCF pressure (A) (approximately 186 bar) was used in combination with a high drug: excipient ratio (1:10) (p-value = 0.044). Once again this was confirmed in the surface curves, where a higher pressure (A) had a positive response on yield, while a low and high temperature (B) did not appear to influence the process yield.

A major drawback in the use of tubing between the sample cylinder and precipitation chamber, is the absence of spraying ability. Nevertheless, this limitation may have been partly overcome but using various tubing diameters, such as 1/4” and 1/16”, in order to increase or decrease the release orifice size and rate and hence mimic spraying. The model experiment found that orifice diameter (G) only had a significant main effect on t_{1/2} and two possible interactions with pressure (A) for both the extent dissolution after 20 minutes and t_{1/2}. This indicates that the process yield was not affected by the orifice diameter and that dissolution may be improved further with use of more sophisticated nozzles with better spraying capabilities.
In vitro dissolution

In order to compare the PGSS and cosolvent (CS), comelt (CM) and physical mixing (PM) performances, data obtained from the $2^3$ experiments with the highest dissolution extents after 60 minutes and drug loading within 3% of original amount of PGN added for each excipient: PGN ratio inside the experimental domain were compared with those obtained by performing a conventional set of methods on the same ingredients. Figure 4-14 shows the release profiles of PGN from Gelucire 44/14 systems prepared using various methods.

ANOVA carried out on the data obtained by SCF processing (PGSS) and various conventional methods; ANOVA results did show evidence of significant differences between sets of data. Statistical analysis of the extent after 60 minutes and the area under the curve of Gelucire 44/14 semisolid dispersions illustrated the amount of PGN released was significantly (p-value $<$ 0.05) lower from the controls than SCF formulations, shown in Table 4-12.

![Figure 4-14. Percentage of cumulative PGN amount released from Gelucire 44/14 systems over 60 minutes. Data points represent mean ± SD, n = 3.](image-url)
Chapter 4. Optimisation

The ability of the SCF formulations to outperform or at least equal the conventional formulations confirms the effectiveness of the PGSS method for PGN with Gelucire 44/14 (Table 4-12). In addition, PGSS preparation proved to be faster (excluding PM) than CS and CM, and thus more advantageous in time required to make dispersions.

Table 4-12.Extent dissolution after 60 minutes and area under the percentage drug released vs time curve (AUC) of PGN and Gelucire 44/14 dispersions.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Extent after 60 minutes ± SD</th>
<th>AUC % min·mL⁻¹ ± SD</th>
<th>Main SCF conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCF 1:1</td>
<td>97.14 ± 2.3</td>
<td>4421.6 ± 217.3</td>
<td>Pressure (A) 186.2 bar, Temperature (B) 60°C</td>
</tr>
<tr>
<td>SCF 1:5</td>
<td>91.83 ± 4.6</td>
<td>4452.9 ± 47.10</td>
<td>Pressure (A) 135.1 bar, Temperature (B) 40°C</td>
</tr>
<tr>
<td>SCF 1:10</td>
<td>96.25 ± 1.5</td>
<td>4601.6 ± 61.70</td>
<td>Pressure (A) 89.9 bar, Temperature (B) 60°C</td>
</tr>
<tr>
<td>CS 1:1</td>
<td>71.84 ± 2.1</td>
<td>3044.7 ± 116.9</td>
<td>Pressure (A) atm, Temperature (B) 25°C</td>
</tr>
<tr>
<td>CM 1:1</td>
<td>46.93 ± 2.2</td>
<td>1790.9 ± 149.4</td>
<td>Pressure (A) atm, Temperature (B) ≥ 135°C</td>
</tr>
<tr>
<td>PM 1:1</td>
<td>76.67 ± 1.5</td>
<td>3732.8 ± 128.8</td>
<td>Pressure (A) atm, Temperature (B) 25°C</td>
</tr>
</tbody>
</table>

SCF = supercritical fluid, CS = cosolvents, CM = comelt, PM = physical mixing, and atm = atmospheric pressure. Data are mean ± SD, n = 3

Release profiles demonstrated that after 60 minutes none of the formulations had fully dissolved. The effect of having Gelucire 44/14 at a ratios of 1:1 and 1:5 was investigated but release data showed no significant difference to the formulations with higher Gelucire 44/14 content (p-value > 0.05). The controls demonstrated slower release profiles to the SCF samples, even though two of the SCF based formulations had less Gelucire 44/14 present at 1:1 and 1:5 ratios. This is suggestive of a rate-limiting step in the semi-solid matrix of the Gelucire 44/14 which may or may not be desirable. It is proposed that improved distribution of PGN throughout Gelucire 44/14 using the PGSS method increased the wettability of PGN.

4.3.10.1. Zero and first order

Release data obtained generally did not fit the zero or first-order models ($R^2 \leq 0.99$) for all formulations. Nevertheless, statistical analysis of the data showed that rate release of PGN from the controls and SCF formulations was significant (p-value < 0.05). Furthermore, the SCF 1:10 with more Gelucire 44/14 showed significantly (p-value < 0.05) higher rates over the lower Gelucire 44/14 content dispersions, shown in Table 4-13.
Chapter 4. Optimisation

Table 4-13. Release parameters for the PGN dispersions obtained from zero and first order models

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Pressure (A)</th>
<th>Zero Rate ± SD</th>
<th>Adjusted $R^2$</th>
<th>First Rate ± SD</th>
<th>Adjusted $R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated PGN atm</td>
<td>0.49 ± 0.05</td>
<td>0.995</td>
<td>0.16 ± 0.05</td>
<td>0.986</td>
<td></td>
</tr>
<tr>
<td>CS 1:1 atm</td>
<td>1.01 ± 0.03</td>
<td>0.916</td>
<td>0.48 ± 0.03</td>
<td>0.911</td>
<td></td>
</tr>
<tr>
<td>CM 1:1 atm</td>
<td>1.34 ± 0.04</td>
<td>0.938</td>
<td>0.47 ± 0.02</td>
<td>0.982</td>
<td></td>
</tr>
<tr>
<td>PM 1:1 atm</td>
<td>1.04 ± 0.02</td>
<td>0.785</td>
<td>0.55 ± 0.04</td>
<td>0.825</td>
<td></td>
</tr>
<tr>
<td>SCF 1:1a High</td>
<td>1.59 ± 0.03</td>
<td>0.927</td>
<td>0.62 ± 0.04</td>
<td>0.947</td>
<td></td>
</tr>
<tr>
<td>SCF 1:1b Low</td>
<td>1.64 ± 0.08</td>
<td>0.906</td>
<td>0.55 ± 0.02</td>
<td>0.976</td>
<td></td>
</tr>
<tr>
<td>SCF 1:5a Med</td>
<td>1.63 ± 0.02</td>
<td>0.953</td>
<td>0.43 ± 0.03</td>
<td>0.950</td>
<td></td>
</tr>
<tr>
<td>SCF 1:5b Med</td>
<td>1.57 ± 0.03</td>
<td>0.896</td>
<td>0.54 ± 0.07</td>
<td>0.939</td>
<td></td>
</tr>
<tr>
<td>SCF 1:10a High</td>
<td>1.97 ± 0.09</td>
<td>0.887</td>
<td>0.62 ± 0.07</td>
<td>0.989</td>
<td></td>
</tr>
<tr>
<td>SCF 1:10b Low</td>
<td>1.88 ± 0.05</td>
<td>0.847</td>
<td>0.52 ± 0.06</td>
<td>0.984</td>
<td></td>
</tr>
</tbody>
</table>

SCF = supercritical fluid, CS = cosolvents, CM = comelt, PM = physical mixing, and atm = atmospheric pressure (~0.86 bar). High pressure = 186 bar, low pressure = 90 bar. Data are mean ± SD, n = 3.

4.3.10.2. Higuchi

Release data obtained for the Higuchi model generally fitted ($R^2 = 0.97$ or higher), except for the several SCF formulations ($R^2 \geq 0.944$), and the control employing a cosolvent method fitted well ($R^2 = 0.995$). Statistical analysis of the data showed the rate of release of PGN from Gelucire 44/14 was higher for SCF formulations (p-value < 0.01) than the controls, shown in Table 4-14.

Table 4-14. Release rate constants for the PGN dispersions obtained from the Higuchi model

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Pressure (A)</th>
<th>$k_H$ ± SD</th>
<th>Adjusted $R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated PGN atm</td>
<td>4.03 ± 0.5</td>
<td>0.959</td>
<td></td>
</tr>
<tr>
<td>CS 1:1 atm</td>
<td>9.42 ± 0.4</td>
<td>0.995</td>
<td></td>
</tr>
<tr>
<td>CM 1:1 atm</td>
<td>11.34 ± 0.4</td>
<td>0.982</td>
<td></td>
</tr>
<tr>
<td>PM 1:1 atm</td>
<td>9.40 ± 0.8</td>
<td>0.944</td>
<td></td>
</tr>
<tr>
<td>SCF 1:1a High</td>
<td>13.67±0.9</td>
<td>0.985</td>
<td></td>
</tr>
<tr>
<td>SCF 1:1b Low</td>
<td>14.43 ± 0.8</td>
<td>0.976</td>
<td></td>
</tr>
<tr>
<td>SCF 1:5a Med</td>
<td>13.91 ± 0.3</td>
<td>0.972</td>
<td></td>
</tr>
<tr>
<td>SCF 1:5b Med</td>
<td>13.76 ± 0.5</td>
<td>0.974</td>
<td></td>
</tr>
<tr>
<td>SCF 1:10a High</td>
<td>13.95 ± 1.1</td>
<td>0.946</td>
<td></td>
</tr>
<tr>
<td>SCF 1:10b Low</td>
<td>13.63 ± 0.5</td>
<td>0.984</td>
<td></td>
</tr>
</tbody>
</table>

SCF = supercritical fluid, CS = cosolvents, CM = comelt, PM = physical mixing, and atm = atmospheric pressure (~0.86 bar). High pressure = 186 bar, low pressure = 90 bar. $k_H$ is the release constant of the dosage form. Data are mean ± SD, n = 3.
Chapter 4. Optimisation

4.3.10.3. Korsmeyer-Peppas

To further explain the mechanism of PGN release from the Gelucire 44/14 systems, data was fitted to the Korsmeyer-Peppas model and dual-first order release models. A significant difference was observed between the release exponent of the CS and PM controls and the SCF formulations with the Korsmeyer-Peppas model (p-value < 0.01), as shown in Table 4-15. All of the SCF formulations (except SCF 1:1a samples), had release exponents above 0.5, demonstrating an engagement of more than one release process occurs in the dissolution of PGN from Gelucire 44/14. The data generally fitted into the Korsmeyer-Peppas model, although not well in all cases with the least ($R^2 = 0.92$) to best ($R^2 = 0.99$) for the fitted data.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>$k_{K-P}$ ± SD</th>
<th>$t_{1/2}$ (minutes) ± SD</th>
<th>$n$ ± SD</th>
<th>Adjusted $R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated PGN</td>
<td>-</td>
<td>137.8 ± 1.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CS 1:1</td>
<td>13.1±2.0</td>
<td>25.1 ± 0.2</td>
<td>0.48±0.04</td>
<td>0.998</td>
</tr>
<tr>
<td>CM 1:1</td>
<td>7.86±0.7</td>
<td>22.3 ± 0.4</td>
<td>0.69±0.03</td>
<td>0.964</td>
</tr>
<tr>
<td>PM 1:1</td>
<td>28.7±2.5</td>
<td>12.4 ± 0.5</td>
<td>0.33±0.02</td>
<td>0.989</td>
</tr>
<tr>
<td>SCF 1:1a</td>
<td>12.8±2.3</td>
<td>14.3 ±0.4</td>
<td>0.42±0.05</td>
<td>0.961</td>
</tr>
<tr>
<td>SCF 1:1b</td>
<td>19.2±2.1</td>
<td>9.70 ± 0.3</td>
<td>0.58±0.02</td>
<td>0.971</td>
</tr>
<tr>
<td>SCF 1:5a</td>
<td>13.8±1.9</td>
<td>14.5 ±0.4</td>
<td>0.63±0.03</td>
<td>0.962</td>
</tr>
<tr>
<td>SCF 1:5b</td>
<td>22.4±3.1</td>
<td>8.70 ± 0.2</td>
<td>0.57±0.02</td>
<td>0.985</td>
</tr>
<tr>
<td>SCF 1:10a</td>
<td>21.1±2.8</td>
<td>8.10 ± 0.2</td>
<td>0.78±0.04</td>
<td>0.980</td>
</tr>
<tr>
<td>SCF 1:10b</td>
<td>26.2±3.3</td>
<td>7.40 ± 0.1</td>
<td>0.68±0.03</td>
<td>0.919</td>
</tr>
</tbody>
</table>

SCF = supercritical fluid, CS = cosolvents, CM = comelt, PM = physical mixing, a = high pressure (186 bar), and b = low pressure samples (90 bar). $k_{K-P}$ is a constant characteristic of the dosage form and $n$ is the release exponent indicative of the release mechanism. Data are mean ± SD, n = 3.

4.3.10.4. Dual first order

To test the hypothesis that at least two pathways control the release of PGN from Gelucire 44/14, a dual first order model was constructed, shown in Table 4-16. It was noted that this model showed generally improved regression ($R^2 ≥ 0.98$) over the previously examined models, except for the PM control ($R^2 = 0.964$). In fact seven of the nine formulations showed well fitted profiles to the dual first-order release model ($R^2 ≥ 0.99$). Statistical analysis of the initial rate of release ($H_{1}$) of PGN from the formulations showed significant difference compared with the controls (p-value < 0.05). However the same model illustrated mixed results for the second rate constant ($K_1$), with some significant and others not
significant compared with the controls. A representative example of a non-linear plot is shown in Appendix IX.

Table 4-16. Release parameters for the PGN dispersions obtained from dual first order model

<table>
<thead>
<tr>
<th>Formulation</th>
<th>( H_1 ) (%diffusion) ± SD</th>
<th>( K_1 ) erosion (%·minute(^{-1})) ± SD</th>
<th>( t_{1/2} ) (minutes) ± SD</th>
<th>( \chi^2/\text{DoF} ) ± SD</th>
<th>Adjusted ( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated PGN</td>
<td>-</td>
<td>-</td>
<td>137.8±1.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CS 1:1</td>
<td>30.1±0.7</td>
<td>0.11 ± 0.03</td>
<td>7.1±1.3</td>
<td>1.1±0.3</td>
<td>0.991</td>
</tr>
<tr>
<td>CM 1:1</td>
<td>30.5±0.7</td>
<td>0.28 ± 0.02</td>
<td>8.15±1.3</td>
<td>1.6±0.2</td>
<td>0.998</td>
</tr>
<tr>
<td>PM 1:1</td>
<td>40.8±0.5</td>
<td>0.39 ± 0.05</td>
<td>5.4 ± 1.1</td>
<td>1.9±0.6</td>
<td>0.964</td>
</tr>
<tr>
<td>SCF 1:1a</td>
<td>58.1±2.1</td>
<td>0.65 ± 0.05</td>
<td>6.6 ± 0.84</td>
<td>1.6±0.4</td>
<td>0.993</td>
</tr>
<tr>
<td>SCF 1:1b</td>
<td>74.7±2.8</td>
<td>0.42 ± 0.02</td>
<td>9.34±1.2</td>
<td>0.7±0.5</td>
<td>0.992</td>
</tr>
<tr>
<td>SCF 1:5a</td>
<td>86.7±14.3</td>
<td>0.18 ± 0.06</td>
<td>16.0±1.5</td>
<td>0.9±0.3</td>
<td>0.991</td>
</tr>
<tr>
<td>SCF 1:5b</td>
<td>85.8±7.1</td>
<td>0.76 ± 0.02</td>
<td>4.5 ± 0.4</td>
<td>1.5±0.4</td>
<td>0.997</td>
</tr>
<tr>
<td>SCF 1:10a</td>
<td>66.1±2.4</td>
<td>0.56 ± 0.03</td>
<td>6.5 ± 2.9</td>
<td>0.8±0.2</td>
<td>0.988</td>
</tr>
<tr>
<td>SCF1 1:10b</td>
<td>83.1±1.9</td>
<td>0.20 ± 0.04</td>
<td>5.9 ± 0.6</td>
<td>0.9±0.6</td>
<td>0.982</td>
</tr>
</tbody>
</table>

SCF = supercritical fluid, CS = cosolvents, CM = comelt, PM = physical mixing, a = high pressure (186 bar), and b = low pressure samples (90 bar). Data are mean ± SD, n = 3.

Statistical analysis of the \( t_{1/2} \) values obtained from the dual first-order model shows no significant difference between SCF formulations and the controls (\( p \)-value > 0.05), except for SCF 1:5 prepared under high pressure conditions (\( p \)-value < 0.01). The fastest \( t_{1/2} \) value obtained from the dual first-order model was 4.5 minutes, which is 30.6 times quicker than PGN untreated with SCF and without Gelucire 44/14, however the time to dissolve half the PGN present varied considerably between SCF formulations. It is possible viscosity plays a role in drug release from Gelucire 44/14 matrices, however due to the semi-solid nature of these formulations no investigations produced useful data to determine any meaningful information.

The exponent of the Korsmeyer-Peppas model is recognized to be indicative of diffusion release mechanisms from matrix systems [577, 583]. As described by Peppas, [584] an \( n \)-value of 0.5 reflects Fickian diffusion, and an \( n \)-value between 0.5 and 1 can explain non-characteristic transport where more than one release process occurs. Fitting experimental data to non-linear curves was able to show the inclusion of more than one dissolution process in the release of PGN from Gelucire 44/14. It follows that the use of a multiple release model is necessary to describe at least two processes. Unfortunately, where both processes follow first-order kinetics no straightforward model is available [583]. Currently, iterative curve-fitting techniques such as the dual first order model, are useful ways to determine the required parameters [583]. This study demonstrates the dual-first order equation to be a sufficient
Chapter 4. Optimisation

empirical model to describe the release of molecules from Gelucire 44/14 matrices prepared by either conventional or SCF methods.

Furthermore, the degree of viscosity may have been another factor in the observed profiles. The formulations manufactured in this research were of semi-solid nature, meaning the thickness or resistance to flow was high, which was observed. The high viscoelastic properties of Gelucire 44/14 systems was likely to have influenced the release rates [316, 318]. Gelucire 44/14 is known to form hexagonal and lamellar networks, where the viscosity maximum occurs between PEG diesters connecting the lamellar networks [316]. The rate of drug release may have been reduced with increasing the viscosity, however these networks melt below physiological temperatures [316]. Given the experimental temperature was at 37°C, it was expected that the Gelucire 44/14 dissolution was not affected by the PEG networks. There are numerous studies that have found viscosity to directly influence dissolution, thus bioavailability [585-588]. As described by the Stokes-Einstein equation, the diffusion of a drug is inversely proportional to the viscosity of the system [320]. Gelucire 44/14 is also known to form micelles at relatively low concentrations approximately 0.1 g·mL⁻¹, which could build an entangled network creating an increase in viscosity [319, 561]. Thus, at some point in the dissolution of Gelucire 44/14, the viscosity of the Gelucire 44/14 systems may control the release of PGN loaded throughout the Gelucire 44/14. Further investigations are required into Gelucire 44/14 micelle formation, PGN solubility and SCF processing, and viscosity, and how each of these factors effect PGN dissolution, thus bioavailability of PGN.

4.4. Conclusions

In the investigation of the effects of the seven tested parameters in the model formation of Gelucire 44/14 dispersions, the use of an experimental design approach was able to identify relationships between variables and responses. The optimal or near optimal experimental conditions for the developed and constructed SCF unit employing the PGSS method were also found for PGN inside the experimental domain considered. It was found all seven factors were significant for one or more of the responses investigated, but not all seven factors mattered for each response. High pressure (186 bar), temperature (60°C), and processing time (30 minutes) all had positive effects on yield, $E_{20}$ dissolution, and $t_{1/2}$, except for $t_{1/2}$ where a shorter processing time of 10 minutes was more ideal. The higher loading amount of 9 g and longer sonication time of 10 minutes was only significant $E_{20}$ dissolution, while the larger
orifice size during expansion only affected $t_{1/2}$. The lower drug to excipient ratio of 1 to 10 had a statistically significant influence on only the processing yield. It may be concluded that a higher pressure and temperature, larger sample loading, longer processing time, longer sonication duration, a lower drug to excipient ratio, and larger orifice size during expansion are the optimal conditions for the preparation of PGN loaded Gelucire 44/14 dispersion systems.

The results show the significance not only of the main effects of pressure and temperature in the mixing of Gelucire 44/14 with PGN, but also of the interaction and curvature effects that would have been lost in the use of a conventional one-variable at a time approach. The PGSS method proved to some extent, to be a more or at least equally effective mixing procedure, when compared to conventional methods.

The double simultaneously occurring diffusion and erosion processes may contribute to the release of PGN, thus established deterministic models (viz Higuchi and Korsmeyer-Peppas) are only partially useful. It has been shown that a non-linear derivative dual first order model is capable of determining the simultaneous release pathways. These models therefore provide another tool in the explication of release mechanisms.

Finally, this chapter shows that, SC-CO$_2$ processing under various SCF conditions, is more effective at mixing PGN and Gelucire 44/14 uniformly and often with smaller particle size compared to that of conventionally prepared dispersion systems as illustrated by the increased release rate of PGN. Given the physical and release properties investigated so far in this chapter and previous chapters, TPGS and Gelucire 44/14 were promising excipients for PGN loaded semi-solid dispersion systems for improving the transdermal delivery of PGN. The following chapter explores the ability of the developed formulations to deliver PGN across mouse skin.
Chapter 5:
Permeability Study of Progesterone
Chapter 5. Permeability Study of Progesterone

5.1. Introduction

Up to here the formulation and characterization has been examined for SCF processing of various excipients with PGN. The next step was to investigate the permeability of PGN using one of the selected excipients and optimised SCF conditions. It is hypothesised that the final SCF dispersion will be prepared using either Gelucire 44/14 or TPGS based on the \textit{in vitro} dissolution results, although PEG will be studied to confirm that the PEG 400/4000 system has poor PGN permeability. It is also predicted that the final SCF delivery system will form micelles when engaged with the skins surface. Water present on the skins surface may help disperse the supersaturated semi-solid system providing more thermodynamic activity and improve the permeation rate of PGN. In addition, it has been determined that the optimal permeation for drugs across the skin is between logP values 1 – 3 \cite{97}. Given the logP of PGN is 3.5, its permeability across the skin may pose difficulty. An oil and penetration enhancer was added to the SCF systems employed so far, and it was hypothesized that the oil would enhance solubility while the penetration enhancer would improve partitioning, increasing the rate of permeation through the skin. The common ways to study drug absorption are to consider how molecules penetrate through both an artificial membrane and \textit{ex vivo} membrane.

Skin from animals is easier to obtain than from humans, and usually in larger sample numbers, although the age and gender of the animals cannot always be controlled \cite{589}. Skin excised from rodents and other animals have been used quite often for many years by other researchers \cite{590}. The main \textit{in vitro} method is the use of excised skin from, to name a few, rats, mice, rabbits, hamsters, pigs, snake, and monkeys, which have been mounted on vertical glass diffusion cells \cite{591}. Ideally human skin from autopsies, amputations or cosmetic surgery can be used but it is not always obtainable so mammalian animals are used as a practical alternative.

The primary problem with using animal skin as a human model is that it could under or over estimate permeation \cite{590}. This problem is partly caused by hydration effects from extended exposure (generally 24 h or longer) to an aqueous environment. Presence of donor
and receptor water has been associated with a diminished skin barrier, and hairless mice are known to be greatly influenced by hydration properties [590]. The important difference between skins of different species is the lipid composition and arrangement in the SC [590]. To help reduce the effects of hydration, and hence lipid organization, studying permeation is said to be ideal if the total exposure time is kept within 12h [590]. Specifically for hairless mouse skin it has been recommend that it is not used as *in vitro* permeation model for human tissue under long-term hydration, viz greater than three days [592].

In addition to there being several other types of diffusion methods, there are also some other useful skin analysis methods including bioassays, differential scanning caloimetry, ultrasound, and spectral analysis using infrared and Raman spectroscopy [97]. In addition to quantifying the amount of drug permeated across the skin, the mechanism of permeation can be assessed by evaluation of the skin structure. These are *ex vivo* methods and include histology, electron microscopy of the skin surface and conformational changes in the skin lipids.

As eluded to above, the diffusion (Franz) cell is a common and useful tool for obtaining transdermal drug delivery information. In a typical Franz setup, shown in Figure 5.1, the skin is applied between a donor and receptor chamber and the test formulation added to the skins surface [593]. At pre-determined time intervals, samples are removed from the sample port and analysed for drug content.

![Figure 5-1. Schematic of a Franz cell used to evaluate drug permeation. Reproduced and adapted from [593].](image)

Table 5-1 shows a comparison between different PGN delivery systems that were investigated for skin permeation. It is important to note that comparisons made between
different animals and formulations are incomenserable. However, it is interesting to find that there is generally a dramatic difference in values obtained for PGN permeation across a range of skin types. Scheuplein, et al. (1969) did a lot of the original work on percutaneous absorption of steroids and found that PGN permeation was approximately $4.1 \times 10^{-7}$ cm·s$^{-1}$ using hairless mouse [594]. In another study using PGN with a thin-filmed patch over shed snake skin, the permeability of PGN was $43.6 \times 10^{-7}$ cm·s$^{-1}$ [595]. Table 5-1 shows the range of permeability values obtained from various studies and formulations. There were likely to be different concentrations utilised, thus thermodynamic activity of drugs in the transdermal vehicles was different between the formulations, which can significantly influence drug delivery [174]. There are a range of different formulations used, including gels, creams, liposomes, polymer matrixes, patches, and aqueous solutions. Other differences between the permeation studies were the skin type, including snakes, mouse, porcine, rat, and synthetic membrane, and use of penetration enhancers.

Table 5-1. Comparison of PGN permeation data across the skin or synthetic membranes from lowest to highest values.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Model (skin or membrane)</th>
<th>Permeation $\times 10^{-7}$ (cm·s$^{-1}$)*</th>
<th>Penetration enhancer</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>commercial gel</td>
<td>shed python, cobra &amp; viper</td>
<td>est. 0.03, 0.02 &amp; 0.009</td>
<td>no</td>
<td>[596]</td>
</tr>
<tr>
<td>commercial cream</td>
<td>shaved porcine ear</td>
<td>0.15</td>
<td>no</td>
<td>[597]</td>
</tr>
<tr>
<td>SMEDDS</td>
<td>shaved porcine ear</td>
<td>0.29</td>
<td>tween 85</td>
<td>[597]</td>
</tr>
<tr>
<td>spray vehicle</td>
<td>shed python &amp; clipped porcine</td>
<td>1.7 &amp; 6.2</td>
<td>no</td>
<td>[598]</td>
</tr>
<tr>
<td>saturated aqueous solution methacrylic films</td>
<td>nude mouse</td>
<td>4.1</td>
<td>no</td>
<td>[594]</td>
</tr>
<tr>
<td>liposome</td>
<td>PTFE membrane</td>
<td>7.1</td>
<td>no</td>
<td>[599]</td>
</tr>
<tr>
<td>thin-filmed patch polyurethane &amp; silicone films</td>
<td>shaved rat snake membrane soaked in bovine plasma</td>
<td>44.1</td>
<td>no</td>
<td>[595]</td>
</tr>
<tr>
<td>aqueous/ethanol solution polymer matrix (PVA, PVP &amp; PMA)</td>
<td>nude male mouse</td>
<td>210</td>
<td>no</td>
<td>[601]</td>
</tr>
<tr>
<td></td>
<td>shaved female rat</td>
<td>314, 241 &amp; 53.4</td>
<td>urea or pantothenol</td>
<td>[602]</td>
</tr>
</tbody>
</table>

*where multiple values are given this is due to the use of more than one formulation or skin model that has been tested. **reported as cm$^2$·s$^{-1}$

The skin is a very effective barrier, as described in Chapter 1 and will often require a number of physicochemical strategies to overcome. Some of these strategies may include skin hydration, temperature, pH, diffusion, partition coefficient, drug concentration, and molecular size and shape [97]. In this chapter, several strategies are investigated such as
improving drug concentration at the surface of the skin, use of a penetration enhancer, and preparation of unique dispersion systems by a novel PGSS method using SC-CO\textsubscript{2}. The PGSS prepared PGN dispersions systems with Gelucire 44/14, TPGS and PEG were also compared to that of several conventional preparation methods, which were the CS, PM, and CM methods and controls: (1) aqueous PGN suspension and (2) PGN market cream.

5.2. Experimental

5.2.1. Materials

Industrial grade PGN was purchased from PCNZ (Auckland, NZ) which was sourced from Pharmaca & UpJohn-Pfizer (New York, USA). Progesterone Cream™ was purchased from PCNZ (Auckland, NZ). PEG 400 and 4000 was brought from BDH, VMR International Ltd. (Poole, England). EDTA, Tween 80, sodium deoxycholate, aniseed oil (Pimpinella anisum), and Span 20 were purchased from Sigma-Aldrich (USA). Vitamin E TPGS was obtained from Zhejiang Shiner Chemical Company Ltd. (Zhejiang, China). Gelicure 44/14 was received by donation from Gatiffossé Corporation, (New Jersey, USA). Myritol 318 was obtained from Cognis Car Chemicals (Monheim, Germany). Urea and transcutol P was generously donated by Gatiffossé Corporation (Saint Priest Cedex, France). Sesame seed oil was from Fluca (Buchs, Switzerland). Evening primrose oil was from Soap Kitchen (Devon, UK). Cellulose acetate© membrane was purchased from Whatman Healthcare Ltd. (Queenstown, Singapore). Propylene glycol (PG) was purchased from Midwest pharmaceuticals (Hastings, NZ). Methanol and acetonitrile were HPLC grade obtained from Sigma-Aldrich (Auckland, NZ). Liquid CO\textsubscript{2} (purity 98%) was purchased from BOC Gas (Auckland, NZ). Triple-distilled MillQ water was obtained in-house by reverse osmosis (Millipore, USA). All samples were used without any further purification steps.

Full thickness mice skin from fresh nude white CD1 mouse skin (ex-breeders) was collected from the Vermon J Unit (VJU) at the University of Auckland (Auckland, NZ). Any underlying excessive fat on the skin was carefully removed using a scalpel (size 20) and discarded. Each mouse skin section was cut into four pieces of roughly 2 to 2.5 cm\textsuperscript{2} and placed immediately on a Franz cell or stored at -20°C.

Male porcine ears were obtained from Auckland Meat Processing (AMP) Limited (Auckland, New Zealand), within an hour post-sacrifice. Full thickness skin (epidermis, dermis and subcutaneous tissue) was prepared by carefully separating skin from the basement cartilage layer and cutting the skin into approximately 2.5 cm\textsuperscript{2} sections.
Chapter 5. Permeation

5.2.2. SCF processing

The dispersions made from SCF processing were formed using the PGSS method. The general method has been outlined previously in section 3.5.2 and the optimised method outlined in section 4.3. The conditions used to prepare the SCF dispersions for permeation analysis were those determined in the optimised process. An important difference to note from previous SCF processing, is that two new components were added. The resulting dry semi-solid mass was removed and stored at 4°C in glass vials for later analysis. In addition, to the selected excipients, there was a preparation fabricated with an oil and penetration enhancer; myritol 318 and transcutol P, respectively.

5.2.3. Conventional methods for preparation of dispersion systems

Dispersion systems were once again prepared by three conventional methods: (1) the solvent (cosolvents – CS) evaporation method, PGN and excipient were dissolved in a minimum volume of methanol in a conical flask and heated, (2) the melting (comelt – CM) recrystalization method, which involved stirring PGN and excipient in a beaker on a hot stage at temperature (135°C), and (3) the physical mixing (PM) method, involving the mixing of PGN and excipient to the required ratio (e.g. 1 to 25), in a mortar and pestle. The methods used for conventionally prepared formulations are outlined in more detail previously in Chapter 3, section 3.6.1.

5.2.4. Oil and enhancer selection

The ability of several enhancers to increase the permeation of PGN over 24 hours was conducted to screen for the best penetration enhancer. This was conducted in two phases: (1) determine a suitable oil for both PGN and the penetration enhancers using birefringence, and (2) screen the selected penetration enhancers for their ability to permeate PGN across the skin. The oil selection involved dissolving the selected excipients into various concentrations of each oil with excess PGN and left to stir for 15 hours at 45°C. The enhancer screening occurred using a Franz cell unit and nude mouse skin membrane. Collection samples were made accordingly, and analysed using HPLC. The penetration enhancer with the highest permeation extent after 24 hours and highest flux was selected for the final formulation. The Franz cell method is outlined in the following section.
Chapter 5. Permeation

5.2.5. PGN solubility

An important factor of in vitro skin permeation studies is the selection of the receptor fluid. PGN is practically insoluble in water ($3.79 \times 10^{-5}$ M at 25°C) [249]. The use of aqueous only receptor fluids are unsuitable for drugs with a water solubility lower than 10 μg·mL$^{-1}$ [604]. Hence, a mixture of propylene glycol in water, 40 and 60% v/v, respectively was chosen. The solubility of PGN in this receptor fluid has been established for ex vivo animal skin diffusion studies [604-606].

5.2.6. Evaluation of skin integrity

Each skin membrane was checked for any obvious holes, rips or surface damages. The integrity of the membranes was determined by measurement of the electrical resistance (ER) across the skin. Resistance was measured by passing a fixed current across the skin using an e-Corder 410 and eDAQ Potentiostat recorder (New South Wales, Australia) connected with three electrodes, using a setting of 100 kHz. Silver/silver chloride (Ag/AgCl) electrodes were used with current set to 300 mV. This was undertaken at least 30 minutes after the Franz cell was loaded with phosphate buffered saline (PBS) to ensure temperature and humidity equilibration. One electrode probe, at least 10 mm long, was inserted into the receptor chamber via the sample port, below the saline level. The donor chamber probe was positioned in the saline above the skin, taking care not to touch the skins surface. After several seconds of stabilization, the measurement was recorded. ER was expressed as both kΩ·cm$^{-2}$ and kΩ based on Central Toxicology Laboratory (CTL) standards for static diffusion cells. The ER cut-off values for mouse whole skin must be equal to or above 5 kΩ [607-609].

5.2.7. Skin storage

After excision and removal of fatty extraneous tissue, the skin membranes were either immediately used or stored frozen (-20°C) in aluminum foil until used. The effects of freezing on the permeability of animal skin have been studied [607, 608]. On this basis and other tests performed in the laboratory, skin permeability was not found to be altered. Studies have used varying lengths of storage time, including human skin being used within twelve months of being frozen, and porcine and mouse after 6 weeks of being frozen [607, 610]. As a rule for this research, whole skin membranes were used within four weeks of being frozen at -20°C.
5.2.8. Ex vivo permeation studies

The ex vivo permeation of PGN from each dispersion was determined by using Franz (vertical) diffusion cell; VTC 200 manufactured by Logan Instruments Corporation, (New Jersey, USA). The mouse skin was mounted on the receptor compartment with the SC side facing upwards into the donor compartment. After 5 to 10 minutes the skin membranes were tested for integrity using electrical resistance (see section 5.2.6). A 40% v/v propylene glycol was used as the receptor medium to maintain a sink condition. The top of the diffusion cell was covered with Parafilm® (Chicago, USA) to provide an occlusive effect. The available diffusion area of cell was 1.77 cm². The receptor compartment was maintained at 37 ± 0.5°C and stirred by a magnetic bar at 600 rpm. At periodic intervals, samples (400 µL) were withdrawn and immediately replaced by an equal volume of fresh receptor medium. The samples were then analyzed by HPLC method. All dispersions were tested at least three times, while the controls were tested 6 times for the market cream and 7 times for the water control.

5.2.9. Synthetic release model

PGN from each dispersion was determined by using Franz (vertical) diffusion cell with a synthetic membrane in accordance to the procedure outlined in section 5.2.8. Cellulose acetate membrane is semi-permeable allowing substances with a molecular weight cut off between 10,000 to 12,000 Da. The samples were stored at 4°C in a refrigerator, and analysis of the samples was by HPLC assay. The cumulative amount of drug released was calculated as a function of time.

5.2.10. Permeation theory

Fick's diffusion laws (First Law and Second Law) have been widely used over the last four decades to analyze skin permeation data. It has been 40 years since Scheuplein and Blank explicitly reported that Fick's First Law can adequately describe transdermal diffusion of various drugs [611]. The drug can be in different forms, such as a gas, an ion, or a nonelectrolyte, with the exception of drugs that damage the skin. In the literature, Fick's laws have been used to both estimate the intrinsic transport properties of the skin tissue for a given drug, and predict the skin permeability in vivo based on short-term skin permeation measurements, thus reducing the experimental time associated with the in vivo skin studies [611]. The following equation has been used and is based on a practical solution to Fick's
Second Law, for a single layered skin membrane. It is assumed that (a) the skin acts as an inert homogeneous membrane, (b) the drug traverses the skin via pure diffusion, and (c) no binding or enzymatic activity of the drug occurs inside the skin (viz, the drug molecules that enter the skin remain intact and are freely available for the diffusion process). Sophisticated mathematical modeling of transdermal transport has also been investigated using relaxed versions of the above assumptions. Studies have used, for example, simultaneous diffusion and bio-conversion models to test enzyme effects [611]. In these studies “viable skin is used to predict the plasma concentration of transdermally applied drugs in vivo, as well as to determine the diffusion and metabolism-related parameters of the different skin layers (SC and viable skin tissue)” [611].

The cumulative amount (Qt) of PGN permeated through the skin was calculated using the following equation:

\[ Qt = [VrCt + \sum_{t=0}^{t-1} VnCn] \frac{1}{A} \]

Equation 29

where \( Vr \) is the volume of the receptor chamber (12 mL), \( Ct \) is the drug concentration in the receptor chamber at each time interval, \( Vn \) and \( Cn \) are the volume and concentration for the cumulated number of samples withdrawn and \( A \) is the relative diffusion surface area (1.77 cm²). The amount of PGN permeated over 24 hours was plotted over time (hours). Regression analysis was carried out on linear regions of each plot. The lag time, \( \text{Lag}_t \), was then calculated using the steady state flux (Jss) by measuring the linear portion of the cumulative penetration curve to the time axis where drug release was equal to zero, such that Equation 20 can be deducted:

\[ \text{Lag}_t = \frac{h^2}{6D} \]

Equation 20

where, \( h \) is the skin membrane thickness (μm) and \( D \) is the diffusion coefficient provided that the membrane thickness is available. On the permeation profile the Flux (μg·cm⁻²·h⁻¹) was represented by the y-axis and time \( t \) was plotted on the x-axis. The calculation of the in vitro lag time from the back-extrapolation of the linear portion (steady-state slope) of the plots to its interception with the time axis has been done elsewhere in the literature [170, 612, 613]. The apparent permeability coefficient (\( P_{app} \)) was calculated using Equation 21, as used in the literature:[614, 615]
Chapter 5. Permeation

\[ P_{\text{app}} = \left( \frac{dX_r}{dt} \frac{1}{A} \right) \cdot C_0 \]  \hspace{1cm} \text{Equation 21}

where \( P_{\text{app}} \) is determined with the final units as cm·s\(^{-1}\), \( X_r \) is the amount of PGN in the receptor chamber, \( A \) is the surface area of mouse skin exposed (cm\(^2\)), and \( C_0 \) is the initial PGN concentration at specific time point (µg·mL\(^{-1}\)).

5.2.11. Histology

Histological micrographs of the dermis were made to view for swelling and gross anatomy changes of the dermis that may have occurred from contact with the formulations. Each formulation was applied to excised mouse skin mounted on the diffusion cell at 37°C for 24 hours. After removing the skin it was washed with, and fixed, with 10% v/v buffered (pH 7.4) formalin for 24 hours. This was followed with a further 24 hours of fixing in 70% v/v of ethanol. The skin samples were then prepared in paraffin blocks and vertically cut into 4 – 5 µm thick cross-sections. Each sample was then stained with hematoxylin-eosin (H&E) and observed under light microscope. Untreated skin and skin in contact with water were used as controls. Each formulation was completed in triplicate.

Brightfield microscopic images were made using a Leica DMR upright microscope (Solms, Germany). The images were taken using a Nikon DS-U1 Digital Sight cooled colour camera with EclipseNet software for standard image acquisition (Tokyo, Japan). The shutter speed was set at 1/350 seconds and live mode captures taken with full 640×480 pixel images. Objectives higher than 10× were produced using an oil immersion above the sample skin.

5.2.12. FTIR analysis

Abdominal mouse skin was cut into 4 cm\(^2\) sections with practical amounts of fat removed without damaging the dermis and SC layers. Suitable skin samples had approximately 600 mg of each formulation applied for 24 hours at 37°C. The sections were then rinsed with MilliQ water to remove remaining formulation and gently tapped dry with a paper towel. The infrared spectrum of the resulting samples was measured using FTIR spectroscopy with the SC facing the diamond surface. Every sample was scanned 30 times at a resolution of 4 cm\(^{-1}\) from 3000 to 600 wavenumbers. Untreated skin and skin immersed in MilliQ water worked as the controls. A positive control to confirm a change in CH stretching was conducted using fresh skin immersed in MilliQ water heated at 60 – 63°C for 48 hours.
5.2.13. Stability test

Partial physical stability testing was completed according to ICH guidelines section Q1A (R2) under accelerated storage conditions. The guideline states that the storage conditions in the general case for an accelerated study must be at a temperature of 40 ± 2°C and relative humidity of 75 ± 5% RH over 6 months. This study was conducted for physical stability testing, rather than in situ or in vivo skin tissue, i.e. conditions where PGN has not been exposed to enzyme degradation.

5.2.14. Statistical analysis

Statistical analyse was performed with Minitab® Release 15.0 software (Minitab Inc. State College, PA, USA). The data was tested for significance using analysis of variance (ANOVA) with a 95% confidence interval (p-value < 0.05). Raw data was collected in Excel 2007 (Microsoft® Corporation, CA, USA) on an Intel Core™ 2 Dell computer.

5.3. Results and discussion

5.3.1. Oil solubility

Birefringence and dissolution was used to test the solubility of PGN in several oils including myritol 318, sesame seed oil, evening primrose oil (EPO), and aniseed oil. After several in vitro tests and review of the literature, myritol 318 was selected. Using the preliminary PGN permeability results, see section 5.3.4, Figure 5-4, from each of the selected excipients; Gelucire 44/14, TPGS, and PEG 400/4000, it was found that TPGS had the highest PGN permeability rate and extent over 24 hours. TPGS was therefore selected for progress toward a final dispersion system. Before myritol 318 was added to the formulation process using PGSS, three concentrations of myritol 318 were examined with TPGS using birefringence. Figure 5-2 shows the microscopy images under polarised light. There was an obvious difference in the amount of visible PGN crystals with increasing amounts of myritol 318. The mixtures (without PGSS) show less PGN crystals present with higher amounts of myritol 318. The physical mixture of TPGS with PGN is shown in Figure 5-2(d), with visible uniformity and similar particle size. The PM is without myritol 318 present.
Chapter 5. Permeation

Figure 5.2. Microscopy pictures under polarised light (200 × magnification);
(a) 10% myritol, (b) 20% myritol, (c) 35% myritol, (d) physical mixture of TPGS with 4% PGN, (e) SCF processed TPGS with PGN and 35% myritol 318, and (f) aqueous PGN suspension.

Figure 5-2(e) also shows a marked decrease in the visible PGN crystals, although not completely dissolved into the oil. The TPGS and myritol 318 (35%) w/w were used for the final formulation which would also contain a penetration enhancer and be processed with PGSS. The next section discusses the method for penetration enhancer selection.

5.3.2. Penetration enhancer

Before the permeation study was conducted, a penetration enhancer was added to what would become the final SCF formulation. There were six penetration enhancers selected based on their ability to dissolve in myritol 318 (the oil previously selected to dissolve PGN). The penetration enhancers selected were tween 80, sodium deoxycholate (NaDC), urea, transcutol P, EDTA, and span 20. Appendix X gives the molecular structures for the six penetration enhancers investigated. Figure 5-3 shows the PGN permeation across nude mouse skin after 24 hours for each penetration enhancer at two concentrations. The permeation kinetic parameters of the penetration enhancers are given in Table 5-2. The two highest cumulative amounts of PGN from the urea and transcutol P systems were 413.3 ± 45.9 µg·cm⁻², and 505.5 ± 20.3 µg·cm⁻², respectively. Transcutol P also had the shortest lag time and highest flux, 0.36 ± 0.09 hours and 20.3 ± 1.4 µg·cm⁻²·h⁻¹, respectively. Therefore, transcutol P was selected for incorporation into the final SCF formulation in the presence of myritol 318.
Figure 5-3. Extent of PGN permeation with selected penetration enhancers after 24 hours.

- ■ Represents the control (water) group (147.1 µg·cm⁻²).
  * Statistically significant groups compared to the control (water) group.
  Results are provided as mean ± SD, n = 3.

It was noted that the flux of the Tween 80 at 1% was lower than the water control. This is interesting and in theory the Tween 80 would increase PGN permeation. However, in this case, the flux may have been affected by several variables;

(a) The surface area of the Franz cell unit was too small (1.77 cm²), so the complete effect of PE enhancement was not possible at only 1% concentration of Tween 80;

(b) The receptor fluid can form bubbles (overnight), reducing the effective surface area available for permeation, hence the PGN amount was low in the receptor chamber; and

(c) The PGN and Tween 80 may interact, reducing the freely available amounts of both PGN and Tween 80, as they could have formed a complex. This complex in the donor compartment meant that partitioning into the skin surface was much lower.
Table 5-2. Parameters for the penetration enhancers.

<table>
<thead>
<tr>
<th>Penetration Enhancer</th>
<th>Extent (24 hrs) ±SD</th>
<th>Lag time (hours) ±SD</th>
<th>Flux (µg·cm$^{-2}$·h$^{-1}$) ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (water)</td>
<td>147.1 ±80.1</td>
<td>6.1 ±1.20</td>
<td>7.8 ±0.9</td>
</tr>
<tr>
<td>Tween 80 (1%)</td>
<td>86.6 ±22.4</td>
<td>3.1 ±0.89</td>
<td>4.0 ±0.3</td>
</tr>
<tr>
<td>Tween 80 (2%)</td>
<td>229.3 ±21.7</td>
<td>5.6 ±1.34</td>
<td>11.8 ±0.9 *</td>
</tr>
<tr>
<td>NaDC (0.075%)</td>
<td>185.9 ±23.5</td>
<td>2.8 ±0.97</td>
<td>8.6 ±0.6</td>
</tr>
<tr>
<td>NaDC (0.15%)</td>
<td>129.5 ±5.6</td>
<td>3.9 ±1.10</td>
<td>6.3 ±0.7</td>
</tr>
<tr>
<td>Urea (0.5%)</td>
<td>192.4 ±29.3</td>
<td>1.9 ±0.23</td>
<td>8.5 ±0.6</td>
</tr>
<tr>
<td>Urea (1%)</td>
<td>413.3 ±45.9</td>
<td>2.4 ±0.95</td>
<td>18.4 ±1.1 *</td>
</tr>
<tr>
<td>Transcutol P (5%)</td>
<td>140.9 ±15.3</td>
<td>5.5 ±1.29</td>
<td>7.4 ±0.7</td>
</tr>
<tr>
<td>Transcutol P (10%)</td>
<td>505.5 ±20.3</td>
<td>0.36 ±0.09</td>
<td>20.3 ±1.4 *</td>
</tr>
<tr>
<td>EDTA (0.25%)</td>
<td>250.1 ±19.2</td>
<td>0.81 ±0.05</td>
<td>13.1 ±0.9 *</td>
</tr>
<tr>
<td>EDTA (0.5%)</td>
<td>196.7 ±11.2</td>
<td>4.9 ±0.94</td>
<td>10.2 ±0.8 *</td>
</tr>
<tr>
<td>Span 20 (1%)</td>
<td>204.7 ±9.5</td>
<td>1.2 ±0.52</td>
<td>13.8 ±1.1 *</td>
</tr>
<tr>
<td>Span 20 (4%)</td>
<td>334.1 ±23.4</td>
<td>1.8 ±0.21</td>
<td>9.1 ±0.8</td>
</tr>
</tbody>
</table>

Results are provided as mean ± SD, n = 3
* Statistically significant groups compared to the control (water). Highlighted row is the selected penetration enhancer for the PGN formulation. Highlighted row is the selected penetration enhancer and concentration.

5.3.3. Electrical resistance test

Electrical resistance (ER) measurements of each skin preparation taken over 5 to 10 minutes were very similar. A comparison over longer time periods was not conducted, although some studies have shown differences between 30 minutes and 6 hours has little or no difference in ER measurements [607]. Hence, short time data was only used for this investigation. As ER across the membrane is dependent on the area of the membrane used, the data obtained was presented both as kΩ·cm$^{-2}$ and kΩ. Table 5-3 provides the integrity results for the nude mouse skin using ER. The investigations were based on acceptance and rejection criteria previously published [607, 609]. The pass rate was 92.9% which was higher than some reported tests in the literature. For example, Davies, et al. (2004) showed that for whole mouse skin a pass rate of 77% was achieved [607, 609]. The ER cut off values for mouse whole skin was equal to or above 5 kΩ, rendering the skin acceptable for the ex vivo permeability studies [607].
Table 5.3. Electrical resistance for mouse skin membranes.

<table>
<thead>
<tr>
<th>Specie</th>
<th>Skin type</th>
<th>ER*</th>
<th>kΩ·cm(^{-2}) ± SD</th>
<th>kΩ ± SD**</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>Whole</td>
<td>3.98±0.56</td>
<td>6.8±0.95</td>
<td>28</td>
<td></td>
</tr>
</tbody>
</table>

* The ER cut-off values for mouse are equal to or above 5 kΩ.
** Standard diffusion cells and skin area = 1.77 cm\(^2\).
Results are provided as mean ± SD, n = 28.

Measuring ER was a relatively time efficient method compared to using dyes or water leaking methods, where the skin samples are required to soak for at least 60 minutes. Also dyes and water may influence the surface of the skin, through SC disruption and swelling. In this investigation it has been shown that ER is equally suitable and has the advantage of requiring less time.

5.3.4. Skin permeation profile

Figure 5-4 compares the permeation profiles of the SCF formulations prepared using PGSS. Each of these formulations contain 4% w/w of PGN and mixed separately with either Gelucire 44/14, TPGS or PEG 400/4000 (50:50), which was processed accordingly under SCF conditions. None of the SCF formulations, excluding the formulations with myritol and transcutol P, significantly increased the extent of PGN permeation when compared to the controls of saturated water and market cream (p-value > 0.05). Interestingly, the flux from the SCF formulations with TPGS (7.5 ± 1.3 µg·cm\(^{-2}\)·hr\(^{-1}\)) and Gelucire 44/14 (6.8 ± 1.6 µg·cm\(^{-2}\)·hr\(^{-1}\)) was higher than that of the PEG-based formulation (2.8 ± 0.5 µg·cm\(^{-2}\)·hr\(^{-1}\)), and this was significantly different (p-value < 0.05). The extent of PGN permeation over 24 hours, however, was not statistically significant between the formulations (p-value > 0.05). The highest flux of the SCF based formulations was the TPGS containing dispersion of which the mean was 7.5 µg·cm\(^{-2}\)·h\(^{-1}\).
Based on the permeation results, the TPGS dispersion prepared from SCF processing gave the highest extent of permeation over 24 hours and strongest flux, averaging 164.2 (µg·cm⁻²), and 7.5 µg·cm⁻²·hr⁻¹, respectively. Previously Gelucire 44/14 was used in the optimisation of the SCF process as a model excipient, although the permeation of Gelcure 44/14 and TPGS is statistically insignificant (p-value > 0.05), the TPGS-based formulation has a higher permeation extent after 24 hours. Therefore, TPGS was selected to undergo further formulation incorporating transcutol P and myritol 318.

This led to the final SCF formulation containing 4% w/w PGN, 10% w/w transcutol P, 35% w/w myritol 318, and 51% w/w TPGS. Figure 5-5 shows the flux of PGN permeated from the final SCF prepared dispersion, and controls using the ex vivo Franz cell apparatus.
Chapter 5. Permeation

The control group formulations were: (1) a market cream, containing PGN dispersed in non-ionic cream, and (2) an aqueous suspension of PGN.

The extent and flux of PGN permeated from the final TPGS/myritol/transcutol P and SCF prepared dispersion system was significantly higher than that of the controls, shown in Figure 5-5 (p-value < 0.05). However, no significant difference in flux was observed in *ex vivo* skin permeation between the final SCF-TPGS formulation and SCF-TPGS and CM-Gelucire 44/14 based formulations (p-value > 0.05). The flux from the final SCF with myritol and transcutol P is 150.7% more when compared to that of the PGN suspension (water control) (p-value < 0.05).

![Figure 5-5](image)

Figure 5-5. Skin PGN permeation profile for the final SCF and control formulations (water and market cream).

Figure 5-6 shows the permeation profiles for cosolvent (CS) formulations. There was no difference between the formulations when comparing flux and extent of PGN permeation over 24 hours. With respect to lag time, there was a significant different between the market cream and all three CS formulations, although there was no difference of statistical
significance between the saturated water control and the Gelucire 44/14 and PEG based dispersions. Only the lag time from the TPGS formulation prepared using a cosolvent was statistically significant to both the controls (p-value < 0.05), the TPGS formulation had a lag time of 2.5 hours compared to the saturated water of 6.1 hours. Also of note was the relatively unusual observation seen in the first 2 hours, where there was an immediate release of PGN from both the TPGS and Gelucire 44/14 based formulations. In general, this was observed with the other formulations, however it was most obvious with the CS prepared formulations. The highest transdermal flux of the CS formulations was the Gelucire 44/14 containing dispersion of which the mean was 4.5 µg·cm⁻²·h⁻¹.

![Graph showing skin PGN permeation profile for conventional cosolvent (CS) formulations.](image)

Figure 5-6. Skin PGN permeation profile for the conventional cosolvent (CS) formulations.

Figure 5-7 compares the permeation profiles of the various comelted formulations. Once again there was little observable difference in the profiles, and neither the flux, nor permeation over 24 hours was statistically significant. The lag times of the CM formulations
Chapter 5. Permeation

however, were all significantly faster than the saturated water. The shortest lag time of 1.8 hours was observed for the TPGS formulation with a flux of 4.3 µg·cm⁻²·h⁻¹.

Figure 5-7. Skin PGN permeation profile for the conventional comelted (CM) formulations.

Figure 5-8 gives the permeation profile of the formulations prepared with the physical mixing method. When comparing the extent of permeation over 24 hours, there was no significant difference between the Gelucire 44/14 and PEG-based formulations, and saturated water (control) (p-value < 0.05). The PEG-based formation in the case of PM preparations, was the leading formulation with highest extent of PGN permeation at 157.2 µg·cm⁻², and highest flux of 4.3 µg·cm⁻²·h⁻¹.
Figure 5-8. Skin PGN permeation profile for the physically mixed (PM) formulations.

Overall, the permeation profile of the final SCF formulation (Figure 5-5) delivered more PGN over 24 hours compared to the controls and conventional formulations. There was a 2-fold increase in PGN delivered across the skin from the final SCF-TPGS dispersion than the control (market cream) over 24 hours, 245.7 and 126 µg·cm$^{-2}$, respectively. In comparison with the final SCF formulation and all formulations, except CM-Gelucire 44/14 and SCF-TPGS formulations, there was a significant difference using a one-way ANOVA statistical analysis (p-value < 0.05, F = 2.73). Table 5-6 and Table 5-7 give the complete statistical results for both cumulative permeation over 24 hours and transdermal flux.

The SCF-TPGS and CM-Gelucire 44/14 dispersions also showed increases over the water and market controls but this was not significant (p-value > 0.05). Interestingly, there was also no significant difference between these two formulations and the final SCF formulation. No other formulations over 24 hours showed any significant differences when
compared to the control. There was also no difference of significance between the conventional dispersions and the SCF dispersions (p-value > 0.05).

Table 5-4 represents the pharmacokinetic parameters of each formulation as determined through the investigations prepared using a SCF method, controls and conventional methods. Based on the findings, the SCF prepared PGN dispersions with TPGS/myritol 318/transcutol P (the final SCF formulation) delivers the largest rate of PGN across skin over 24 hours in comparison with the control (water) formulation. This rate is 150.7% more when compared with the control over 24 hours. This improvement in the delivery rate of PGN was statistically significant (p-value < 0.05), whereas none of the other formulations had an advantage in delivering PGN across the skin over 24 hours in comparison with the control.

Table 5-4. Pharmacokinetic parameters for the controls and PGN dispersion systems.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Excipient</th>
<th>Cumulative amount within 24 hours (µg·cm⁻²) ± SD</th>
<th>Lag time (hours) ± SD</th>
<th>Flux (µg·cm⁻²·h⁻¹) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>Water 12 hours #</td>
<td>n/a</td>
<td>n/a</td>
<td>3.1 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Water 24 hours #</td>
<td>147.2 ± 40.1</td>
<td>6.1 ± 1.2</td>
<td>7.7 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>Cream</td>
<td>126.1 ± 24.1</td>
<td>0.8 ± 0.3</td>
<td>5.4 ± 0.8</td>
</tr>
<tr>
<td>ComeltS</td>
<td>Gelucire 44/14</td>
<td>133.3 ± 18.7</td>
<td>3.1 ± 0.5</td>
<td>6.4 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>TPGS</td>
<td>108.3 ± 10.0</td>
<td>1.8 ± 0.6</td>
<td>4.3 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>PEG</td>
<td>130.8 ± 23.1</td>
<td>4.6 ± 0.7</td>
<td>5.7 ± 1.3</td>
</tr>
<tr>
<td>CosolventS</td>
<td>Gelucire 44/14</td>
<td>82.9 ± 15.6</td>
<td>5.5 ± 0.9</td>
<td>4.5 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>TPGS</td>
<td>90.8 ± 24.2</td>
<td>2.5 ± 0.8</td>
<td>4.2 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>PEG</td>
<td>88.1 ± 11.6</td>
<td>5.9 ± 1.1</td>
<td>4.8 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>Gelucire 44/14</td>
<td>138.8 ± 19.5</td>
<td>5.1 ± 0.9</td>
<td>7.2 ± 1.9</td>
</tr>
<tr>
<td>Physical Mixing</td>
<td>TPGS</td>
<td>86.8 ± 17.8</td>
<td>1.2 ± 0.3</td>
<td>3.7 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>PEG</td>
<td>157.2 ± 23.1*</td>
<td>5.4 ± 0.7</td>
<td>8.1 ± 1.1</td>
</tr>
<tr>
<td>SCF</td>
<td>Gelucire 44/14</td>
<td>125.3 ± 12.4</td>
<td>5.1 ± 1.2</td>
<td>6.8 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>TPGS</td>
<td>164.2 ± 46.2</td>
<td>4.5 ± 1.6</td>
<td>7.5 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>PEG</td>
<td>67.2 ± 22.3</td>
<td>1.8 ± 0.9</td>
<td>2.8 ± 0.5</td>
</tr>
<tr>
<td>Final SCF</td>
<td>TPGS</td>
<td>246.2 ± 58.9*</td>
<td>1.1 ± 0.3</td>
<td>10.9 ± 2.7**</td>
</tr>
</tbody>
</table>

# Representing the two linear phases (dual flux) observed for the control-water.
* Representing significantly higher extent of PGN permeated after 24 hours compared to the market cream (p-value < 0.05).
** Representing significantly higher flux to market cream (p-value < 0.05).
Results are provided as mean ± SD, n ≥ 3.

Table 5-5 gives the permeability parameters for the formulations. The measured skin permeability of PGN from water (4% PGN) and market cream across the skin were ~1.04 ± 0.15 × 10⁻⁷ cm·s⁻¹ and ~0.71 ± 0.30 × 10⁻⁷ cm·s⁻¹, respectively. The control (water) has been
stated with two values due to the dual phase permeation of PGN across the skin. Each of the two linear sections of its profile has been determined and the flux calculated for both. This was thought to be due to the long lag time observed (6.1 hours). It is possible the first 8 to 12 hours gave a slower permeation as the skin remained intact, while over the 12 to 24 hours there was a faster permeation due to the absorption of water which was swelling the epidermis, increasing transdermal flux. There is substantial evidence the water is able to increase the fluidity of the bilayer region. In one study using endotherms to measure lipid melting it was found that water lowered the lipid endotherms [616]. “As lipids provide a significant part of the skin’s barrier function, any reduction in the relevant intermolecular forces will allow drug to migrate more easily” [616]. Skin hydration is able to increase the polar and non-polar permeant fluxes due to the reduced density of the lipophilic region in the bilayer; thus molecules will be more mobile and free to move [616]. There are several other studies also concluding water is associated with diminishing the skin’s lipid arrangement [613, 617, 618]. Furthermore, for water, which is the most rapidly permeating species studied, and is in high concentration in stratum corneum (approximately 40%), the apparent diffusivity, according to Scheuplein, et al. (1969) was about $6 \times 10^{-10} \text{ cm}^2\cdot\text{s}^{-1}$ [613]. Therefore, water is also likely to precede any drug molecule in its absorption into the skin, affecting the passage of the drug molecule across the skin. This helps to explain why PGN was able to have improved permeation over 24 hours from an aqueous suspension.

The experimental skin permeability values reported in this research were compared with the literature values. In one study, using aqueous ethanol formulations and hairless mouse tissue, PGN had a permeability of $2.1 \times 10^{-5} \text{ cm}\cdot\text{s}^{-1}$ [602]. Research using saturated solutions, was able to determine the permeability of PGN as $4.1 \times 10^{-7} \text{ cm}\cdot\text{s}^{-1}$. As reported by Johnson, et al. (1995), numerous groups have examined the skin permeabilities and many of them contain too many experimental differences leading sometimes to data differences by as much as 10 orders of magnitude [619]. For example, the use of ethanol could both improve drug solubility and act as a penetration enhancer. In addition, the use of hairless skin could have involved the shaving of hair prior to the permeation study, thus disrupting the SC layer, leading to higher permeation results. In this study there was no organic solvent used, and the mouse skin used was naturally hairless (nude). Regarding the CS, CM, PM, and SCF dispersion systems and selected excipients investigated in this thesis, there was no literature found to compare against the measured permeability of PGN. The final SCF-TPGS/myritol/transcutol P dispersion system of PGN gave the highest permeability value of $1.47 \pm 0.28 \times 10^{-7} \text{ cm}\cdot\text{s}^{-1}$, reported in Table 5-5.
All of the formulations showed a shorter lag time than the water control (6.1 hours). In fact all the formulations, except the CS-PEG dispersion, showed some level of immediate permeation over the first 2 hours. The ability of the formulations to show improved PGN delivery over the initial 2 hours is possibly due to the semi-solid nature of the formulations. Dissolved PGN near the skin’s surface is readily released and freely available to permeate the skin, and Gelucire 44/14 and TPGS are able to form micelles, which can improve the delivery of PGN across the skin. The near immediate release and permeation of PGN from Gelucire 44/14 and TPGS is also consistent with the aqueous dissolution results, where both Gelucire 44/14 and TPGS showed rapid dissolution. From the literature such immediate release profiles are not unusual [620, 621]. Both Lim, et al. (2008) and Chai, et al. (2010) produced profiles with immediate results, sometimes also with higher rates over the first 2 hours compared to remaining time over 24 hours [610, 620, 621]. It is possible that the PGN dispersed in the formulation nearer the skin surface crossed the skin promptly, while the remaining PGN was unable to diffuse through the formulation quickly, hence was unable to cross the skin freely. Results from the porcine skin studies are shown in Appendix XI(a),

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Type</th>
<th>$P_{\text{app}}$ (×10^{-7} \text{cm}\cdot\text{s}^{-1}) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>Water</td>
<td>1.04 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>Cream</td>
<td>0.71 ± 0.30</td>
</tr>
<tr>
<td>Comelts</td>
<td>Gelucire 44/14</td>
<td>0.83 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>TPGS</td>
<td>0.56 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>PEG</td>
<td>0.74 ± 0.16</td>
</tr>
<tr>
<td>Cosolvents</td>
<td>Gelucire 44/14</td>
<td>0.59 ± 0.34</td>
</tr>
<tr>
<td></td>
<td>TPGS</td>
<td>0.54 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>PEG</td>
<td>0.63 ± 0.19</td>
</tr>
<tr>
<td>Physical</td>
<td>Gelucire 44/14</td>
<td>0.94 ± 0.29</td>
</tr>
<tr>
<td>Mixing</td>
<td>TPGS</td>
<td>0.48 ± 0.23</td>
</tr>
<tr>
<td></td>
<td>PEG</td>
<td>0.11 ± 0.25</td>
</tr>
<tr>
<td>SCF</td>
<td>Gelucire 44/14</td>
<td>0.89 ± 0.32</td>
</tr>
<tr>
<td></td>
<td>TPGS</td>
<td>0.98 ± 0.24</td>
</tr>
<tr>
<td></td>
<td>PEG</td>
<td>0.37 ± 0.31</td>
</tr>
<tr>
<td>Final SCF</td>
<td>TPGS</td>
<td>1.47 ± 0.28</td>
</tr>
</tbody>
</table>

* Representing significantly increased flux compared to the controls (water and cream) (p-value < 0.05).

Results are provided as mean ± SD, n ≥ 3.
Chapter 5. Permeation

showing mixed profiles (similar and different to the mouse studies) and the market cream (control) outperforming all the other formulations, although due to the pre-treatment procedures involving steam at the meat processors it was difficult to make conclusive statements about PGN permeability.
Table 5-6. Statistical comparison of cumulative extent of PGN permeated over 24 hours using one–way ANOVA.

<table>
<thead>
<tr>
<th>Controls:</th>
<th>Water</th>
<th>Cream</th>
<th>Final SCF</th>
<th>SCF-G</th>
<th>SCF-T</th>
<th>SCF-P</th>
<th>CS-G</th>
<th>CS-T</th>
<th>CS-P</th>
<th>CM-G</th>
<th>CM-T</th>
<th>CM-P</th>
<th>PM-G</th>
<th>PM-T</th>
<th>PM-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cream</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Final SCF</td>
<td>S</td>
<td>S</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SCF-G</td>
<td>ns</td>
<td>ns</td>
<td>S</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SCF-T</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SCF-P</td>
<td>ns</td>
<td>ns</td>
<td>S</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CS-G</td>
<td>ns</td>
<td>ns</td>
<td>S</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CS-T</td>
<td>ns</td>
<td>ns</td>
<td>S</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CS-P</td>
<td>ns</td>
<td>ns</td>
<td>S</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CM-G</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CM-T</td>
<td>ns</td>
<td>ns</td>
<td>S</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CM-P</td>
<td>ns</td>
<td>ns</td>
<td>S</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PM-G</td>
<td>ns</td>
<td>ns</td>
<td>S</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PM-T</td>
<td>ns</td>
<td>ns</td>
<td>S</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PM-P</td>
<td>ns</td>
<td>ns</td>
<td>S</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Abbreviations: S = Statistically significant, ns = statistically not significant, p-value < 0.05, F = 2.73; G = Gelucire 44/14, T = TPGS (d-α-tocopheryl PEG 1000 succinate), P= PEG (polyethylene glycol 4000 and 400 in 50:50 mix), SCF = supercritical fluid, CM = co-melted, CS = co-solvent and PM = physically mixed.
Table 5-7. Statistical comparison of PGN flux data using one–way ANOVA.

<table>
<thead>
<tr>
<th>Controls:</th>
<th>Water</th>
<th>Cream</th>
<th>Final SCF</th>
<th>SCF-</th>
<th>SCF-</th>
<th>SCF-</th>
<th>CS-</th>
<th>CS-</th>
<th>CS-</th>
<th>CM-</th>
<th>CM-</th>
<th>CM-</th>
<th>PM-</th>
<th>PM-</th>
<th>PM-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>G</td>
<td>T</td>
<td>P</td>
<td>G</td>
<td>T</td>
<td>P</td>
<td>G</td>
<td>T</td>
<td>P</td>
<td>G</td>
<td>T</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>Water*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cream</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Final SCF</td>
<td>S</td>
<td>S</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SCF-G</td>
<td>ns</td>
<td>ns</td>
<td>S</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SCF-T</td>
<td>ns</td>
<td>ns</td>
<td>S</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SCF-P</td>
<td>ns</td>
<td>ns</td>
<td>S</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CS-G</td>
<td>ns</td>
<td>ns</td>
<td>S</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CS-T</td>
<td>ns</td>
<td>ns</td>
<td>S</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CS-P</td>
<td>ns</td>
<td>ns</td>
<td>S</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CM-G</td>
<td>ns</td>
<td>ns</td>
<td>S</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>S</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CM-T</td>
<td>ns</td>
<td>ns</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CM-P</td>
<td>ns</td>
<td>ns</td>
<td>S</td>
<td>ns</td>
<td>ns</td>
<td>S</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PM-G</td>
<td>ns</td>
<td>ns</td>
<td>S</td>
<td>ns</td>
<td>ns</td>
<td>S</td>
<td>ns</td>
<td>ns</td>
<td>S</td>
<td>ns</td>
<td>S</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PM-T</td>
<td>ns</td>
<td>ns</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>S</td>
<td>ns</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PM-P</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>S</td>
<td>ns</td>
<td>-</td>
</tr>
</tbody>
</table>

Abbreviations: S = Statistically significant, ns = statistically not significant, SCF = supercritical fluid, CS = cosolvents, CM = comelt, PM = physical mixing, p-value < 0.05, F = 17.41, G = Gelucire 44/14, T = TPGS (d-α-tocopheryl PEG 1000 succinate), P= PEG (polyethylene glycol 4000 and 400 in 50:50 mix).

*based on 24 hour data
5.3.5. Release profile

The *in vitro* release of PGN was studied at the same skin permeation experimental conditions, replacing the full thickness mouse skin with artificial semi-permeable membrane with the goal of correlating the release data with the skin permeation data. The *in vitro* release profiles of PGN obtained from different formulations are shown in Figure 5-9 revealing linear release profiles and no lag time in all tested formulations. The release shows no significant difference between the formulations tested (p-value > 0.05).

![Graph showing release profile of PGN](image)

**Figure 5-9.** Release profile of PGN from water, SCF-TPGS and market cream.

The calculated release rates are presented in Table 5-8. Similar release profiles were previously recorded for a lipophilic drug from microemulsions [588]. The rate of drug release may have depended on the viscosity of formulations with the release rate decreasing with increasing the viscosity. Thus the non-ionic cream formulation produced the lowest release
rate. However, the saturated drug solution in water and the final SCF formulation did not produce significantly higher release rates (p-value > 0.05).

Table 5-8. Release kinetic data for controls and final SCF-TPGS formulations.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Cumulative amount within 24 hours (µg) ± SD</th>
<th>Lag time (hours) ± SD</th>
<th>Release rate (µg·cm⁻²·h⁻¹) ± SD</th>
<th>P_app (×10⁻⁷ cm·s⁻¹) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>Water</td>
<td>242.2±39.7</td>
<td>0.25±0.03</td>
<td>10.1±1.9</td>
</tr>
<tr>
<td></td>
<td>NIC</td>
<td>164.9±25.5</td>
<td>0.06±0.01</td>
<td>6.94±1.5</td>
</tr>
<tr>
<td>Final SCF</td>
<td>TPGS</td>
<td>197.2±20.0</td>
<td>0.27±0.03</td>
<td>8.21±1.7</td>
</tr>
</tbody>
</table>

Results are provided as mean ± SD, n = 3.

There was very short lag times for all the formulations tested. This was expected, as PGN freely passes across synthetic membrane. The permeation rates from skin and release rates from synthetic membrane were not the same. A comparison of the different rates (µg·cm⁻²·h⁻¹) are shown in Figure 5-10. The release rates were higher for the final SCF formulation from mouse skin, compared to the synthetic membrane, 10.9 ± 2.7 µg·cm⁻²·h⁻¹, and 8.2 ± 1.7 µg·cm⁻²·h⁻¹, respectively. The skin rate for the final SCF formulation is almost 25% higher than the rate from the synthetic membrane. This is similar to a study that found hydrocortisone (a lipophilic drug) permeation was 10 times more through mouse skin than a synthetic membrane [622]. This may indicate that the final SCF formulations are rate limiting, in other words, the release of PGN from the final SCF formulation was limiting the permeation rate through the mouse skin. This was in contrast to the control formulations, where both formulations had higher release rates than the skin permeation rates. The control (water) formulation release rate was 10.1 ± 1.9 µg·cm⁻²·h⁻¹, while flux for the two linear segments (first and last 12 hours) of the profile were 3.1 ± 0.4 and 7.7 ± 0.9 µg·cm⁻²·h⁻¹, respectively. This indicates that the water formulation was rate not limiting, especially over the first 12 hours, which is consistent with the argument that the skin was less permeable over the first 12 hours, when the skin had not yet absorbed water, which leads to SC swelling, and SC lipid bilayer disruptions, thus leading to higher permeation. As with the water control, the cream formulation result suggests that permeability was affected by the skin membrane and not the formulations that controlled drug release.
Figure 5-10. Comparison of transdermal flux and formulation release rate within 24 hours for the market cream, water control and final SCF dispersion.
* Represents significant difference compared to that of the market cream control (p-value < 0.05)

5.3.6. Histology observations

Microscopic examination of nude mouse skin was able to evaluate the effect of various formulations on the skin integrity. It is important to note that although the skin was from nude mouse, the skin has hair and hair follicles throughout. Untreated skin, water, and market cream were used as the control groups. Skin sections taken from untreated mouse skin are shown in Figure 5-11, where relatively well defined dermal layers can been seen. Skin sections immersed in water and applied with a market cream are shown in Figure 5-12. The dermis contains cells held between the collagen fibers and may be with or without any skin appendages and hair follicles. The water control shows collagen disruption due to dermal swelling, see Figure 5-12 (a) and (b). General epidermal degradation is also visible. Dermal oedema and epidermal impairment is also viewed in the skin samples applied with the final SCF-TPGS dispersion. Figure 5-13 shows the skin images treated with the final SCF formulation from three magnification settings (100, 250 and 400x magnifications). The conventional dispersions were also tested for their ability to disrupt the skin layers, see Figure 5-14. No major visible difference was seen between the water control and other formulations, both SCF and conventional. All formulations showed some degree of SC fragmentation, and dermal and epidermal oedema, causing swelling. The observed skin swelling could offer a possible mechanism for the permeation of PGN through the skin. Appendix XI(b) shows
Chapter 5. Permeation

selected histology results from a study conducted during this research with porcine ears. These results from the porcine skin match well with the mouse histology results.

![Figure 5-11](image)

Figure 5-11. Transverse sections taken from a light microscope of untreated hairless mouse skin:
(a): 100× magnification showing hair follicles (HF), hair follicles with associated sebaceous glands (HFS), adipose tissue (Ad), and muscle tissue (MS); (b) 250× magnification showing the subcutaneous (Sb) region, and; (c) 400× magnification, showing stratum corneum (SC) epidermis (Ep), dermis (Dr), and showing SC fragmentation (Fr), and dermal oedema (Oe). The sections were stained with haematoxylin and eosin.
Figure 5-12. Transverse sections taken from a light microscope of water treated (a) and (b), and market cream (c) and (d) treated hairless mouse skin:

(a): 100× magnification showing dermis (Dr) and subcutaneous adipose tissue (Sb), and; (b) 400× magnification showing intercellular oedema (Oe), and epidermal fragmentation (Fr), and; (c): 100× magnification showing full cross-sectional thickness skin, hair follicle (HF), muscle tissue (MS), and SC fragmentation (Fr), and (d): 400× magnification showing adipose tissue (Ad), stratum corneum (SC), SC fragmentation (Fr), and dermal oedema (Oe). The sections were stained with haematoxylin and eosin.
Figure 5-13. Transverse sections taken from a light microscope of nude mouse skin treated with the final SCF – TPGS dispersion:
(a) 100× magnification showing epidermis dermis (Dr) and surrounding hair follicles (HF); (b) 250× magnification showing the stratum corneum (SC), oedema (Oe), and epidermal fragmentation (Fr) occurring in the SC, and artery (Ay), and; (c) 400× magnification showing intercellular oedema (Oe), and nerve bundle (Nb). The sections were stained with haematoxylin and eosin.
5.3.7. FTIR spectroscopy

A representative FTIR spectrum is shown in Figure 5-15 for untreated mouse skin. The profile is characteristic of hydrated skin tissue with three main regions. Peaks at approximately 1540 and 1640 cm\(^{-1}\) indicate amide II and amide I stretching, respectively. Figure 5-16 shows an expanded view of the amide shifting regions form representative FTIR
spectra. The amide I and II bands are complex materials with contributions from a range of molecular vibrations from peptide groups and proteins in the epidermis and SC layers [304]. Peaks at approximately 2850 and 2920 cm\(^{-1}\) correspond to symmetric and asymmetric CH stretching, respectively. Figure 5-17 shows an expanded view of the CH stretching regions from representative FTIR spectra. The wide peak region between approximately 3100 and 3700 cm\(^{-1}\) is a relative indicator of water content.

![FTIR spectrum of untreated mouse skin](image)

Figure 5-15. FTIR spectrum of untreated mouse skin; (a) amide II stretching \(\approx 1540\) cm\(^{-1}\), (b) amide I stretching \(\approx 1640\) cm\(^{-1}\), (c) symmetric CH stretching \(\approx 2850\) cm\(^{-1}\), (d) asymmetric CH stretching \(\approx 2920\) cm\(^{-1}\), and (e) relative water content curve \(\approx 3100 – 3700\) cm\(^{-1}\).

The amide and CH stretching peaks before and after application of controls, final SCF and conventional formulations are shown in Table 5-9 and 5-10, respectively. The CH stretching regions of interest were reproducible for the controls that were untreated and treated with water. There was no statistically significant difference (p-value > 0.05) between these controls for either CH stretching regions. Significant red shifts (p-value < 0.05) were observed in the CH stretching regions between the water control formulation and all other formulations. There is various research showing amide and CH stretching changes that are
Chapter 5. Permeation

associated with structural changes in the lipid bilayer [623-625]. A change of just 1.0 cm\(^{-1}\) is said to be large enough to suggest significant intercellular lipid disruption has occurred [623]. In another study, there were changes observed from the controls to the test formulations of between 2.0 and 25 cm\(^{-1}\) [304].

In Figure 5-16 the amide stretching regions generally showed a higher degree of deviations compared with the CH stretching areas, see Figure 5-17, but still relatively reproducible. There were significant red shifts (p-value < 0.05) in the amide II stretching region. This was seen with the market cream and final SCF – TPGS formulation, where amide II stretching shifted towards a higher wavenumber, indicating a possible change in the subcutaneous (SC) lipids. The FTIR analysis suggests that the conventional formulations also act as penetration enhancers. All formulations tested were able to partially change the packing arrangement of the SC lipids at a molecular level.

![Figure 5-16. Expanded FTIR spectrum of untreated mouse skin showing amide shifts.](image-url)

```latex
\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5-16}
\caption{Expanded FTIR spectrum of untreated mouse skin showing amide shifts.}
\end{figure}
```
Table 5-9. FTIR summary of amide stretching regions for specific peaks for various formulations and controls.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Water content AUC</th>
<th>Amide II stretching (cm(^{-1}))</th>
<th>Amide I stretching (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>untreated skin</td>
<td>101.3 ± 40.5</td>
<td>1551.0 ± 2.4</td>
<td>1632.1 ± 1.2</td>
</tr>
<tr>
<td>positive</td>
<td>78.4 ± 7.60</td>
<td>1552.1 ± 0.8</td>
<td>1634.3 ± 1.4</td>
</tr>
<tr>
<td>water</td>
<td>269.9 ± 5.70</td>
<td>1541.6 ± 1.9</td>
<td>1645.3 ± 1.5</td>
</tr>
<tr>
<td>cream</td>
<td>193.5 ± 24.1</td>
<td>1542.1 ± 0.9</td>
<td>1642.4 ± 0.8</td>
</tr>
<tr>
<td>CS</td>
<td>243.4 ± 30.9</td>
<td>1543.9 ± 1.2</td>
<td>1643.2 ± 0.9</td>
</tr>
<tr>
<td>CM</td>
<td>261.2 ± 37.9</td>
<td>1542.0 ± 0.6</td>
<td>1643.0 ± 0.9</td>
</tr>
<tr>
<td>PM</td>
<td>241.7 ± 32.5</td>
<td>1542.4 ± 0.8</td>
<td>1643.1 ± 1.1</td>
</tr>
<tr>
<td>Final SCF - TPGS</td>
<td>206.2 ± 29.5</td>
<td>1547.0 ± 1.3</td>
<td>1642.5 ± 0.7</td>
</tr>
</tbody>
</table>

Formulation key: SCF = supercritical fluid, CS = cosolvent, CM = comelt, and PM = physical mix.

Figure 5-17. Expanded FTIR spectra of mouse skin showing the CH stretching regions.
Chapter 5. Permeation

Profiles of the asymmetrical and symmetrical CH stretching peaks for the hydrocarbon chains of lipids in the skin treated with different formulations were compared. In a comparison of the skin treated with the water, the peak shapes of the formulations generally became wider and broadened, and less intensive. This was similar to a study using ethanol and TPGS systems, there was also splitting on the top portion of the peak for the skin treated with increasing concentrations of TPGS in the cosolvent system [304]. It was suggested that this could be due to the presence of TPGS having a physical interactions with lipids of the SC [304].

Table 5-10. FTIR summary of CH stretching regions for specific peaks for various formulations and controls.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Symmetric CH stretching (cm(^{-1}))</th>
<th>Asymmetric CH stretching (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>untreated skin</td>
<td>2854.2 ± 0.9</td>
<td>2927.9 ± 2.2</td>
</tr>
<tr>
<td>positive</td>
<td>2925.2 ± 4.8</td>
<td>2923.4 ± 5.7</td>
</tr>
<tr>
<td>water</td>
<td>2850.3 ± 0.5</td>
<td>2918.3 ± 0.9</td>
</tr>
<tr>
<td>cream</td>
<td>2851.9 ± 0.7</td>
<td>2921.5 ± 1.4</td>
</tr>
<tr>
<td>CS</td>
<td>2851.9 ± 1.1</td>
<td>2921.2 ± 0.4</td>
</tr>
<tr>
<td>CM</td>
<td>2852.3 ± 0.7</td>
<td>2922.0 ± 1.3</td>
</tr>
<tr>
<td>PM</td>
<td>2852.4 ± 0.5</td>
<td>2922.5 ± 1.8</td>
</tr>
<tr>
<td>Final SCF - TPGS</td>
<td>2853.8 ± 1.2</td>
<td>2920.7 ± 0.9</td>
</tr>
</tbody>
</table>

Formulation key: SCF = supercritical fluid, CS = cosolvent, CM = comelt, and PM = physical mix

5.3.8. Stability test

Partial physical stability of PGN was tested according to the ICH guidelines once every four weeks over 6 months at 40°C at 75% relative humidity. Figure 5-18 gives the calculated concentration changes observed during this study period. Time zero was taken as the initial PGN concentration, and subsequent PGN content was compared with this time point.
Figure 5-18. PGN stability data over 6 months for the final SCF-TPGS formulation.

The physical stability of the final drug-loaded SCF-TPGS formulation was analysed by HPLC. The findings show that the mean content of PGN remained at 93% during the observation period. It has been suggested that variations in drug content were probably due to problems in analysis rather than to instability of the steroid hormone [604, 626]. No degradation products were detected by HPLC; similar to other literature results [627]. The incorporation of the penetration enhancer (transcutol P) or the oil (myritol 318) did not seem to influence the recovery of PGN.

Although, PGN is not susceptible to water degradation, the selected excipients may draw in water at higher RH. The myritol 318 and Vitamin E are not in themselves hygroscopic, however the presence of the PEG in TPGS may facilitate an interaction with water [628]. Upon exposure to 75% RH at 40°C, the maximum water uptake has been found to be approximately 6.80%, and is achieved after 36 hours [561, 628].

5.3.9. General discussion

As outlined in Chapter 1, the delivery of molecules across the skin can be improved by increasing the amount of freely available drug at the surface of the skin, and reducing the barrier nature of the skin [170, 629, 630]. TPGS prepared dispersions using PGSS improved the transdermal delivery of PGN using both of these actions.
Chapter 5. Permeation

The final SCF – TPGS/myritol/transcutol P dispersion system significantly increased the permeation rate of PGN compared to the control: market cream (p-value < 0.05). Amongst other factors, the degree of saturation has played a role in the permeation of PGN across the skin. The saturation solubility tests conducted in Chapter 2, shown in Figure 2-6, showed that TPGS is able to incorporate a large amount of PGN (410.3 ± 16.2 µg·mL⁻¹) at TPGS concentrations of 30% w/w, while PEG 400/4000 was only able to saturate 67.5 ± 10.5 µg·mL⁻¹ of PGN at 30% w/w. The PEG-based dispersion prepared using SCF also gave the lowest transdermal flux of 2.8 µg·cm⁻²·h⁻¹. Utilising the saturated solubility curve, a graph has been drawn to determine the relationship with skin permeation of PGN from the SCF prepared systems absent myritol 318 and transcutol P, see Figure 5-19. The lowest flux value was selected for comparison with the excipients regardless of the method used and excluded the final SCF formulation with enhancers or myritol present. The transdermal flux of PGN was directly proportional to the excipient saturation of PGN for the Gelucire 44/14 and PEG-based dispersion systems. The Gelucire 44/14-based dispersion had a high flux across the skin while also having a high degree of PGN saturation, while for PEG had both the lowest saturation solubility and lowest flux. The TPGS-based dispersion system was unusual because it was found to have the highest flux but much lower saturation solubility compared to that of Gelucire 44/14, although almost 3 times higher compared to that of PEG’s saturation solubility. This means that, although improving the saturation solubility of PGN has contributed to the improved flux across the skin, it is not the only factor that has contributed to the observed results.
Chapter 5. Permeation

Figure 5-19. Transdermal flux of PGN across the skin (left axis) as a function of solubility saturation (right axis) for excipients at 30% w/w; (a) Gelucire 44/14, (b) TPGS, and (c) PEG 400/4000 prepared from the SCF method absent myritol 318 and transcutol P.

It is important to note that the dispersion systems used to determine flux were supersaturated solid dispersions, rather than saturated aqueous solutions, as used in the saturation solubility test. One study has found, using estradiol in different concentrations of propylene glycol/water cosolvent systems, supersaturation is able to effect flux across the skin. It was shown that the uptake and flux across human skin and silastic membrane increased with increasing degree of saturation and for supersaturation [139]. Flux through human skin from an 18 times saturated solution was able to increase about 13-fold when compared with the flux from saturated solution [139]. This increase was attributed to the increased drug solubility in the skin membrane [139]. Furthermore, a higher saturation solubility can help increase the release ability of PGN from formulation, giving rise to a lower transdermal flux. The final SCF formulation used in this chapter was prepared in order to increase the saturation solubility of PGN. Compared to the aqueous suspension, the solubility of PGN was increased in the selected oil. The higher concentration of dissolved PGN in this formulation was the main driving force for improved transdermal bioavailability.

In an in vivo study, supersaturatable systems were designed to incorporate PNU-91325 into a water soluble cellulosic polymer called hydroxypropyl methylcellulose (HPMC) and PEG 400 for oral delivery [631]. Although this research was preliminary, the importance
Chapter 5. Permeation

of the supersaturated state on absorption of poorly water soluble drugs was evident. The study found a five-fold higher bioavailability using improved saturated formulations compared to a PEG 400 based formulation. The supersaturation is claimed to be due to the high free drug concentration *in vivo* from generation and stabilization of the supersaturated state, that can prevent or lower the precipitation of the drug [631]. The low bioavailability of the PEG 400 formulation was possibly due to the uncontrolled precipitation of drug on administration, which is a commonly observed problem with an aqueous environment [631]. This also was consistent with the data found in this research, where the PEG-based formulation showed the lowest *in vitro* dissolution rate (see Chapters 3 and 4) and generally underperformed in the permeation of PGN across the skin. This is similar to other studies using PEG, where the low bioavailability of the PEG 400 formulation is attributed to the uncontrolled precipitation of PNU-91325 upon dosing, a commonly observed phenomenon with the cosolvent approach [631]. In another study, the type of polymer used in the matrix systems has a significant influence on PGN permeation using PVP, PVA, and PMA. It was found that PVP and PVA have higher PGN release and permeation rates than does PMA. A comparison of 24 hour release data to permeation data through rat skin shows a good correlation when no additives are included in the matrix systems [605]. Reviews of polymer based systems such as patches and matrixes have argued that these systems are the backbone of transdermal drug delivery [632]. Other studies have shown non-vehicle based systems such as those with penetration enhancers are ideal for passive diffusion and partitioning into the skin [134]. These studies suggest a vehicle based system to penetrate the skin is not required. However, it is possible, that an ideal formulation uses both approaches, as done in this research, where a vehicle type method and penetration enhancer method were used.

Interestingly, the flux (or permeation rate) of PGN generally did not seem to depended on the method of mixing and excipient used. Figure 5-20 shows the comparisons of flux values between all the formulations, except the water control where the 1 – 12 and 12 – 24 hour values are provided. The only formulation that is outside the general range of flux’s was the final SCF formulation, with a flux over 3-fold higher than the water control after 12 hours and 2-fold higher than the market cream. Also only the TPGS based formulations appeared to show improved flux with the SCF based formulations without myritol 318 and transcutol P.
Figure 5-20. The effect on permeation rate (flux) of PGN across the skin between different methods of preparation.

PM = physical mixing, CS = Cosolvent, CM = comelting, and SCF = supercritical fluid.

The water control was prepared to form a saturated aqueous suspension. This was similar yet different to other studies investigating the permeation of PGN from various skin models. Further, in relation to the supersaturation technique, the main limitation is the instability of these systems. The drug can rapidly crystallise leading to a decrease in the initial high activity, this problem has been overcome by the use of antinucleant polymers to stabilise the supersaturated systems [633]. Results suggest that optimisation of supersaturation and polymer content is one way to achieve both high permeation rates and inherent stability [175]. The degree of saturation was not obvious from the material sections of all the studies found, thus it is difficult to comment further on how much of a factor the saturation may have been in terms of explaining the differences in PGN permeation.

The degree of viscosity may have been another factor in the observed profiles. The formulations manufactured in this research were of semi-solid nature, meaning the thickness or resistance to flow was high, thus viscosity could not be measured using viscometers in the laboratory. Although not measured in this research there are numerous studies that have found viscosity to directly influence permeation of drugs across the skin [585-587]. Furthermore, the experimental temperature of the skin study was set at 37°C and the melting
point of TPGS is approximately 33°C, therefore the viscosity of the TPGS formulations may have dropped from approximately 1,000 mPa·s to 600 mPa·s [335]. However, TPGS can form micelles at relatively low concentrations, which would build an entangled network with a higher viscosity [561].

TPGS and Gelucire 44/14 may both form micelles on exposure to the aqueous regions of the skin. The water content of SC is 30 to 50% (w/w) of dry weight in vivo and is varied when occluded by a water impermeable membrane and stored into water [115]. TPGS forms micelles at concentrations ≥ 0.02 g·mL⁻¹ in water and Gelucire 44/14 undergoes micelle formation at approximately 0.1 g·mL⁻¹ [314, 319]. Both excipients are the main ingredient (> 40% w/w) of their respective formulations. These concentrations are higher than the critical micellar concentration (CMC) of the excipients suggesting that PGN was likely to be included into micelles in order to increase its absorption. Figure 5-21 shows how the individual surface active agents (SAA) can arrange themselves into micelles. Formation of vehicles to enhance transdermal drug delivery is controversial, however, with different studies stating that vehicles may or may not be required for absorption to occur. There are numerous studies showing that nano- and micro-emulsion based systems are excellent vehicles for drug delivery into the skin [200, 588, 634, 635]. The various potential mechanisms to enhance drug penetration via these emulsions include directly affecting the skin, creating pores for drug to pass through, and modifying the formulation so the partition, diffusion, or solubility are changed [634].

![Figure 5-21. Schematic illustration of monomer-micelle equilibrium.](image)

(a) surfactant monomers, (b) micelle, (c) hydrophobic tail groups, and (d) hydrophilic head groups.

The availability of PGN to permeate across the skin depends on the vehicle phases containing PGN. An oil-in-water cream, shown in Figure 5-22, requires PGN to first move into the water environment from the oil phase, and since PGN is more lipophilic in nature, it is more likely to stay in the oil phase, reducing the amount available for delivery to the skin surface [636]. However, a micellar system or formulation containing SAA may help deliver more PGN and help penetrate the skin surface, by lowering the interfacial tension between
the oil and water phases, allowing increased PGN partitioning and release, increasing the bioavailability of PGN. The effect of oil/water ratio and presence of SAA are important factors when considering delivery of drugs through the skin [637]. As eluded to earlier, the SC is a source of water, in fact the thickness of hydrated SC (52 µm) is 3.3 times that of dehydrated skin (16 µm) [115]. Sweating can therefore also affect the phase behaviour of a vehicle once in contact with the skin. The formation of micelles and effects on PGN solubility must be further studied in human volunteers.

Figure 5-22. The structural aspects of a semi-solid cream:
(a) aqueous environment(s), (b) hydrophobic tail region(s), (c) hydrophilic head region(s), and (d) lipid/oily region(s). Redrawn from [636].

Figure 5-23 illustrates the possible movements of PGN molecules from the semi-solid with TPGS/myritol 318 and transcutol P components and shows the multiple aspects of skin absorption. Any drug crystals must initially dissolve in order for the drug molecules to diffuse through the formulation towards the skin's surface. The penetration enhancer must also follow this step and act on the surface of the skin. The PGN partitions into the oil, diffuses through water, and partitions into any micelles forming, where the micelles themselves will also help deliver PGN into the skin. Once the drug reaches the skin surface, it partitions into the skin's surface membrane (or stratum corneum), and diffuses across it. Some PGN may bind to receptors, fat/adipose tissue, and muscle; the remaining PGN travels further into the skin, and partitions into the viable epidermis where PGN would meet blood vessels and be carried away into systemic circulation. As discussed earlier, the logP of PGN (3.5) is higher than the optimal value for transdermal delivery (logP 1 – 3). This means PGN is likely to form depots throughout the different skin regions. For the research conducted in this thesis, the PGN content in the receptor fluid was low and was not observed to be any higher than 63.8 µg·mL⁻¹. Although SAA such as transcutol P can increase the transdermal diffusivity of
Chapter 5. Permeation

PGN, it appears, in this case, may not have prevented PGN from accumulating in the skin. Further research must be conducted in terms of PGN skin content in order to determine the skin deposit effects on PGN.

![Diagram showing the process of PGN delivery from a transdermal formulation into the skin and into systemic circulation.](image_url)

**Figure 5-23.** Major stages in PGN delivery from a transdermal formulation into the skin and into systemic circulation. Micelles sourced from Gelucire 44/14 or TPGS formulations.

From the membrane permeation experiment it can be concluded that the drug flux might be increased supra-proportionally with increasing donor concentration, drug (super-)saturation (proportional), beyond what would be anticipated based on ideal donor concentration and partition coefficient considerations only. These findings could not be confirmed in the *in situ* investigation. This could probably be due to additional vehicle effects (e.g. enhancement, irritation, drug binding) which have to be expected and could have altered the integrity of the SC and therewith transdermal bioavailability of the drug. Different concentrations of PGN formulations were not prepared and examined.

The studies in this research were conducted with the skin membranes occluded with parafilm. Occlusion is known to increase the transdermal flux of drugs across the skin [589, 591, 638]. The occlusion mechanism involves the maintained hydration of the SC layer of the skin [639]. The use of occlusion also slows down the recovery of the SC layer that has been
disrupted by lipid compromising penetration enhancers [640]. Several studies have attributed slower SC recovery from occlusive methods [641]. In one study, barrier repair depended on the occlusive ability of preparations [642]. Damaged or compromised skin from a variety of ways showed improved barrier recovery, when the skin did not shut off the metabolic processes. The more occlusive a preparation, the more the metabolic function of the skin decreased, delaying barrier recovery. Other studies have also observed that skin occluded for 3 days had slower recovery times compared to the non-occluded skin after exposure to surfactants and tape stripping [641]. It was also found that the lipid bilayer of the SC was able to recover once the occlusive material had been removed. No studies have observed the occlusive effects on PGN permeation, however it is possible that the occlusion of the skin surface can also improve the transdermal delivery of PGN.

**5.4. Conclusion**

In this chapter, the SCF method known as PGSS was used to form unique dispersion systems, which were investigated to deliver PGN across nude mouse skin. Various dispersion systems using the selected excipients were prepared using a PGSS method, conventional CM, CS, and PM methods, and compared to that of the controls; aqueous suspension and market cream based on permeation studies. The flux of the TPGS/myritol 318/transcutol P formulation (10.9 µg·cm⁻²·h⁻¹) prepared using SCF processing was statistically significantly compared to that of the controls; aqueous suspension and (7.7 µg·cm⁻²·h⁻¹) market cream (5.4 µg·cm⁻²·h⁻¹) (p-value < 0.05). FTIR studies of treated skin revealed changes in the SC lipid bilayer structure of the skin compared to that of untreated skin. Further examination of the histology showed swelling and fragmentation of the skins SC and epidermis had occurred with the tested formulations, explaining how PGN permeation occurred. The advantage of this dispersion system was its ability to solubilise PGN, form micelle vehicles, and disrupt the SC bilayer leading to improved PGN permeation, thus likely to offer improved bioavailability. Further investigation must be conducted into optimization of the SCF-based formulation with myritol 318 and transcutol P and examine the in vivo permeation of PGN across the human skin.
Chapter 6:

Conclusion and future direction
Chapter 6. Conclusion and future direction

6.1. Final overview

The fall in PGN levels during perimenopause and subsequent low levels of PGN and estradiol are known to cause a variety of symptoms such as hot flushes, and is associated with pathological conditions such as osteoporosis. It is estimated that approximately 9% of the American population has osteoporosis, and the percentage in elderly (> 65 years) women is almost 60%. In NZ, more than 60% of the 84,000 fractures in 2007 were estimated to occur from women of postmenopausal age. The pathophysiology of osteoporosis is well understood, although there remains no cure other than by treatment with hormones such PGN and estradiol.

There is a variety of treatments available designed to slow the progression of osteoporosis that are separated into three groups: HRT, non-hormonal, and non-drug therapies. HRT remains the primary form of management of postmenopause, however it is now conventional practice to restrict HRT use to under 5 years as longer durations are linked to, amongst others, breast cancer and heart disease. The use of non-hormonal therapies for osteoporosis such as bisphosphonates, vitamin D₃, and calcium have had reasonable success but require regular dosing and have range of adverse effects with varying degrees of severity. The other treatments such as non-hormonal and non-drug options have conflicting evidence including over efficacy.

It has been proposed that endogenous PGN delivered transdermally may offer a substitute to oral HRT. Extracted from Mexican wild yam, PGN can be manufactured into a fine white powder. The efficacy of transdermal PGN is controversial, and current clinical research has demonstrated that PGN bioavailability is low from delivery systems across the skin. However, when taken orally, the PGN doses are typically 200 mg or more daily in order to reach therapeutic levels. This is because PGN is heavily metabolised in the GIT and liver, i.e. first-pass metabolism. A transdermal delivery system would be able to bypass the liver, thus allow for lower doses to be used. In addition to these advantages, the transdermal formulation of PGN can be drastically improved. As outlined in Chapter 1, although the physicochemical properties of PGN are near optimum, the low bioavailability is associated
Chapter 6: Conclusion

with the chemical (poor aqueous solubility) and physical (SC skin layer) hurdles. A novel method has been developed for improving transdermal PGN delivery using SC-CO₂ processing and penetration enhancer. An advantage of SCF processing was that it could improve the formulation solubility of PGN, while the penetration enhancer would help overcome the SC barrier.

The basis of Chapter 2 was to establish the physicochemical properties of PGN in order to help direct the development of a SCF formulation. The preformulation tests carried out included the selection of excipients using a literature search, solubility parameters, saturation solubility, and birefringence. Finding that Gelucire 44/14, TPGS, and PEG 400/4000 had compatible solubility parameters and were used extensively in the literature for SCF formulations, meant that these excipients were good candidates. Melting point behaviour of PGN was also tested to examine the effects of mixing with the selected excipients. Significantly improved aqueous solubility using these excipients further indicated that PGN could be formulated as a lipid-water based delivery system. The release of a lipophilic drug such as PGN is higher in an environment with less well defined phases, c.f. o/w or w/o creams, because the drug is not required to first partition into the aqueous phase. Improved release meant more PGN was freely available to overcome the SC barrier which was expected to help increase the permeation rate. Once the selection of the excipients was complete, numerous characterisation studies were performed. The identity of PGN and selected excipients was determined using FTIR, XRPD, and DSC, and polymorphism was investigated using Raman spectroscopy. It was found that SC-CO₂ does not induce transformational changes to the molecular structure of PGN, while melting causes the shift into a less stable morphology. Given the MP of PGN is approximately 132°C and the SCF process used did not exceed 70°C, it was concluded that polymorphism would not be of concern in this research. The preformulation studies included the development and validation of an HPLC method for PGN. The C₁₈-HPLC method used was a rapid, sensitive, reproducible, and specific assay for determination of PGN. PGN maintained a steady elution without interference whether it was spiked with the selected excipients or non-ionic cream.

The knowledge gained at this stage was used to plan experiments for the formulation and characterisation of semisolid PGN dispersions, and formed the basis of investigations in Chapter 3. Before formulation could begin, a SCF processing unit had to be designed, built, and operating conditions determined. The SCF unit had to be capable of preparing PGSS using SC-CO₂, and this was the main purpose of Chapter 3. Prior to the construction of the SCF unit, three SCF methods were tested for their suitability and usefulness in PGN
Chapter 6: Conclusion

formulation. As PGN crystals were already micronized, the RESS method offered little opportunity to further reduce particle size, moreover PGN was too soluble in SC-CO₂, meaning the maximum capability of RESS was not possible. The degree of PGN solubility in SC-CO₂ also meant that the GAS method was not feasible, as CO₂ cannot act as antisolvent, underminding the principle effect of the GAS methods. This left the PGSS method to be explored which allowed for the convenience of loading additional excipients and/or solvents into the processing step, leading to the foundation for a possible formulation. In addition, the PGSS had the advantage of not being dependent on the solubility parameters of SC-CO₂. Whether the PGN is in solution or suspended in SC-CO₂, or both, is not critical to the supercritical processing conditions of the PGSS method. A novel PGSS unit was then constructed and developed to prepare unique PGN dispersion systems.

The process was summarised into three stages: preparation, processing, and recovery. The high process yields (> 90%) for all the selected excipients meant that further elimination steps would be required in choosing a final excipient. Novel solid and semi-solid dispersions of PGN were prepared with elementary experiments using SC-CO₂ and compared to that of conventional methods; comelting, cosolvent, and physical mixing. All dispersions were further characterised by XRPD, FTIR, DSC, and in vitro dissolution. The rapid in vitro dissolution profiles of PGN containing either Gelucire 44/14 or TPGS, labelled them both as possible candidates for further development. The Gelucire 44/14 based system was investigated for the optimization of SCF processing due to its high dissolving capacity and improvement to PGN’s aqueous dissolution.

In Chapter 4, a factorial design experiment was developed to optimise the unique SCF process and produce a practical product to be tested for transdermal delivery of PGN. This was appropriate due to the non-standardarized approaches typical of SCF research. This study provided essential information about the behaviour of the ternary system (SCF:PGN:Gelucire 44/14). The seven variables measured near or within SCF conditions were temperature, pressure, sample loading, processing time, sonication time, PGN:excipient ratio (1:1, 1:5 and 1:10), and orifice diameter. There were three responses measured: the process yield, extent of PGN dissolution after 20 minutes, and time to reach 50% PGN dissolved. The results showed that the use of higher pressure (A) of 186 bar, higher temperature (B) of 60°C, a longer processing time (D) of 30 minutes, and lower PGN ratio to excipient (Gelucire 44/14) (F) of 1:10 were all important features to the increasing yield of PGN dispersions using the PGSS method. While pressure, temperature, and processing time were also pivotal factors for the
dissolution extent after 20 minutes and $t_{1/2}$, the sample loading size and sonication only factored into the dissolution extent after 20 minutes, while orifice diameter only had a significant effect on $t_{1/2}$. Therefore, the ideal SCF processing conditions were determined as being a high pressure (186 bar), high temperature (60°C), long processing time (30 minutes), high sample load (9 g), sonication (at least 10 minutes), a lower PGN to excipient ratio (at least 1:10), and smaller orifice diameter (1/16\textsuperscript{th} inch). The maximum and minimum levels of the experimental domain were selected based on the P-T phase diagram and S-L-V curves of CO\textsubscript{2}, restrictions of the equipment, and conditions that have been used from the literature. In addition, surface response curves were constructed by fixing 5 of the 7 factors tested in the $2^{p-k}$ model, where only pressure and temperature remained changeable. This basically confirmed the extensive effects of pressure and temperature on four responses: process yield, PGN dissolution extent after 20 minutes, $t_{1/2}$, and PGN dispersion uniformity. Generally a higher pressure and higher temperature produced a positive response, except on PGN dissolution after 20 minutes, where either a low or high temperature produced a positive response. In order to understand the mechanisms behind the dissolution of PGN, several dissolution models were utilised including zero and first-order, Higuchi, Korsmeyer-Peppas, and dual-first order. This was also investigated in Chapter 4.

It was found that diffusion, as well as erosion processes, contribute to the release of PGN from a Gelucire 44/14 formulation prepared using SC-CO\textsubscript{2}. The zero, first, Higuchi, and Korsmeyer-Peppas models generally did not fit well, although the relative order of these models was zero < first < Korsmeyer-Peppas < Higuchi, indicating that there was some degree of controlled release mechanism occurring. The Korsmeyer-Peppas model ($R^2 = 0.919$ to 0.998) gave similar results to the Higuchi model ($R^2 = 0.944$ to 0.995), and was able to identify the relative release mechanisms of PGN. The SCF based formulations produced results showing that more than one process of PGN release was occurring, which is likely to be more or less a function of diffusion and erosion. However, the Korsmeyer-Peppas cannot directly define or indicate which release process is more significant. Fitting experimental data to non-linear curves was also able to show the inclusion of more than one dissolution process in the release of PGN from Gelucire 44/14. An iterative curve-fitting technique such as the dual first order model, was a useful way to determine the extent of dissolution parameters: erosion and diffusion. The conventional mixing methods: CS, CM, and PM showed diffusion accounted for 30.1%, 30.5%, and 40.8%, respectively. The SCF dispersions mixed with the same amount of excipient showed a diffusion range from 66.1 to 83.1% was occurring. The remaining release of PGN was occurring as a function of erosion, which may
Chapter 6: Conclusion

or may not have occurred first, as the diffusion and erosion processes occur simultaneously. The reason for a higher extent of diffusion occurring in the SCF dispersions may have been a phenomenon due to increased dispersion of PGN crystals and possibly a higher extent of dispersed PGN molecules throughout the formulation compared to the conventional preprepared formulations. This study was also able to demonstrate that the dual first order equation was a sufficient empirical model to describe the release of molecules from Gelucire 44/14 dispersion prepared by either conventional or SCF methods.

The final aim of this thesis was to investigate the permeation rate of PGN from a SCF manufactured TPGS dispersion (final version), selected conventionally prepared dispersions, selected market cream, and aqueous suspension. Based on an initial permeation study, the selected excipients were screened for their permeability of PGN, which found TPGS based dispersions gave the highest flux and extent of PGN permeation. At this stage, an oil (myritol 318) and penetration enhancer (transcutol P) were also added to form PGN loaded TPGS dispersion systems. The final SCF formulation contained 4% w/w PGN, 10% w/w transcutol P, 35% w/w myritol 318, and 51% w/w TPGS.

Nude ex vivo mouse skin was used to study permeation of PGN because it was readily available in excellent quality, cost-sensitive, and relatively similar to human skin. Compared to the market cream, the final SCF-TPGS dispersion increased the permeation rate of PGN by 2-fold and reduced the lag time almost 6-fold. Compared to the other TPGS based dispersions: CM, CS, and PM, the final SCF-TPGS dispersion increased the permeation rate of PGN by 2.5, 2.6, and 2.9 times, respectively (p-value < 0.05). The increased permeation rate can be attributed to several mechanisms, the increased solubility of PGN due to TPGS, SCF processing, and presence of oil, increasing thermodynamic activity, the formation of micelles acting as a vehicle at the interface between the formulation and skin’s surface increasing partitioning, and as the result of oil and surfactant components acting as penetration enhancers. Through the use of FTIR studies, changes in the amide and CH stretching regions suggested that the presence of TPGS, myritol 318, and transcutol P were having physical interactions with lipids of the SC bilayer. These interactions were able to disrupt and swell the skins surface, allowing for improved solubility and partitioning into the skin. Whilst FTIR spectra showed that the final SCF formulation was able to shift the amide and CH stretching regions, studies of the conventional formulations showed that the degree of amide and CH stretching was not always drastic, and most of the results between the final SCF formulation and conventional formulations were similar. Generally the application from the SCF and conventional formulations, gave similar changes indicating that the test
dispersion systems may be safe to use on skin. The histological examination of the skins surface suggested that the enhanced permeation rate of PGN was a result of the oil’s ability to solublise PGN and transcutol P’s ability to disrupt the SC and epi/dermis layers of the skin. Microscopic evaluation of the skin indicated that the effects of the final SCF-TPGS based formulation on the integrity of the skin was more or less similar to the aqueous control. Therefore, the final SCF formulation was considered generally safe to use on the skin, however this must be further tested in animal models before human investigation can proceed.

The final SCF formulation could be applied directly to the skin in its current form, added into a patch system, or incorporated into an alcohol (liquid) reservoir rollon or spray. The patch would act as a controlled release system, affecting the permeation rate of PGN, while the roll-on or spray would act in a similar way to the current form, but further studies would be required to reassess the effects on PGN permeation. An investigation into the use of a microemulsion prepared using a SCF method would also be interesting. Several studies, although not all of them are pharmaceutical, have demonstrated highly efficient manufacturing of microemulsions [530, 643-645]. It is known that PGN rapidly precipitates when in the presence of water. This may be avoided or slowed by use of a mixture of TPGS and PEG because polymers such as PEG have antinucleating properties. The effects of saturation vs. supersaturation would also be interesting to evaluate the differences in the thermodynamic activity and permeation rate. It would also be essential to have a stability indicating test for PGN in different formulations according to ICH guidelines. As the PGN content in the final SCF formulation was around the saturation solubility, it would not be difficult to cause supersaturation. Thus it would be important to determine the optimal PGN amount with high permeation, which is also stable over a range of storage conditions.

The main objective of this thesis was to enhance the transdermal permeation rate of PGN using a SCF based method. The final SCF formulation was also able to achieve the objective, however the formulation could be further improved to increase the permeation of PGN. Following is an outline of the research limitations which are followed by the future directions that could be taken from this research.

### 6.2. Limitations

This project describes the early stage development of a SCF method to improve transdermal delivery of PGN, and the following limitations were experienced:
although PGN and the selected excipients do not have ionisable functional groups, the
dissolution medium did not mimic the *in vivo* circumstance (the skin’s surface has pH 5.5);

- no study was conducted into the formation of micelles at the interface between the
  skins surface and release of SAAs (Gelucire 44/14 and TPGS) from the formulations;
- although histological examination and FTIR showed minimal effect on the skins
  surface occurred from all the formulations investigated, lack of extensive toxicity
  testing using CaCo-2/LD$_{50}$ and/or LDH leaking from skin tissue;
- the choice of the PE was from mixing PGN and PE directly. The effects of the other
  excipients was not examined;
- the use of multiple SCFs were not explored in combination or separately
- restricted experimental designs mostly within the SCF regions only. Subcritical P-T
  regions would have allowed investigative research to measure high-pressure gas
  effects and establish the binary S-L-V projections between excipients and SC-CO$_2$ in
  comparison to SCF processing;
- there was no loading of organic cosolvents, e.g. methanol, or other excipients to
  investigate effects on PGN solubility in SC-CO$_2$;
- the effects of using a nozzle were not explored properly (lack of time and funding);
- limited research was done to investigate the optimisation of a TPGS formulation
  under different SCF processing conditions;
- limited pre-screening data of a comprehensive list of possible influencing variables
  was conducted;
- restricted response forms for measuring optimisation e.g. using permeation as a
  response to optimise the formulation, rather dissolution to optimise the SCF process;
- limited study was completed with the final formulation, regarding different ingredient
  concentrations with the myritol 318 and penetration enhancer (transcutol P),
- characterisation studies were not conducted to determine the phase behaviour effects
  of excipients on exposure to water and their micelle formation abilities,
- no scale up tests were performed, nor associated problems examined such as
  management of large-scale liquid CO$_2$ and effects on the properties of SC-CO$_2$,
- that although PGN is susceptible to enzyme degradation in the skin, it was not
  examined.
6.3. Future direction

There are numerous possibilities for the best direction in which to take this research further, however following is an outline that could be of some use. One of major setbacks was the monopoly that CO$_2$ had on the processing of the PGN dispersions. It would be interesting to investigate the use of multiple SCFs and explore the effects of SCF combinations or other SCF separately, on PGN solubility, dispersability, and permeation. In addition, some research suggests that more pure (SCF grade) CO$_2$, possibly as high as 99.99% is required to optimise the SCF process [224]. One study found that a linear dependence of critical temperature with critical pressure over a range of concentrations for the solvents and drug components that may be influenced by the CO$_2$ purity [513]. Future research could be conducted to complete experiments using different CO$_2$ purities.

Whilst the P-T phase regions of CO$_2$ were utilised to ensure SCF status was achieved for the PGN formulations in this research, it would be important in the future to establish knowledge on the S-L-V curves of binary systems containing the selected excipients and the effects of these curves compared to pure CO$_2$. In addition to this, the use of other solvents and cosolvents, and excipients could be used to test their abilities to improve PGN solubility.

Although controversial, the effects of using different nozzle types could be explored, although the research would need to be well funded, as some nozzles can cost as much as $2,000 NZD. The nozzle effects would be expected to influence the dispersibility of PGN throughout the formulation. In relation to this, further research could be done to investigate the optimisation of a TPGS formulation under different SCF processing conditions. As various parameters potentially affect the PGSS process, the optimization of the experimental conditions represents a critical step in the development of a SCF method [566]. It is known, for example, solubility of the drug [470, 521] can be controlled by the composition, density and temperature of the SCF, moreover the recovered product is not only dependent on the operating conditions but also on the sample characteristics, e.g. water content, matrix type, particle size, viscosity, etc., making selection of experimental conditions difficult, especially with subsequent reliable quantification [216, 243, 244, 369, 447, 521]. Another common area of development could surround the expansion phase of PGSS where mixing plays an important role [519, 520]. There could be extensive work required in this area regarding the ‘mixing tee’, capillary flow, restrictor size and type, and so forth. Another area may include the method of mixing in the SCF, for example use of built-in stirring bars or rods, resins, and ultra-sonication. This could lead to the need for more screening tests using an experimental
Chapter 6: Conclusion

design because there are numerous variables that could have been tested, i.e. the seven variables tested in this thesis were not a comprehensive list. For example, use of continuous CO₂ feeding flow into the precipitation vessel during expansion, or slower release of SC-CO₂ expansion, or higher pressures and temperatures, e.g. above 200 bar or above the melting point of PGN, and analyse the effects each of these could have on PGN precipitation and dispersion/uniformity. This would raise questions about possible responses to best measure the optimisation of PGN formulation. In this research, dissolution responses and process yield were used to help optimise the PGSS method, but using *ex vivo* permeation, for example, could be an ideal way to optimise the formulation.

Another important step would be to evaluate the use of different concentrations of myritol 318 and transcutol P. It would be interesting to investigate the effects of a range of myritol 318 and transcutol P concentrations, and SCF conditions, on dissolution and permeation across the skin. In addition, combinations of Gelucire 44/14, TPGS, and PEG in a range of concentrations could also be investigated. This would lead to characterisation studies conducted to determine the phase behaviour effects of these excipients on exposure to water and their micelle formation abilities. A number of formulation parameters need to be optimised and validated to achieve increased levels of drug solubility and release.

The transformation from laboratory curiosity to commercial production requires the scale-up to manufacture large batch sizes. Scale-up tests could be performed and associated problems examined such as the management of large-scale liquid CO₂ and effects on the properties of SC-CO₂, especially at higher pressures and temperatures. The potential for SCF processes to be scaled up has been successfully achieved in the food industry. An example, is the decaffeination of coffee beans, where a closed SCF system has been engineered without moving parts and built with low maintenance high-grade stainless steel [323]. SCF methods used by drug making companies, such as Bradford Plc., have reported on the use of 1 ton of ingredients having more or less equal manufacturing costs to conventional methods. The improvements in dissolution rate, time efficiency in preparation, and stability of PGN dispersions prepared by SCF processing provide significant advantages in this alternative to conventional methods.

As one of the final points, although this research focused in a formulation approach to improving PGN bioavailability, another way could be a pharmacological approach. Due to the presence of enzymes in the skin there could be an effect on PGN permeation, although there is some preliminary evidence suggests that PGN bioavailability is not drastically
Chapter 6: Conclusion

influenced by enzymatic activity [646]. Therefore, future studies need to future investigate the use of enzyme inhibitors or enzyme saturation of PGN.

In summary, future work could involve the development of the described PGSS method towards a clinically relevant product and to address some of the limitations of this project. Furthermore, by developing a platform into which a range of steroids and other drugs from different therapeutic classes, with varying physicochemical parameters, can be loaded and released, the versatility of the delivery system would be established. More studies are required to determine to what extent these factors play a role in drug absorption in order to reach an accurate correlation with PGN permeation across the skin.

6.4. Conclusion

From the various experiments conducted, it is concluded that TPGS-based dispersions prepared using PGSS can improve the transdermal permeation rate of PGN. The main mechanism of PGN delivery through the skin is modification of the SC barrier. Dispersions based on Gelucire 44/14 and PEG 400/4000 showed limited improvements but may be modified more to enhance the bioavailability of PGN. The main experiments were able to show that:

1) FTIR and Raman spectroscopy, DSC, birefringence, and XRD are all capable technologies to characterise PGN;
2) Chromatography is a reliable analytical method to separate PGN from its formulation components;
3) SC-CO$_2$ does not affect the polymorphism of PGN;
4) PGSS can form uniform dispersions using SC-CO$_2$ with Gelucire 44/14, TPGS and PEG and the dispersion systems were optimised using factorial design experiments, and utilitise a dual first order model to explain the mechanisms of PGN release;
5) TPGS/myritol 318/transcutol P-based dispersions can increase the permeation rate of PGN across the skin by 2-fold compared to the market non-ionic cream;
6) The improvement of PGN permeability could be explained by the dispersion system’s ability to disrupt the SC bilayer and improve the solubility of PGN in the skin; and
7) A transdermal delivery system of PGN platform using SC-CO$_2$ processing was established and it could be used for other steroids and other poorly aqueous drug study in the future.
References


References


References


63. Drugs.com, Menopause Center, M.W. Kluwer™, Editor. 2011, Copyright © 2000-2012. All rights reserved.


References


References


References


References


References


References


References

References


273. Box, K.J., Völgyi, G., Baka, E., Stuart, M., Takács-Novák, K., Comer, J.E.A., Equilibrium versus kinetic measurements of aqueous solubility, and the ability of


References


References

References


References


References

References


References


References


References


References


References


References


References


References


References

545. Varona, S., Kareth, S., Cocero, M.J. Encapsulation of essentials oils using biopolymers for their use in ecological agriculture. in International Symposium on Supercritical Fluids. 2009. Arcachon, France.
References


References


References


References


References


Figure I. Representative light transmittance graph for PGN powder.
Appendix IIa

Result Analysis Report

Sample Name: ProgesteroneBeforeRESS - Average
Sample Source & type: Pharmacy = 27/05/08
Sample bulk lot ref: Averaged

SOP Name: Measured by: particle
Measurements: Tuesday, 27 May 2008 4:04:38 p.m.
Analysed: Tuesday, 27 May 2008 4:04:39 p.m.

Particle Name: Progesterone
Particle RI: 1.450
Dispersant Name: Water
Dispersant RI: 1.330

Accessory Name: None
Absorption: 0

Analysis model: General purpose
Size range: 0.020 to 2000.000 um
Weighted Residual: 1.007 %

Sensitivity: Normal
Obscuration: 15.80 %
Result Emulation: Off

Concentration: 0.1739 %Vo
Span : 1.251
Uniformity: 0.393
Result units: Volume

Specific Surface Area: 0.0775 m²/g
Surface Weighted Mean D[3,2]: 77.395 um
Vol. Weighted Mean D[4,3]: 117.464 um

Particle Size Distribution

Operator notes: Particle Test
## Appendix IIb

### Result Analysis Report

<table>
<thead>
<tr>
<th>Sample Name:</th>
<th>SOP Name:</th>
<th>Measured:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progesterone after RESS - Average</td>
<td>Measured by: particle</td>
<td>Tuesday, 27 May 2008 4:10:30 p.m.</td>
</tr>
<tr>
<td>Sample Source &amp; type: Pharmacy</td>
<td>27/05/08</td>
<td>Analysed: Tuesday, 27 May 2008 4:10:31 p.m.</td>
</tr>
<tr>
<td>Sample bulk lot ref:</td>
<td>Result Source: Averaged</td>
<td></td>
</tr>
</tbody>
</table>

### Analysis Model

- **Particle Name:** Progesterone
- **Particle RI:** 1.450
- **Dispersant Name:** Water
- **Dispersant RI:** 1.330
- **Accessory Name:** None
- **Analysis model:** General purpose
- **Size range:** 0.020 to 2000.000 um
- **Weighted Residual:** 0.638 %
- **Result Emulation:** Off
- **Concentration:** 0.0493 %Vol
- **Span:** 2.259
- **Uniformity:** 0.703
- **Result units:** Volume
- **Specific Surface Area:** 0.139 m²/g
- **Surface Weighted Mean D[3.2]:** 43.319 um
- **Vol. Weighted Mean D[4,3]:** 88.469 um

### Particle Size Distribution

#### Progesterone after RESS - Average, Tuesday, 27 May 2008 4:10:30 p.m.

<table>
<thead>
<tr>
<th>Size (um)</th>
<th>Volume %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.010</td>
<td>0.00</td>
</tr>
<tr>
<td>0.011</td>
<td>0.00</td>
</tr>
<tr>
<td>0.015</td>
<td>0.00</td>
</tr>
<tr>
<td>0.020</td>
<td>0.00</td>
</tr>
<tr>
<td>0.025</td>
<td>0.00</td>
</tr>
<tr>
<td>0.030</td>
<td>0.00</td>
</tr>
<tr>
<td>0.035</td>
<td>0.00</td>
</tr>
<tr>
<td>0.040</td>
<td>0.01</td>
</tr>
<tr>
<td>0.045</td>
<td>0.01</td>
</tr>
<tr>
<td>0.050</td>
<td>0.01</td>
</tr>
<tr>
<td>0.060</td>
<td>0.01</td>
</tr>
<tr>
<td>0.070</td>
<td>0.01</td>
</tr>
<tr>
<td>0.090</td>
<td>0.01</td>
</tr>
<tr>
<td>0.105</td>
<td>0.01</td>
</tr>
</tbody>
</table>

### Operator notes:

- Particle Test
## Appendix III

Table III. Materials and equipment used for the PGSS project setup

<table>
<thead>
<tr>
<th>Part Description</th>
<th>Supplying Company</th>
<th>Type/Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progesterone (powder)</td>
<td>Pharmacia &amp; UpJohn-Pfizer Company (USA)</td>
<td>Commercial grade</td>
</tr>
<tr>
<td>98% Liquid CO₂</td>
<td>BOC Gas (NZ)</td>
<td>Industrial grade</td>
</tr>
<tr>
<td>99.99% CO₂</td>
<td>BOC Gas (NZ)</td>
<td>Supercritical grade</td>
</tr>
<tr>
<td>Gelucire 44/14</td>
<td>Gattefossé SAS Corporation (France)</td>
<td>3051PP1</td>
</tr>
<tr>
<td>TPGS</td>
<td>Zhejiang Shiner Chemical Company Ltd. (China)</td>
<td>USP29 compliant</td>
</tr>
<tr>
<td>PEG 400 &amp; 4000</td>
<td>BDH, VMR International Ltd. (England)</td>
<td>Commercial grade</td>
</tr>
<tr>
<td>Syringe pump</td>
<td>Teledyne (USA)</td>
<td>Isco Series 260D</td>
</tr>
<tr>
<td>Sonnicating water bath</td>
<td>Bandelin-SonoRex Digital (Germany)</td>
<td>DX-1028P</td>
</tr>
<tr>
<td>Double-ended sample cylinder</td>
<td>Swagelok (NZ)</td>
<td>316L-50DF4-300-18406</td>
</tr>
<tr>
<td>Non-rotating stem valve</td>
<td>Swagelok (NZ)</td>
<td>SS-16DKM4F-2</td>
</tr>
<tr>
<td>Pressure transducers</td>
<td>WIKA (Germany)</td>
<td>ECO-1</td>
</tr>
<tr>
<td>Temperature probes</td>
<td>WIKA (Germany)</td>
<td>T8907-6</td>
</tr>
<tr>
<td>Data logging software</td>
<td>MeterMaster NZ Ltd. (NZ)</td>
<td>PicoLog</td>
</tr>
<tr>
<td>Street tee</td>
<td>Swagelok (NZ)</td>
<td>SS-4-ST</td>
</tr>
<tr>
<td>Micrometering valve</td>
<td>Hoke (NZ)</td>
<td>86041</td>
</tr>
<tr>
<td>Precipitation vessel</td>
<td>UOA - Engineering (NZ)</td>
<td>various</td>
</tr>
<tr>
<td>Male/ female connectors</td>
<td>Swagelok (NZ)</td>
<td>SS-100-1-2RT</td>
</tr>
<tr>
<td>Needle valve</td>
<td>Swagelok (NZ)</td>
<td>Integral-bonnet SS-ORS2</td>
</tr>
<tr>
<td>1/16” joint union</td>
<td>NZ Alltech Inc. (NZ)</td>
<td>ZU1PK</td>
</tr>
<tr>
<td>1/8” &amp; 1/16” tubing</td>
<td>NZ Alltech Inc. (NZ)</td>
<td>30107 &amp; 30212</td>
</tr>
<tr>
<td>Pressure gauge</td>
<td>Swagelok (NZ)</td>
<td>PGI-50M-PG5000-LAOX</td>
</tr>
<tr>
<td>PTFE O-ring kit</td>
<td>Swagelok (NZ)</td>
<td>EP-P4T-K2</td>
</tr>
<tr>
<td>Plug (ball) valve</td>
<td>AIScientific (NZ)</td>
<td>SS-4P4T1</td>
</tr>
<tr>
<td>Reducing connector</td>
<td>AIScientific (NZ)</td>
<td>P/N 209-0161-63</td>
</tr>
<tr>
<td>CO₂ connector</td>
<td>DKSH NZ Ltd. (NZ)</td>
<td>68-1247-043</td>
</tr>
<tr>
<td>Relief valve</td>
<td>Swagelok (NZ)</td>
<td>SS-RL3S4</td>
</tr>
<tr>
<td>Rupture Disc</td>
<td>Swagelok (NZ)</td>
<td>SS-RDK-16-1900</td>
</tr>
<tr>
<td>Ethylene propylene rings</td>
<td>Swagelok (NZ)</td>
<td>OD, 14D series</td>
</tr>
</tbody>
</table>
Appendix IV

Figure IV. CO$_2$ cylinder connection package for Series D pump from DKSH Limited/ AI Scientific (Auckland, NZ).
Appendix V

Sample Cylinders

Ordering Information, Technical Data, and Dimensions
Select an ordering number.
Dimensions are for reference only and are subject to change.

### Single-Ended Cylinders

<table>
<thead>
<tr>
<th>Material Grade/</th>
<th>Pressure Rating</th>
<th>Internal Volume</th>
<th>P in.</th>
<th>Ordering Number</th>
<th>Dimensions, in. (mm)</th>
<th>Weight (lb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cylinder Specification</td>
<td>psig (bar)</td>
<td>cm³ at 5 %</td>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>304L SS/</td>
<td>500 (34.4)</td>
<td>150</td>
<td>1/4</td>
<td>304L-05SF4-150</td>
<td>4.88 (124)</td>
<td>1.1 (0.50)</td>
</tr>
<tr>
<td>DOT-4B 500</td>
<td></td>
<td>300</td>
<td></td>
<td>304L-05SF4-300</td>
<td>8.62 (219)</td>
<td>1.8 (0.82)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td></td>
<td>304L-05SF4-500</td>
<td>12.6 (324)</td>
<td>2.7 (1.2)</td>
</tr>
</tbody>
</table>

### Double-Ended Cylinders

<table>
<thead>
<tr>
<th>Material Grade/</th>
<th>Pressure Rating</th>
<th>Internal Volume</th>
<th>P in.</th>
<th>Ordering Number</th>
<th>Dimensions, in. (mm)</th>
<th>Weight (lb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cylinder Specification</td>
<td>(1 gil)</td>
<td>cm³ at 5 %</td>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>304L SS/</td>
<td>1800 (124)</td>
<td>40</td>
<td>1/8</td>
<td>304L-HDF2-40</td>
<td>1.25 (31.8)</td>
<td>0.31 (0.14)</td>
</tr>
<tr>
<td>DOT-3E 1800</td>
<td></td>
<td>50</td>
<td></td>
<td>304L-HDF4-50</td>
<td>3.75 (96.2)</td>
<td>0.38 (0.17)</td>
</tr>
<tr>
<td>TC-3EM 124</td>
<td></td>
<td>75</td>
<td></td>
<td>304L-HDF4-75</td>
<td>4.94 (125)</td>
<td>0.62 (0.28)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>150</td>
<td></td>
<td>304L-HDF4-150</td>
<td>5.25 (133)</td>
<td>0.94 (0.43)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300</td>
<td></td>
<td>304L-HDF4-300</td>
<td>8.94 (227)</td>
<td>1.6 (0.73)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>400</td>
<td></td>
<td>304L-HDF4-400</td>
<td>11.4 (290)</td>
<td>2.1 (0.95)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td></td>
<td>304L-HDF4-500</td>
<td>13.8 (351)</td>
<td>2.6 (1.2)</td>
</tr>
<tr>
<td>304L SS/</td>
<td>1800 (124)</td>
<td>1000</td>
<td>1/4</td>
<td>304L-HDF4-1000</td>
<td>3.50 (88.9)</td>
<td>6.5 (2.9)</td>
</tr>
<tr>
<td>DOT-3A 1800</td>
<td></td>
<td></td>
<td></td>
<td>304L-HDF8-1000</td>
<td>10.9 (277)</td>
<td>14 (6.4)</td>
</tr>
<tr>
<td>TC-3ASM 124</td>
<td></td>
<td></td>
<td></td>
<td>304L-HDF8-2250</td>
<td>17.2 (437)</td>
<td>21 (9.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>304L-HDF2-2250</td>
<td>20.6 (520)</td>
<td>21 (9.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>304L-HDF4-10AL</td>
<td>26.7 (676)</td>
<td>21 (9.5)</td>
</tr>
<tr>
<td>316L SS/</td>
<td>1800 (124)</td>
<td>150</td>
<td>1/4</td>
<td>316L-HDF4-150</td>
<td>5.25 (133)</td>
<td>0.94 (0.43)</td>
</tr>
<tr>
<td>DOT-3E 1800</td>
<td></td>
<td></td>
<td></td>
<td>316L-HDF4-300</td>
<td>8.94 (227)</td>
<td>1.6 (0.73)</td>
</tr>
<tr>
<td>TC-3EM 124</td>
<td></td>
<td></td>
<td></td>
<td>316L-HDF4-500</td>
<td>13.8 (351)</td>
<td>2.6 (1.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>316L-SDF4-150</td>
<td>8.00 (203)</td>
<td>3.0 (1.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>316L-SDF4-300</td>
<td>14.5 (368)</td>
<td>5.6 (2.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>316L-SDF4-500</td>
<td>23.5 (597)</td>
<td>9.1 (4.1)</td>
</tr>
<tr>
<td>316L SS/</td>
<td>5000 (344)</td>
<td>150</td>
<td>1/4</td>
<td>316L-SDF4-300</td>
<td>1.90 (48.2)</td>
<td>0.24 (0.1)</td>
</tr>
<tr>
<td>DOT-3A 5000</td>
<td></td>
<td></td>
<td></td>
<td>316L-SDF4-300</td>
<td>14.5 (368)</td>
<td>5.6 (2.5)</td>
</tr>
<tr>
<td>TC-3ASM 344</td>
<td></td>
<td></td>
<td></td>
<td>316L-SDF4-500</td>
<td>23.5 (597)</td>
<td>9.1 (4.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>316L-SDF4-500</td>
<td>23.5 (597)</td>
<td>9.1 (4.1)</td>
</tr>
<tr>
<td>Alloy 400/</td>
<td>150</td>
<td>M-HDF4-150</td>
<td>2.00 (50.8)</td>
<td>5.25 (133)</td>
<td>0.94 (0.43)</td>
<td></td>
</tr>
<tr>
<td>DOT-SP7-68 1500</td>
<td></td>
<td></td>
<td></td>
<td>M-HDF4-300</td>
<td>8.94 (227)</td>
<td>1.8 (0.82)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M-HDF4-500</td>
<td>13.8 (351)</td>
<td>2.9 (1.3)</td>
</tr>
</tbody>
</table>
Appendix VI

Table VI. Summary of test runs and operating parameters using RESS, SAS, and PGSS methods.

<table>
<thead>
<tr>
<th>Run No.</th>
<th>Loading weight (g)</th>
<th>Drug: excipient ratio</th>
<th>CO$_2$ or DME volume (mL)</th>
<th>Sample cylinder pressure (bar)</th>
<th>Sample cylinder temperature (°C)</th>
<th>Run time (min)</th>
<th>Results/ comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
<td>15</td>
<td>-</td>
<td>-</td>
<td>95.5</td>
<td>42</td>
<td>15</td>
<td>RESS</td>
</tr>
<tr>
<td>2</td>
<td>2.8</td>
<td>-</td>
<td>-</td>
<td>82.7</td>
<td>46</td>
<td>10-12</td>
<td>N$_2$/DME, RESS</td>
</tr>
<tr>
<td>3*</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>~82.0</td>
<td>40</td>
<td>10</td>
<td>SAS</td>
</tr>
<tr>
<td>4*</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>55.2 - 95.5</td>
<td>40</td>
<td>16</td>
<td>SAS</td>
</tr>
<tr>
<td>5*</td>
<td>20</td>
<td>1:10</td>
<td>-</td>
<td>83.4</td>
<td>50</td>
<td>30</td>
<td>PEG8000, low yield</td>
</tr>
<tr>
<td>6*</td>
<td>10</td>
<td>1:1</td>
<td>1500</td>
<td>89.9</td>
<td>40 ± 2.5</td>
<td>20</td>
<td>PEG4000, low yield</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>1:1</td>
<td>1550</td>
<td>89.9</td>
<td>35 ± 3.5</td>
<td>19.5</td>
<td>PEG4000, low yield</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>1:1</td>
<td>-</td>
<td>135</td>
<td>42 ± 2</td>
<td>69</td>
<td>PEG4000, RESS, methanol</td>
</tr>
<tr>
<td>9</td>
<td>10</td>
<td>1:1</td>
<td>-</td>
<td>100</td>
<td>65 ± 7.5</td>
<td>58</td>
<td>PEG4000, PGSS, subcritical</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>1:1</td>
<td>-</td>
<td>31.4</td>
<td>30</td>
<td>16</td>
<td>DME, PEG4000, subcritical</td>
</tr>
<tr>
<td>11</td>
<td>12</td>
<td>1:5</td>
<td>-</td>
<td>120</td>
<td>56 ± 2</td>
<td>17</td>
<td>PEG4000, moderate yield</td>
</tr>
<tr>
<td>12</td>
<td>12</td>
<td>1:5</td>
<td>-</td>
<td>124.1</td>
<td>37 - 41</td>
<td>25</td>
<td>TPGS, CO$_2$ flow off, low recovery</td>
</tr>
<tr>
<td>13</td>
<td>12</td>
<td>1:5</td>
<td>-</td>
<td>124.1</td>
<td>52 - 56</td>
<td>15</td>
<td>Gelucire 44/14, moderate yield</td>
</tr>
<tr>
<td>14</td>
<td>5</td>
<td>1:4</td>
<td>-</td>
<td>110.3</td>
<td>34 - 36</td>
<td>10</td>
<td>low yield</td>
</tr>
<tr>
<td>15</td>
<td>5</td>
<td>1:5</td>
<td>-</td>
<td>100.3</td>
<td>40 - 41</td>
<td>15</td>
<td>low yield</td>
</tr>
<tr>
<td>16</td>
<td>5</td>
<td>1:4</td>
<td>-</td>
<td>93.1</td>
<td>39 - 42</td>
<td>22</td>
<td>Gelucire44/14, low yield</td>
</tr>
<tr>
<td>17</td>
<td>5</td>
<td>1:5</td>
<td>-</td>
<td>106.9</td>
<td>35 - 37</td>
<td>14</td>
<td>TPGS, low yield</td>
</tr>
<tr>
<td>18</td>
<td>5</td>
<td>1:4</td>
<td>-</td>
<td>110.3</td>
<td>30</td>
<td>31</td>
<td>moderate yield</td>
</tr>
<tr>
<td>19</td>
<td>5</td>
<td>1:5</td>
<td>-</td>
<td>131</td>
<td>41 - 43</td>
<td>24</td>
<td>moderate yield</td>
</tr>
</tbody>
</table>
## Appendix VI

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>5</td>
<td>1:4</td>
<td>-</td>
<td>121</td>
<td>65 - 68</td>
<td>35</td>
</tr>
<tr>
<td>21</td>
<td>5</td>
<td>1:5</td>
<td>-</td>
<td>103.4 - 110.3</td>
<td>59 - 62</td>
<td>44</td>
</tr>
<tr>
<td>22</td>
<td>5</td>
<td>1:4</td>
<td>-</td>
<td>110.3</td>
<td>44</td>
<td>15</td>
</tr>
<tr>
<td>23</td>
<td>5</td>
<td>1:5</td>
<td>-</td>
<td>110.3</td>
<td>39 - 42</td>
<td>20</td>
</tr>
<tr>
<td>24</td>
<td>5</td>
<td>1:4</td>
<td>-</td>
<td>110.3</td>
<td>40</td>
<td>19</td>
</tr>
<tr>
<td>25</td>
<td>5</td>
<td>1:5</td>
<td>-</td>
<td>124.1</td>
<td>36</td>
<td>22</td>
</tr>
<tr>
<td>26</td>
<td>7</td>
<td>1:1</td>
<td>900</td>
<td>86.2</td>
<td>40 - 41</td>
<td>48</td>
</tr>
<tr>
<td>27</td>
<td>7</td>
<td>1:1</td>
<td>848</td>
<td>82</td>
<td>40 - 42</td>
<td>52</td>
</tr>
<tr>
<td>28</td>
<td>4</td>
<td>1:1</td>
<td>757</td>
<td>75.8</td>
<td>45 - 55</td>
<td>20</td>
</tr>
<tr>
<td>29</td>
<td>4</td>
<td>1:1</td>
<td>934</td>
<td>76</td>
<td>36 - 60</td>
<td>30</td>
</tr>
<tr>
<td>30</td>
<td>6</td>
<td>1:5</td>
<td>1034</td>
<td>82.7</td>
<td>31 - 54</td>
<td>55</td>
</tr>
<tr>
<td>31</td>
<td>3</td>
<td>1:5</td>
<td>1167</td>
<td>91.3</td>
<td>30 - 55</td>
<td>22</td>
</tr>
<tr>
<td>32</td>
<td>3</td>
<td>1:5</td>
<td>1150</td>
<td>67.3</td>
<td>60</td>
<td>18</td>
</tr>
<tr>
<td>33</td>
<td>3</td>
<td>1:5</td>
<td>1344</td>
<td>95.7</td>
<td>43</td>
<td>35</td>
</tr>
<tr>
<td>34</td>
<td>3</td>
<td>1:5</td>
<td>1300</td>
<td>82.7 - 87</td>
<td>46</td>
<td>36</td>
</tr>
<tr>
<td>35</td>
<td>3</td>
<td>1:5</td>
<td>1180</td>
<td>83</td>
<td>47</td>
<td>33-60</td>
</tr>
<tr>
<td>36</td>
<td>3</td>
<td>1:5</td>
<td>1230</td>
<td>80</td>
<td>49</td>
<td>46</td>
</tr>
<tr>
<td>37</td>
<td>9</td>
<td>1:5</td>
<td>1450</td>
<td>83 - 89.6</td>
<td>39</td>
<td>26</td>
</tr>
<tr>
<td>38</td>
<td>9</td>
<td>1:5</td>
<td>-</td>
<td>Subcritical</td>
<td>41</td>
<td>26</td>
</tr>
<tr>
<td>39</td>
<td>9</td>
<td>1:5</td>
<td>1000</td>
<td>&gt; 83</td>
<td>26</td>
<td>20</td>
</tr>
<tr>
<td>40</td>
<td>3</td>
<td>1:5</td>
<td>-</td>
<td>83</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>41</td>
<td>2.5</td>
<td>1:10</td>
<td>-</td>
<td>137.9</td>
<td>37</td>
<td>27</td>
</tr>
<tr>
<td>42</td>
<td>3</td>
<td>1:10</td>
<td>-</td>
<td>137.9</td>
<td>41 ± 5</td>
<td>34</td>
</tr>
</tbody>
</table>
Appendix VI

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>43</td>
<td>3</td>
<td>1:10</td>
<td>-</td>
<td>131</td>
<td>26 ± 1</td>
<td>26</td>
</tr>
<tr>
<td>44</td>
<td>3</td>
<td>1:10</td>
<td>1400-1500</td>
<td>124.1</td>
<td>27 ± 3</td>
<td>31</td>
</tr>
<tr>
<td>45</td>
<td>3</td>
<td>1:10</td>
<td>-</td>
<td>137.9 – 144.8</td>
<td>40 ± 5</td>
<td>22</td>
</tr>
<tr>
<td>46</td>
<td>9</td>
<td>1:10</td>
<td>-</td>
<td>131-137.9</td>
<td>39 - 40</td>
<td>26</td>
</tr>
<tr>
<td>47</td>
<td>9</td>
<td>1:10</td>
<td>1200-1300</td>
<td>110.3 – 117.2</td>
<td>38 - 40</td>
<td>15</td>
</tr>
<tr>
<td>48</td>
<td>9</td>
<td>1:10</td>
<td>950</td>
<td>79.3</td>
<td>40 ± 2</td>
<td>6</td>
</tr>
</tbody>
</table>

*run with 99.99% (SCF grade) CO₂.*
Appendix VII

Figure VII. Shows untreated PGN *in vitro* dissolution under sink conditions showing constant dissolution into aqueous solution over 180 minutes. The solubility of pure PGN is approximately 7 to 9 µg·mL⁻¹, therefore in 900 mL dissolution units the maximum amount of PGN that could be used is 8.1 mg. For this study, to ensure sink conditions for PGN dissolution, there was 30 to 50 %w/v of the maximum amount used.
Appendix VIII

Figure VIII. Photos of recovered samples from the experimental design. X1 for example shows loose solid state particles, while X3 is a semi-solid.
Figure IX: Representative dual first order plot for SCF dispersion system containing Gelucire 44/14 showing non linear fit which in this case used 8 iterations and had a regression of 0.987
Appendix IX

Appendix X

Figure X. Molecular structures for selected penetration enhancers.
Appendix XI

Figure XI(a). Permeation profiles of PGN delivery from various formulations across porcine ears. Control = market cream (non ionic o/w cream).

Figure XI(b). The observed dermal swelling could offer a possible mechanism for the permeation of PGN through porcine skin. Light microscopic images (200 × magnification) of porcine skin. 5A = Untreated skin, 5B = water control, 5C = conventional representative (CS sample), 5D = market cream, 5E = SCF-Gelucre 44 dispersion, 5F = SCF-Gelucre 44 (4 times magnification). SC = stratum corneum, Fr = fragmentation, Oe = edema and Dr = dermis.