Development of an Intra-peritoneal Implant for the Sustained Release of Lidocaine Following Abdominal Surgery

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Abstract

Introduction: Patients experience post-operative pain and fatigue following abdominal surgery due to damage to the peritoneal lining of the abdominal cavity. Recent studies have demonstrated that it is possible to decrease pain and fatigue effectively through the continuous infusion of local anaesthetic into the peritoneal cavity for 72 h following abdominal surgery. It seems likley that additional benefits may be evident if delivery of local anaesthetics could be prolonged over 10 days. Poly(ethylene-co-vinyl acetate) (EVA) has a long history of clinical use as a polymeric carrier to provide sustained release of drug over an extended period of time. However, drug solubility in EVA matrices is often limited, leading to instability issues with the potential for dispersed drug to recrystallise. The aim of this thesis was to develop a controlled release EVA based formulation capable of releasing lidocaine in a sustained manner, for use following abdominal surgery. **Methods:** A stability indicating high-performance liquid chromatography (HPLC) method was developed for the quantification of lidocaine from forced degradation samples, EVA matrices and biological fluids using an Agilent Series 1260 HPLC system. The compatibility of lidocaine with EVA was then assessed using various analytical tools, including thermo gravimetric analysis, differential scanning calorimetry, nuclear magnetic resonance spectroscopy, Fourier transform infrared spectroscopy (FTIR) and HPLC. Additionally, potential interactions between lidocaine and EVA, alongside fatty acid additives, were investigated using FTIR. A detailed characterisation of human peritoneal fluid collected following abdominal surgery was performed to determine its composition, physicochemical properties and rheological parameters to build an understanding of the intra-peritoneal environment. The key physicochemical properties of peritoneal fluid were then compared with phosphate buffered saline, a commonly used synthetic media for the evaluation of the *in vitro* release performance of intra-peritoneal drug delivery systems. Finally, the differences between peritoneal fluid and phosphate buffered saline were further explored by comparing lidocaine solubility and the release performance of the EVA based formulation in the two media. **Results and Discussion:** An efficient and cost-effective quantification method was developed to determine lidocaine from force degradation samples, EVA matrices and biological fluids. Compatibility studies confirmed the stability of lidocaine in the presence of EVA, regardless of vinyl acetate composition. An interaction was demonstrated between EVA and lidocaine by FTIR, possibly in the form of hydrogen bonding. This interaction was more pronounced in the presence of myristic acid, resulting in the formation of a transparent formulation thereby demonstrating the fatty acid's ability to prevent the recrystallisation of lidocaine in EVA matrices. The composition and physicochemical properties of peritoneal fluid varied between different patients and within the same patient over time. Inter-patient variations were observed with regard to pH ($p<0.001$), buffer capacity ($p<0.05$), osmolality ($p<0.001$) and surface tension ($p<0.05$). Rheological examination of peritoneal fluid demonstrated non-Newtonian shear thinning behaviour and the fluid predominantly exhibited the characteristics of an entangled network. The investigated physicochemical properties of peritoneal fluid all differed from phosphate buffered saline ($p<0.001$). The solubility of lidocaine was significantly higher in peritoneal fluid compared to phosphate buffered saline. The release of lidocaine occurred in a sustained manner from EVA matrices in both peritoneal fluid and phosphate buffered

saline, with a relatively higher release rate of lidocaine observed in peritoneal fluid. When the experimental set-up was changed from sink to non-sink conditions the release rate slowed, possibly due to the formation of a boundary layer. **Conclusions:** Detailed investigations were performed supporting the development process for an implantable formulation for the sustained delivery of lidocaine into the peritoneal cavity. EVA was shown to be a suitable polymer to disperse lidocaine and to achieve controlled release over 10 days. The properties of peritoneal fluid are markedly different to phosphate buffered saline, and release of lidocaine from the formulation occurred at a faster rate into peritoneal fluid than the synthetic phosphate buffered saline. Future work could seek to develop a bio-relevant media for intra-peritoneal drug delivery systems. To translate the data in this thesis into an implant ready for patients, future studies should focus on evaluating formulation performance in animal models to assess local and systemic toxicity before proceeding into human clinical trials.

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Nature of contribution by PhD candidate	Compiling articles, writing first draft and work on the commentes made by the co-authors
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Certification by Co-Authors

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Dr Darren Svirskis		20/04/2017

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Table of Contents

List of Figures

List of Tables

LIST OF ABBREVIATIONS

AF	Atrial fibrillation
ANOVA	Analysis of variance
API	Active pharmaceutical ingredient
CHL	Chloramphenicol
CNS	Central nervous system
COPD	Chronic obstructive pulmonary disease
CR	Controlled release
DCM	Dichloromethane
DSC	Differential scanning calorimetry
ECF	Extracellular fluid
EVA	Poly(ethylene-co-vinyl acetate)
FA	Fluocinolone acetonide
FDA	Food and drug administration
FTIR	Fourier transform infrared spectroscopy
G´	Elastic modulus
G˝	Viscous modulus
GT	Gordon-Taylor
GX	Glycine xylidide
HDEC	Health and Disability Ethics Committee
HDL	High density lipids
HME	Hot melt extrusion
HPLC	High performance liquid chromatography
HPMC	Hydroxyl propyl methyl cellulose

HTN	Hypertension
IAF	Intra-articular fluid
ICH	International conference on harmonization
IHD	Ischemic Heart Disease
IP	Intraperitoneal
IPDDS	Intra-peritoneal drug delivery systems
LA	Local anaesthetics
LDL	Low density lipids
LOD	Limit of detection
LOQ	Limit of quantification
LVR	Linear viscoelastic region
MEC	Minimum effective concentration
MEGX	Mono-ethyl glycine xylidide
MFI	Melt flow index
MIC	Minimum inhibitory concentration
MTC	Minimum toxic concentration
NMR	Nuclear magnetic resonance
NOES	Nuclear overhauser effect spectroscopy
NSAIDs	Non-steroidal anti-inflammatory drugs
NTI	Narrow therapeutic indices
PBS	Phosphate buffered saline
PCA	Patient controlled analgesia
PCTDDS	Patient controlled transdermal drug delivery system
PDA	Photo-diode array
PDMS	Polydimethylsiloxane

PEEK	Polyether ether ketone
PF	Peritoneal fluid
PLGA	Poly(lactic-co-glycolic acid)
POF	Post-operative fatigue
PONV	Post-operative nausea vomiting
SA	Sodium azide
SSI	Surgical site infection
T2DM	Type 2 Diabetes mellitus
TDDS	Transdermal delivery systems
TDS	Transdermal delivery system for scopolamine
T_g	Glass transition temperature
TG	Triglycerides
TGA	Thermo-gravimetric analysis
THF	Tetrahydrofuran
ULAR	Ultra-low anterior resection
VA	Vinyl acetate
XRD	X-ray diffraction spectroscopy

CHAPTER 1. INTRODUCTION

Most surgeries result in the formation of a somatic wound, but in the case of abdominal surgery the somatic wound is accompanied by an autonomic wound due to incision of peritoneal tissue. The resulting post-operative pain and fatigue following abdominal surgery are distressing to the patient, and in the case of postoperative fatigue can last for months (1). Recent studies have shown that it is possible to decrease pain and post-operative fatigue and enhance post-surgical recovery, by the continuous infusion of local anaesthetic (LA) into the peritoneal cavity over 72 h (2-4). It is hypothesised that additional benefit could be gained if the delivery of LA could be prolonged for a period of 10 days. Uninterrupted infusion of LA into the peritoneal cavity is generally achieved by an elastomeric pump or some other infusion device. However, the risk of infection due to prolonged catheter placement and the potential for the catheter to dislodge from its intended administration site are of concern (5). Considering the inconvenience associated with these devices, a more suitable alternative for the prolonged delivery of LA is needed.

Controlled release (CR) systems have been employed to overcome the limitations of conventional LA delivery due to their ability to release LA over a prolonged period of time. Various types of CR delivery systems containing LA for local application have been investigated and commercialised, including liposomes (6), gels (7), films (8) and patches (9). EXPAREL® is liposomal CR system which delivers LA over a period of 72 h (10), but reports have surfaced demonstrating no improved efficacy compared to traditional injectable solution (11). Further, the incidence of premature leakage of particulate CR systems like liposomes from the peritoneal cavity make them inappropriate for our purpose (12). Issues around dose dumping and the inability to remove the local anaesthetic in the event of toxicity are additional limitations (13). Accordingly, there is a need for a CR system that can be recovered from the body after achieving clinical goal, or in cases of LA related toxicity.

Solid implantable systems are widely recognised for being simple to produce and scale up and for their capacity to release a drug in a controlled manner for a prolonged period of time. Non-biodegradable polymers don't erode inside the body. They retain their physical and mechanical properties and are thus well suited be developed as recoverable implants. There is a long clinical history of using non-biodegradable polymers to develop recoverable implants such as Norplant® (14) and Implanon® (15). A range of non-degrading biocompatible materials have been used to achieve constant levels of drug release, including silicon (16) and meth-acrylate based co-polymers (17). Poly(ethylene-co-vinyl acetate) (EVA) is a non-biodegradable polymer with proven biocompatibility, and has been used in numerous biomedical applications (18, 19). EVA is the carrier selected for this study for the development of a sustained release formulation for LA because of its non-biodegradable nature and flexibility for addressing implantation and removal issues.

Lidocaine has been chosen as the model LA for this study due to its short-acting nature and suitable safety profile compared to other LAs. However, lidocaine may undergo various transformations during formulation preparation or storage. A suitable quantifying method is necessary to monitor formulation efficacy and stability of lidocaine at each step of formulation development. Further, the method should enable the quantification of lidocaine in the presence of degradation related impurities, EVA and

biological fluids. Previously reported methods have limitations as they are specific to a particular analytical purpose. A stability indicating HPLC method with broader applications will need to be developed for lidocaine before moving into the preformulation studies.

Assessment of the compatibility of a drug with polymeric excipients is crucial before developing formulations for CR systems. The main challenges to prepare a stable EVA formulation containing lidocaine are the incidence of degradation, phase separation and recrystallisation of lidocaine in the EVA matrix. Interactions between lidocaine and EVA could make the formulation stable by sustaining the supersaturated state of lidocaine in EVA matrices. An understanding of lidocaine compatibility with EVA and potential interactions will provide useful information for preventing or slowing down lidocaine recrystallisation in the EVA matrices.

The developed formulation will be surrounded by peritoneal fluid (PF) following its intra-peritoneal administration into which the release of lidocaine will occur. The release performance of a formulation will be partially governed by the environment in which it performs. Therefore, an understanding of PF is necessary to predict the true *in vivo* release performance of the developed formulation following implantation. Generally, the release performance of any CR system is determined *in vitro* in bio-relevant media to predict the outcomes expected *in vivo*. Phosphate buffered saline (PBS) is typically used as a synthetic release media for intra-peritoneal drug delivery systems (20). However, it is not known how well PBS mimics the PF environment. Very few investigations into the properties of PF have been reported and no bio-relevant media for intra-peritoneal drug delivery systems have been described previously. Therefore, a detailed investigation of PF will be helpful in understanding the composition and properties of PF with relevance to the performance of lidocaine containing EVA-based formulation.

1.1 Thesis aims

The overall aim of this project is to develop a controlled release EVA-based formulation loaded with lidocaine. As EVA is non-biodegradable and has a long history of clinical use as a CR implant, this polymer is suitable for this purpose. It is hypothesised that EVA can be loaded with lidocaine to provide sustained release of lidocaine into the peritoneal cavity over a period of ten days.

The objectives of my thesis are to:

- Develop and validate a stability indicating HPLC method to quantify lidocaine in EVA matrices and biological fluids
- Perform preformulation studies of lidocaine and EVA to support the formulation of a CR formulation
- Investigate human peritoneal fluid to determine its composition, physicochemical properties and rheological parameters
- Characterise the solubility of drugs and the release performance of a controlled release formulation into peritoneal fluid and phosphate buffered saline.

1.2 Thesis structure

A literature review presented in **Chapter 2** first provides a broad overview of contemporary pharmacotherapy employed in post-operative care, including current limitations. Focus has been made on our intended application to treat pain and post-operative fatigue following abdominal surgery. The local anaesthetic lidocaine, the polymeric carrier EVA, intra-peritoneal drug delivery systems and the need for an in-depth investigation of peritoneal fluid are then discussed accordingly.

Chapter 3 describes the development of an analytical method for lidocaine and demonstrates its wide application to quantify lidocaine from forced degradation samples, EVA matrices and biological fluids.

A description of preformulation studies of lidocaine and EVA is outlined in **Chapter 4**. Compatibility of lidocaine with EVA followed by an interaction study between lidocaine and EVA are then presented.

Chapter 5 provides a comprehensive investigation of the composition, physicochemical properties and rheological parameters of human peritoneal fluid.

The release performance of developed EVA formulation in phosphate buffered saline and peritoneal fluid is described in **Chapter 6**.

Finally, **Chapter 7** summarises the outcomes from this thesis and provides future directions.

CHAPTER 2. LITERATURE REVIEW

2.1 Controlled release drug delivery systems

Controlled release (CR) technologies logically combine various approaches to regulate the release rate of a drug at a target site for a particular duration of time (21). Unlike immediate release dosage forms, special polymers or encapsulating agents are used in CR systems by which the drug is released safely in a controlled fashion for a prolonged period of time (Figure 2.1) (22).

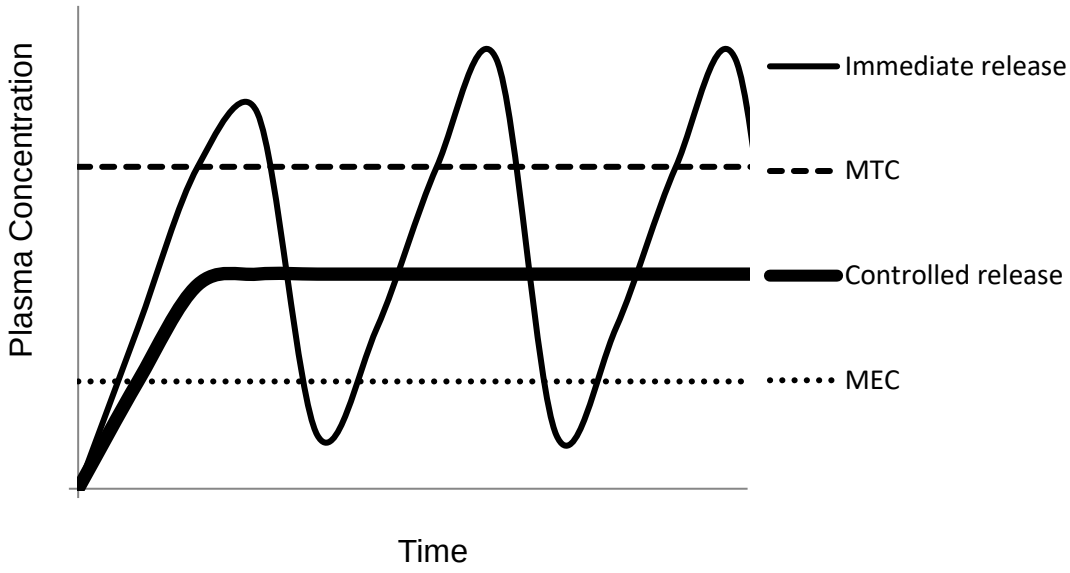

Figure 2.1. Medicine plasma concentrations resulting from repeated administration of an immediate release dosage form and from the single administration of a controlled release system.

The choice of the polymer/encapsulating agent is crucial in designing a controlled release formulation because of its influence over drug release (23). Traditional CR systems include macro carrier systems such as matrix, reservoir and osmotic systems (24) (Figure 2.2). In matrix systems, the drug is dispersed in a polymeric matrix and drug elution occurs via erosion and/or diffusion of the drug. Reservoir systems consist of a drug concentrate core encapsulated with a membrane and release occurs via diffusion (25). Osmotic systems consist of a drug concentrate core surrounded by a semi-permeable membrane which only allows water to permeate through to generate osmotic pressure and thereby render the controlled release of the drug through an aperture (26).

Advanced CR systems consist of micro CR and nano CR systems. Micro CR system are often called microparticles and include the carriers microspheres, microcapsules and microemulsions, where the drug is entrapped/dispersed in polymeric or lipid carriers with a diameter in the micrometer range (27). Nano CR systems include liposomes, nanoparticles, polymeric micelles and nanosuspensions, all of which are in the nanometer range (24).

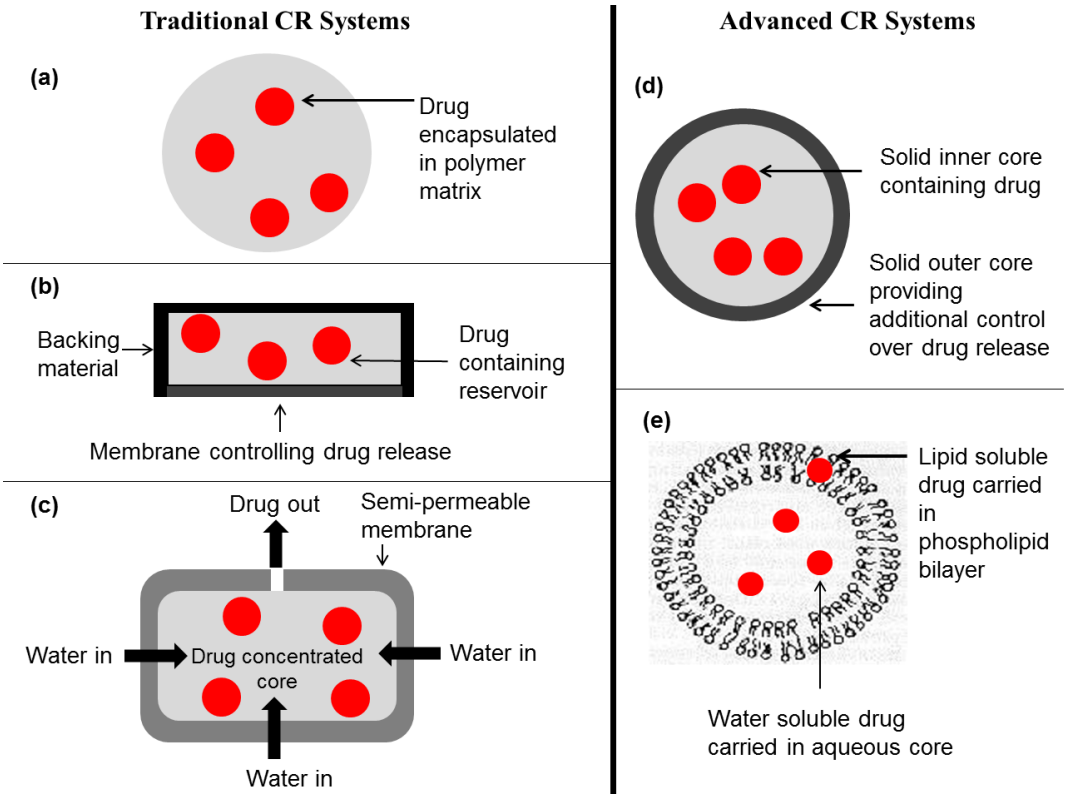

Figure 2.2. Schematic diagram of (a) matrix system; (b) reservoir system and (c) osmotic system; (d) core-shell microsphere and (e) structure of unilamellar liposomal vesicle where drug can be loaded into the aqueous core or the lipid bilayer

Implantable CR systems can be made from biodegradable or non-biodegradable materials. Biodegradable implants degrade slowly inside the body and release of drugs occurs both by erosion and diffusion. Meanwhile, non-biodegradable implants do not degrade inside the body and drug is released by diffusion. These classes of CR systems are particularly useful when controlled release of a drug over a relatively longer period is required. For instance, Implanon® and Norplant® can release contraceptives over a period of five years (14, 15). They have an additional advantage in that they can be removed when their therapeutic activity is no longer required. It is also possible to engineer implantable devices as 'smart systems' where release of drugs can be tuned. Recent research has demonstrated CR systems which allow drug release to be increased or decreased as required. These CR systems can alter the release rate of drugs in response to a trigger which can be in the form of heat, light, pH, magnetic field, ultrasound, mechanical force (28), electric current (29) or biochemical species (30).

2.2 Controlled release drug delivery systems in post-operative pharmacotherapy

Each year more than 230 million surgical procedures are conducted worldwide and this number is expected to increase in the future (31). Surgery results in the alteration of various physiological and

psychological conditions due to which the patient may experience a range of symptoms including pain, post-operative nausea and vomiting (PONV), inflammation, infection and post-operative fatigue, collectively called post-operative complications. These complications negatively influence a patient's wellbeing and delay post-operative recovery, resulting in prolonged hospital stays and ultimately placing a burden on patients and incurring societal costs (32). The appropriate management of these post-operative complications is essential to promote a patient's post-operative recovery and shorten their hospital stay. With significant decreases in operative mortality over the last 50 years (33), the emphasis in post-operative care has increasingly been on the management of these post-operative complications. Pharmacotherapy is an essential domain of post-operative care but predominantly relies on immediate release dosage forms which are sub-optimal in their design. Immediate release dosage forms release their active drug load rapidly following their administration, thus requiring frequent administrations to achieve the desired therapeutic effect. Typically, frequent administration results in fluctuating levels of drugs in the body, thereby risking toxicity during times of peak plasma concentrations and treatment failure during troughs. CR dosage forms of medicine have the ability to maintain drug levels within the therapeutic range for a prolonged period of time and thus improve treatment efficacy and reduce adverse effects (Figure 2.1) (34).

The controlled delivery of pharmacotherapy can improve the management of post-operative pain, inflammation and infection, promoting faster post-operative recovery. Certain CR dosage forms of medicines are widely used in post-surgical settings, including oral controlled release opioid tablets and capsules. They have the obvious benefit of providing up to 24 hours of continuous analgesic cover by releasing their drug load slowly (34). In post-operative pharmacotherapy, opioids, local anaesthetics (LA), potent non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids and antibiotics can and do provide benefit following their formulation into CR dosage forms. A range of controlled delivery systems have been employed in post-surgical settings, ranging from simple matrix systems and drug reservoirs with rate controlling membranes to those relying on nanotechnology. Oral, transdermal and injectable dosage forms are often used to administer these CR systems to prolong the delivery of drugs for one or a few days. In addition to dedicated drug delivery systems, medical devices augmented with CR drug delivery functionality have also been used. Examples of these include surgical sutures (35, 36), wound dressing (37, 38), hernia repair mesh (39, 40), central lines and catheters (41, 42), bone cements (43) and orthopaedic implants (44). This thesis is focused towards the development of a controlled release formulation to treat pain and post-operative fatigue following abdominal surgery. However, there are other common post-operative complications in addition to pain and post-operative fatigue such as inflammation and infection. It is possible that the developed formulation described in this thesis could be adapted to deliver drugs for the treatment of infection range of post-operative complications. Therefore, common post-operative complications are discussed next to give a broader view of current options for post-operative pharmacotherapy including CR systems.

2.2.1 Pain

Following surgery, pain reduces patient quality of life, slows recovery and prolongs hospital stays. Somatic wounds result in multiple sympathetic and inflammatory responses activating peripheral nociceptors which transmit information to the central nervous system resulting in the sensation of pain (45). It has been reported that 50 to 70% of patients experience moderate to severe pain following surgery (46). Effective pain management after surgery has become a focal point of post-operative care.

Opioids which act on the central nervous system are the first-line analgesic agents for controlling post-operative pain. However, due to their adverse effects, such as drowsiness, respiratory depression, gastrointestinal and bladder dysfunction, and their potential for misuse and addiction, alternative treatment options have been actively sought (47). Opioids have short elimination half-lives (48), thus conventional dosage forms require regular administration to achieve prolonged pain relief. The resulting fluctuations in plasma concentrations may cause patients to experience breakthrough pain when concentrations fall below the minimum effective concentration (MEC), and adverse reactions when concentrations exceed the minimum toxic concentration (MTC). Patient-controlled analgesia (PCA) enables patients to self-administer analgesic agents based on pain levels, with the aid of infusion pumps or electronic devices, and can help maintain plasma concentrations within the therapeutic window (49). However, in one study up to 12% of patients reported finding PCA systems complex and difficult to use, resulting in inadequate pain relief or dose related adverse events (50). A retrospective study conducted with surgical patients revealed that patients exhibiting opioid-related adverse events had longer hospital stays with higher total health care costs, alongside a higher probability of hospital readmission (51). To reduce the dosing requirements of opioids, a combination of NSAIDs or local anaesthetics (LAs) with opioids can be an effective way to manage post-operative pain while decreasing total opioid consumption and opioid related adverse effects (52). Through their direct action on affected tissues, NSAIDs are effective analgesics widely used on their own or in combination with other analgesics (53). However, irritation to local tissues and gastric mucosa following both local and systemic administration of NSAIDs is of concern, as well as their potentially harmful effects on the renal system. Endoscopic evidence of gastric and duodenal ulcers has been reported in 15 to 25% of NSAID users (54). NSAID use is contraindicated in many patient groups due to these adverse effects. LAs are widely used as nerve blocking agents to prevent the transmission of pain signals from disturbed tissue, but their short half-lives limit the period of effect. LAs are commonly administered by injection or infiltration at the surgical site to provide temporary analgesia. Elastomeric pumps have been used to prolong analgesic effects. However the infection risks due to prolonged catheter placement and the potential of the catheter to be dislodged require frequent monitoring by trained staff, typically in an in-patient setting, which increases the total health care costs (5). To achieve sustained effects large bolus doses of LAs can be administered to local tissues, however there are risks of systemic toxicity and local ischaemia with this approach (55).

CR systems for post-operative pain are primarily formulated to either increase the duration of effect or decrease adverse effects. Oral CR tablets and capsules containing opioids and NSAIDs are well known and frequently prescribed post-operatively. The first commercially available controlled release delivery

system for opioids was MSContin® (48). It is a tablet comprising a polymeric mix where morphine sulfate is blended with both hydrophilic (hydroxypropyl methylcellulose) and hydrophobic polymers (hydroxyl ethyl cellulose). The hydrophilic component allows rapid release of morphine sulphate, whereas the hydrophobic component achieves sustained release. Similarly, other commercially available examples of CR formulations for opioids include Oramorph® SR (morphine sulphate extended release tablets), Avinza® (morphine sulphate extended release capsules) and Kadian® (morphine sulphate extended release capsules). Oramorph® SR is an extended release tablet, where morphine sulfate is blended with hydroxyl propyl methyl cellulose (HPMC). This hydrophilic polymer forms a gel-like structure in the aqueous environment after ingestion which retains its structure for some time to slow the release rate of morphine sulfate from the tablet (48). Avinza® comprises a mixture of immediate-release and extended-release components in hard gelatin capsules. The immediate-release component consists of sugar/starch spheres that enable rapid release of morphine, whereas the extended-release component is the same sugar/starch spheres coated with an ammonium methacrylate copolymer. The penetration of fluids is slowed due to the coating and is mediated by a local pH modifier (fumaric acid) to achieve sustained release of morphine sulfate (48). Kadian® is also an extended release capsule consisting entirely of a polymeric blend of water insoluble ethyl cellulose and water soluble polyethylene glycol and methacrylic acid copolymer (48). The efficacy of these CR systems has been demonstrated in terms of maintaining plasma drug levels, reducing dosing frequency and improving treatment adherence (34). However, oral CR systems containing opioids are less flexible compared to fast acting dosage forms when dosing adjustments are required; safe and effective regimes are difficult to achieve with CR forms alone when pain levels are changing. In addition there are concerns around incorrect use leading to toxicity; if the dosage form is broken, chewed or crushed, the full dose may be rapidly absorbed rather than absorbed slowly over an extended time, resulting in concentrations in the body reaching toxic levels.

Not only are NSAIDs formulated into oral CR systems to achieve sustained release, but enteric-coated formulations are also designed to reduce the cumulative exposure of NSAIDs on the upper GI tract epithelium. However, it is important to note that enteric-coated systems which do not release the drug into the stomach are only partially successful in reducing NSAID related GI complications due to the distal portion of the intestine being exposed to the drug as it released locally, and the entire GI tract being exposed to NSAIDs following systemic distribution (56). An indomethacin containing oral CR system (Osmosin®) was developed commercially based on an osmotic platform. But, it was withdrawn from the market after reports of intestinal ulceration in elderly patients, possibly due to high local concentrations of indomethacin following lodging of the tablet in intestinal diverticula (57).

While the oral route is frequently preferred by both patients and health care professionals, it is not always a practical option in patients unable to swallow, or where gastro-intestinal function is compromised. Transdermal delivery systems (TDDS) for opioids such as Durogesic® (fentanyl matrix or reservoir systems) and Butrans® (buprenorphine matrix systems) can be beneficial in providing baseline cover by opioids. However, a prolonged lag time following the positioning of the TDDS on the skin can be an issue in their post-operative use. Lag times and absorption rates may differ according to the site of application and during activities of daily living as the rate of blood flow changes across various body parts (58),

leading to inter- and intra-patient variability. Meanwhile, removal of a patch in the event of side effects does not result in rapid resolution of the side effects as drug partitioned into the skin continues to be distributed systemically for some time. A patient controlled transdermal delivery system (PCTDDS) has been designed to overcome the problem of conventional TDDS for opioids. IONSYS® (fentanyl HCl PCTDDS) is a FDA approved TDDS which can be applied to the upper arm or chest (59). An electric current, triggered by the patient, is used to charge the drug molecule and drive it through the skin by iontophoresis (58).

Administering pain relief parenterally allows treatment to reach the site of action rapidly. While the invasive nature of parenteral administration is of concern, the development of injectables with controlled release properties can reduce the required frequency of administration, and therefore the total number of injections. Parenteral controlled release systems such as microspheres and liposomes have been explored as a way of prolonging post-operative analgesia (60). These micro- and nano-carriers can solubilise a wide range of drugs allowing them to be injected and achieving prolonged analgesic effects. A range of analgesics and anaesthetics, such as morphine, noroxymorphone, bupivacaine, lidocaine and ibuprofen, have been developed into liposomal formulations to alleviate post-operative pain. DepoDur®, an injectable liposomal formulation containing morphine sulphate, was approved by the Food and Drug Administration (FDA) for epidural use in 2004 (61). This formulation is based on the proprietary extended release platform technology DepoFoam®, which is comprised of multivesicular lipid particles with nonconcentric aqueous chambers to encapsulate morphine sulphate and render sustained release of the drug (62). DepoDur® was developed with the aim of providing post-operative analgesia for up to 48 hours (63). Its efficacy has been demonstrated in a wide range of surgeries including hip replacement surgery (64), lower abdominal surgery (65) and elective caesarean section delivery (66). EXPAREL®, another liposomal formulation containing bupivacaine and based on the same DepoFoam® platform (10), was approved by the FDA in October 2011. A bolus injection of EXPAREL® (266 mg of bupivacaine) has been reported to provide analgesia for up to 72 hours following knee replacement surgery (67). However, research results for EXPAREL® are mixed. Bagsby et al. conducted a retrospective cohort study to compare the efficacy of EXPAREL® against traditional periarticular injections to control pain following total knee replacement surgery (11). Inferior pain control was reported for EXPAREL® and it is considerably more expensive than traditional injections.

Injectable CR systems for pain relief must address concerns over local toxicity caused by the drug. McAlvin et al. reported inflammation with nerve and tissue injury following the injection of lidocaine and bupivacaine loaded microspheres into rats for sciatic nerve blockade (68). In addition to local toxicity, there are safety concerns following their inevitable systemic distribution. Recovery of these injectable CR systems is extremely difficult and practically often impossible, which is of concern in the event of toxicity or adverse drug reaction. In a randomised controlled trial, around 12.5% of patients required an opioid antagonist following DepoDur® administration to manage post-operative pain after caesarean surgery due to the adverse effects caused by systemic distribution of opioids (69).

Combining drugs with medical devices such as surgical sutures (70, 71), wound dressings (37) and hernia repair mesh (72) to provide sustained pain relief have been investigated. Developing surgical sutures as CR systems is challenging as mechanical strength must be retained following drug incorporation. In a recent study, a sheet of biodegradable polymeric film comprising poly(lactic-co-glycolic acid) (PLGA) and ibuprofen was separately prepared and then later braided around commercially available VICRYL W9114® sutures (Figure 2.3. a). (70). A burst of 75% to 85% release was observed over the first day followed by sustained release over a further six days (Figure 2.3.b). Wound dressing was prepared from silicon oxide (xerogel) microparticles embedded in copolymer matrices to provide near zero order release of bupivacaine and mepivacaine over a period of seven days (37).

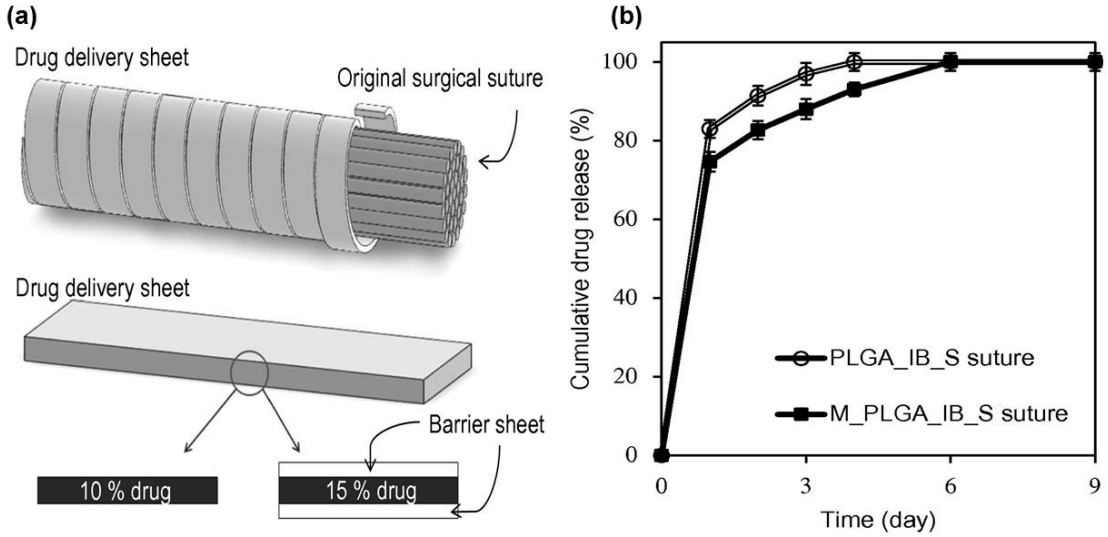

Figure 2.3. Schematic diagram showing preparation of drug-loaded sutures. (a) An ibuprofen loaded polymeric sheet is separately prepared and physically braided around the suture. Suture coated with single-layered sheet of PLGA loaded with 10% w/w ibuprofen (PLGA_IB_S) and suture coated with multi-layered sheets of PLGA loaded with 15% w/w ibuprofen and sandwiched between the sheets of PLGA (M-PLGA_IB_S). (b) In vitro drug release from PLGA_IB_S and M_PLGA_IB_S sutures. Reprinted with permission from Elsevier (70).

However, with all these CR systems drug release occurs at a predetermined rate which cannot match the changing analgesic needs of individual patients. Future CR systems for post-operative pain should be engineered in such a way that release rates can be tuned according to an individual patient's needs.

2.2.2 Post-operative nausea and vomiting (PONV)

Following surgery one third of all patients experience PONV (73) and it commonly lasts for 24 h (74, 75). PONV affects patients' quality of life and in extreme cases may lead to dehydration, electrolyte imbalance, venous hypertension, bleeding, haematoma formation, and suture and oesophageal rupture

(76). Irritants directly trigger the vomiting centre, however indirect signals from the gastrointestinal tract, cerebral cortex, thalamus and vestibular region may also trigger the chemoreceptor trigger zone (CTZ) leading to episodic nausea (77). Anticholinergics, phenothiazines, antihistamines, butyrophenones and benzamides are the traditional medicines used to manage PONV. Newer classes of antiemetic medicines include 5-HT_3 receptor antagonists such as ondansetron and granisetron (78).

As conventional regimens with single or multimodal regimens of antiemetics can readily manage common PONV, there is no great need for CR systems to address PONV. However, there are some CR systems for the management of PONV such as Transderm Scop®, a transdermal delivery system for scopolamine (TDS). It is generally administered 24 h before surgery and delivers scopolamine in a constant manner over a period of 72 h.

2.2.3 Inflammation

After surgery, alterations in the neuroendocrine, metabolic and immune systems stimulate the systematic release of inflammatory modulators including cortisol, catecholamines, acute phase reactants and cytokines (79). An appropriate balance between pro-inflammatory and anti-inflammatory modulators is essential to promote wound healing and tissue repair. Exaggerated pro-inflammatory or anti-inflammatory responses can prolong surgical recovery and, if extreme, can be fatal (80).

Corticosteroids, NSAIDs (81) and LAs are all effective for down-regulating inflammation. Corticosteroids are highly effective medicines for reducing inflammation and are used following ophthalmic, oral and dental surgery. Systemic administration of corticosteroids requires high doses to achieve the required concentrations at the intended site of action. This is exaggerated when the intended site of action has poor blood supply, such as ophthalmic tissues and bone. Exposure of systemic tissues to steroids results in well recognised adverse events including delayed wound healing, metabolic disturbances, hypertension and osteoporosis. Post-operative dosing with corticosteroids occurs over days and up to several weeks (82, 83). In the eye, direct sub-conjunctival injection is an option to avoid non-specific drug accumulation following ocular surgery and to increase ocular permeability, but this route of administration requires a highly skilled health professional and has poor acceptability among patients. Achieving therapeutic concentrations of corticosteroids in poorly vascularised tissues without compromising side effects is the major challenge with conventional regimens. This challenge indicates the need for an ideal anti-inflammatory dosage form, which preferentially releases medicines locally at the site of surgery.

CR systems are used to deliver corticosteroids locally and over a prolonged period following surgery to tissues with a limited blood supply. Surodex® is a dexamethasone containing biodegradable implant developed to control post-operative inflammation following cataract surgery (84). It contains 60 µg of dexamethasone incorporated into a poly(lactic-glycolic)-acid (PLGA) matrix that controls the release of dexamethasone over seven days, achieving higher intraocular drug levels than conventional dexamethasone drops (84).Ozurdex®, Retisert® and Iluvien® are all ocular implants which release

corticosteroids over six, 30 and 36 months respectively, and are indicated for use in macular edema and uveitis (85). Ozurdex® is based on the NOVADUR® CR platform, where dexamethasone is fabricated with the biodegradable PLGA to form a small rod shaped implant. The implant slowly degrades following administration, allowing controlled release of dexamethasone as it diffuses through the polymer and as the polymer erodes (86). Retisert® and Iluvien® are flucinolone acetonide (FA) containing non-biodegradable implants, based on silicone elastomers and polyimide/poly vinyl alcohol respectively, which allow the slow release of FA through diffusion. These CR systems deliver anti-inflammatory compounds locally to tissues hard to access from the blood supply, while minimising systemic exposure and thereby reducing systemic adverse events. There is potential for similar approaches to be used to modulate undesirable inflammation following joint and bone surgeries where blood supply is poor or compromised.

2.2.4 Infection

There is an increased risk of infection during and following surgery as the body's natural defenses are compromised. Approximately 2% of surgical patients are affected by surgical site infection (SSI), although incidence rates vary by surgery type (87). The overall cost of SSI treatment in the US is estimated at $US 10 billion annually (88). Development of antimicrobial resistant microbes is a serious concern, as they significantly increase both mortality and morbidity. Infections caused by antimicrobial resistant strains are often very difficult to treat, which ultimately increases the length of hospital stay and treatment costs (89). Peri-operatively, frequent administration of high doses of antibiotics orally and/or parenterally are required to achieve and maintain minimum inhibitory concentrations (MIC) in the target tissues. However, certain target tissues, such as within joints, have a poor blood supply with high systemic concentrations required to achieve MIC at the target site. For some avascular sites, such as bone, it is virtually impossible to achieve anti-infective treatment success through systemic drug delivery. Antibiotic concentrations below MIC can result in the development of antibiotic resistant strains (90). Further, antibiotics such as aminoglycosides have narrow therapeutic indices (NTI) and conventional delivery methods are associated with the risk of systemic toxicity, including nephrotoxicity and ototoxicity (90). Localising the delivery of these antimicrobial agents to the infected tissues would avoid unnecessary exposure of the antibiotics to non-target tissues and reduce or eliminate toxic reactions.

CR systems can be used to achieve and maintain MIC of anti-microbial agents at the site of infection for an extended period of time (90). For infection sites with a good blood supply, oral sustained release tablets are available to reduce the required dosing frequency and to minimise fluctuations in blood levels. Such systems include Moxatag® (amoxycilin), Augmentin® XR (amoxicillin/clavulanate potassium) and Cipro XR® (ciprofloxacin).

Infection of prosthetic devices such as orthopaedic implants and hernia repair mesh following their implantation is a serious concern, with systemic treatment challenging if the tissues have a poor supply of blood. Systemic treatment is also difficult as bacterial adhesion onto implanted devices results in biofilm formation at the device-tissue interface, through which drugs have limited penetration. In bone

infections, it is often difficult to achieve microbicidal concentrations of an antibiotic at the target site without exceeding toxic systemic concentrations. The local delivery of antibiotics, such as following release from an implanted material, can achieve high local concentrations without raising serum concentrations. Local delivery of an antibiotic to reduce the rate of post-operative infection is not a new concept. Jansen et al. reported the implantation of sulphanilamide crystals in compound fractures in 1939 (91). More recently, local CR antibiotic delivery systems have been developed and refined. Anti-infective CR systems to control post-operative infections are available in the form of bone cements, bone implants, fillers, intra-vascular devices, various device coatings, wound dressings, surgical sutures and vascular grafts (92). A range of antimicrobial agents such as gentamicin, tobramycin, vancomycin, amoxicillin and carbenicillin have been incorporated into CR systems to control post-surgical infections (44, 93). Various bone cements with antibiotic agents incorporated have been approved by the FDA, including Palacos® G (gentamicin), Cemex® Genta (gentamicin), Simplex® P (tobramycin), Combalt® G-HV (gentamicin), Samrtset® GHV (gentamicin), and Versabond® AB (gentamicin) (17). The major limitation of these bone cements is the rapid burst of release following implantation over the first 24 to 48 hours, followed by much lower levels of sustained release associated with the inability to release the entire drug payload. When the local antibiotic concentration falls below the MIC threshold, the cement becomes susceptible to microbial growth leading to secondary infections as a result of biofilm formation, with associated concerns around developing antimicrobial resistant microbial strains due to the presence of sub-therapeutic concentrations of antimicrobial agents (94). Furthermore, the addition of antibiotic to the bone cement results in mechanical weakening, leading to the rapid elution of antibiotic upon implantation and an increased risk of aseptic loosening of the implant. As a result, research effort has focused on designs to improve overall release of antibiotics without compromising mechanical strength. Benoit et al. decreased the burst release by coating the material with a biodegradable polymer. They achieved sustained release of vancomycin over five weeks when an antibiotic loaded plaster of paris was coated with polylactide-co-glycolide (43). Similarly, other options for additives to improve antibiotic release without compromising the mechanical strength of bone cement have been reported. Jackson et al. demonstrated the addition of sodium chloride and dextran into bone cement improved drug release while maintaining mechanical strength (95).

Bacterial infection and biofilm formation can also be prevented by either coating the implantable devices with an antimicrobial loaded matrix (39, 40, 44, 96), or by covalently bonding the antimicrobial agent directly to the surface of the device (97). Importantly, the surface morphology and chemistry should not be altered significantly or integration with tissue will be affected. A recent study described the controlled release of antibiotic from a perforated metallic implant without altering the surface (98). Linezolid and cefazolin were packed into the hollow tubular reservoir and release occurred through previously drilled pinholes (Figure 2.4.a). Controlled release of antibiotic from this orthopaedic implant was demonstrated from set positions *in vitro* without altering mechanical integrity or surface morphology (Figure 2.4.b). However, the time course of release for this approach will need to be extended in the future and desirable locations of release be identified for successful *in vivo* translation.

Wound dressings are designed to provide both a barrier to pathogens and a suitable environment to accelerate the healing process (99). However, the dressing itself can act as an environment for microbial growth, particularly following the absorption of wound discharge. Wound dressings containing antimicrobial agents can release antibiotics/antimicrobial in a controlled fashion to the tissue they are in contact with. Current wound dressings available for this purpose include Contreet® Foam (silver), PolyMem Silver® (silver), Urgotul SSD® (silver sulphadiazine particles), Silvercel® (silver), Aquacel® (silver) and Acticoat® (silver) (100). Recently, interest has increased in the controlled release of bioactives from surgical sutures to control post-operative infections (101). A retrospective controlled trial in patients undergoing abdominal surgeries (35) demonstrated the antimicrobial efficacy of triclosan coated polyglactin 910 sutures (VICRYL® Plus) compared to conventional VICRYL® sutures in decreasing the incidence of SSI. But the evidence for the efficacy of surgical sutures coated with antibiotics for controlling SSI in wider post-surgical applications is inconclusive. A recent systematic review and meta-analysis conducted by Chang et al. found that the triclosan coated sutures didn't significantly reduce SSI or wound breakdown following surgery (102). In addition to sutures, antibiotic impregnated catheters and central lines have also been developed and are recommended for use by the Centre of Disease Control and Prevention (CDC) (103). A study conducted by Ramos et al. concluded that antibiotic coated catheters significantly reduce the incidence of infections associated with central lines (104). However, other studies have questioned the ability of these antibiotic coated medical devices to control infection (41, 42).

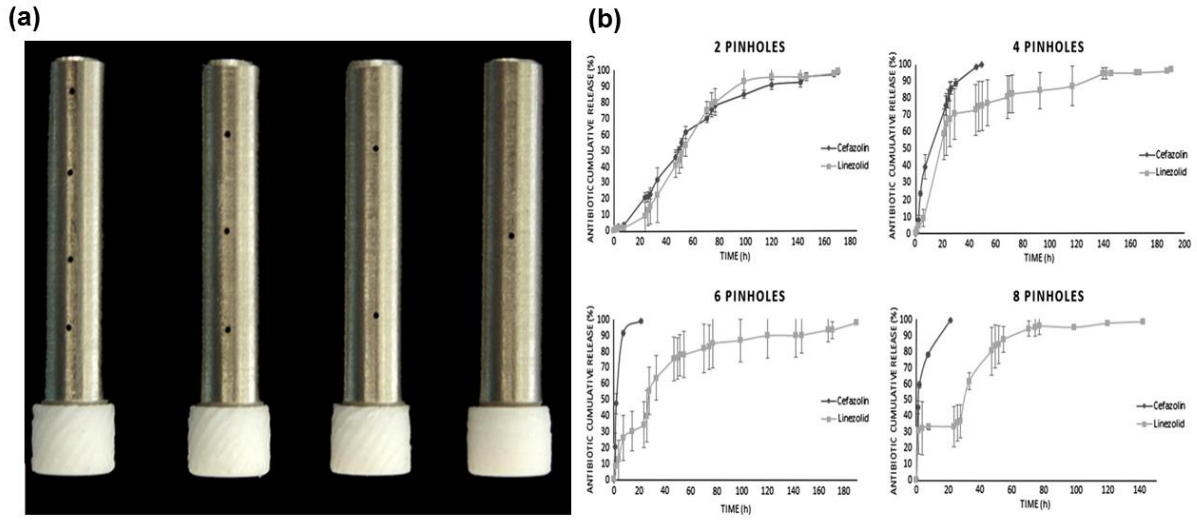

Figure 2.4. (a) Lateral image of the perforated orthopaedic implant (From right to left: one, two, three and four pin holes). (b) Percentage release of linezolid and cefazolin from orthopaedic implants (98).

SSI is multifactorial in origin and the ability of CR systems to control infection depends upon a number of factors. A predetermined steady release of antibiotic at the site of infection is not the ultimate solution

to achieving maximum efficacy. The antimicrobial effect can be time dependant, requiring sustained release, or concentration dependant, requiring pulsatile release. The rationale guiding the design of CR anti-infective formulations will depend upon the antibiotic type and the ability of the CR formulation to release its bioactive payload in accordance to the levels of microbes present.

2.3 Abdominal surgery

The term abdominal surgery refers broadly to any surgical procedure involving the abdominal region including appendectomy, caesarean section, hernia repair and the removal of cancerous tissue. Abdominal surgery results in the formation of two wounds, one on the abdominal wall (a somatic wound) and another in the peritoneal cavity (an autonomic wound) (Figure 2.5) (105). The peritoneum is richly innervated with functionally diverse neuronal fibers. Any disruption of local tissues is directly related to the central nervous system (CNS) via vagal signalling and results in patients experiencing tiredness, lethargy, altered emotional state and the desire to sleep. This collection of somewhat non-specific symptoms is collectively referred to as post-operative fatigue (POF) (1). Managing post-operative pain following abdominal surgery has been a long-standing goal, but treatment to relieve POF has been largely ignored. For a large number of surgical patients, POF persists longer than pain and negatively affects their quality of life. The incidence changes depending on the surgical procedure, however in one study almost 74% of patients reported experiencing moderate to severe POF after undergoing abdominal surgery (106). Post-operative fatigue has been found to persist for several weeks, and even as long as 90 days, after abdominal surgery (1).

Significant advances have been made to minimise somatic wounds, including the development of key-hole surgery, however there are far fewer reports of treatment measures to address POF caused by the peritoneal wound. Recent investigations by Hill et al. found that it is possible to address the pain and resulting post-operative fatigue by reducing vagal nerve activation with the application of LA (Figure 2.5). It was shown that continuous intraperitoneal infusion of LA over 72 h not only controls pain, but can reduce POF symptoms and enhance recovery (3). Constant infusion of LA into the peritoneal cavity is generally achieved with the aid of an elastomeric pump or other infusion device. However, the long-term use of infusion devices requires monitoring by trained health professionals and is associated with risks of infection or displacement. Alternative treatment strategies enabling controlled local release of LA into the intra-peritoneal region are required for the effective treatment of both pain and POF.

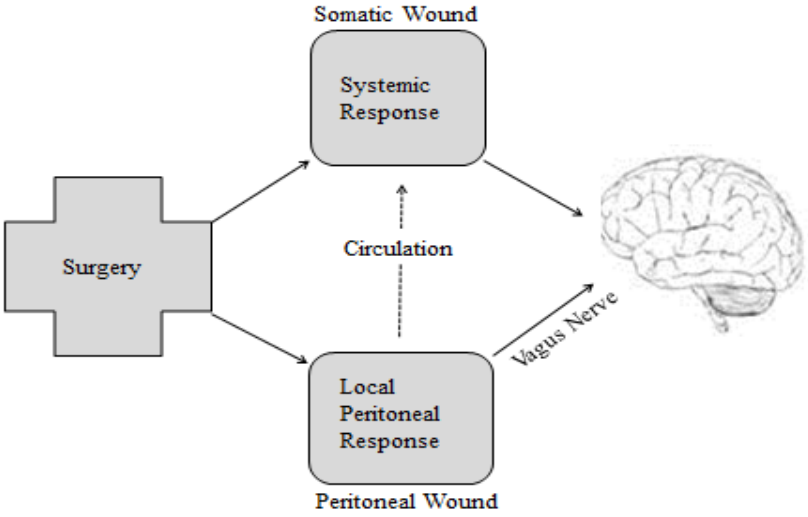

Figure 2.5. Two wound model hypothesis following visceral surgery. Local anaesthetic can be administered to peritoneal cavitys to block vagal transmission to the CNS. Reprinted with permission from Elsevier (105)

2.3.1 Current intra-peritoneal controlled release drug delivery systems (IPDDS)

Recently, interest has grown in the development of intra-peritoneal drug delivery systems (IPDDS), particularly for the treatment of intestinal and ovarian cancer. CR systems such as micelles (107) liposomes (12), microspheres (108, 109), nanoparticles (110) and gels (111) have all been studied for their potential application in the peritoneal cavity. The major limitation of particulate CR systems like liposomes and nanoparticles is their rapid clearance from the intra-peritoneal cavity. Clearance of liposomes into the lymphatic system were reported by Hirano and Hunt, when 50 -720 nm sized liposomes were injected into the intra-peritoneal section of Sprague-Dawley rats (12). Similarly, the clearance of PLGA based nanoparticles from the peritoneal cavity into the systemic circulation was reported by Kohane et al. (110). Meanwhile, microspheres were cleared relatively slowly demonstrating their better ability to retain drug in the peritoneal cavity. In a study conducted by Tsai et al., the retention time of three kinds of particulate CR systems for paclitaxel was investigated in mice (112). Auto-radiographic examination showed that the microsphere based CR system was retained for longer period of time when compared to micellar (five times longer) and nanoparticle (22 times longer) based CR systems. Dispersing particulate CR systems into a carrier medium consisting of hydrogel or polymeric solution has been reported to prevent rapid clearance of particle based CR systems from the peritoneal cavity. Nanoparticles dispersed into in-situ crosslinking hydrogel were retained for one week, as compared to free nanoparticles which were cleared in just two days (110, 111).

Solid implants are attractive for use as IPDDS because of their ability to concentrate drug locally and sustain required therapeutic concentrations for a long period of time. The safety of polymeric materials

for use in intra-peritoneal implants was investigated in a recent study. A biocompatibility study of intra-peritoneal implants based on polydimethylsiloxane (PDMS), poly-hydroxy metha-acrylate and EVA was conducted in different animal models (113). Based on the evaluation of histological data and the levels of inflammatory markers present following implantation, these polymers were confirmed to be biocompatible and safe for intra-peritoneal application. An intra-peritoneal implantable system based on PDMS and EVA was developed recently for local delivery of anastrazol (20). *In vivo* evaluation of this implant confirmed its greater ability to localise anastrazol in the intra-peritoneal region when compared to subcutaneous implants.

2.3.2 Potential use of IPDDS to deliver LA

Various controlled release systems for LA have been reported to prolong anaesthetic effect and minimise toxicity, including gels (114), microspheres (115), liposomes (116), films (8) and patches (117). Given the symptoms of pain and post-operative fatigue following peritoneal disruption, prolonged release of LA to reduce vagal tone over 7–10 days post-surgery would be beneficial. To achieve treatment in this time frame, the CR system would need to be loaded with large doses of LA. Attention must be paid to the appropriate formulation of a CR system loaded with high doses of LA to alleviate concerns of system failure releasing a potentially toxic dose. As discussed in previous sections, previously reported CR systems for LA are not suitable for our intended application to improve patient recovery after surgery, due to their potential for dose dumping, their inability to terminate LA release and/or their inability to achieve the required local concentrations of LA (118).

Solid implantable systems are attractive due to being simple to produce and scale up, and their capacity to release drugs in a predictable controlled manner for a long period of time. Biodegradable implants start eroding after implantation and hence would be less preferable for our application where recovery of the implant is essential, and the maintenance of mechanical strength is required. Non-biodegradable materials don't erode inside the body, retaining their physical and mechanical properties. Non-biodegradable polymers are thus well suited for developing recoverable implants, with a long history of clinical use for this purpose (15).

2.3.2.1 Local anaesthetics

Local anesthetics (LA) are widely used in post-operative settings to provide temporary analgesia following surgery. Analgesia is achieved upon the interruption of nerve conduction, which occurs when LA diffuse into the axoplasm through the neuronal membrane to prevent neurons from acting. LA consist of an aromatic ring, an intermediate ester or amide chain and a terminal amine (119). Lidocaine, bupivacaine, ropivacaine and mepivacaine are local anaesthetics commonly administered post-surgery. Despite bupivacaine being the most widely used anaesthetic, it is associated with cardio-toxicity and is considered less safe than other LAs for CR applications (120). Meanwhile, lidocaine is comparatively safe and has a high threshold for systemic toxicity (121). Blood levels of lidocaine below 5 µg/mL are

usually safe, with no associated toxicity (120). In extreme cases of dose dumping, lidocaine is also preferable because of its shorter half-life (90 min) (122, 123). In view of these concerns of safety and efficacy, lidocaine was selected as the model LA for our study.

2.3.2.1.1 Lidocaine

Lidocaine was first synthesised by a Swedish chemist at the University of Stockholm in 1943. It is chemically known as 2-(diethyl-amino)-N-(2,6-dimethylphenyl)-acetamide and has a molecular weight of 234.34 g/mol. It is basic drug and consists of a benzene ring and tertiary amine group linked by an amide bond (Figure 2.6). The benzene group renders the molecule to be partially lipophilic, while the tertiary amine group contributes to its hydrophilicity. It is a white crystalline powder and melts at around 68 °C (124). Lidocaine has poor aqueous solubility (4.2 mg/ml) and has a pK_a value of 7.9. It is generally prepared as lidocaine hydrochloride to increase its aqueous solubility. It is also known as xylocaine or lignocaine. It is available commercially in various dosage forms including transdermal systems, injectables, ointments and creams.

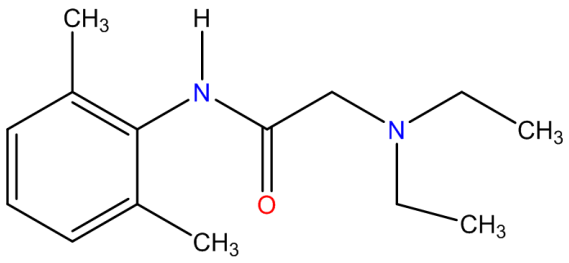

Figure 2.6. Chemical structure of lidocaine

Lidocaine has a very rapid onset of action of between one and 15 minutes, depending upon the route of administration and the final preparation. It distributes quickly and moderately binds with plasma proteins (125). Lidocaine has high affinity towards alfa-acid glycoproteins, which accounts for 70% of its total protein binding. Like other LA, the mechanism of action involves the blockade of voltage-gated sodium channels by which the flux of sodium ions across the neuron is prevented. This results in decreased neuronal depolarisation and failure to transmit an action potential. An anaesthetic effect is then created due to the interruption of pain signal transmission to brain (126).

Lidocaine undergoes enzymatic degradation primarily in the liver, although some degradation occurs in other tissues as well. The major degradation pathway involves oxidative de-ethylation of lidocaine to form mono-ethyl glycine xylidide (MEGX) and glycine xylidide (GX) via CYP3A4 and CYP1A2. It is followed by subsequent hydrolysis to produce 2,6-xylidine, then converted to 4-hydroxy-2,6-xylidine and excreted in urine. Up to 10 % of lidocaine is not metabolised and excreted unchanged. It has a relatively short half-life (1–1.5 h), hence is rapidly cleared from the body (126).

Elastomeric pumps can achieve long term delivery for lidocaine but are associated with complications which have already been discussed in previous sections. Like other LAs, lidocaine can result in local ischaemia and systemic toxicity at high doses. Systemic toxicity occurs once the plasma concentration start exceeding 5 µg/mL, resulting in various adverse events. At plasma levels of 5–8 µg/mL, the patient may experience twitches and hallucinations. Convulsion, comma and cardiac arrest can occur if plasma levels of lidocaine exceed over 8 µg/mL (119, 126).

The toxicity issues of lidocaine and the clinical requirements of treating post-surgical pain and POF require a CR system enabling controlled release of lidocaine over a period of 10 days which can be recovered if necessary. As discussed in previous sections, the available CR systems are not suitable due to dose dumping issues.

Development of a sustained release formulation for lidocaine utilising non-biodegradable polymers is possible. Such a formulation would not only provide controlled release of lidocaine for a ten-day period, but could also be recovered when desired. A range of non-degrading, biocompatible materials have been used to achieve constant levels of drug release, including silicon (16) and meth-acrylates based co-polymers (17). Poly(ethylene- co-vinyl acetate) (EVA) is a non-biodegradable polymer and has been widely used to develop sustained release formulations. More importantly, EVA has been previously tested and commercialised for use as a recoverable implant (15, 127), which makes it an appropriate polymeric carrier for our purpose.

2.3.2.2 Poly(ethylene-co-vinyl acetate) (EVA)

In the 1930s, poly(ethylene-co-vinyl acetate) (EVA) was the first ethylene copolymer to be synthesised (128). Since 1950 this co-polymer has been used extensively in the cable industries and medical field. As shown in Figure 2.7, it consists of ethylene and vinyl acetate (VA) components. The physical properties of EVA depend upon the proportions of these two components, with the polymer varying from crystalline to rubbery forms. When the VA composition of the ethylene chain increases, the amorphous state of EVA increases which then decreases the overall rigidity of the polymer. Thus, the composition of VA governs the melt flow index (MFI) of EVA. The MFI of EVA is important in determining its melt extrusion feasibility. If the MFI of EVA is high it will have superior melt extrusion properties.

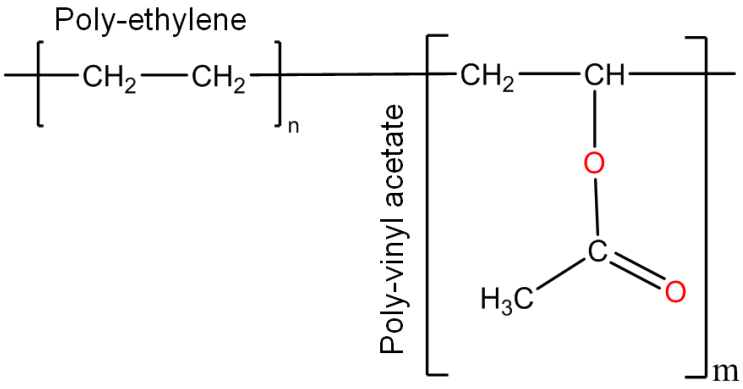

Figure 2.7. Chemical structure of poly(ethylene-co-vinyl acetate) (EVA)

Various forms of EVA are available in the market, depending upon the proportions of the VA component (2–50 wt/wt%). EVA becomes increasingly amorphous when the proportion of VA in the polymer increases. Beyond 50 wt/wt% VA, EVA becomes completely amorphous. When the VA composition of EVA is increased, the permeability of drug is increased leading to higher drug release rates (129). Therefore, the choice of EVA in relation to proportion of VA is important when formulating CR delivery systems. EVA has been approved by the FDA and is widely used as a polymeric carrier in commercially available implants and CR devices (Table 2.1) (15, 18, 19, 130).

Table 2.1. Commercial drug eluting systems based on EVA

Name	Drug
Ocusert® (131)	Pilocarpine
Progestasert® (132)	Progesterone
Implanon® (15)	Etonogestrel
NuvaRing® (19)	Etonogestrel/Ethinylestradiol
Actisite® (133)	Tetracycline
Cypher® (134)	Sirolimus
Vitrasert® (135)	Ganciclovir

2.4 Compatibility and interactions between drug and polymeric carrier

The role of the polymeric carrier in a CR system is to aid in the formulation process and to modulate the release rate of drugs. The carrier must be pharmacologically inert and should not interfere with the stability of drugs. However, bioactive drugs may undergo various transformations during preparation or upon storage, ultimately decreasing the actual drug content. In addition to this, there is also a possibility of physical or chemical interaction between the drug and excipient, by which the stability and therapeutic efficiency of CR formulations may be altered. However, these interactions are not always disadvantageous. In some CR formulations, interactions between drug and polymeric carrier are helpful in making the formulation physically stable and sustaining a supersaturated state of drug in the polymer matrix. The main challenges to developing a CR formulation with a sustained amorphous state of drug are phase separation and recrystallisation of drug in the polymer matrix. A drug in the amorphous state is in a higher energy state and will tend to spontaneously release energy by recrystallisation (136). Strong drug-polymer interactions enhance drug-polymer miscibility which is essential to make a formulation stable (137). An in-depth molecular level understanding of interactions is therefore necessary to prevent or slow down recrystallisation of drug in the polymeric matrix. Commonly occurring interactions between drug and polymeric carriers include hydrogen bonding, ionic bonding, dipole-dipole interaction and Van der Waal interactions (137). A thorough investigation of drug and excipient, their compatibility and potential interactions is crucial before going into the formulation process (138, 139). The commonly used tools for this purpose include Fourier transform infrared spectroscopy (FTIR) (140), differential scanning calorimetry (DSC) (140), thermo-gravimetric analysis (TGA), nuclear magnetic resonance (NMR) (141) and X-ray diffraction spectroscopy (XRD) (140).

FTIR is an important tool used to elucidate drug-polymeric carrier interactions. It provides information on structural and molecular conformation by probing the band vibrations in infra-red regions. Each functional group/bond from the pure molecule gives a characteristic absorption band upon irradiation by infrared light (142). Any changes in the characteristic bands of either drug or polymer in the formulation/mixture indicate the presence of molecular interactions between drug and polymer. DSC and TGA are thermal techniques that provide useful information about the melting point of a drug, glass transition temperature (T_g), crystallisation, polymorphic changes and drug-polymeric miscibility (143). DSC is a powerful tool for identifying drug conversion or interaction with the excipients. Any change in the conformation and structure of the drug molecule is exhibited by change in position, sharpness, and shape of transition(s) in DSC scans (144). Meanwhile, TGA can determine the mass loss of material as a function of temperature. This tool is important for identifying any volatile degradation of drug at high temperature. Generally, in the event of interactions between drug and polymer, there is an experimental deviation of T_g from that predicted theoretically by the Gordon-Taylor (GT) equation (145). Next, NMR is a powerful tool for investigating drug-polymer interaction both in solid and solution state, as it allows the study of interactions at a molecular level. In any event of drug degradation or interaction, the electron density at the interacting atom changes, resulting in variation and changes in chemical shift (146). Both solid state ^{13}C NMR and ^{1}H NMR are widely used in the pharmaceutical arena to study chemical interactions (147). Finally, XRD is often used to characterise the amorphous state, crystalline states and different polymorphic forms of drug in the polymeric carrier (148).

2.5 Understanding biological fluids

While majority of efforts are accentuated in preformulation and formulation design, less attention is given to understand the environments at drug release site. It is important to note that the performance of CR systems depends not only on formulation design, but also upon the local environment they perform within. Following patient administration, CR systems become surrounded by body fluids into which drug release occurs. The release performance of drug delivery systems do not solely depend upon its intristic properties but also with the physicochemical and rheological properties of the body fluids. Therefore, a detailed understanding of biological fluid at the drug release site will be crucial in predicting the release performance of CR systems. Determination of key parameters such as composition, physiochemical properties and the rheological parameters is essential to understand the fate of CR systems following administrations at *in vivo* conditions.

The solubility and dissolution of the drug, particularly for ionisable drugs, greatly depends upon the pH and the buffer capacity of the media. The pH and buffer capacity not only affect the dissolution of a drug, but also influence the properties of excipients and drug carriers (149). Similarly, different osmolal environments contribute to different release behaviours from the drug delivery systems (150). Osmolality will influence fluid penetration into the drug delivery system, resulting in different release behaviour. Naturally occurring surfactants in biological systems vary across patients and may influence drug release from drug delivery systems (151). Surfactants reduce the surface tension and can increases the

dissolution of lipophilic drugs by improving the wettability of the drug (151). The viscosity of biological fluids also influences the release performance of CR systems. As predicted by the Noyes Whitney equation (Equation **2**.**1**), the rate of drug dissolution is dependent upon multilple parameters including diffusion coefficient, surface area, and dissolution volume, solubility of drug and thickness of the diffusion layer.The thickness of the diffusion layer is dependant upon viscocity of release medium, where the drug release rate decreases when viscosity of media is increased. Biological fluids typically consist of water, proteins (collagens), non-collagenous glycoproteins, proteoglycans, and polysaccharides (152). Variations in composition among patients alter the physiochemical properties and rheological parameters, ultimately influencing the release performance of CR systems. A thorough investigation of biological fluids at various administration sites is therefore extremely important to predict the release performance of CR systems. Biological fluids such as human gastric fluid (153-155), mucus (156), synovial fluid (157) and saliva (158, 159) have been widely studied for composition, physicochemical properties and rheological parameters.

Equation 2.1. Noyes Whitney equation

$$dm/dt = \frac{DA}{\delta}(Cs - C)/V$$

Where,

dm/dt= rate of drug dissolution

D= molecular diffuction coefficient

A=surface area

V=volume of media

C=instantaneous concentration of drug

Cs=saturated concentration of drug

δ=thickness of diffusion layer

In a study conducted by Daikidou et al., ascending colonic fluids collected under both fasting and fed states were characterised for composition and physicochemical properties (153). Free water content, pH, surface tension and the concentration of iso-butyrate were all lower in fed volunteers when compared to the fasting ones. Meanwhile, buffer capacity and osmolality, as well as levels of acetate, butyrate, cholate and chenodoxycholate were all higher in the fed subjects. The rheological parameters and lipase activities of human gastric fluid in fasting state was reported recently by Pederson et al. (155). Both inter- and intra-variations in physicochemical properties and rheological parameters were reported in the gastric fluid samples. The apparent viscosity of the gastric fluid was found to be higher than 0.1 M HCl and simulated gastric fluid. The rheological characterisation of synovial fluid obtained from the knees of

osteoarthritic patients compared to visco-supplements was described in a recent study (157). Variation in viscoelastic properties was not only limited to different patients and visco-supplements, but also found between the two knees of the same patient. Detailed studies of biological fluids are essential to develop substitutes, supplements and bio-relevant media for *in vitro* testing.

Bio-relevant media are designed to create a similar environment to that of biological fluids for *in vivo* conditions. *In vitro* release testing performed in bio-relevant media is assumed to closely correlate with the performance of CR systems *in vivo* conditions. Various kinds of bio-relevant media for different administration sites have been described, including saliva (158, 159), gastric fluid (155, 156), intestinal fluid (154), synovial fluid (157), mucus (156) and tears (160). However, phosphate buffered saline (PBS) is typically used to evaluate intraperitoneal drug delivery systems (108, 161). No literature towards the development of bio-relevant media for peritoneal fluid has been reported to date. Therefore a detailed description of peritoneal fluid is necessary to predict the release performance of IPDDS and in the design the bio-relevant media in the future.

2.6 Peritoneal fluid

The cavity between the parietal peritoneum and the visceral peritoneum is filled with fluid and consists of blood vessels, lymphatics, fibroblasts, and fats. The fluid is termed peritoneal fluid (PF) and it lubricates surface tissues lining the abdominal wall and pelvic cavity. Very few investigations into the properties of PF have been performed, despite PF being widely used as a diagnostic tool to examine pathological conditions such as ascites and peritonitis (162). One study analysed the PF of 134 patients for pH, electrolyte concentrations (Na^+, K^+, Ca^{2+} and HCO_3^-), partial pressure of carbon dioxide and oxygen (pCO_2 and pO_2) and O_2 saturation (163). The pH was measured following midline incisions of the abdominal walls by placing a pH probe into the peritoneal cavity. The recovered PF was also measured for pH after 10 and 20 min of sampling. A relatively higher pH value for PF was recorded when compared to the pH of blood. Similarly, in a study conducted by Kelton et al., the chemical composition of PF collected from patients undergoing peritoneal dialysis was compared with serum (164). It was found that the calcium and albumin content were respectively 20% and 43% lower when compared to serum. However, there are several other physicochemical parameters which need to be considered to fully characterise PF with respect to IPDDS release performance, including buffer capacity, osmolality, surface tension and viscosity. Further, the development of IPDDS for our purpose relates to trauma of the peritoneal tissues as a result of surgery. Surgery causes endocrinological, immunological and haematological changes resulting in sequestration of fluid at the site of injury at levels depending upon individual patients and the magnitude of tissue damage (165). This may result in changes in PF composition which ultimately alter physicochemical properties and rheological parameters of PF. This necessitates a detailed investigation of PF following surgery to better predict the performance of our developed EVA formulation.

CHAPTER 3. A STABILITY INDICATING HPLC METHOD TO DETERMINE LIDOCAINE FROM POLY(ETHYLENE- CO-VINYL ACETATE) MATRICES AND BIOLOGICAL FLUID

3.1 Background

Development of a stability indicating analytical method is essential before going into any formulation and testing. A reliable quantification method is required at every step to investigate any drug degradation during preparation and storage, and monitor the release performance of the formulation. The Food and Drug Administration (FDA) guidelines state the necessity of generating stability data to understand the fate of drugs in various environmental conditions (166). Generally, forced degradation is conducted to accelerate reactions under stress conditions and enable the identification of degradation pathways and products. Samples subjected to forced degradation studies can be used to develop a stability indicating method that can be applied for conducting longer term stability studies in less extreme conditions (166). According to the FDA, a stability indicating method is a validated method for accurately quantifying an active ingredient without interference from degradation products (167). The forced degradation conditions recommended by the FDA and ICH (International conference on harmonization) include acid and base hydrolysis, thermal degradation, photolysis and oxidation (168-170). Degradation products generated during the process of manufacturing or storage have structures related to the active pharmaceutical ingredient (API), and can often co-elute with the drug peak on HPLC (171). Therefore, the drug peak should be free from interference to enable accurate analysis.

Several HPLC methods have been reported in the literature for quantifying lidocaine and its degradation products. In a recent study lidocaine and its degradation products were separated using a gradient elution method (172) through continuous change in the composition of the mobile phase at predetermined times. However, gradient methods may not be suitable for every HPLC set up. An earlier study investigated lidocaine stability in aqueous solutions by subjecting lidocaine to varying conditions of pH, temperature, buffer and metal ion concentrations (173). However, the applicability of this method was limited as the ability to resolve and quantify lidocaine from oxidative and thermally stressed samples was not demonstrated. Stability indicating methods for lidocaine have also been developed with other drugs in combination formulations, including oxycodone (174) and ephedrine (175). However, the studies using these methods did not investigate all the required stress conditions (168-170). Similarly, methods for the quantification of lidocaine salt by HPLC have been reported recently (176, 177). But, these methods are specific to the salt form of lidocaine and their ability to extract lidocaine efficiently from biological fluids was not investigated.Very few studies have reported on extracting and quantifying drug from a poly(ethylene-co-vinyl acetate) (EVA) matrix. A process for the determination of content uniformity of UC781, a non-nucleotide reverse transcriptase inhibitor (NNRTI), from an EVA matrix was described in a recent study (178). However, the extraction procedure was time consuming as it involved refluxing the EVA matrix for 2 h using dichloromethane (DCM) and required further cooling for an additional 1 h.

Lidocaine is administered at a range of body sites to achieve analgesia during and following surgical procedures (2, 179, 180). An appropriate quantification method, including an extraction procedure, is essential for determining the release performance and safety of lidocaine releasing systems. While there are numerous published reports on the extraction and quantification of lidocaine from plasma, there are

no reports of extraction from other biological fluids. Recently, Qin et al. reported an extraction procedure and HPLC method to quantify lidocaine along with other local anaesthetics from human plasma (181). The extraction procedure involved liquid-liquid extraction with aqueous sodium hydroxide solution (1 M) and ethyl ether. This was followed with evaporation and then further dilution of the dried residue with a methanol water mixture before injection onto HPLC. Similarly, another recent study described a HPLC-tandem mass spectrometric (LC-MS-MS) method for assaying lidocaine in human plasma, where liquid-liquid extraction from alkalinised plasma with *tert*-butylmethyl ether was used, followed by drying of the supernatant by vacuum before injection into the column (182). These previously described methods for the quantification of lidocaine from plasma are either labour intensive or costly, requiring additional set-ups such as LC-MS.

3.2 Aim and objectives

The overall aim of the work described in this chapter was to develop a stability indicating HPLC method suitable for quantifying lidocaine in different settings. The specific objectives were to:

- Force the degradation of lidocaine using FDA and ICH recommended stressors
- Develop a rapid and isocratic stability indicating HPLC method for lidocaine capable of separating lidocaine from degradation products
- Validate the developed method for linearity, accuracy, precision, LOD and LOQ
- Apply the developed method to quantify lidocaine extracted from EVA matrices
- Apply the developed method to quantify lidocaine extracted from plasma, intra-articular fluid (IAF) and peritoneal fluid (PF)

3.3 Materials

Lidocaine was purchased from Sigma (Auckland, New Zealand). EVA containing 25% and 33% vinyl acetate content was purchased from Dupont (USA). All reagents and solvents were of HPLC or analytical grade. Water was obtained by reverse osmosis (MilliQ unit, Millipore) of demineralised water. Human plasma samples with the reference number 2013/32 were obtained from the New Zealand Blood Service, Auckland. Human intra-articular fluid and peritoneal fluid were obtained from patients at Middlemore Hospital, Auckland under Health and Disability Ethics Committee (HDEC, Ministry of Health Wellington, New Zealand) approvals 14/STH/182 (closed 20 June 2016) and 15/CEN/30 (closed 26 March 2017), respectively.

3.4 Methods

3.4.1 Chromatographic conditions and method optimization

An Agilent Series 1260 system (Agilent Corporation, Germany) consisting of a quaternary pump, an auto sampler and photo-diode array (PDA) detector were used, with data acquisition achieved using Chemstation software (Agilent Corporation, Germany). Several columns, mobile phase combinations, and column temperatures were trialled to achieve satisfactory retention of lidocaine peak.

3.4.2 Forced degradation studies of lidocaine

Forced degradation was attempted with lidocaine in both solution and solid state. Acid, base, hydrolytic and oxidative stress were achieved in the solution state by dissolving lidocaine in 0.1 M HCl, 0.1 M NaOH, water and 0.02% H_2O_2 respectively to prepare a lidocaine solution of 100 µg/mL. Samples were stored at both 25 °C and at the elevated temperature of 60 °C. For solid state, lidocaine powder was exposed to artificial ultraviolet light at 10000 lux and elevated temperature of 60 °C. At predetermined time intervals, samples were withdrawn and diluted with mobile phase before analysis by HPLC. Studies were conducted for 10 days or until significant degradation (>5 %) was observed, whichever occurred first (183). Peak purity was calculated as a purity index using Chemstation software (Agilent, Germany), where the similarity curve was compared to threshold curve. It was ensured that the purity index of lidocaine always remains above the threshold limit to confirm the absence of degradation peaks within the lidocaine peak.

3.4.3 Method Validation

The developed HPLC method was validated for linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy and precision. For linearity, a stock solution was diluted using mobile phase to produce working solutions ranging from 0.4–50.0 µg/mL. LOD and LOQ were determined by injecting serially lower concentrations of lidocaine. LOD was determined at the lidocaine concentration where the signal to noise ratio was greater than 3 (184). LOQ was determined as the lowest standard on the calibration curve whose response was at least five times the blank response and reproducible with a precision of less than 20 % and accuracy between 80–120% (168). Intra-day accuracy and precision were determined through analysis of five replicates of 0.4, 6.25, 12.5, 25, and 50 µg/mL lidocaine in mobile phase. The inter-day accuracy and precision were determined by the analysis of the same concentrations with three replicates over three days. The precision was determined as % relative standard deviation (RSD), while the accuracy was determined by comparing the measured concentration against the standard solution.

3.4.4 Application of the HPLC method to quantify lidocaine

The utility of the developed method was tested by determining lidocaine from release studies and following extraction from EVA polymer and biological fluid. The methods used to prepare the EVA based formulation in this thesis involved both solvent casting and melt extrusion. It was therefore necessary to conduct release studies with both solvent cast and melt extruded samples to confirm the ability of the developed method to quantify lidocaine without any interference from degradation products. Hot melt extrusion involves exposure of the drug-polymer mixture to a high temperature for a certain period of time; the stability indicating analytical method should be able to determine lidocaine stability to this process. In addition, the ability of the method to resolve lidocaine in the presence of the primary metabolite in the human liver, 2,6-dimethyl aniline, was also tested. An aqueous solution of lidocaine and 2,6-dimethyl aniline was prepared at concentrations of 12.5 and 2 µg/mL respectively. The solution was centrifuged at 5844 g for 4 min before analysis with HPLC.

3.4.4.1 Determination of content uniformity and assay and release of lidocaine from EVA films

Solvent cast EVA (33% VA) films loaded with lidocaine (10% w/w) were prepared by weighing out predetermined quantities of EVA and lidocaine and adding them to dichloromethane (DCM). The mixture was stirred at room temperature (25 °C) until dissolved. The mixture was then poured into a precooled glass petri dish at 4 °C, and maintained at 4 °C overnight to slow down the rate of solvent evaporation and promote uniform film formation. The film was dried until a constant weight was obtained to ensure complete solvent evaporation. For content uniformity, 10 sections were cut from different locations, weighed and individually extracted and analysed by HPLC. For the assay, samples weighing approximately 10 mg were taken from five different portions of the cast film and lidocaine extracted from all the pieces together. Lidocaine was extracted from the samples using DCM as a solvent. The samples were added to 50 mL of DCM and sonicated for 40 min. The volume was adjusted by DCM before one mL of the resulting DCM solution was withdrawn with a glass pipette and dried at 60 °C for an hour in a volumetric flask. Methanol was added to the resulting lidocaine containing EVA film and sonicated for 30 min. The methanolic solutions containing lidocaine were centrifuged at 5844 g for 4 min and injected into HPLC against the standard in methanol for quantification.

In vitro release of lidocaine from the EVA film was performed in phosphate buffered saline (PBS) (pH 7.4) in Falcon tubes. Falcon tubes containing EVA films (10 mm diameter 0.5 mm thickness) (10% w/w lidocaine) with PBS medium were agitated at 40 oscillation per minute in a water bath maintained at 37 °C. Samples were withdrawn at predetermined time intervals and replaced with fresh medium. The samples were again diluted suitably with mobile phase and centrifuged at 5844 g for 4 min before analysis with HPLC. Sink conditions were maintained throughout the experiment, with the concentration of drug in release media below 10% of the saturated solubility at all times.

3.4.4.2 Determination of lidocaine release from EVA melt extrudates

Extrudates containing EVA (25% vinyl acetate) and lidocaine (10% drug loading) were prepared using a Mini Lab co-rotating twin screw extruder from Thermo Fisher Scientific. The predetermined weight of each material was weighed out using an analytical balance and manually mixed. The mixture was then fed gradually into the hopper of the extruder. The operational temperature was set at 100 °C with the screw speed set at 20 rpm during the entire manufacture. The extrudate passing through the die had a diameter of 2.3 mm.

In vitro release of lidocaine from the melt extrudate was performed in Falcon tubes conaining 20 mL of pH 7.4 phosphate buffered saline. The Falcon tubes containing melt extrudate with medium were agitated at 40 cpm in a water bath maintained at 37 °C. Samples of 0.5 mL were withdrawn at predetermined time intervals and replaced with fresh medium. The samples were again diluted suitably with mobile phase and centrifuged at 5844 g for 4 min before analysis with HPLC. Release studies were conducted under both sink and non-sink conditions. For sink conditions, the sample was treated in such a way that the final concentration of drug in medium remained below 10% of the saturated solubility if the entire drug was released; whereas for non-sink conditions concentration reached approximately 40% of saturated solubility when the entire drug was released.

3.4.4.3 Quantification of lidocaine from biological fluids

The linearity of lidocaine response in biological fluids was determined following serial dilution to prepare concentrations of 1.6, 3.1, 6.2, 12.5, 25 and 50 µg/mL. Recovery was determined by taking blank plasma, human intra-articular fluid (IAF) and peritoneal fluid (PF) and spiking them with known amounts of lidocaine dissolved in mobile phase (1 mg/mL) to concentrations of 12.5, 25 and 50 µg/mL, with 3 replicates for each concentration. Extraction was achieved by adding 800 µL of acetonitrile and 100 µL of water to 100 µL of plasma samples to precipitate the proteins. The resulting solutions were vortexed and allowed to stand overnight at 4-6 °C. The next day the stored samples were centrifuged at 5844 g for 10 min. The supernatant was then injected onto the HPLC.

3.5 Results and Discussion

An isocratic and low cost stability indicating HPLC method was developed and validated to quantify lidocaine from forced degradation samples, EVA matrices, plasma, PF and IAF. The extraction procedure used to quantify lidocaine from EVA matrix and biological fluids was found suitable for routine analysis of quality control procedures and *in vivo* evaluation.

3.5.1 Chromatographic conditions and method optimization

The experimental variables included the column, injection volume, pH of buffer, percentage of organic phase and column temperature. Initially, a shorter column of 150 mm and 4.6 mm internal diameter was tested with an injection volume of 20 µl and a mobile phase consisting of a potassium di-hydrogen phosphate buffer (pH 4.5, 0.02 M) and acetonitrile in the (v/v) ratio 45:65. The column temperature was set at 35 °C. A broad lidocaine peak was present with a retention time around 2 min (Figure 3.1.a). As the peak was close to the solvent front, there was a high chance of peaks arising from degradation products and biological fluids co-eluting with the lidocaine peak. Accordingly, the method was adjusted to prolong the retention time of the lidocaine peak.

Next, a longer column of 250 mm and 4.6 mm internal diameter was tested with an injection volume of 10 µl while keeping the other variables the same in order to achieve a peak of better shape and acceptable retention time. The peak obtained was relatively sharp but appeared around three minutes (Figure 3.1.b). As the peak obtained was still close to the solvent front, the co-elution of peaks derived from the degradation products and biological fluids was likely, further adjustment of the method was necessary.

Finally, changes were made to column temperature, which was increased to 40 °C, and composition of the mobile phase, where the pH of the buffer was adjusted to 5.5 by dropwise addition of 0.1 M NaOH solution. The pH of 5.5 was still sufficient to keep lidocaine predominantly ionised (pK_a=7.6) and to allow partition of lidocaine into the mobile phase. Once the pH was fixed at 5.5, the acetonitrile to buffer ratio was readjusted to 26:74 to achieve a lidocaine peak at the desired retention time. The chromatogram of lidocaine with optimised chromatographic conditions is displayed in Figure 3.1.c.

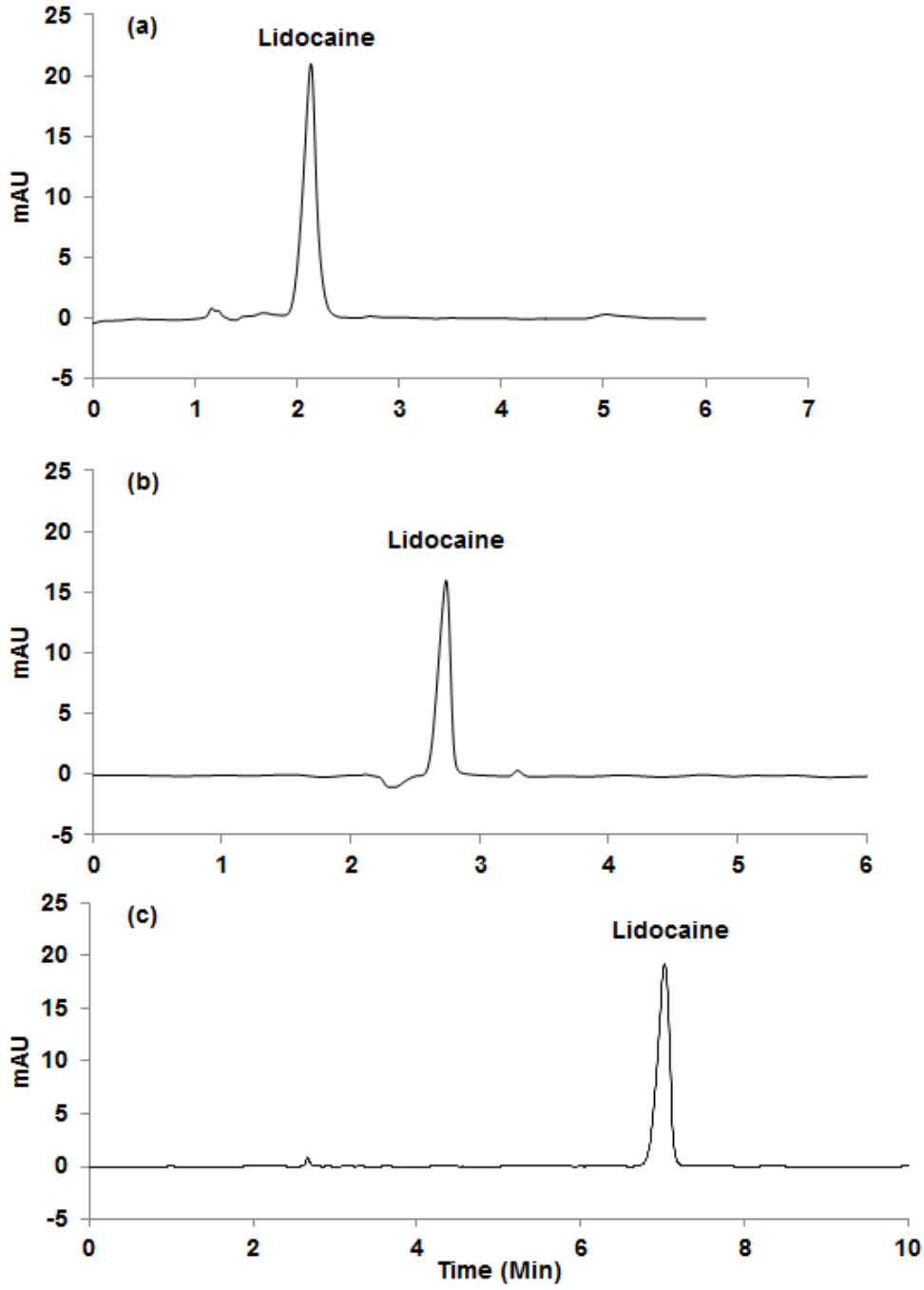

Figure 3.1 Chromatograms of lidocaine obtained from method optimisation (a) Phenomenex Luna column (150X4.6 mm) with a mobile phase consisting of a potassium di-hydrogen phosphate buffer (pH 4.5, 0.02 M) and acetonitrile (45:65), (b) Kintex (250X4.6 mm) with a mobile phase consisting of a potassium di-hydrogen phosphate buffer (pH 4.5, 0.02 M) and acetonitrile (45:65) and (c) Kintex (250X4.6 mm) with a mobile phase consisting of a potassium di-hydrogen phosphate buffer (pH adjusted to 5.5 by 0.1 M NaOH, 0.02 M) and acetonitrile (74:26).

Column temperature also played a significant role in the separation of lidocaine stressed conjugates. A column temperature below 40 °C was not sufficient to separate lidocaine from the degradation products produced by peroxide. The major reason behind this phenomenon is due to the increment of diffusivity of mobile phase at elevated temperature. When the temperature is increased the viscosity of the mobile phase is reduced which increases the solute transfer from mobile phase to stationary phase (185). This phenomenon increases the plate number and resolution which increases the overall efficiency of the column. Table 3.1 summarises the optimised chromatographic parameters for the separation of lidocaine. The parameters were kept the same for the entire analysis.

Table 3.1. Chromatographic parameters for the determination and separation of lidocaine from its degradation products

Parameter	**Details**
Column	Kinetex C18 ODS (Phenomenex, USA), 250 X 4.6 mm i.d., and 5 µm particle size
Flow rate	1.0 mL/min
Detection	UV detector, 230 nm PDA detector, 200 to 400 nm for peak purity testing
Column temperature	40 °C
Injection volume	10 µL
Mobile phase	Potassium di-hydrogen phosphate (pH 5.5; 20 mM):Acetonitrile (74:26 % v/v)
Retention time	7 min

3.5.2 Forced degradation studies of lidocaine

The forced degradation of lidocaine was achieved under several stress conditions. Using the chromatographic parameters described in Table 1, the lidocaine peak remained pure at all instances with no interference from the degradation products or the solvent front. Lidocaine was stable in the aqueous solution, acidic solution and aqueous solution exposed to UV light over a period of 10 days. However, degradation of lidocaine was observed in alkaline and peroxide environment. The results from the forced degradation studies are summarised in Table 3.2.

Table 3.2. Forced degradation of lidocaine

	Stressors	**Storage temperature (°C)**	**Days**	**Assay (%) Mean±SD (n=3)**
	Aqueous solution	60	10	99.0 ± 0.76
	UV light (Aqueous)	25	10	100.1 ± 0.69
	0.1 N HCl	60	10	100.1 ± 1.22
	0.1 N NaOH	60	10	85.3 ± 3.02
	0.02% H_2O_2	25	10	84.8 ± 2.28
	0.02% H_2O_2	60	3	13.4 ± 0.04
	UV light	60	10	100.8 ± 1.56
	Heat	60	10	99.0 ± 0.76

The chromatograms of blank solutions and the corresponding peak of lidocaine from the forced degradation studies are displayed in the following figures. Figure 3.2 shows the chromatograms of a water injection and lidocaine in water stressed at different conditions. As displayed in Table 3.2, lidocaine was stable in the aqueous solution exposed to UV light over 10 days, with between 99–101% of the initial concentration remaining.

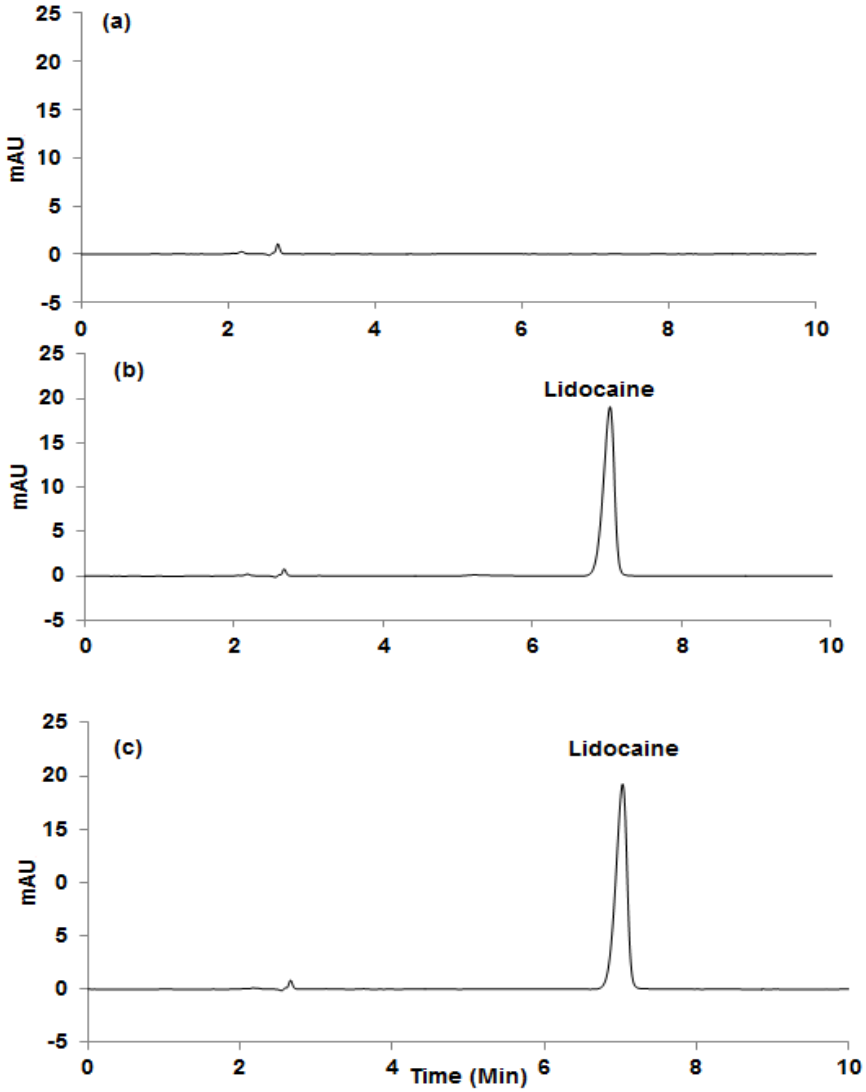

Figure 3.2. Chromatograms of (a) blank water and lidocaine in water stressed at (b) UV conditions at 10000 lux and (c) 60 °C for 10 days.

Lidocaine remained stable in an acidic environment, with 100.1 ± 1.22% being recovered when lidocaine solution in 0.1 M HCl stored at 60 °C for 10 days was examined. Figure 3.3 displays the chromatograms of blank 0.1 M HCl and lidocaine in 0.1 M HCl stressed at 60 °C for 10 days.

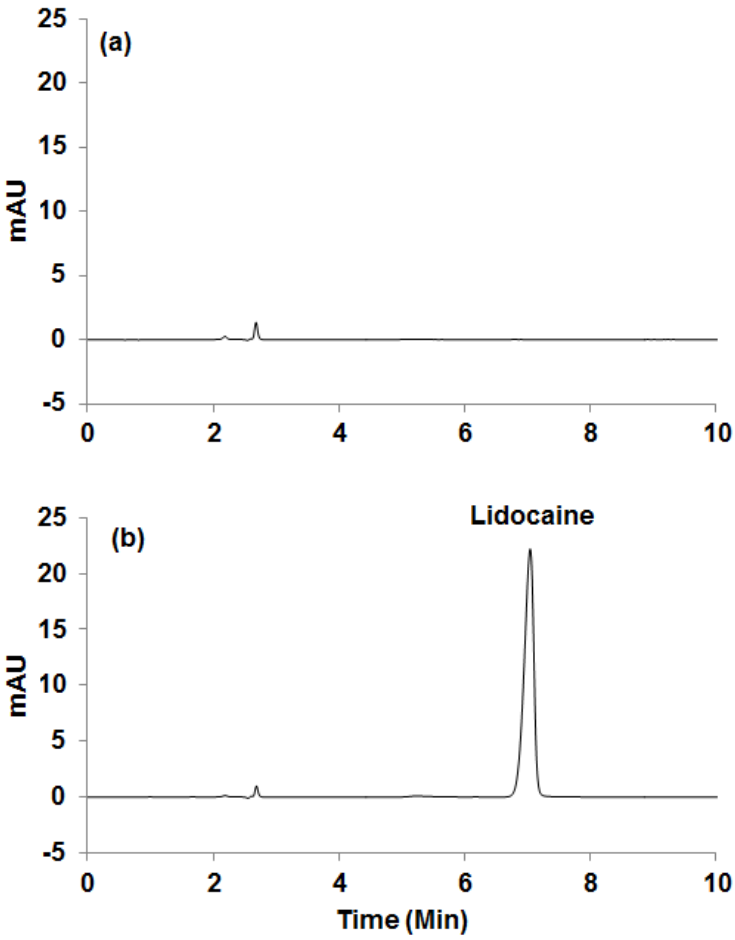

Figure 3.3. Chromatograms of (a) blank 0.1 M HCl and (b) lidocaine in 0.1 M HCl stressed at 60 °C for 10 days.

However, degradation of lidocaine was observed in an alkaline medium at 60 °C, with 84.8 ± 3.0% of lidocaine remaining after 10 days. As displayed in Figure 3.4.a, NaOH produced a relatively large solvent front, however this didn't interfere with the lidocaine peak. The results obtained are in agreement with previously published literature, where the instability of lidocaine in alkaline environments has been reported (186).

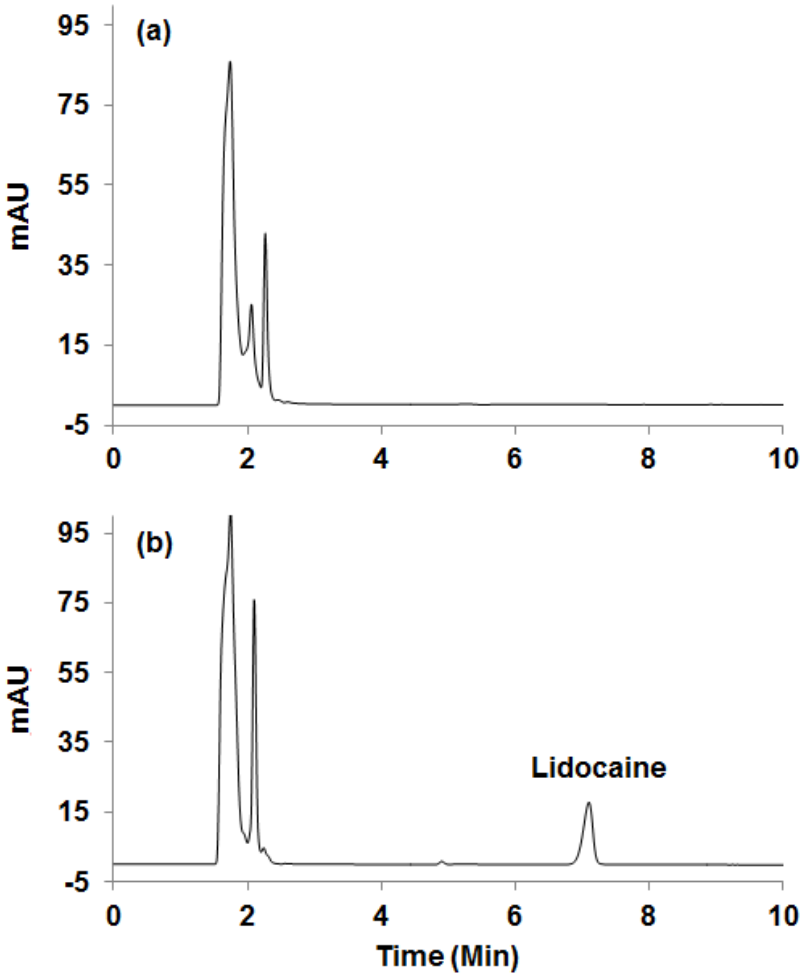

Figure 3.4. Chromatograms of (a) blank 0.1 M NaOH and (b) lidocaine in 0.1 M NaOH stressed at 60 °C for 10 days.

Meanwhile, significant degradation of lidocaine was seen in H_2O_2 solution. Due to the rapid degradation of lidocaine in peroxide medium at 60 °C (13.9 ± 0.0% remaining after 3 days), studies were also conducted at 25 °C with 84.83 ± 2.2% found remaining after 10 days. In a recent report, Ngwa outlined the significance of conducting oxidative stress studies at both room and elevated temperatures. At elevated temperature, alkoxyl radical is formed (2HO.) due to increased homolytic cleavage of the OH-OH bond. This radical is highly reactive and dominates the degradation pathway (167). Our data also support this argument. There was a lower extent of degradation at room temperature under peroxide stress, with only one main degraded product observed, whereas a greater extent of degradation and multiple degradation peaks were observed with the stressed sample exposed at 60 °C (Figure 3.5). The appearance of two degradation peaks with oxidative degradation at 60 °C could be due to formation of two new N-oxide products, as previously reported by Pascuet et al. (186).

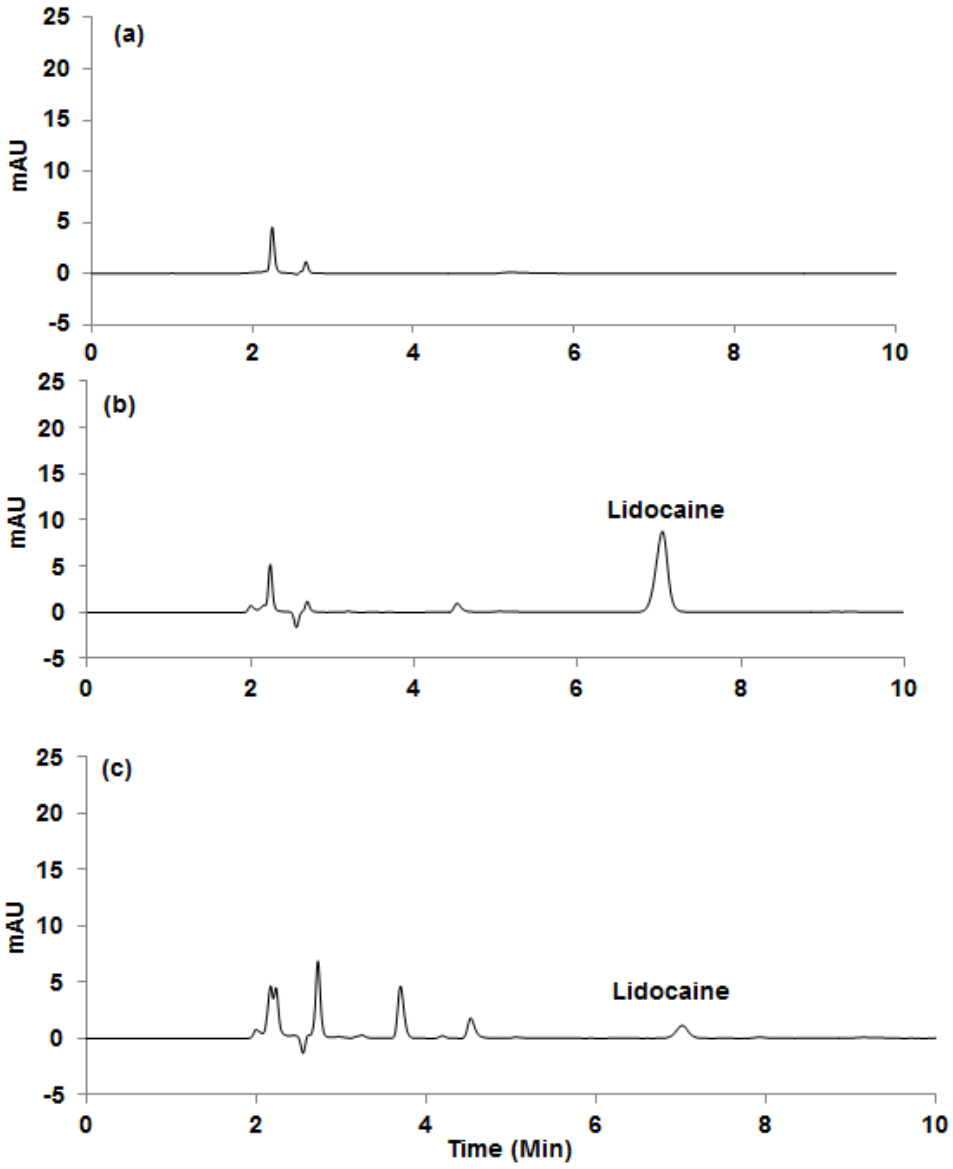

Figure 3.5. Chromatograms of (a) blank 0.02% H_2O_2, (b) lidocaine in 0.02% H_2O_2 stressed at 25 °C and (c) 60 °C for 3 days.

Lidocaine didn't show any degradation in solid state when powder was exposed to a temperature of 60 °C, with or without UV light. Figure 3.6 (a) and (b) display the chromatograms of lidocaine powder stressed at 60 °C and under UV light for 10 days.

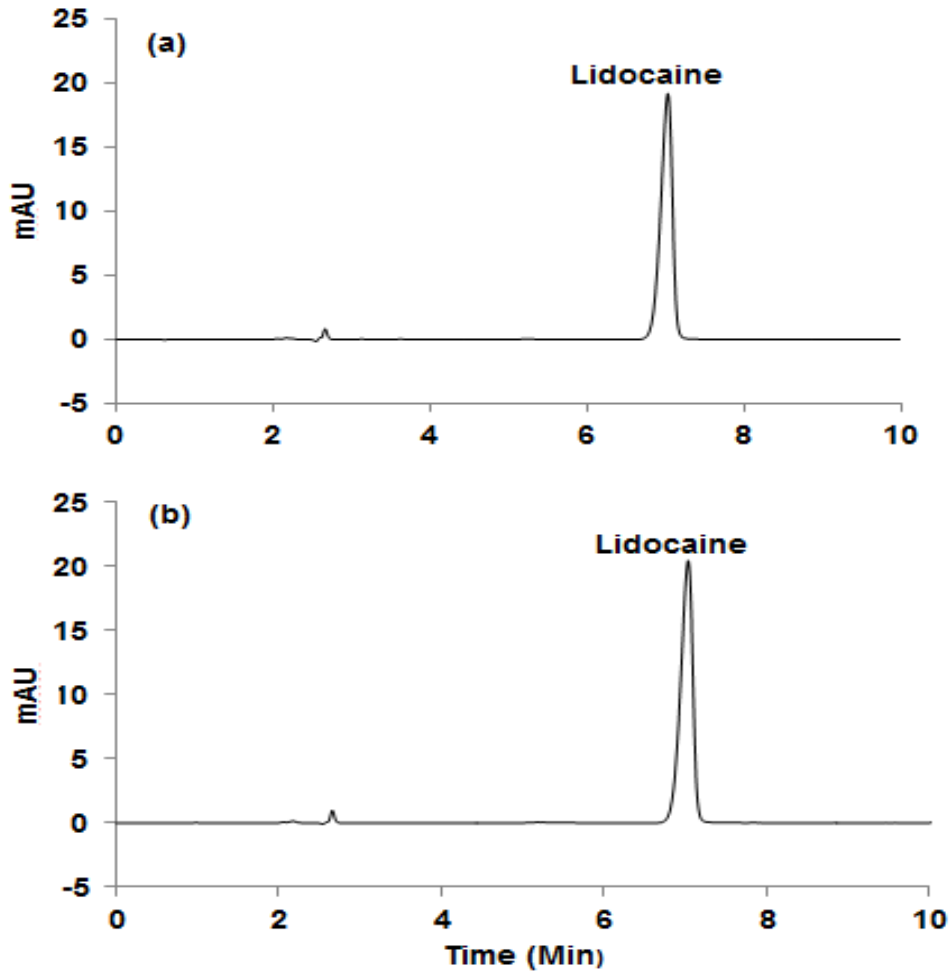

Figure 3.6. Chromatograms of lidocaine (a) stressed at 60 °C and (b) stressed under UV light for 10 days.

This method was also utilised to check its ability to resolve 2,6 di-methyl lidocaine in the presence of lidocaine. Di-methyl aniline, also known as 2,6-xylidine, is one of the primary metabolites of lidocaine obtained following enzymatic degradation in the liver. As displayed in Figure 3.7, the peak of 2,6-dimethyl aniline appeared around 15 min and remained separated from the lidocaine peak, thus demonstrating the wider applicability of this method.

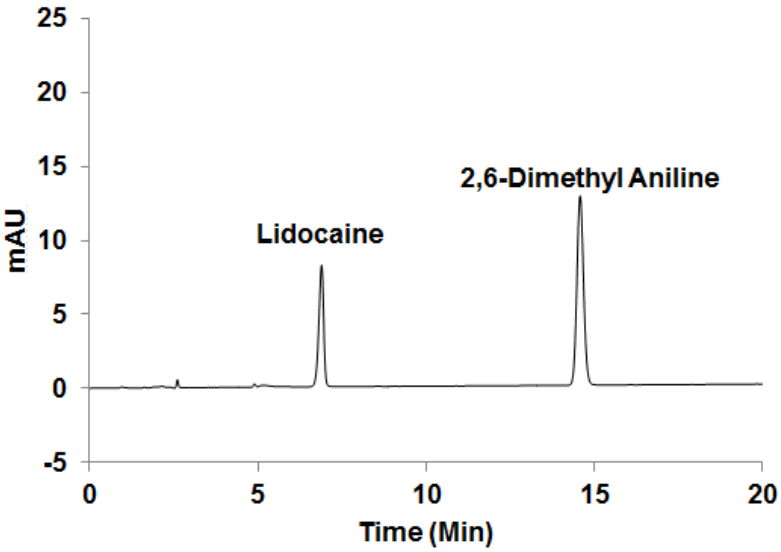

Figure 3.7. Chromatograms showing separation of lidocaine and its primary metabolite in humans, 2, 6-Dimethyl aniline

With these HPLC parameters, the lidocaine peak remained pure for all samples.

3.5.3 Method validation

The standard curve for lidocaine was linear from 0.40 to 50 µg/mL with a linear equation of y = 8.36x + 0.78 and a correlation coefficient (*r*) of 0.9999 (Figure 3.8). The LOQ and LOD obtained were 0.40 µg/mL and 0.025 µg/mL, respectively.

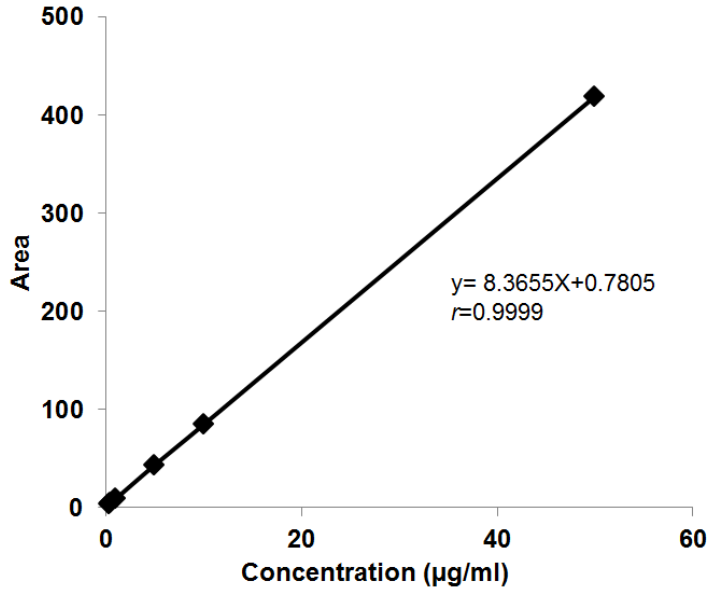

Figure 3.8. Calibration curve of lidocaine standard solution. (value represent the average ± SD, n=3 of area obtained by HPLC from the five different concentrations of lidocaine)

The accuracy and precision results obtained from the optimised HPLC method are summarised in Table 3.3. At the concentration tested, intra-day accuracy was between 97.3 and 99.3% with % RSD less than 4.2%. The inter-day accuracy was between 95.9 and 98.8%, with % RSD less than 6.8%. The accuracy and precision results obtained complied with the recommended range, as all the accuracy values were in the range of 80–120%.

Table 3.3. Accuracy and precision data for the presented HPLC method

Accuracy and Precision				
	Intra-day (n=3)		Inter-day (n=3)	
Conc (µg/mL)	Accuracy (%)	Precision RSD (%)	Accuracy (%)	Precision RSD (%)
0.40	97.7	4.16	95.9	6.77
6.25	97.3	0.23	96.8	0.35
12.5	98.2	0.21	97.8	0.67
25	99.4	0.68	98.9	0.41
50	98.7	0.47	98.7	0.44

3.5.4 Application of the HPLC method to quantify lidocaine from EVA films

The developed HPLC method was applied to estimate lidocaine release from EVA carriers. In addition, the method was employed to estimate the assay and content uniformity of lidocaine following extraction from EVA films. The developed method was tested for recovery and linearity in biological fluids by extracting lidocaine from the fluids.

3.5.4.1 Assay and content uniformity and release of lidocaine from EVA films

The assay and content uniformity of lidocaine in solvent casted EVA films was found to be 103.5 ± 3.6% and 100.3 ± 2.6%, respectively. The RSD for content uniformity was 2.6%, which confirmed the suitability and applicability of this proposed method to extract and analyse lidocaine from EVA films. Along with this, the low RSD value indicated the uniform distribution of lidocaine within the EVA matrix as is expected from a matrix prepared by solvent casting. The extraction method presented is attractive compared to previously described methods which require reflux of the EVA films for 2 h. Refluxing lidocaine with DCM should be avoided due to the flammable solvent and the chance of degradation of lidocaine at high temperature.

The release profile of lidocaine from solvent cast EVA film is shown in Figure 3.9, with release occurring in a sustained manner over a period of 24 h. The release was rapid from the solvent casted films. An

initial burst of release was observed over 2 h with 36% of lidocaine released; this was followed by slower release rates.

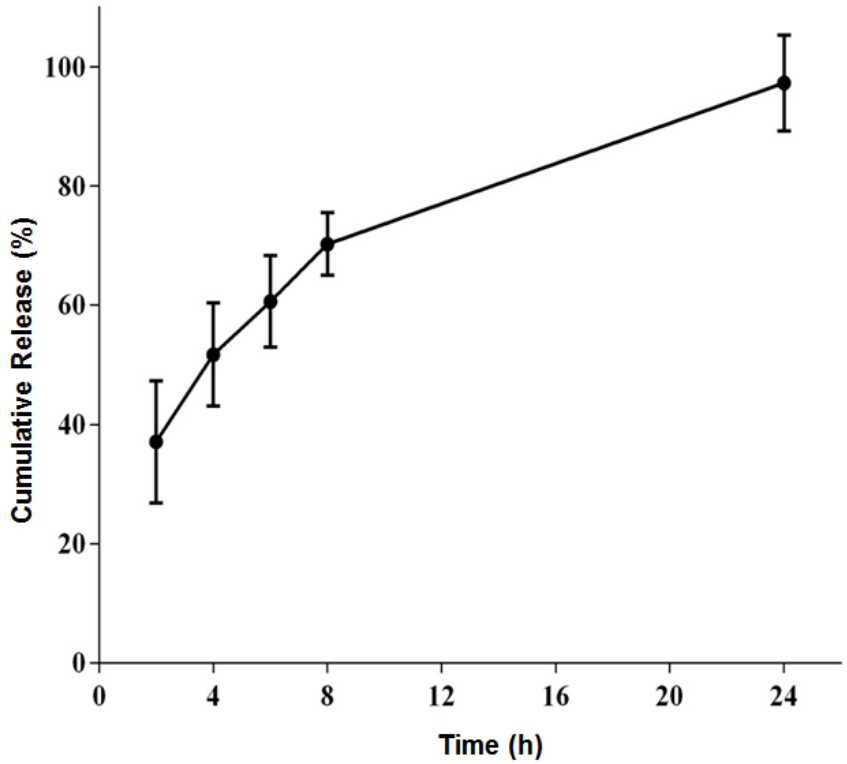

Figure 3.9. Release of lidocaine from solvent cast EVA film (n=3).

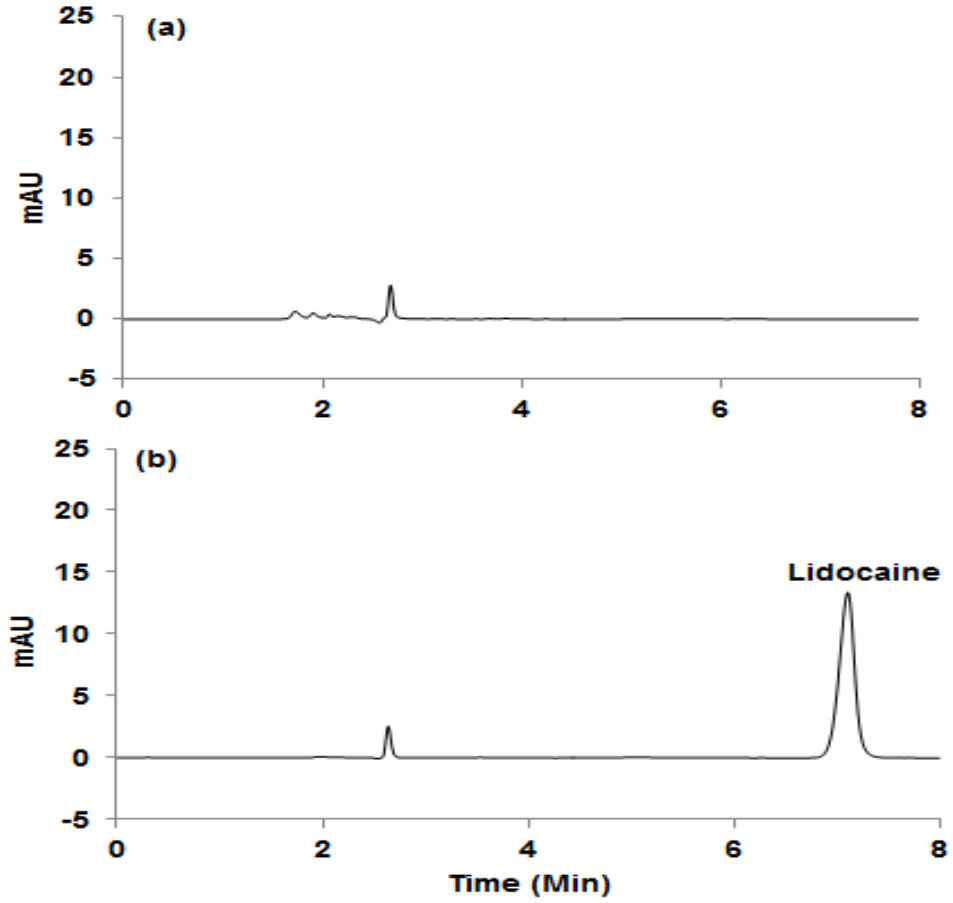

Figure 3.10. Chromatograms of (a) blank 0.01M phosphate buffered saline (PBS) of pH 7.4 and (b) lidocaine released in PBS from EVA film.

There were no additional peaks co-eluting with the lidocaine peak on the chromatograms of the samples (Figure 3.10), demonstrating the utility of the HPLC method for quantifying lidocaine release from EVA matrices

3.5.4.2 Release of lidocaine from EVA melt extrudate

As the release was rapid from the EVA film prepared by solvent casting method, hot melt extruded rods of EVA were examined. The rods prepared by melt extrusion had a smaller surface area (2.3 mm diameter) when compared to the solvent cast films (10 mm diameter X 0.5 mm thickness). Hence, a slower release rate of lidocaine could be expected from the melt extrudates. Although the studies were conducted over seven days, the cumulative release of lidocaine from the rods didn't exceed 70 % (Figure 3.11). There was visible precipitation of lidocaine over the surface of the melt extrudates, meaning the actual content of lidocaine could have decreased if recrystallised lidocaine was being lost from the surface between extrusion and determining release.

The release of lidocaine from ME under sink and non-sink conditions is shown in Figure 3.11.a. The release rates were similar for the first 6 h, after which faster release was observed for sink conditions compared to non-sink conditions. The difference in release became more apparent over the course of time. Lidocaine is continuously removed from the surface of melt extrudates under sink conditions. Meanwhile under non-sink conditions, interfacial kinetics result in the sufficient formation of a boundary layer over time so as to decrease the release of drug from the melt extrudate (187). This phenomenon becomes clear when a curve is plotted between the ratios of percentage release between non-sink and sink conditions versus the time (Figure 3.11.b) (187). It is apparent from the plot that the ratio is initially close to unity. After 6 h, as the concentration in the media rose above 20% of the saturated solubility, this movement away from sink conditions resulted in a decrease in rate of release. This resulted in a decrease in the ratio of percentage release between non-sink and sink conditions. As no peaks eluted to interfere with analysis from the formulation or PBS, this method was deemed suitable to quantify lidocaine from the both the solvent cast and melt extrudate EVA.

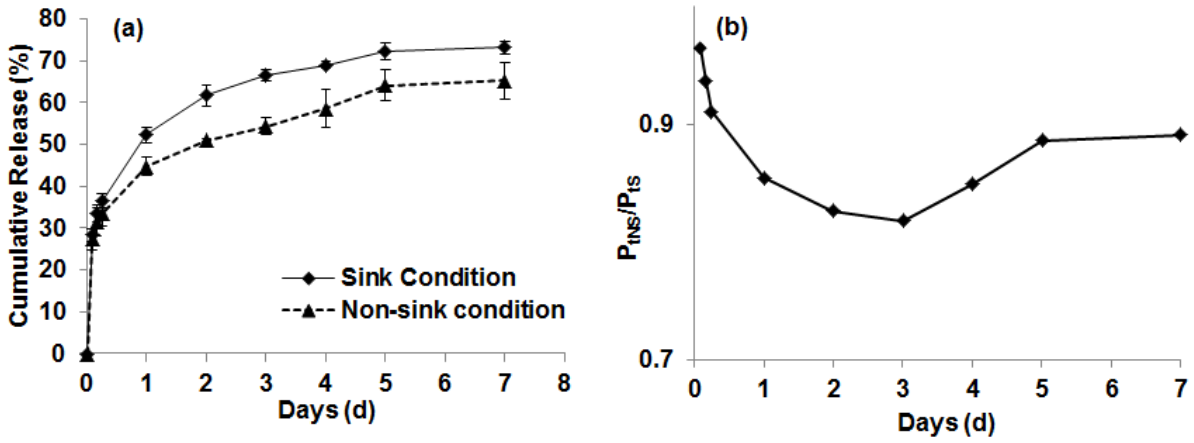

Figure 3.11. (a) Release of lidocaine from ME under sink and non-sink conditions (n=3); (b) Comparison of sink versus non-sink conditions. Pt_{NS} and Pt_S refer to percentage release under non-sink and sink conditions respectively.

3.5.4.3 Quantification of lidocaine from biological fluids

Recovery from plasma, IAF and PF is shown in Table 3.4. The mean recovery of lidocaine from different biological fluids ranged from 91.1–100.6% with linearity values greater than 0.998.

Table 3.4. Recovery of lidocaine from biological fluids.

Conc (µg/mL)	**Plasma**		**Intra-articular fluid**		**Peritoneal fluid**	
	Recovery (%)	Linearity (*r*)	Recovery (%)	Linearity (*r*)	Recovery (%)	Linearity (*r*)
12.5	91.2 ± 0.09		92.3 ± 2.45		93.8 ± 1.62	
25	93.5 ± 0.19		92.7 ± 2.23		97.5 ± 1.69	
50	100.7 ± 1.50		91.5 ± 1.52		97.0 ± 0.64	

Generally, 80 to 120% accuracy of recovery from any biological fluids is acceptable (188). The chromatograms of lidocaine recovered from plasma, IAF and PF are illustrated in Figure 3.12. There were no impurities derived from the biological fluids co-eluting with lidocaine. The peak purity calculated from the biological samples confirmed that the lidocaine peak was pure and free from any interference. The calibration plots were found to be linear from 1.6 to 50 µg/mL, with correlation coefficients (*r*) of 0.9980, 0.9998 and 0.9983, for plasma, IAF and PF respectively.

Generally, plasma concentrations of lidocaine below 5 µg/mL are safe (55). The calibration curve plotted for the plasma samples (1.6–50 µg/mL) indicates the ability of the method to assess the threshold limit of lidocaine when any drug delivery system containing lidocaine is studied *in vivo* conditions. Lidocaine is administered locally into intra-peritoneal and intra-articular regions to alleviate post-operative pain following abdominal and knee surgery respectively (3, 189). This quantification method can be employed to assess the release performance of any lidocaine based drug delivery system in intra-articular fluid and peritoneal fluid at in situ and/or *in vivo* settings. The utility of this method to estimate lidocaine release into human peritoneal fluid from an intra-peritoneal implant will be further explored in Chapter 6.

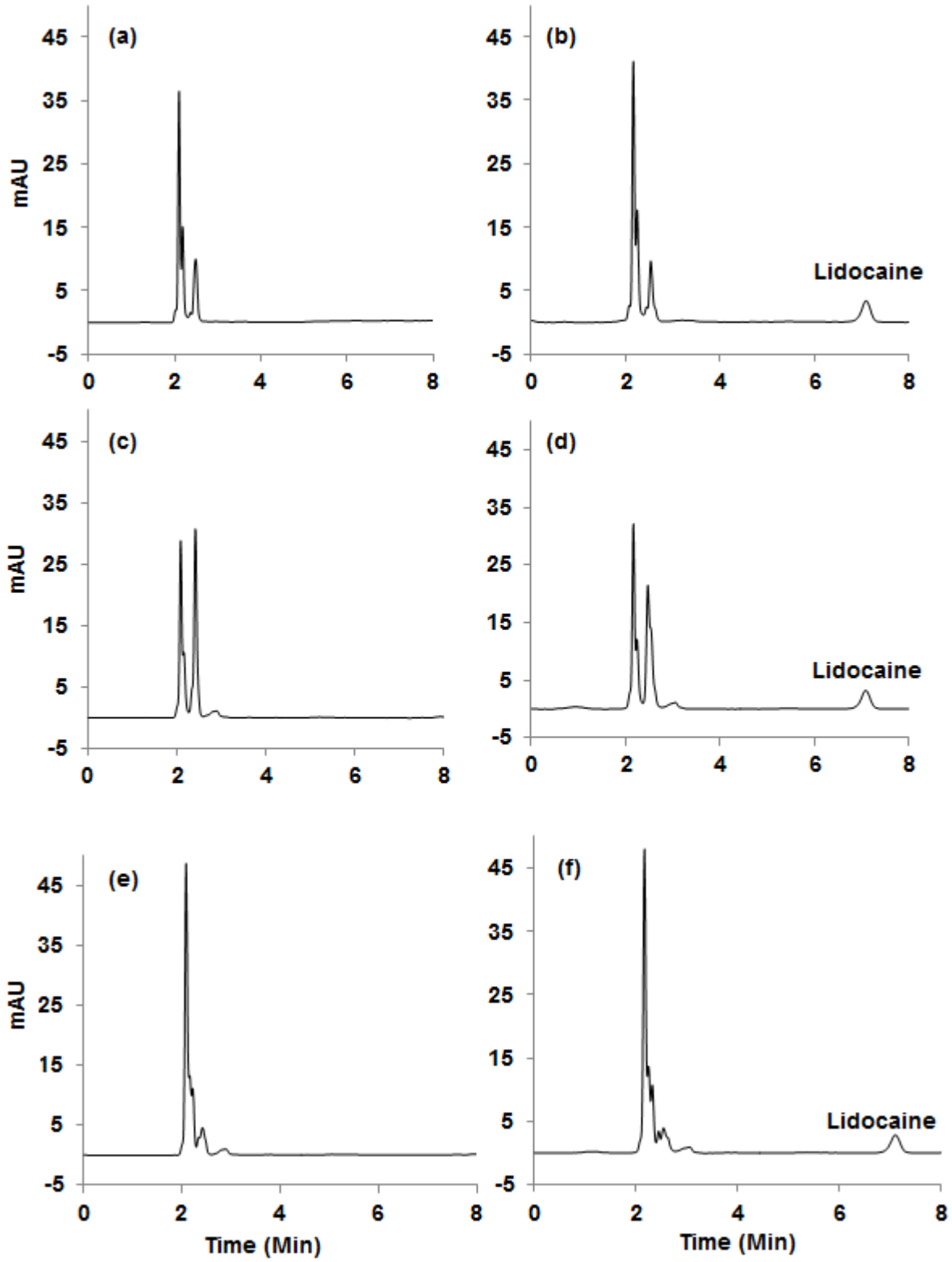

Figure 3.12. Chromatograms of blank (a) plasma, (c) intra-articular fluid and (e) peritoneal fluid and lidocaine (RT ~7 min) recovered from (b) plasma (50 µg/mL), in (d) intra-articular fluid (50 µg/mL) and (f) peritoneal fluid (50 µg/mL)

3.6 Conclusions

A simple, rapid and economical HPLC method with isocratic elution was developed for the quantification of lidocaine from stressed samples, EVA matrices and biological fluids. Lidocaine was found to be unstable in peroxide medium and an alkaline environment with degradation products well resolved from the lignocaine peak on HPLC analysis. The proposed HPLC method has specific advantages over previously published methods including its simplicity, cost-effectiveness and isocratic conditions. The ability to quantify the released lidocaine from EVA solvent cast films and melt extrudates was also demonstrated. The release of lidocaine from EVA films occurred at faster rates when compared to melt extrudates. A simple extraction method to quantify lidocaine from EVA matrices biological fluids has been described, with wide applicability for routine analysis and to study the performance of lidocaine formulations *in vitro* and *in-vivo* studies.

CHAPTER 4. PREFORMULATION STUDIES OF LIDOCAINE AND POLY(ETHYLENE-CO-VINYL ACETATE)

4.1 Background

The main role of poly(ethylene-co-vinyl acetate) (EVA) for the purpose of this study will be to encapsulate and modulate the release rate of lidocaine to provide controlled release over a period of 10 days. EVA has a long history of use in drug eluting implants. Its safety and biocompatibility have been investigated and it has been approved by the FDA for use as a biomaterial in a range of body sites (15, 18, 19). Despite EVA's widespread use, confirmation is required that lidocaine, the drug of interest, will remain stable with EVA. A few investigations have reported the stability of drugs with EVA, including chlorhexidine, doxycycline, tetracycline and nystatin (190). However, there are no reports describing compatibility studies between local anaesthetics and EVA. Detailed investigations of the properties of lidocaine and EVA alone and in combination will help in the rational development of a CR drug delivery system. There are various approaches to assess the compatibility of drugs with polymeric carriers. Isothermal testing is a convenient approach whereby a binary mixture of drug and polymer is taken and stressed under elevated temperatures before analysis by various means (138). Various analytical tools are used to characterise the compatibility of drugs with polymeric carriers, including thermo-gravimetric analysis (TGA), differential scanning calorimetry (DSC), nuclear magnetic resonance (NMR), Fourier transform infrared spectroscopy (FTIR) and high performance liquid chromatography (HPLC) (191-193). The specific objectives of using these analytical tools to elucidate drug-polymer compatibility have been described in detail in Section 2.4 of Chapter 2.

The preparation of our intended implant will involve either solvent casting, melt extrusion or injection moulding. The major challenge to preparing a stable implant is the incidence of drug crystallisation in the polymer matrices. EVA has a long polyethylene chain with a vinyl acetate group. Lidocaine consists of a benzene ring and tertiary amine group linked by an amide bond. Thus, EVA and lidocaine have contrasting hydrophobicities. Achieving the required dose of lidocaine dispersed in the EVA matrix is expected to be problematic as lidocaine may recrystallise. Accordingly, possible measures to prevent the recrystallisation of lidocaine should also be investigated in preformulation studies. An understanding of interactions between lidocaine and EVA will be helpful in formulating a stable dispersion of lidocaine in EVA matrices. Based on a previous report on interactions between an amino methacrylate copolymer and nifedipine, which have similar functional groups to those of EVA and lidocaine (148), it is likely that interactions could take place between lidocaine and EVA. However, it is not certain that the interactions between EVA and lidocaine would be sufficient to prevent recrystallisation of lidocaine. The addition of a third component with intermediate properties between lidocaine and EVA may be necessary to prepare a stable formulation. In addition, the physical properties of EVA depend upon its vinyl acetate (VA) composition, as discussed in detail in Chapter 2. The polymer becomes crystalline to rubbery when the VA composition in EVA is increased. The extent of interaction and the release rates of lidocaine will differ across various EVA grades. Therefore, screening of various grades of EVA with lidocaine should be conducted. Of the various approaches which could be explored to disperse lidocaine uniformly into an EVA matrix, cryogenic grinding of EVA and lidocaine could also be tested. This approach has been widely used to prepare uniformly dispersed matrix systems (194-196).

4.2 Aims and objectives

The overall aim of the work presented in this chapter was to perform preformulation studies of lidocaine and EVA to support the development of a stable formulation. The specific objectives were to:

- Elucidate the compatibility and potential interactions of lidocaine with EVA using TGA, DSC, NMR, FTIR and HPLC
- Select potential additives to stabilise lidocaine in EVA matrices
- Elucidate the effect of cryogenic grinding on the crystallinity of lidocaine

4.3 Materials

Lidocaine was purchased from Sigma (Auckland, New Zealand). EVA of 25% VA composition was purchased from Dupont (USA). EVA of 33% VA composition was donated by Celanese. EVA of 40% VA composition was purchased from Sigma (Auckland, New Zealand). Myristic acid ≥98% was sourced from Fluka Analytical. Stearic acid, reagent grade 95%, was purchased from Sigma Aldrich (Dorset, UK).

4.4 Methods

4.4.1 Investigation of compatibility and interaction between lidocaine and EVA

An investigation of compatibility and potential interactions between EVA and lidocaine was carried out with both physical mixtures of EVA and lidocaine (1:1) and films of EVA and lidocaine (4:1) prepared by solvent casting.

4.4.1.1 Preparation of binary physical mixture

Accurately weighed amounts of lidocaine and EVA powder in a fixed 1:1 ratio were blended in a mortar and pestle. EVA with three different VA composition was investigated for this study (25%, 33% and 40% VA). Predetermined weights of powder containing the binary mixture of lidocaine and EVA were stressed in an oven at 60 °C for 10 days. The remaining mixtures were stored in Falcon tubes and kept at 25 °C for further analysis. The samples were subjected to characterisation by TGA, DSC, NMR, FTIR and HPLC to determine the chemical stability of lidocaine in the presence of EVA.

4.4.1.2 Preparation of solvent cast film

A solution of lidocaine and EVA was prepared by dissolving 0.4 g of lidocaine and EVA mixture (1:4 w/w) in chloroform at 50 °C. EVA (33% VA) was selected for this study due to evidence of interactions in the

binary mixture. In addition, two different fatty acids, myristic acid and stearic acid, were added individually to the mixture of EVA and lidocaine and allowed to dissolve in chloroform. EVA, fatty acid and lidocaine were in the ratio of 3:1:1. One mL of the solution was taken by glass pipette and poured onto a glass slide. The glass slide containing the film was allowed to dry overnight. The film was then recovered from the slide and analysed by FTIR.

4.4.2 Investigation of the effect of cryogenic grinding on crystallinity of lidocaine

Cryogenic grinding is widely used to grind EVA to produce small particles (197). It was expected that cryogenic grinding of EVA with lidocaine would result in the formation of fine particles to produce a better dispersion of lidocaine in EVA matrices. Cryogenic grinding of the EVA and lidocaine mixture was performed using a freezer mill (6970 EPM Freezer/Mill SPEX Sample Prep, Inc., USA) consisting of a cylinder immersed in liquid nitrogen. The stainless steel rod within the cylinder was shaken with the lidocaine and EVA mixture to provide an impact for grinding. The freezer mill was pre-programmed to a grinding setting consisting of a 10 min precooling time, followed by cycles of grinding with an impact frequency of 10 Hz for 8 min separated by 10 min cool-down periods. Each cylinder was filled to one third of its volume and sealed with a stainless steel cap. The entire cylinder containing the sample was locked inside the freezer mill before initiating the programme. The powdered mixtures of lidocaine and EVA were prepared at 1:4 w/w and ground for 120 min. EVA with 25% VA content was examined for the study. The cap was removed by an automatic cap remover and the milled powders were transferred to Falcon tubes and stored until further analysis by DSC.

4.4.3 Characterisation

The characterisation of different samples by various analytical tools is summarized in Table 4.1

Table 4.1. Characterisation of samples using various analytical tools

Characterization technique	Lidocaine	EVA	Binary physical mixture	Binary physical mixture stored at 60 °C for 10 days	Solvent cast film	Milled by cryogenic grinding
TGA	√	√	√	√	-----	-----
DSC	√	√	√	√	-----	√
NMR	√	√	√	√	-----	-----
FTIR	√	√	√	√	√	-----
HPLC	-----	-----	-----	√	-----	-----

4.4.3.1 Thermo gravimetric analysis (TGA)

The thermal stability of the samples including lidocaine, EVA polymer and the binary physical mixture of lidocaine and EVA were studied using a TA instrument Q500 TGA (TGA; Leatherhead, UK). Samples were taken in aluminium pans and heated at a scanning rate of 10 °C/min from 10 °C to 300 °C. In order to simulate the conditions during hot melt extrusion, another set of experiments was conducted whereby the samples in the aluminium pans were heated at a scanning rate of 10 °C/min from 10 °C to 120 °C and held for 10 min at 120 °C. Nitrogen was used as the purging gas during all the TGA experiments.

4.4.3.2 Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) of samples was performed using a Perkin-Elmer DSC 4000 (Cambridge, UK) equipped with a refrigerated cooling system (Perkin-Elmer Intra cooler-SP). Data analysis was performed using Pyris Manager software (version 10.1) Nitrogen was used as the purge gas at a flow rate of 20 mL/min. The samples were taken in aluminium pans and crimped before heating at a scanning rate of 10 °C/min from -40 °C to 110 °C. The DSC was calibrated for baseline correction using empty pans, and for temperature and enthalpy using the high purity metals indium and zinc.

Differential scanning calorimetry (DSC) of the various samples including pure lidocaine, EVA powder and the binary physical mixtures of of EVA and lidocaine, both freshly prepared and after storage at 60 °C for 10 days, was performed to characterise the thermal behaviour of EVA and lidocaine.

Thermal analysis of samples milled by cryogenic grinding was carried out using a DSC unit with a Q2000TM module (TA Instruments, USA). Indium was used as the reference material to calibrate the

temperature scale and enthalpic response. Approximately 3 mg of the samples including EVA, the physical mixture and cryomilled mixture were taken in aluminium pans and crimped before heating at a scanning rate of 10 °C/min from -40 °C to 110 °C.

4.4.3.3 Nuclear magnetic resonance spectroscopy (NMR)

Solid state NMR spectra were obtained from the EPSRC National Solid-state NMR service at Durham University. The solid state ^{13}C spectrum of each sample was obtained by magic angle spinning solid-state nuclear magnetic resonance (VNMRS-400 Spectrometer, Varian, Crawley, UK). The spectra were recorded at 100.56 MHz, for which samples were loaded into a 6 mm (rotor o.d.) magic angle-spinning probe. Samples including pure lidocaine, EVA powder, and binary physical mixtures of EVA and lidocaine, both freshly prepared and after storage at 60 °C for 10 days, were analysed.

4.4.3.4 Fourier transform infra-red spectroscopy (FTIR)

The infrared transmission spectra of pure lidocaine, EVA powder, physical mixtures of EVA and lidocaine and solvent cast samples were obtained using a FTIR spectrophotometer (Bruker Tensor 37 FTIR spectrometer; OPUS 6.5, Germany). Samples were analysed above a diamond crystal using ATR mode. Spectra of samples between 4000 cm^{-1} to 400 cm^{-1} at resolution of 4 cm^{-1} were considered to investigate the potential interactions between the active functional groups of lidocaine and EVA.

4.4.3.5 HPLC

The quantification of lidocaine from stressed samples was conducted with the HPLC method developed, as described in Chapter 3. Initially, binary physical mixtures of EVA stressed at 60 °C for 10 days were extracted with dichloromethane by sonicating the isothermally stressed sample in a volumetric flask for about 40 min. One mL of solution was withdrawn and dried in a volumetric flask at 60 °C for an hour. Final extraction was carried by sonicating the dried film for about 30 min using methanol as a solvent. Samples were centrifuged at 5844 g for about 4 min before injecting them into HPLC.

4.4.3.6 Curve fitting of FTIR spectrum

Curve fitting was performed using OPUS 6.5 software. Initially, the spectra were converted into absorption mode. The option of rubber band baseline correction in polynomial mode with 10 iterations was employed for each spectrum. Following this, the peak arising from the N-H stretch of lidocaine (3400-3100 cm^{-1}) in different samples was fitted using the software. Curve fitting was done by iterative least square curve-fitting method using the in-built combination of a Gaussian-Lorentzian (GL) function. An acceptable fit was ensured by keeping the error value within the acceptable range as indicated by the software. The information derived from the central wavenumber was interpreted to investigate potential interactions.

4.5 Results and Discussion

Preparing binary mixtures of drug and excipients and subjecting them to elevated temperature of around 60 °C before analysis by various tools is a conventional way to investigate compatibilities between drug and excipients (138, 198). Generally, the characterisations involve a binary ratio of 1:1 (drug:polymer) mixture when assessing the compatibility of drug with polymeric materials (192, 193). Following this, interactions were further probed on solvent cast samples using FTIR.

4.5.1 Binary physical mixture

The samples prepared under various conditions were analysed using different analytical tools.

4.5.1.1 TGA

The TGA thermogram of lidocaine and EVA having 25, 33 and 40% VA composition across a temperature ramp is displayed in Figure 4.1. The TGA ramp for lidocaine started demonstrating weight loss at 135 °C and it was more than 5% at 160 °C leaving no traces of lidocaine after 250 °C (Figure 4.1.a). Previously published reports also confirmed the volatile nature of lidocaine at higher temperatures (199). Meanwhile, weight loss was not pronounced with EVA although minor weight loss was seen with EVA having 40% VA contents (5% after 200°C) (Figure 4.1. f). The weight loss seen for lidocaine could either be due to degradation or due to its volatile nature at higher temperatures (199). Previous studies have shown that the degradation of EVA becomes evident with increasing VA content (200). .

The process of implant preparation involves either solvent casting, melt extrusion or injection moulding. Melt extrusion and injection moulding involve the exposure of a mixture of drug and polymer to a higher temperature. An assessment of thermal stability was therefore important, so the mixture of lidocaine and EVA was exposed to extrusion temperature. Accordingly, TGA ramps were conducted with binary mixtures of EVA and lidocaine. Volatile degradation (>5 %) was evident in all the samples, but only after 175 °C (Figure 4.1.c , Figure 4.1.e and Figure 4.1.g) which was higher when compared to pure lidocaine possibly be due to the shielding effect of EVA against volatile degradation of lidocaine.

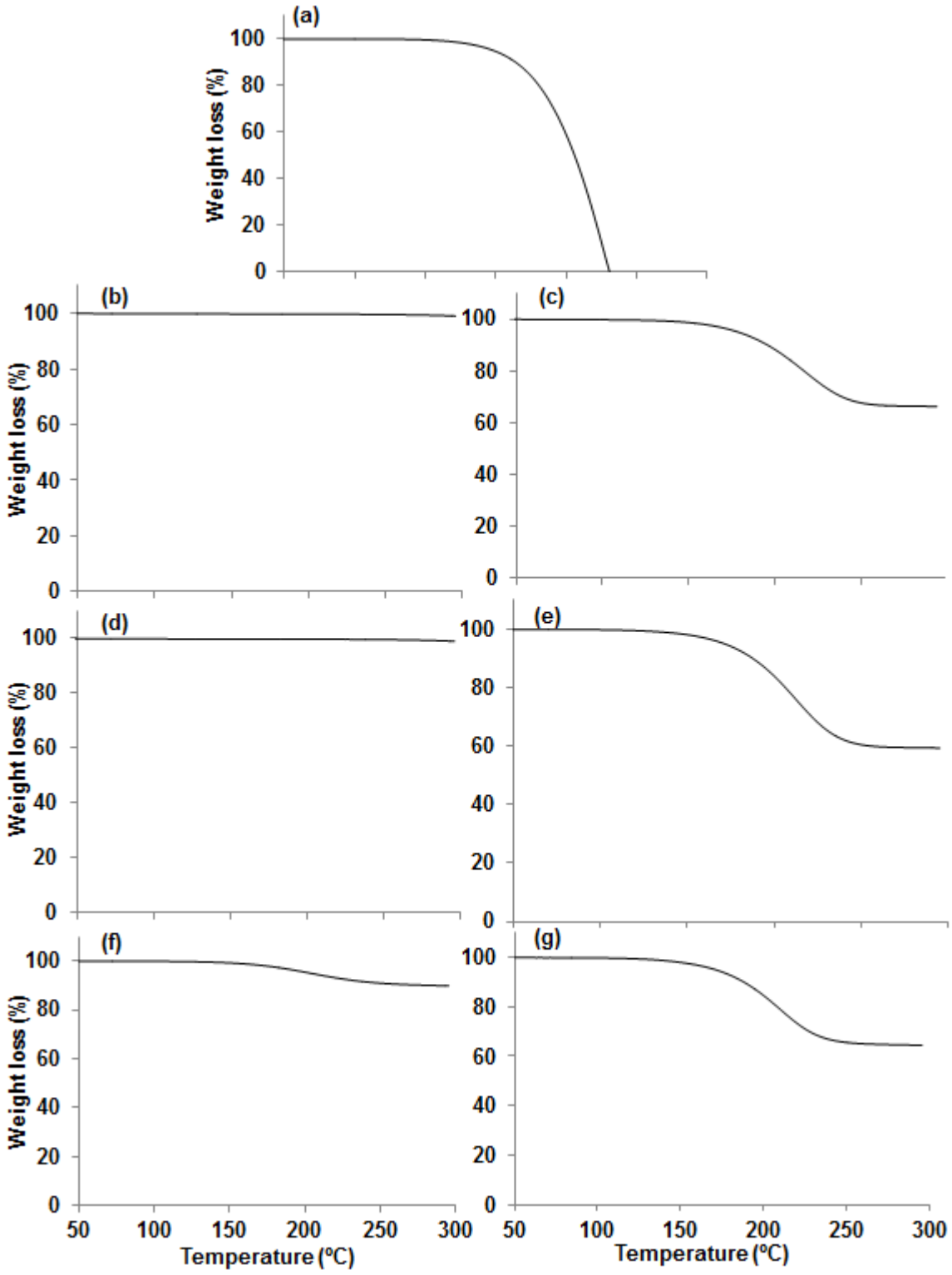

Figure 4.1. TGA thermograms of pure (a) lidocaine, (b) EVA (25% VA), (d) EVA (33%VA) and (f) EVA (40% VA) and binary mixtures of lidocaine and EVA (1:1) related to (c) 25% VA, (e) 33%VA and (g) 40% VA composition

The mean residence time of drug and polymer mixture in hot melt extruder (HME) is around two min (201). Therefore, another TGA ramp was conducted mimicking extrusion conditions by holding the temperature at 120°C. As shown in Figure 4.2, minimal weight loss (>97 % remaining) was seen when samples were exposed to 120 °C held for approximately 10 min. The results obtained from the the TGA experiments suggest that the mixtures were thermally stable under extrusion conditions.

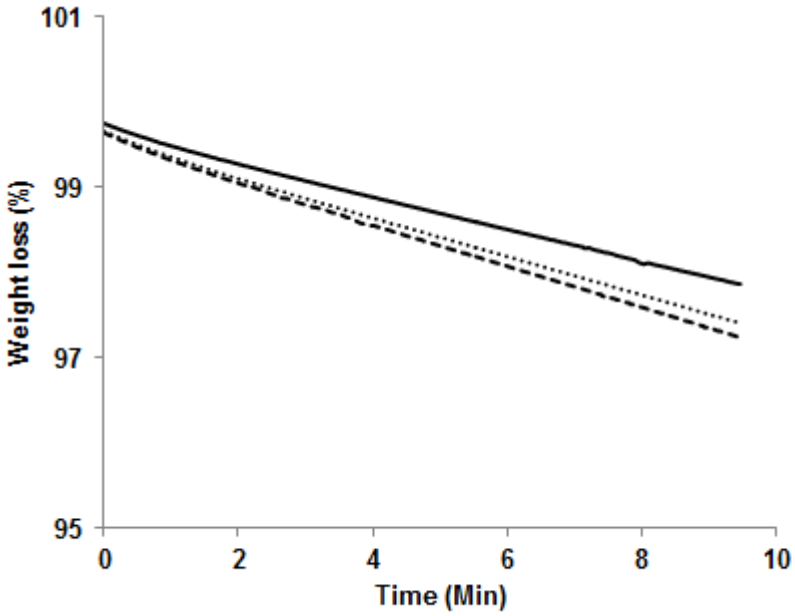

Figure 4.2. TGA thermograms of binary mixture of lidocaine and EVA (1:1) related to 25% VA, 33%VA and 40% VA composition held at 120°C for 10 min as indicated by solid, dotted and dashed curves respectively.

4.5.1.2 Differential scanning calorimetry (DSC)

The DSC thermogram of binary mixtures of lidocaine and EVA related to three different VA contents is displayed in Figure 4.3. For the samples containing EVA with 25% VA, a sharp peak is seen corresponding to the melting point of lidocaine at 69 °C in both the freshly prepared samples and the samples stressed at 60 °C for 10 days (Figure 4.3.a), indicating lidocaine present in a crystalline form in the samples. Similarly, a sharp lidocaine melting point was found for the freshly prepared physical mixtures containing EVA of 33 and 40% VA composition. However, the lidocaine peak is absent for the stressed samples containing EVA having VA compositions of 33 and 40%, implying the amorphous conversion of lidocaine in EVA matrices (Figure 4.3. b and c). The melting point of EVA (25% VA) is higher than the melting point of lidocaine (202), thus, lidocaine did not have the opportunity to be dispersed into the EVA matrices. However, the melting point of EVA (33% VA) and EVA (40% VA) is around 45 °C which is lower than the melting point of lidocaine, thereby favouring the better dispersion of lidocaine in the EVA matrices during the temperature ramp. However, it is important to note that lidocaine exerts a plasticising effect on the polymer (115). This may explain why the melting point of EVA shifted towards lower temperatures. As highlighted in Chapter 2, the lidocaine needs to be in amorphous

state inside the EVA matrix to prepare a stable implant. The results from this experiment suggest that EVA having 33 and 40% VA will be preferable over EVA having 25% VA composition, due to their better ability to disperse lidocaine. However, it is also important to note that the rigidity of polymer decreases with increasing VA content. Accordingly, EVA (33% VA) will have better mechanical strength compared to EVA (40% VA). Thus, EVA with 33% VA composition is an appropriate choice to form a controlled release implant as it can disperse lidocaine while being mechanically robust.

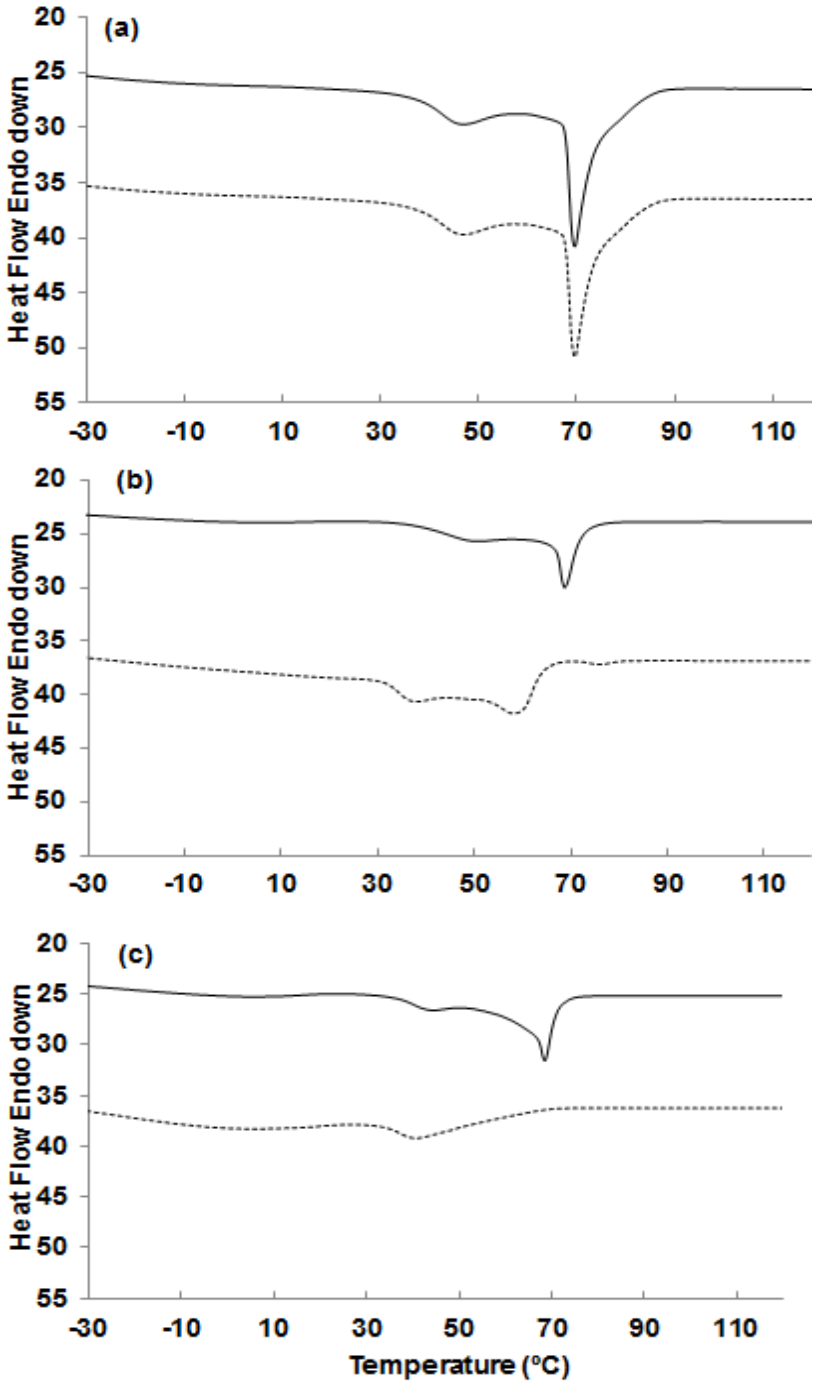

Figure 4.3. DSC thermograms (scanned from -30 to 110 °C) of binary mixture of lidocaine and EVA (1:1) related to (a) 25% VA, (b) 33%VA and (c) 40% VA composition. Solid and dashed lines indicate the respective thermograms of fresh physical mixtures and samples stressed at 60 °C for 10 days.

4.5.1.3 ^{13}C NMR

The ^{13}C spectra of lidocaine and the samples related to lidocaine, EVA (25% VA), EVA (33% VA) and EVA (40% VA) are shown in Figure 4.4, Figure 4.5, Figure 4.6 and Figure 4.7 respectively. Lidocaine gives distinct signals around 170, 138.3, 136.7, 127.9, 127.1, 56.2, 48.7, 18.8 and 13 ppm due to the corresponding carbon atoms, as shown in Figure 4.4.a. There appears to be no significant change in the signals in the spectrum from the lidocaine sample exposed to 60 °C for 10 days, confirming the chemical stability of lidocaine at this stress condition (Figure 4.4.b). The results obtained are in agreement with the forced degradation studies summarised in Chapter 2, which also confirmed the chemical stability of lidocaine when exposed to 60 °C for 10 days.

For all EVA samples, signals from the ethylene component (signals 20 to 40 ppm; labelled i, ii, iii, and iv) dominated over other signals regardless of VA composition (Figure 4.5.a, Figure 4.6.a and Figure 4.7.a). Two signals at around 30 and 33 ppm (labelled iii and iv) were due to contributions from the rigid and flexible ethylene components of EVA (190, 203). The prominent signal (iii) at around 30 ppm was due to the flexible ethylene component, whereas the less prominent signal (iv) at 33 ppm was due to the rigid ethylene component (203). As the rigid ethylene component decreases with increasing VA composition, the signal due to rigid ethylene in EVA (40% VA) is less intense than in EVA having 25% VA composition. A weak signal (vi) seen at around 170 ppm was due to the carbonyl carbon of VA in EVA (204). Overall, there were no major differences in the signals between the VA compositions of 25, 33 and 40%.

For the physical mixtures, the signals derived from lidocaine and EVA were distinct and reasonably well separated (Figure 4.5.b, Figure 4.6.b and Figure 4.7.b). No clear change in signals was seen in any of the samples exposed to 60 °C for 10 days (Figure 4.5.c, Figure 4.6.c and Figure 4.7.c), suggesting that the chemical stability of lidocaine remains unaffected in the presence of EVA regardless of different VA compositions. However, the data obtained were not of much help in identifying possible interactions between the lidocaine and EVA. Lidocaine contains one proton donor (199), while EVA contains a carbonyl oxygen which could accept a proton from the N-H group of lidocaine to form an interaction in a form of hydrogen bonding. As the signals from the carbonyl carbon in the VA of EVA at 170 ppm were poor and dominated by the carbonyl carbon of lidocaine, it was difficult to identify any changes in the signals indicating this possible interaction. An alternative analytical tool was required in order to probe possible hydrogen bonding associated with this carbonyl group in the VA of EVA.

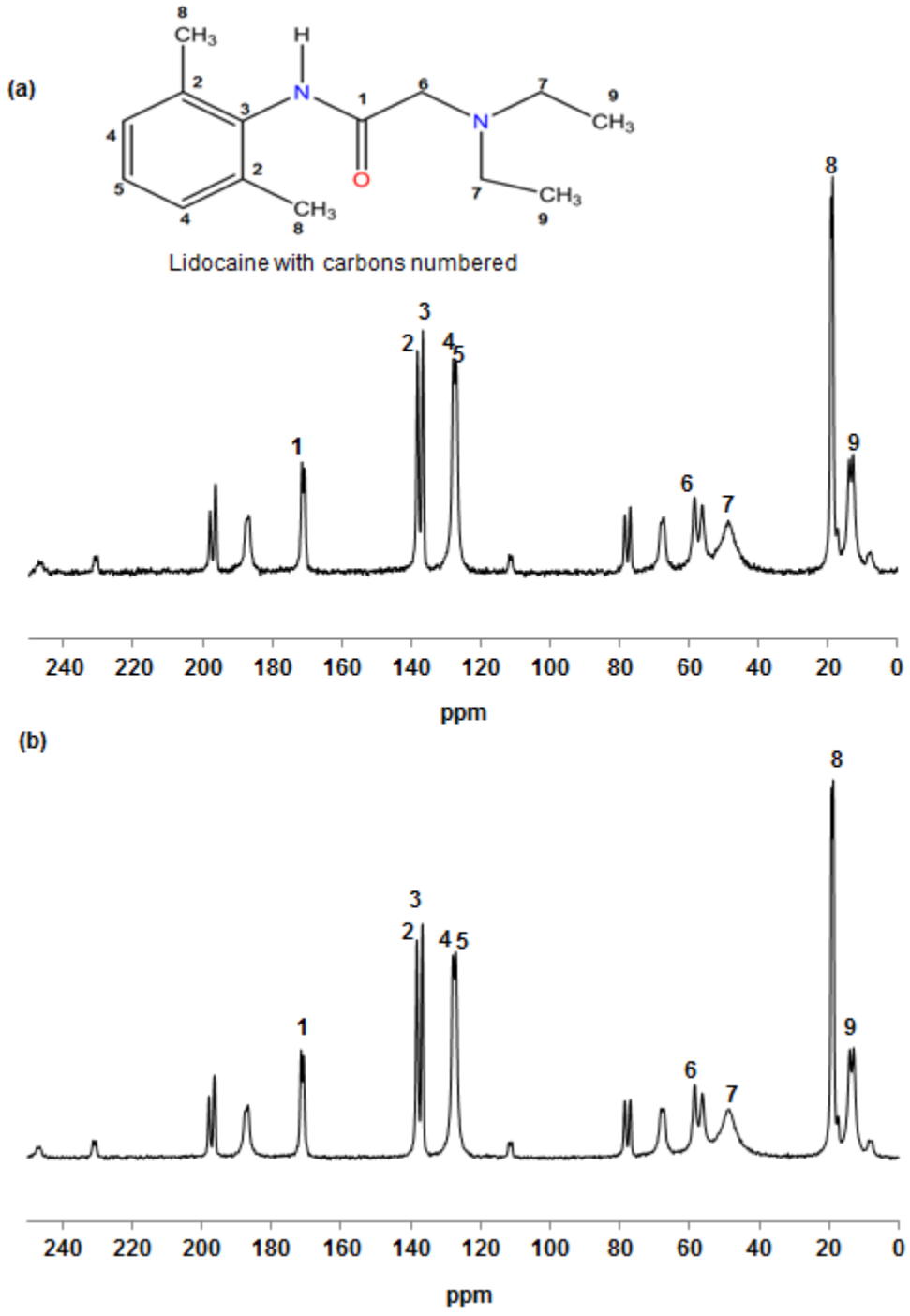

Figure 4.4. ^{13}C spectra of (a) lidocaine and (b) lidocaine stressed at 60 °C for 10 days.

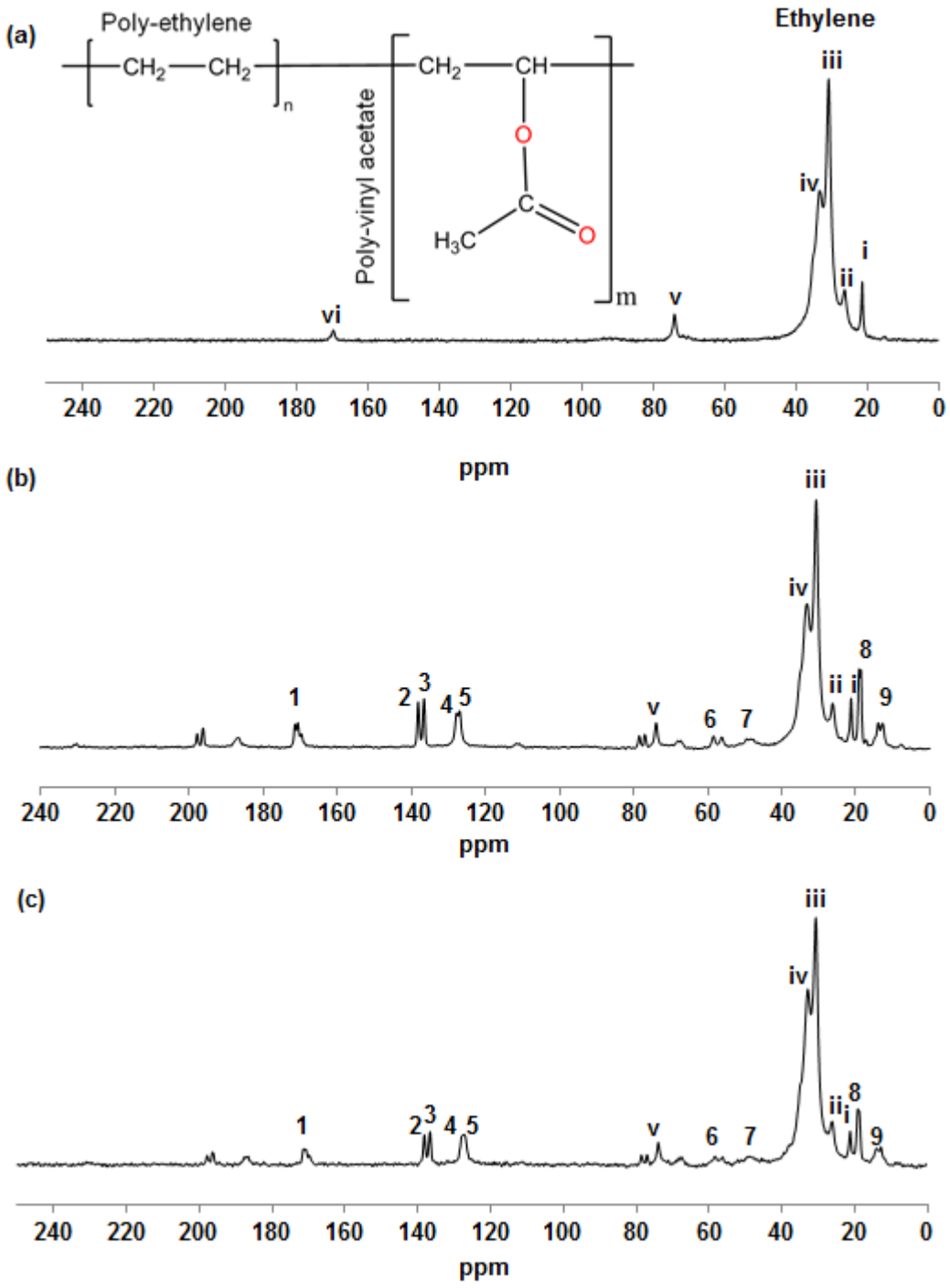

Figure 4.5. ^{13}C spectra of (a) EVA (25% VA), (b) binary mixtures of EVA (25% VA) and lidocaine and (c) binary mixtures of EVA (25% VA) and lidocaine stressed at 60 °C for 10 days

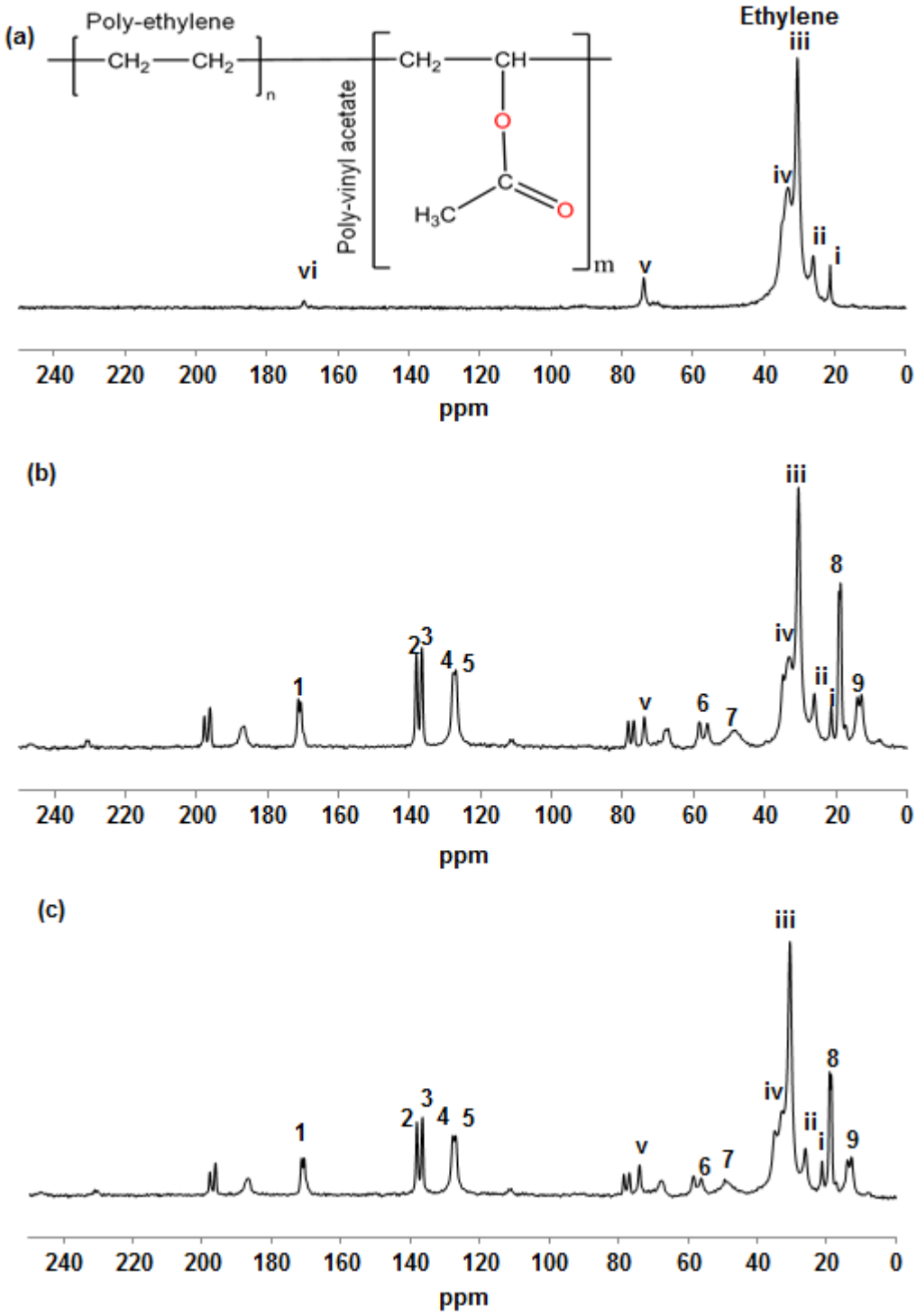

Figure 4.6. ^{13}C spectra of (a) EVA (33% VA), (b) binary mixtures of EVA (33% VA) and lidocaine and (c) binary mixtures of EVA (33% VA) and lidocaine stressed at 60 °C for 10 days

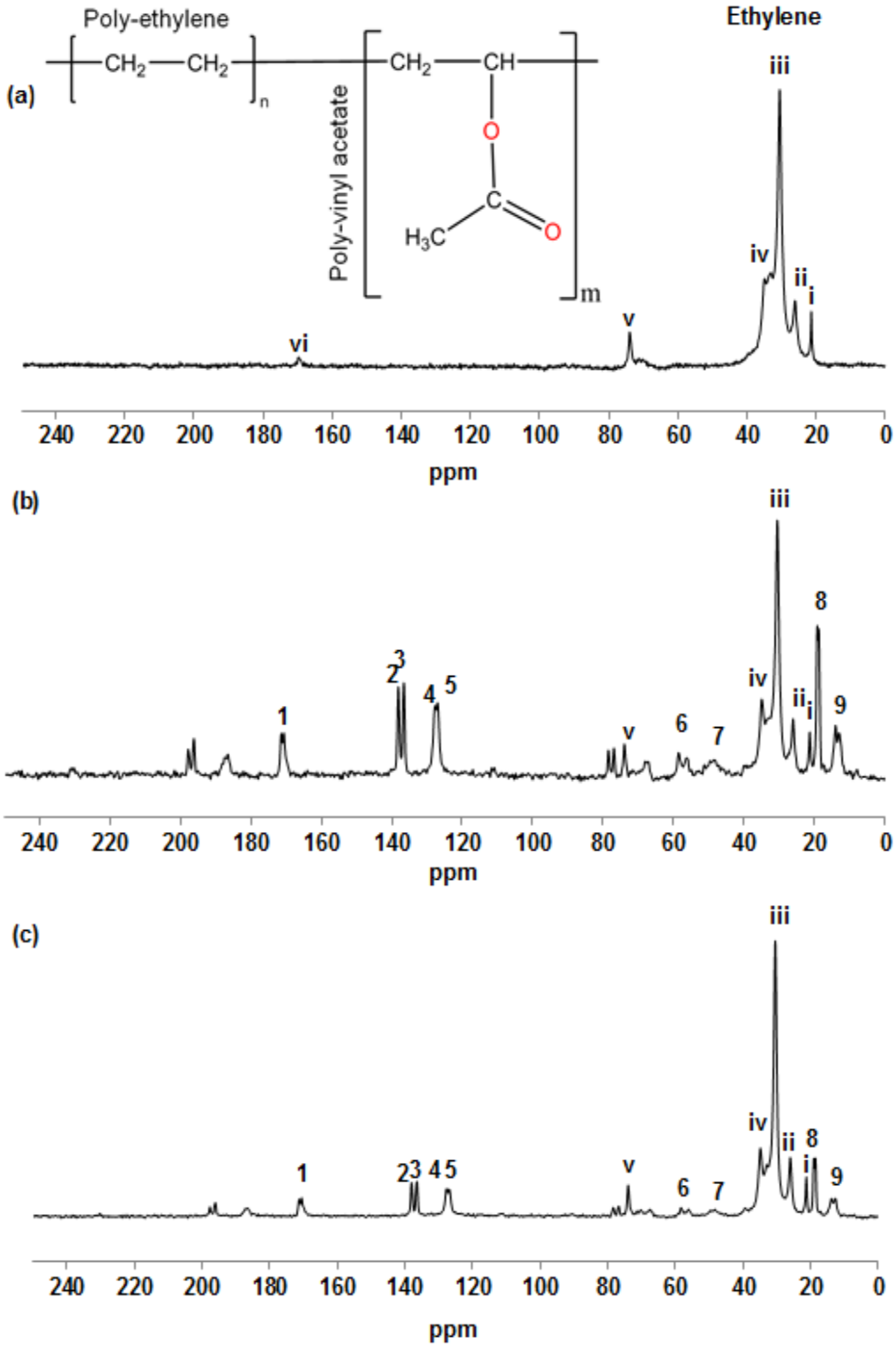

Figure 4.7. ^{13}C spectra of (a) EVA (40% VA), (b) binary mixtures of EVA (40% VA) and lidocaine and (c) binary mixtures of EVA (40% VA) and lidocaine stressed at 60 °C for 10 days

4.5.1.4 FTIR

FTIR is a well-established analytical tool to investigate compatibility and interactions between drug and excipients. FTIR spectra of the various samples are shown in Figure 4.8, Figure 4.9 and Figure 4.10. Lidocaine shows characteristic peaks around 1475 cm^{-1}, 1670 cm^{-1} and 3244 cm^{-1} due to the presence of C-N, C=O and N-H stretching respectively (Figure 4.8.b) (205). The characteristic peaks seen around 1464 cm^{-1}, 1736 cm^{-1} and 2920 cm^{-1} for the EVA sample are due to CH_2 bending, C=O stretching and C-H stretching respectively (Figure 4.8.a) (206). Excipient interactions with the drug generally result in the shifting or absence of characteristic peaks in FTIR spectra (138, 207). Here, the characteristic peaks remained intact and non-shifted for all the physical mixture blends, including the thermally stressed sample with 25% VA composition (Figure 4.8). Notably, a shift in peak position was observed only for thermally stressed binary mixtures with 33% and 40% VA composition (Figure 4.9.d and Figure 4.10.d). For the binary blend of lidocaine and EVA (33 % VA) stressed at 60 °C (10 d), the N-H peak of lidocaine shifted from the original 3244 cm^{-1} to 3249 cm^{-1} (Figure 4.9). Similarly, the N-H peak of lidocaine shifted to 3246 cm^{-1} for the binary blends of lidocaine and EVA (40 % VA) stressed at 60 °C for 10 d (Figure 4.10). Lidocaine contains only one proton donor (199), hence the focus was made on the N-H functional group of lidocaine to probe potential interactions. Lidocaine's N-H functional group may serve as the proton donor in forming an interaction with the electronegative oxygen atom of the carbonyl group in VA of EVA. Therefore, we assumed that the shifting of the lidocaine N-H stretch peak was due to mild intermolecular interaction between these two active functional groups in a form of hydrogen bonding between the electronegative O in the carbonyl group (with a lone pair of electrons) and hydrogen atom on the N-H group of lidocaine (Figure 4.11). Recently, Huang et al. reported a change in wavenumber of the N-H stretch of nifedipine when a dispersion of nifedipine was prepared in a methacrylate polymer (148). The shifting of the peak to a higher wavenumber was attributed to the formation of a hydrogen bond between the amine hydrogen of nifedipine and carbonyl oxygen of Eudragit® RL. Sarisuta et al. reported a similar phenomenon of hydrogen bonding as the $R\text{-}NH^3$ peak of ranitidine shifted slightly to a higher wavenumber when binary mixtures of the drug and Eudragit® E 100 were investigated by FTIR (208).

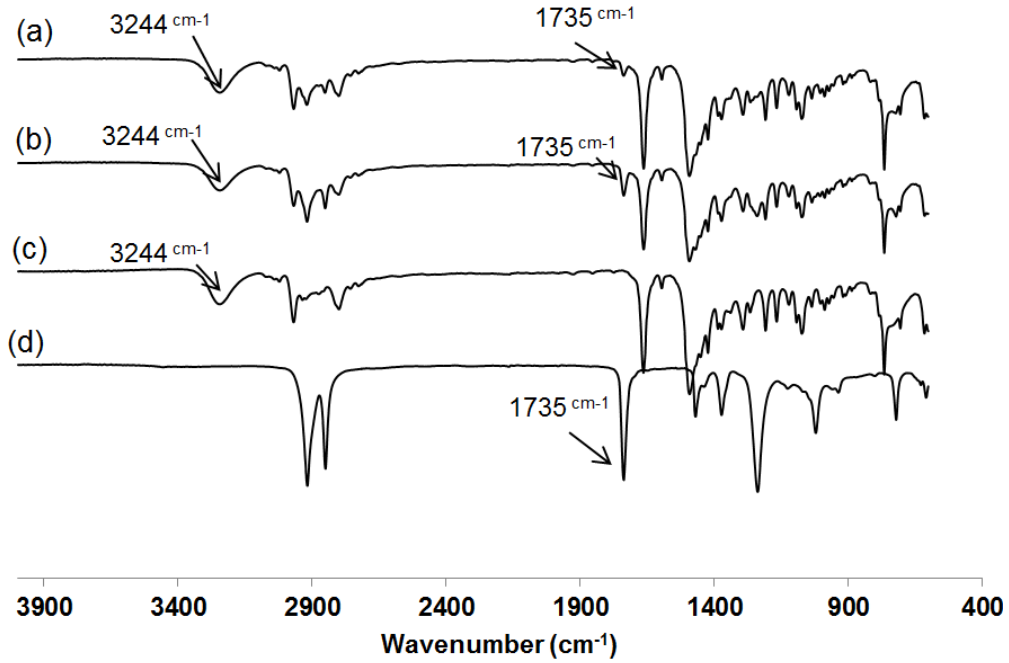

Figure 4.8. FTIR spectra of (a) binary mixtures of EVA (25% VA) and lidocaine stressed at 60 °C for 10 days, (b) binary mixtures of EVA (25% VA) and lidocaine, (c) lidocaine and (d) EVA (25% VA)

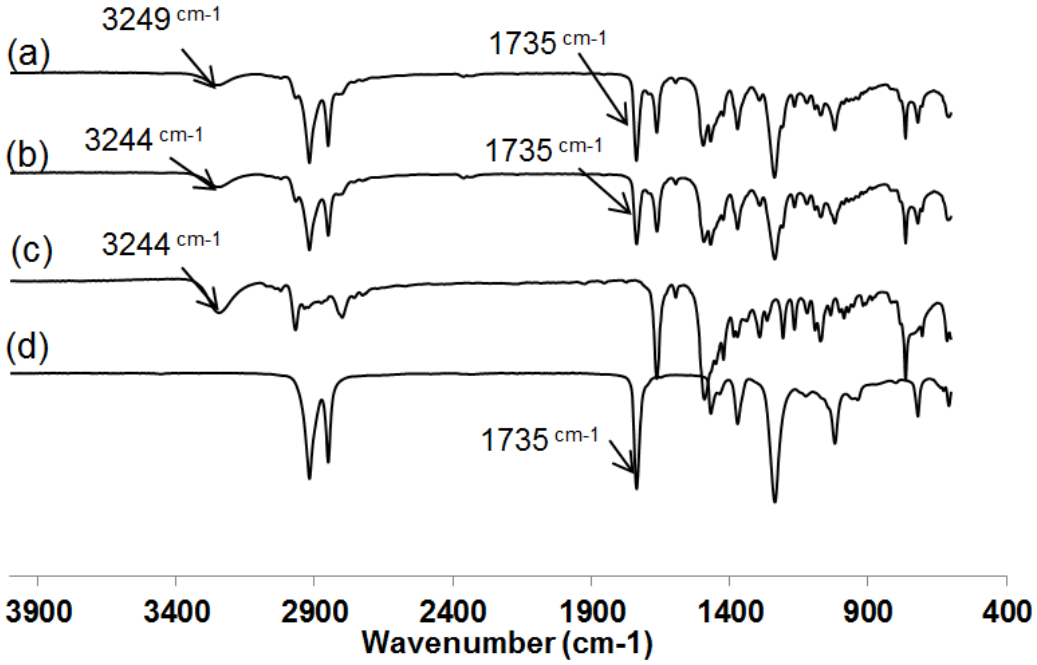

Figure 4.9. FTIR spectra of (a) binary mixtures of EVA (33% VA) and lidocaine stressed at 60 °C for 10 days, (b) binary mixtures of EVA (33% VA) and lidocaine, (c) lidocaine and (d) EVA (33% VA)

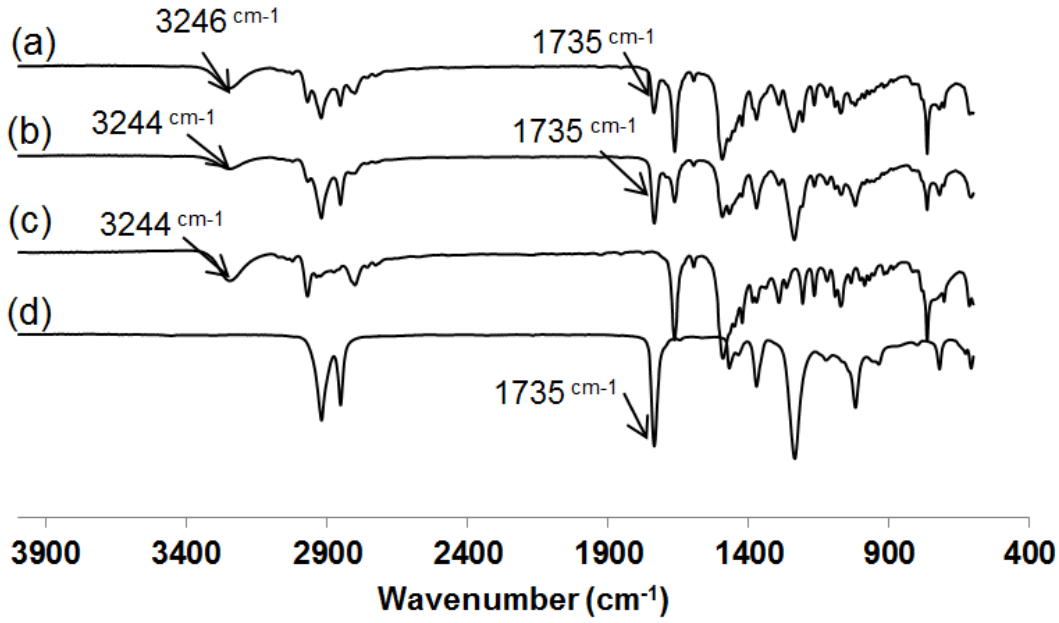

Figure 4.10. FTIR spectra of (a) binary mixtures of EVA (40% VA) and lidocaine stressed at 60 °C for 10 days, (b) binary mixtures of EVA (40% VA) and lidocaine, (c) lidocaine and (d) EVA (40% VA)

The reason peak shifts were only seen in samples stored at 60 °C could be that the melting of EVA led to a better dispersion of lidocaine compared to the physical mixtures. Enhanced dispersion would result in increased exposure between the functional groups and more opportunity for interactions. Meanwhile, the greater fraction of carbonyl groups in VA available for interaction with the N-H group of lidocaine explains why interactions were observed only for EVA with VA composition of 33% and 40%, but not for EVA with a VA content of 25%. Interactions between drug and excipients can be helpful rather than detrimental and can improve the physical stability of a formulation. In the preparation of some formulations, the interactions between the drug and excipients are crucial in making the preparation physically stable and sustaining the supersaturated state of drug in polymer matrix for an extended period of time (137). It was important to demonstrate in this study that the interaction between lidocaine and EVA does not result in drug degradation. The data available from ^{13}C NMR supported the chemical stability of lidocaine in the presence of EVA. However, additional analytical tools enabling the quantification of lidocaine from the stressed sample were required to further confirm that lidocaine remains chemically stable in the presence of EVA regardless of the interactions involved.

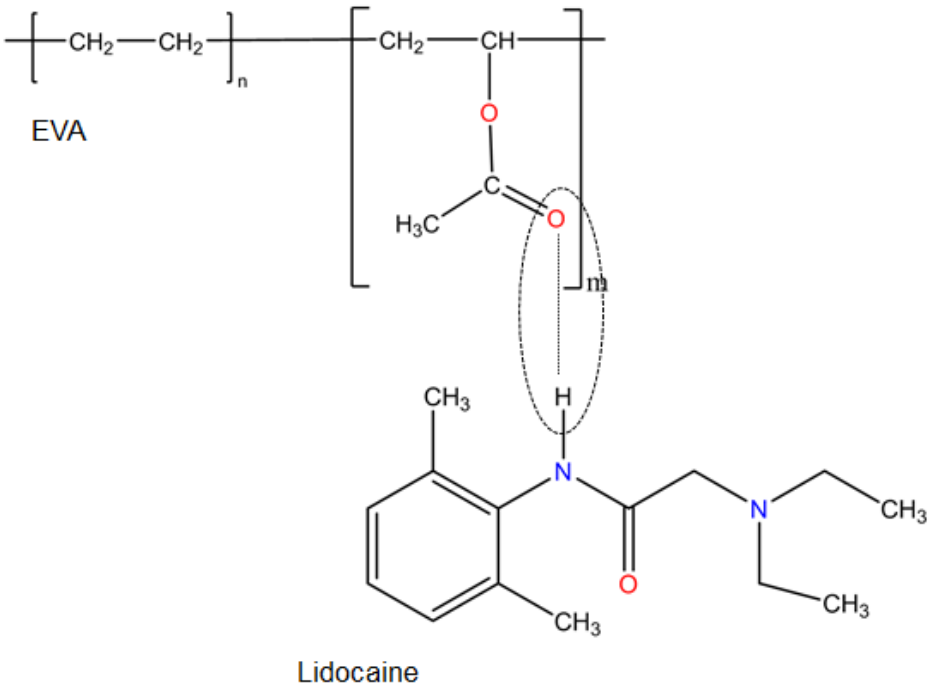

Figure 4.11. Proposed interaction between EVA and lidocaine in the form of hydrogen bonding

4.5.1.5 HPLC

Quantification of drugs by stability indicating HPLC methods give precise results for drug content, hence they have been widely used to investigate the compatibility of drugs with polymeric excipients (138). The stability indicating HPLC method described in Section 3.4.4.1 of Chapter 2 was employed to quantify lidocaine from the stressed samples. As shown in Table 4.2, no degradation of lidocaine was observed for any of the samples stressed at 60 °C for 10 days, as the assay values ranged from 100.6 ± 3.51 to 103.63 ± 1.34 %. The result obtained from the HPLC studies further confirmed the chemical stability of lidocaine in the presence of EVA.

Table 4.2. Assay of lidocaine from the binary mixtures of stressed at 60 °C for 10 days

EVA and Lid (1:1)	Assay (%) (n=3)
EVA (25% VA)	103.63 ± 1.34
EVA (33% VA	102.15 ± 1.34
EVA (40% VA)	100.61 ± 3.51

The sets of data from TGA, DSC, NMR, FTIR and HPLC analyses of the binary mixture of lidocaine and EVA with different VA compositions were helpful in confirming the compatibility of lidocaine in the presence of EVA. Further, FTIR suggested interaction between lidocaine and EVA, possibly in the form of hydrogen bonding. The interactions were specific to EVA with VA content of 33% and 40%. However, because these studies were carried out with physical mixture samples melted at 60°C for 10 days, the complete dispersion of drug and sufficient exposure between functional groups of EVA and lidocaine was not assured. Therefore, a method enabling complete molecular dispersion of lidocaine and EVA was required to allow better exposure between the functional groups of EVA and lidocaine. In addition to this, the shift in wavenumber was determined by manual selection of the lowest intensity of the peak of interest from the raw data. Although, this can be used as supporting evidence of interaction, a detailed study was needed to gain a comprehensive understanding of the interaction.

4.5.2 Intermolecular interactions probed in solvent cast films

Solvent casting is an efficient way to study the interaction between drug and polymer (140). In solvent casting, the drug and polymer are completely dissolved in the solvent, which favours a better dispersion of drug within the polymer matrix when compared to melted mixtures. Accordingly, solvent casting was used to promote exposure between the functional groups of lidocaine and EVA. EVA with 33% VA composition was selected due to previous evidence of interactions. The iterative least-square curve fitting method was employed as this method has previously been used to study drug–polymeric interaction (140). A Gaussian profile is specific to solid samples, whereas a Lorentzian profile is specific to liquid or gases (209). Lorentzian peak shape is typically used to fit infra-red absorption bands (210). However in the case of FTIR-ATR, irregular dispersion results in the formation of infra-red bands which will be neither purely Lorentzian nor Gaussain in shape. Considering the asymmetry and the complexity of the bands, a combination of Gaussian and Lorentzian functions was used to fit FTIR-ATR absorption bands (210).

The curve fitting for pure lidocaine is displayed in Figure 4.12.a. Fitting was specific to wavenumbers in the range 3400 to 3100 cm^{-1} to probe the changes occurring in the N-H stretch of lidocaine which could be attributed to hydrogen bonding (140, 148, 208). Acceptable fitting of the curve was confirmed by the lower error value obtained (0.002) from the fitting procedure. Initially, N-H stretching of pure lidocaine had a prominent peak at 3242 cm^{-1}.

For the solvent cast sample of EVA (33% VA) with 20% lidocaine loading, the peak due to N-H stretch shifted to 3251 cm^{-1} (Figure 4.12.b). Quantum theory states that each molecule has well-defined energy levels (209). Molecules absorb or emit energy giving rise to vibration spectra upon irradiation with IR. Depending upon the functional group of the molecule, a well-defined peak is obtained at a specific location. The location of the peak is also governed by the surrounding environment. In any event of interaction, the bond energy is changed resulting in peak shifting in FTIR spectra. However, the bonding was not enough to prevent the recrystallisation of lidocaine in the film (Figure 4.14.a). Therefore, the addition of a second component was necessary to prevent the recrystallisation of lidocaine in the EVA matrix.

Stearic acid or myristic acid were then included to form a ternary system with lidocaine and EVA. As shown in Figure 4.13, stearic acid and myristic acid have a hydrophobic ethylene chain connected to a carboxylic acid functional group (211). Thus, this class of fatty acid could potentially render an intermediate property between lidocaine and EVA, which might ultimately enhance the stability of lidocaine in the matrix.

As displayed in Figure 4.12.c, the peak due to N-H stretching of lidocaine shifted to 3265 cm^{-1} when stearic acid was included with EVA and lidocaine. However, crystallised lidocaine was still present in the film (Figure 4.14.b). Finally, when myristic acid was included in the mixture, the peak due to N-H stretch shifted to 3292 cm^{-1} (Figure 4.12.d), resulting in the formation of clear film without any visible sign of lidocaine recrystallisation (Figure 4.14.c).

Generally, the shifting of peaks towards lower wavenumbers is reported in the event of hydrogen bonding (212). However, in the data presented here, the N-H peak of lidocaine shifted towards a higher wavenumber for all the samples. A similar phenomenon of the N-H peak shifting towards a higher wavenumber was reported in an investigation of the interaction between nifedipine and methacrylate polymer (148). Huang et al. attributed the shifting of the N-H peak to a higher wavenumber to hydrogen bonding between the N-H functional group of nifedipine and the C=O of methacrylate. Further analysis confirmed that the inter-molecular hydrogen bonds between nifedipine molecules were broken and replaced by the new bond formed between the N-H of nifedipine and the C=O functional group of methacrylate. Lidocaine possesses a similar N-H functional group to nifepidine and moderate hydrogen bonding between two lidocaine molecules has been reported (213, 214). A similar phenomenon might have occurred with lidocaine, as previous reports have also demonstrated peak shifting towards higher wavenumbers when hydrogen bonds were formed between lidocaine and tetracaine (199). We therefore postulate that the interactions observed by FTIR in this thesis are due to hydrogen bonding between the carbonyl functional group of EVA and the N-H functional group of lidocaine.

The ultimate objective of the preformulation studies was to design stable dispersion of lidocaine in the EVA matrix. The sets of experiments described above were beneficial in learning how myristic acid can act to prevent the recrystallisation of lidocaine in EVA matrices.

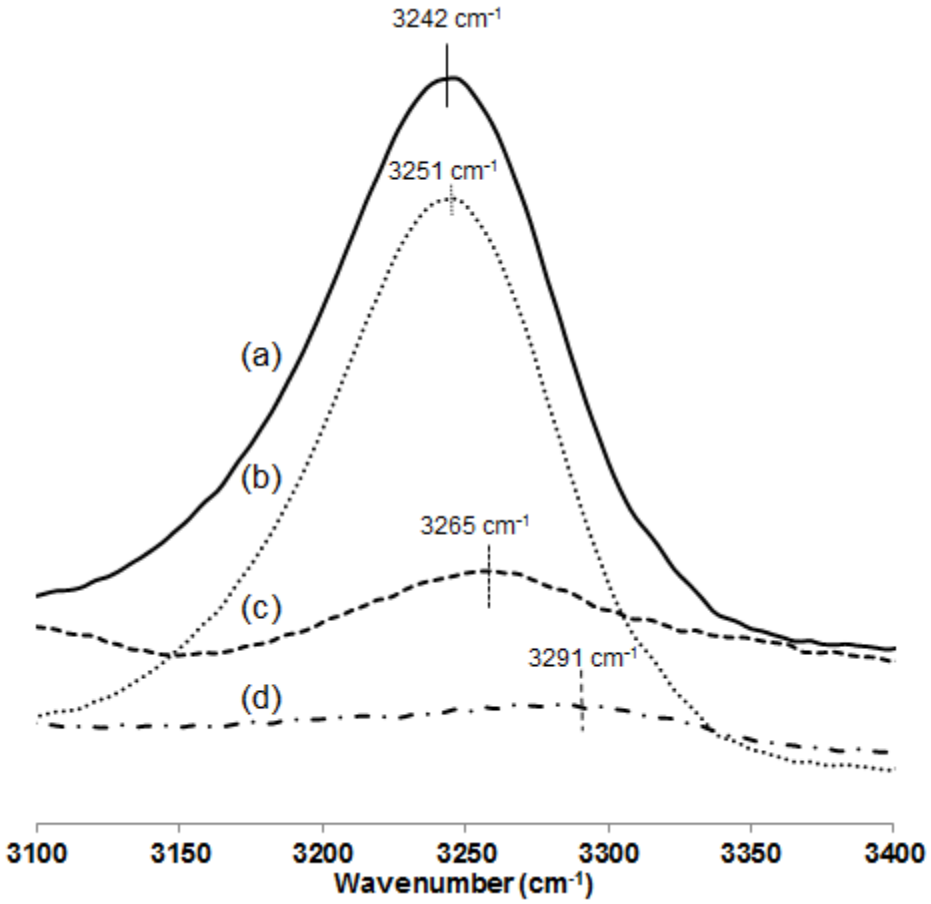

Figure 4.12. FTIR spectra displaying absorbance with the central wavenumber of N-H stretch of lidocaine obtained from (a) pure lidocaine, (b) solvent cast sample of 20% lidocaine in EVA, (c) solvent cast sample consisting of 20% lidocaine, 20% stearic acid and 60% EVA (33% VA), and (d) solvent cast sample consisting of 20% lidocaine, 20% myristic acid and 60% EVA (33% VA)

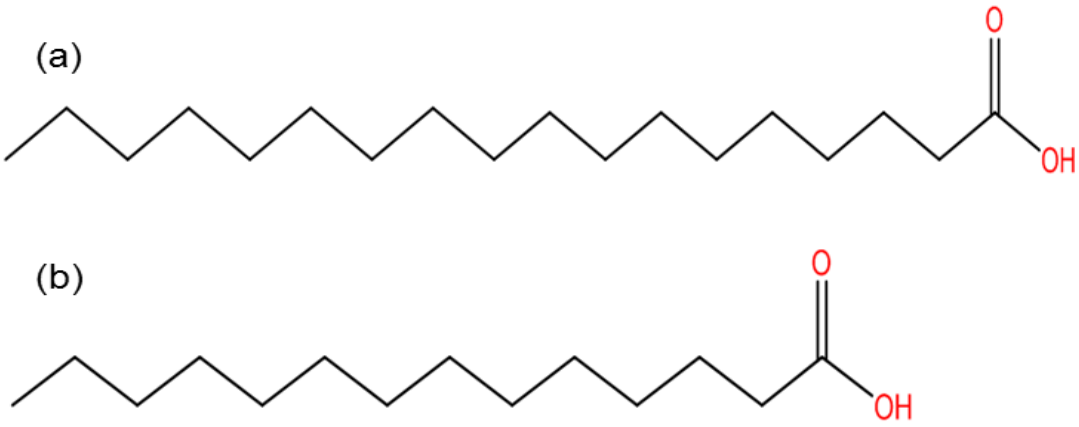

Figure 4.13. Chemical structure of (a) stearic acid and (b) myristic acid

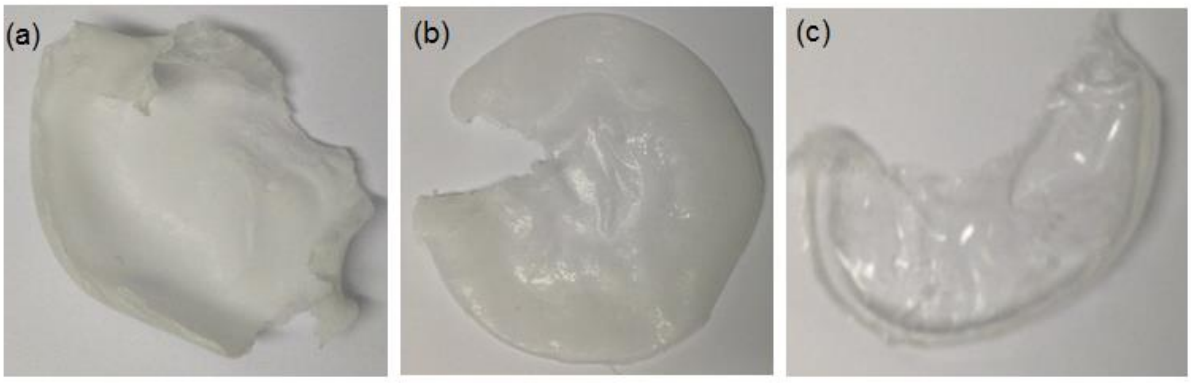

Figure 4.14. Photograph of solvent cast films of EVA and lidocaine: (a) film containing 20% lidocaine and 80% EVA with crystallisation evident, (b) film containing 20% lidocaine, 20% steric acid and 60% EVA with reduced crystallisation, and (c) transparent film containing 20% lidocaine, 20% myristic acid and 60% EVA with no crystallisation

4.5.3 Effect of cryogenic grinding on the crystallinity of lidocaine

The DSC thermogram of pure EVA (25% VA), a physical mixture of EVA (25% VA) and lidocaine, and a milled mixture of EVA (25% VA) and lidocaine at cryogenic temperature are displayed in Figure 4.15. A sharp endothermic peak at around 69° C seen for the physical mixture sample is due to the melting point of lidocaine (215). The DSC curve for EVA shows two broad endothermic peaks around 45 °C and 75 °C. The first endothermic peak corresponds to the transition of vinyl acetate domains, whereas the latter one corresponds to the transition of ethylene domains in the EVA polymer (202). As Figure 4.15.c shows for the milled sample, there is a massive decrease in the intensity of the lidocaine melting peak. This may be due to decrease in the particle size of lidocaine and EVA resulting in better dispersion of lidocaine in EVA matrices. The melting point of vinyl acetate units in the cryogenic sample is slightly shifted towards lower temperature when compared to pure EVA. However, the peak is completely absent with

the sample subjected to re-ramp. This phenomenon could be due to the plasticising effect of lidocaine (115). The phenomenon of drug amorphisation has previously been reported by Descamps et al., when drug and polymer mixtures were milled below the glass transition temperature (T_g) (216). The T_g of EVA (25% VA) is known to be around -27 °C (217), and the temperature inside the cryomill falls below the glass transition temperature of EVA (25% VA) during the process of milling. The EVA therefore becomes brittle at this cryogenic temperature, enabling better dispersion of lidocaine in EVA. The result we obtained indicates the potential of cryogenic grinding to disperse lidocaine in an EVA matrix. In the future various ratios of lidocaine and EVA could be prepared and milled at cryogenic temperatures before extrusion in order to prepare uniformly dispersed drug in the EVA matrices.

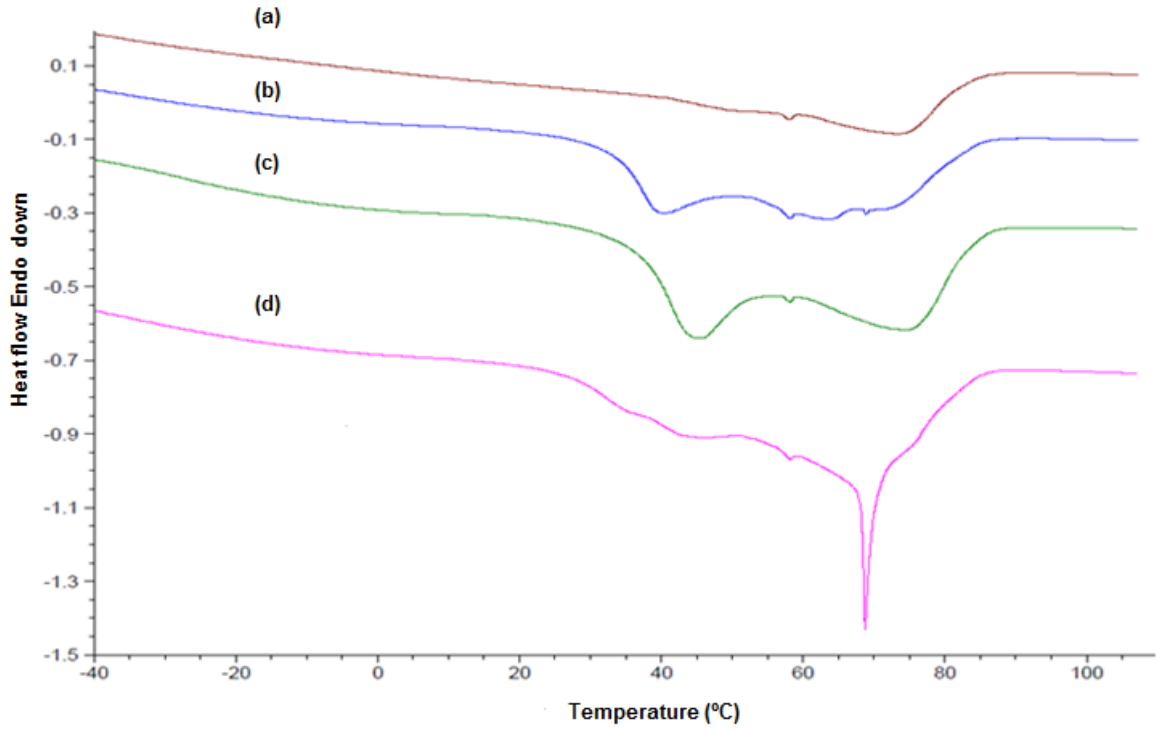

Figure 4.15. DSC thermogram of (a) re-ramp of cryomilled lidocaine and EVA mixture, (b) cryomilled lidocaine and EVA mixture, (c) pure EVA (25% VA) and (d) lidocaine and EVA physical mixture (1:5)

4.6 Conclusions

The preformulation studies performed were of benefit in elucidating the properties of lidocaine and EVA towards the preparation of a controlled release system. Isothermal testing of binary mixtures of lidocaine and EVA confirmed the compatibility of lidocaine with EVA. Lidocaine and EVA were stable at the expected formulation temperature using hot melt extrusion. EVA with 33% VA composition had a better ability to disperse lidocaine when compared to 25 % VA composition. FTIR suggested the presence of interaction between EVA and lidocaine, possibly in the form of hydrogen bonding and this was enhanced by the inclusion of a fatty acid. The addition of myristic acid with EVA and lidocaine resulted in the formation of clear film, suggesting the better ability of myristic acid to prevent recrystallisation of lidocaine in EVA matrices. Finally, milling of EVA and lidocaine at cryogenic temperature resulted in decreased crystallinity of lidocaine which could be explored further in the future as a way of dispersing drug uniformly into an EVA matrix.

CHAPTER 5. INVESTIGATION OF HUMAN PERITONEAL FLUID COLLECTED FOLLOWING ABDOMINAL SURGERY

5.1 Background

The majority of investigations focus on the formulation when designing CR systems. However, it is important to note that the *in vivo* performance of a CR system depends not only on the formulation, but also upon the physiological environment at the site of administration. The physicochemical parameters of the local environment, including pH, buffer capacity, surface tension and viscosity, which influence the solubility of drugs and the release performance of drug delivery systems have been discussed in detail in Section 2.5 of Chapter 2.

Our proposed formulation has been designed for implantation into the peritoneal cavity following abdominal surgery, and is expected to remain there for up to 10 days. Following implantation, sequestration of peritoneal fluid (PF) around the formulation is expected (165), into which lidocaine release will occur. Along with the design of the implant and the properties of polymer and additives used, the *in vivo* release performance will partially depend on the overall properties of PF. Detailed information about the composition and properties of PF is important to understand the *in vivo* fate of EVA formulation. While biological fluids such as saliva (158), gastric fluids (155) and synovial fluids (157) have been widely studied for composition, physicochemical properties and rheological parameters, little is known about PF. Limited data have been briefly reported describing the composition and pH of PF (163, 164), however detailed information with relevance to the solubility and release performance of drug delivery systems is lacking. In addition to pH and composition, other key properties and parameters need to be considered, including buffer capacity, osmolality, surface tension and viscosity. Along with this, it is unknown whether the composition and properties of PF remain constant or change following abdominal surgery. Therefore, a detailed analysis of PF collected from patients at different time intervals following surgery could be helpful to understand the composition and key physicochemical properties of PF *in vivo*.

Generally, *in vitro* release performance of oral dosage forms is tested in bio-relevant media to mimic *in vivo* conditions. However, no specific bio-relevant media has been described for *in vitro* evaluation of the release performance of intra-peritoneal drug delivery systems. Phosphate buffered saline (PBS) solution of pH 7.4 is typically used to evaluate the release performance of intra-peritoneal drug delivery systems (20), but it is not known how well this media simulates the *in vivo* peritoneal environment. Accordingly, a study comparing the composition and key physicochemical properties of PF and PBS would help to identify the similarities and notable differences between these two fluids.

5.2 Aims and Objectives

The overall aim of the work described in this chapter was to characterise human PF over four days following abdominal surgery to obtain a clear understanding of the peritoneal environment after surgery. The specific objectives were to:

- Determine the composition of PF for electrolyte, proteins and lipids
- Evaluate different physicochemical properties of human PF, including pH, buffer capacity, osmolality and surface tension
- Characterise the rheological parameters of PF
- Compare the physicochemical properties of PF with PBS of pH 7.4.

Once these objectives were achieved, a study comparing lidocaine solubility and drug release profiles in PF and PBS was undertaken next, as described in Chapter 6.

5.3 Materials

Phosphate buffered saline (PBS) tablets were bought from Sigma and dissolved in water obtained by reverse osmosis (MilliQ unit, Millipore) to produce 0.01 M PBS of pH 7.4.

5.4 Methods

5.4.1 Collection and handling of PF samples

Human PF was obtained from nine different patients after undergoing abdominal surgery at Middlemore Hospital, Auckland, under Health and Disability Ethics Committee (HDEC, Wellington, New Zealand) approval 15/CEN/30. The PF was collected through surgical drains for up to four days following abdominal surgery. The samples were centrifuged at 3500 rpm for 5 min to separate cells and debris before collecting the supernatant. Aliquots of supernatant were evaluated for composition and the remainder stored at -20 °C until further use. When required, samples were thawed before characterising their physicochemical and rheological properties.

5.4.2 Electrolytes, protein contents and lipid concentrations

PF samples collected from patients were submitted to Labplus (Auckland City Hospital) to determine composition using a Roche Cobas Chemistry Analyser. Levels of sodium, potassium, chloride, phosphate, total protein, albumin, triglycerides, cholesterol, low density lipids (LDL) and high density lipids (HDL) were determined in the PF

5.4.3 Physicochemical properties

The key physicochemical properties of PF were measured against PBS, including pH, buffer capacity, osmolality and surface tension.

5.4.3.1 pH and buffer capacity

Buffer capacity was determined using a Titrando titrator connected to an Unitrode pH electrode and a Metrohm AG stirrer (Model 842, Titrando, Switzerland). After initial measurement of pH, buffer capacity was measured by drop wise addition of 0.05 M NaOH to 10 mL of sample. The additions of 0.05 M NaOH were stopped when the pH changed by one unit. The buffer capacity was calculated using the formula described in Equation 5.1, all measurements were performed in triplicate.

Equation 5.1. Buffer capacity using NaOH

$$Buffer\ capacity\left(\frac{\frac{\mathrm{mM}}{\mathrm{L}}}{\Delta\mathrm{pH}}\right) = \frac{\text{volume of NaOH required X concentration of NaOH}}{\frac{\text{volume of sample}}{\text{change in pH unit}}}$$

5.4.3.2 Osmolality

Osmolality was measured using an Osmometer (Vapro 5600). Ten µL of each sample was inoculated onto a paper disc placed into a sample holder for testing. Analysis of the sample was carried out when the sample holder was locked automatically into the instrument. Calibration of the instrument was performed with standard solutions of 100, 290 and 1000 mOsm/kg. All measurements were performed in triplicate.

5.4.3.3 Surface tension

Surface tension was measured by Du Noüy ring equipment (TLS, UK). Briefly, a thin platinum ring was allowed to submerge slowly into the sample. The ring was then slowly retracted and the force required to detach the ring from the surface of the sample was recorded. All measurements were performed in triplicate.

5.4.4 Rheological characterisation

Rheological characterisation of all the samples was conducted using an AR-G2 rheometer (TA Instruments, Water Corporation, New Castle, USA) with a double gap concentric rotor and cup geometry. The dimension of the rotor used was 17.5 mm (outer diameter) and the gap was set at 2.0 mm. All measurements were performed at 37 °C. The sample volume collected from patient P2 was insufficient to carry out the analysis, therefore rheological characterisation was only carried out on samples from eight of the total nine patients. PF from patients P3 and P8 was characterised over three consecutive days to observe inter-day variation in rheological parameters. Patients P3 and P8 were the only patients from whom sufficient PF volume was collected over three consecutive days to allow for this analysis.

5.4.4.1 Oscillatory rheology

First, the linear viscoelastic region (LVR) was determined though a strain sweep from 0.0001% to 10% at 0.1 Hz. Elastic (G´) and viscous (G´´) moduli were determined across the frequency range of 0.1 to 10 Hz and at a strain rate of 9 %. All tests were performed in triplicate.

5.4.4.2 Steady state flow

The steady state flow of the samples was measured as a function of shear rate from 0.01 to 100 s^{-1}. In each case, shear rate was increased consistently with 10 data points recorded per decade. The tolerance was set to 5%, and the maximum measuring time at each shear rate was 2 min.

5.4.5 Statistics

A series of statistical methods were employed in this study to examine differences in physicochemical properties and rheological parameters. The physicochemical parameters and composition are expressed as mean ± standard deviations (SD). Statistical comparisons of the physicochemical properties of PF and PBS were made using one-way ANOVA followed by Bonferroni post-hoc test. Following this, the physicochemical parameters of PF from different patients were analysed by a single sided variance analysis. The statistical relationship between the patients for viscoelastic parameters at a defined frequency of five Hz was performed by Kruskal-Wallis test followed by Dunn's post-hoc test. The inter-day relationship in viscoelastic parameters of PF obtained from patients P3 and P8 were compared by two-way ANOVA, assuming a coefficient of variation of 10%. In all cases $p<0.05$ was considered significant.

5.5 Results and Discussion

The description of patients who volunteered in the collection of PF is presented in Table 5.1 . The group consisted of five males and four females with an age range of 56 to 85 years. Six of the nine patients

were receiving treatment for rectal cancer. Varying volumes of PF were able to be collected from each patient for between one and four days following abdominal surgery, as displayed in Table 5.2. Some patients produced large volumes of PF (ca. 1 L in total), others very low volumes (ca 0.150 L in total). The PF was characterised for composition, physicochemical properties and rheological parameters.

5.5.1 Electrolytes, protein contents and lipid concentrations

Considerable variation, both between patients and for the same patient over time, was evident for electrolyte, protein and lipid composition, but with no obvious trends (Table 5.2)

Sodium and chloride concentrations in PF ranged between 132–151 mmol/L and 97–116 mmol/L, respectively, and were similar to those of extracellular fluid (ECF) (142 and 108 mmol/L for sodium and chloride, respectively) (218). Meanwhile, the potassium concentration in PF ranged from 3.9 to 15.2 mmol/L, with many values higher than those for ECF (4.2 mmol/L) (77, 218). The raised potassium levels found in the PF may have been due to cellular damage from surgery resulting in the release of intracellular potassium. The phosphate levels in PF ranged from 0.68 to 1.57 mmol/L, with all values lower than ECF (2 mmol/L). Interestingly, the phosphate levels in PF decreased considerably for all the patients after the second day post-surgery.

Total protein and albumin concentration decreased in all patients except for patient P8 after one day post-surgery, and then remained constant over the period evaluated. Despite the inter-patient variation in concentrations, little inter-day variation was observed after the first day. Meanwhile, a reverse trend in total protein and albumin levels was observed in the PF obtained from patient P8, whose total protein and albumin levels increased at day two post-surgery and then remained constant over the time period.

Considerable inter-patient and inter-day variation was evident in the lipid profiles of PF and levels were expected to reflect the lipid profile of each patient going into surgery. Overall, LDL, TG, cholesterol and HDL levels in PF decreased after day one post-surgery. As with the protein levels, patient P8 was the exception; here concentration of LDL remained fairly constant in the post-surgical period, while TG, cholesterol and HDL increased after day one post-surgery and then remained constant out to day four.

Table 5.1. Description of patients from which PF was collected after undergoing abdominal surgery

Patients	Sex	Age (yr)	Comorbidities	Indication	Surgical Procedure	Days of collection
P1	M	85	HTN, prediabetic and prostatism	Rectal cancer	Low anterior resection with loop ileostomy	2
P2	M	56	Ex-smoker 30 years	Sigmoid anastomosis	Revision of sigmoid anastomosis and open re-do anterior resection with loop ileostomy	2
P3	F	73	T2DM, IHD, COPD	Colovesical fistula	Anterior resection and loop ileostomy	3
P4	F	79	AF	Colovesical fistula	Anterior resection and loop ileostomy	2
P5	M	59	Ex- smoker 40 years, non-STEMI	Rectal cancer	Anterior resection and loop ileostomy	1
P6	F	66	Nil	Rectal cancer	ULAR and loop ileostomy	1
P7	M	70	HTN, previous prostate Cancer	Mid-rectal cancer	Low anterior resection with loop ileostomy	2
P8	F	65	Nil	Upper rectal cancer	ULAR and loop ileostomy	4
P9	M	62	Nil	Mid-rectal cancer	Low anterior resection with loop ileostomy	2

Male-M, Female-F, Hypertension-HTN, Type 2 Diabetes Mellitus-T2DM, Ischemic Heart Disease-IHD, Chronic obstructive pulmonary disease-COPD, Atrial fibrillation-AF, Ultra-low anterior resection-ULAR

* Depending upon how much PF each patient produced and could be drained, the biological fluid was collected for between 1 and 4 day

Table 5.2. Description of electrolyte, protein and lipid concentrations in the PF obtained from nine different patients at different days post-abdominal surgery

Patients	Days	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	PO_4^{--} (mmol/L)	TP (g/L)	ALB (g/L)	LDL (mmol/L)	TG (mmol/L)	CHL (mmol/L)	HDL (mmol/L
P1	1	137	4.5	102	1.09	34	20	1.5	0.8	2.5	0.7
P2	1	134	5.6	97	1.2	41	24	1.1	0.6	2.1	0.8
	2	147	10.4	104	0.77	33	23	0.6	0.2	1.4	0.7
P3	1	132	11.1	99	1.43	38	21	0.8	1.6	2.5	1.1
	2	150	13.2	105	0.76	25	16	0.3	0.7	1.4	0.8
	3	140	4.2	101	0.75	25	16	0.2	0.5	1.1	0.7
P4	1	150	15.2	106	1.43	38	20	0.7	0.9	1.7	0.6
	2	151	12.4	101	0.68	29	18	0.4	0.5	1.1	0.5
P5	1	140	6.4	107	1.45	39	22	0.7	1.8	2	0.4
P6	1	133	4.4	97	1.57	28	16	1.2	0.5	1.9	0.5
P7	1	135	4.2	99	1.18	35	20	1.1	0.5	2	0.7
	2	133	4.5	97	0.85	32	19	0.8	0.4	1.6	0.6
P8	1	148	4.3	116	0.92	24	14	0.7	0.3	1.4	0.5
	2	141	4.1	103	0.75	36	21	0.6	0.6	1.6	0.8
	3	141	3.9	104	0.71	36	21	0.5	0.6	1.6	0.8
	4	141	3.9	104	0.72	36	21	0.6	0.6	1.0	0.8
P9	1	138	5.5	102	1.3	41	25	1.7	0.6	2.7	0.8
	2	137	4.2	100	0.91	39	23	1.1	0.4	2	0.7
Mean ± SD		140.4±6.3	6.7±3.8	102.4±3.8	1.0±0.3	33.8±5.5	20±3.0	0.8±0.4	0.6±0.4	1.7±0.4	0.6±0.1
PBS		137	2.7	139.7	10	0	0	0	0	0	0

Sodium-Na+, Potassium-K+, Chloride-Cl-, Phosphates-PO42-, Total Protein-TP, Albumin-ALB, Low density lipids-LDL, Triglycerides-TG, Cholesterol-CHL, High density lipids-HDL and PBS- 0.01 M Phosphate buffered saline (PBS) of pH 7.4.

5.5.2 Physicochemical properties of PF

The physicochemical properties of PF differed from PBS ($p<0.001$), and varied both between patients and over time. As shown in Figure 5.1, the pH of PF ranged from 7.5 to 8.4 which is higher than the pH for ECF (pH 7.4) (219). The pH varied both between patients ($p<0.001$) and within the same patient ($p<0.001$) over time. These results are in agreement with a previous study of the peritoneal fluid of 134 patients which reported higher pH than generally found for physiological pH (163). An increase in pH over consecutive days was observed for all patients. Typically, PBS of pH 7.4 is used as a simulated release medium for studying the release performance of DDS for intra-peritoneal applications (20). This data questions the rationale of using PBS at pH 7.4 as the simulated release medium.

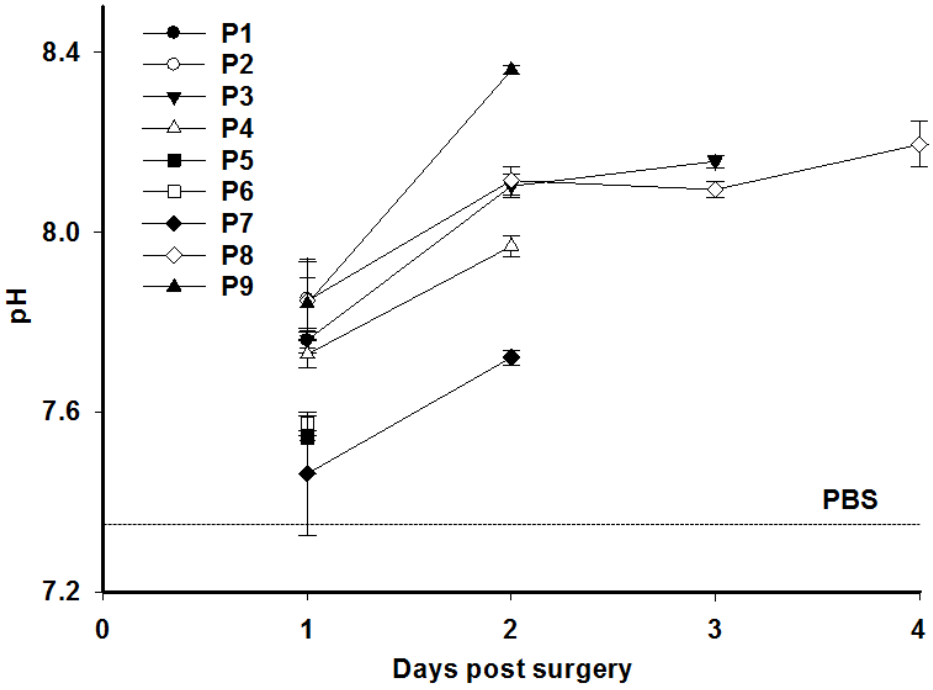

Figure 5.1. pH of peritoneal fluid collected from patients at different days post-surgery (n=3), with the dashed line indicating PBS at pH 7.4

Figure 5.2 illustrates the buffer capacity of patients' PF collected at different days. Inter-patient variation in buffer capacity was evident, as the values ranged from 3.1 to 6.9 $mmolL^{-1}\Delta pH^{-1}$. The estimated mean buffer capacity for PBS was 0.76 ± 0.0 $mmolL^{-1}\Delta pH^{-1}$, which is several folds lower than the value found for buffer capacity of PF. Except for patient P4, increase in buffer capacity was observed in the days following surgery. Significant differences existed in the inter-patient variations in buffer capacity ($p<0.05$). It was only in patient P4 that buffer capacity decreased with statistical significance over time ($p<0.05$).

PF is a complex environment and it was difficult to identify the cause of changes in acid-base balance following surgery which may have contributed to variation in pH and buffer capacity. But, it is important to note that these variations in pH and buffer capacity would be expected to influence drug solubility and the performance of drug delivery systems (149). In addition, the pH and buffer capacity of the medium have been found to influence the ionisation, swelling and erosion behaviour of delivery systems, ultimately affecting the dissolution rate of drug (220, 221).

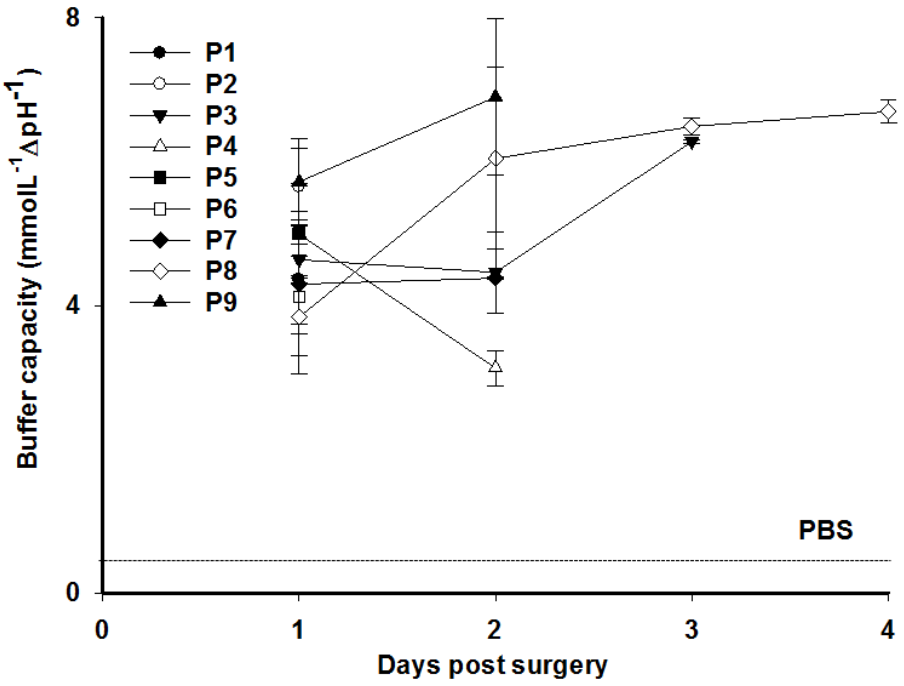

Figure 5.2. Buffer capacity of peritoneal fluid collected from patients at different days post-surgery (n=3), with the dashed line indicating PBS at pH 7.4.

Osmolality varied significantly between patients ($p<0.001$) and over time ($p<0.001$), with the values ranging from 211 to 343 mOsm/kg (Figure 5.3). Meanwhile, the osmolality of PBS solution was 248.0 ± 0.8 mOsm/kg, significantly different to PF ($p<0.001$). As displayed in Figure 5.3, the highest osmolality levels were recorded one day post-surgery with a decrease seen over subsequent days as fluid shifts in the patient's body would result in response to this high osmolality. Patient P4 provided the opposite trend with osmolality increasing from 294 ± 4.6 on day one to 317.33 ± 2.0 mOsm/kg on day two post-surgery. The osmolality of the environment has been found to govern the rate and the extent of medium penetration into the drug delivery systems (150). Faisant et al. reported the increased drug release behaviour of 5-fluorouracil from PLGA-based microparticles when the osmolality of the release medium was increased (150).

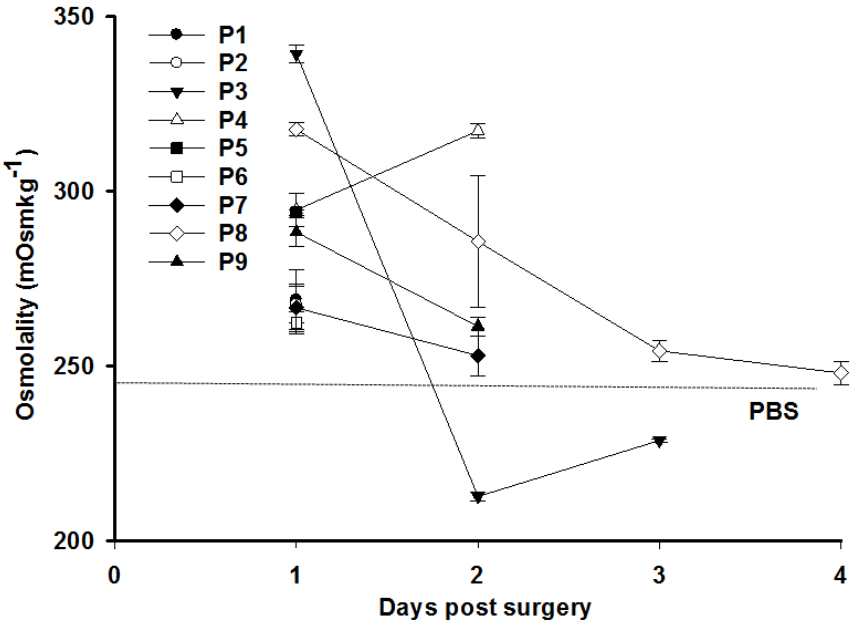

Figure 5.3. Osmolality of peritoneal fluid collected from patients at different days post-surgery (n=3), with the dashed line indicating PBS at pH 7.4.

The surface tension of the collected PF is displayed in Figure 5.4. The values for surface tension of PF collected from different patients at different time intervals were in the range 42.1 to 51.1 mNm^{-1}. The surface tension of PF was significantly lower ($p<0.05$) than PBS, the average value being almost 15 units lower than that of PBS (60.4 ± 1.2 mNm^{-1}). There was significant variation between patients ($p<0.05$), but not within each patient over time. As shown in Figure 5.4, the value of surface tension increased gradually over the days. The estimated value of surface tension of PF was significantly lower when compared to PBS, which can be attributed to presence of natural surfactants in the PF (222). These surfactants may facilitate wetting and penetration of media into the drug delivery systems, thereby altering release performance (151).

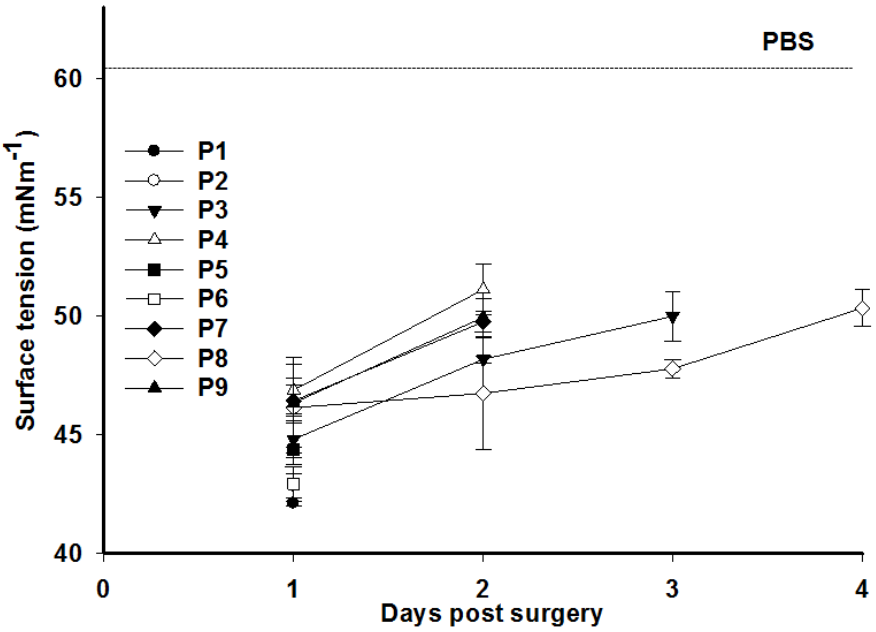

Figure 5.4. Surface tension of peritoneal fluid collected from patients at different days post-surgery (n=3), with the dashed line indicating PBS at pH 7.4.

5.5.3 Rheological properties

The PF samples obtained from patients at day one post-surgery were characterised for oscillatory rheology and flow property. Rheological characterisation of PF samples obtained from patients P3 and P8 were characterised over three consecutive days to observe inter-day variation in rheological parameters. Patients P3 and P8 were the only patients with sufficient PF volume collected over three consecutive days to allow for this analysis. The rheological properties of PF from patient P2 could not analysed as insufficient sample volume was able to be collected.

5.5.3.1 Oscillatory rheology

Frequency sweeps are conducted in order to identify the viscoelastic behaviour of biological fluids as a function of frequency applied. Figure 5.5. and Figure 5.6 show the corresponding oscillatory data for PF collected from different patients at different days post-surgery, expressed as plots of elastic (G´) and viscous moduli (G˝) against frequency. Frequency sweeps were conducted from 0.1 Hz to 10 Hz at a strain rate of 9%. Both moduli were dependent on the frequency of oscillation (Figure 5.5) in all the PF samples collected from patients at day one post-surgery.

The majority of biological fluids behave as entangled networks, weak gels or strong gels (152). In the case of entangled networks, the viscous modulus (G˝) dominates the elastic modulus (G´) until the crossover frequency. Following the crossover frequency, the elastic modulus (G´) dominates over the viscous modulus (G˝). In weak gel systems, elastic moduli dominate over viscous moduli but remain

parallel oven an entire range of frequency. Meanwhile, in strong gel systems the elastic modulus (G´) is several folds higher than the viscous modulus (G˝), with both the moduli showing similar increasing trend over the range of frequency.

In general in our findings, the viscous modulus (G˝) dominated the elastic modulus (G´) at lower frequencies, indicating predominantly viscous behaviour. However following crossover at higher frequencies, G´ was greater than G˝ indicating predominantly elastic behaviour and implying the presence of entangled networks. The crossover frequencies for these patients varied from 0.6 to 2.5 Hz, indicating the boundary line between solid-like and liquid-like behaviour (223). At lower frequencies, the molecular chains release the stress by disentanglement and rearrangement and thus exhibit viscous behaviour. At higher frequencies the molecular chains in PF have insufficient time to disentangle, behaving temporarily as cross-linked networks. Therefore, in patients P1 and P3–P7 the elastic modulus was higher than the viscous modulus at higher frequencies. In contrast, the PF samples collected from patients P8 and P9 exhibited gel like behaviour, as elastic modulus remained larger than the viscous modulus throughout the range of all frequencies (Figure 5.5.g and Figure 5.5.h).

It is difficult to evaluate the ideal magnitude and oscillatory rheological behaviour for a particular biological fluid as different biological fluids vary among and within each other in terms of characterstics. Recently, a study reported by Bhuanantanondh et al (157), suggested the presence of entangled networks in synovial fluid obtained from healthy subjects. The value of G´ and G˝ varied from 0.05 to 9 Pa within a frequency range of 0.01 to 10 Hz. Similarly, huge variations in the magnitude of G´ was observed when human gastric fluid was examined; where, the value of G´ ranged from 0.05 to 1 Pa over a frequency range of 0.01 to 10 Hz (155).

Differences in viscoelastic properties were also observed over time following surgery (Figure 5.6). In the PF from patient P3, the viscous modulus was dominant at day one and day two at lower frequencies. The elastic modulus became dominant by the third day post-surgery at all frequencies, exhibiting gel like behaviour. In contrast, PF obtained from P8 demonstrated a dominant elastic modulus in all instances, indicating gel-like behaviour. Statistical analysis also showed significant inter-patient variation in elastic modulus ($p<0.05$) but this was mostly attributable the marked difference between patients P8 and P9 and the other patients. Similarly, there was significant inter-day variation ($p<0.05$) in elastic modulus of PF recovered from the same patient at three different days post-surgery.

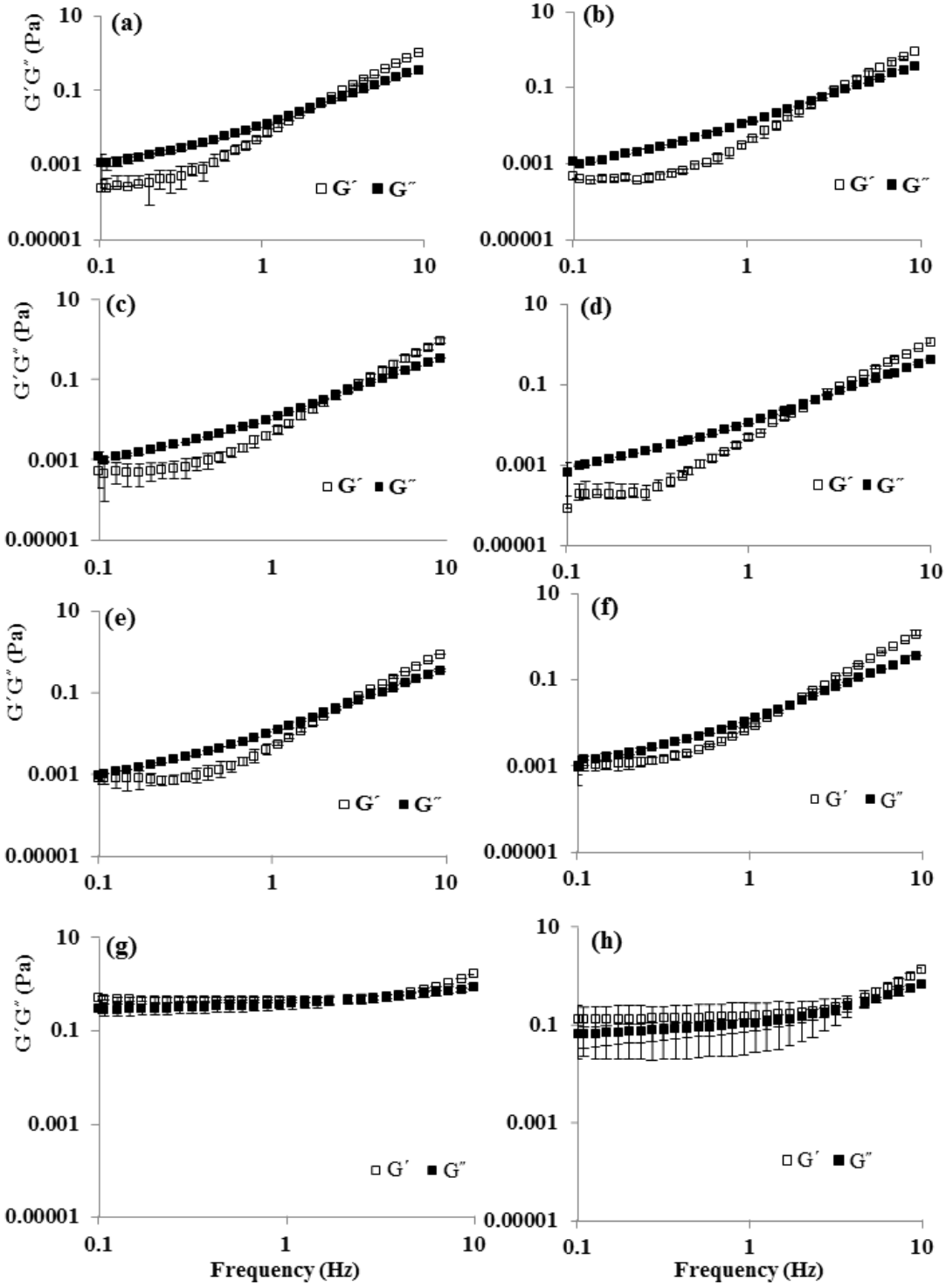

Figure 5.5. Frequency sweep of PF collected from different patients at day one post-surgery. (a) P1, (b) P3, (c) P4, (d) P5, (e) P6, (f) P7, (g) P8 and (h) P9, where the hollow and solid symbols indicate elastic (G´) and viscous moduli (G″) respectively

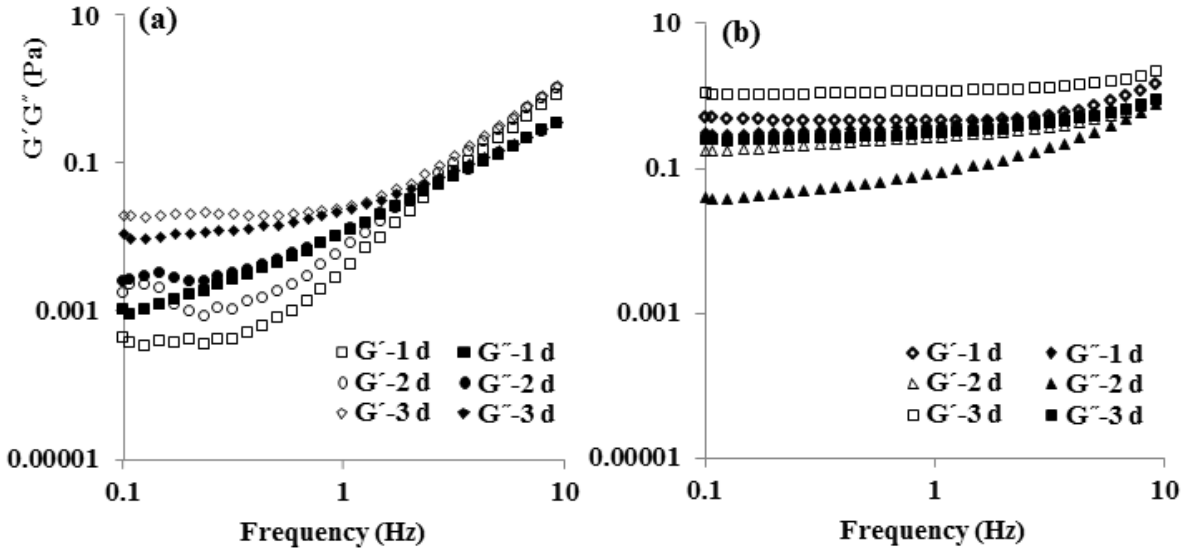

Figure 5.6. Frequency sweep of PF collected from two patients over three days post-surgery: (a) Patient P3 and (b) Patient P8, where the hollow and solid symbols indicate elastic (G´) and viscous moduli (G˝) respectively

5.5.3.2 Flow Rheology

Flow rheology was examined to identify the behaviour of PF at different shear rates. Figure 5.7.a illustrates the viscosity of the PF samples collected from different patients at day one post-surgery. All the PF samples (except patient P5) exhibited shear thinning behaviour, followed by a Newtonian plateau when the shear rate exceeded 1.0 s^{-1}. Large inter-patient differences in viscosity were observed between 0.001–22.87 Pa·s, at a shear rate of 0.1 s^{-1}.

Interestingly, an increase in viscosity was observed over the three-day post-surgery period for the two patients investigated (Figure 5.7.b). At a shear rate of 0.1 s^{-1}, viscosity increased from 0.14 to 22.87 Pa·s and from 0.01 to 0.05 Pa·s over the three-day period for patients P3 and P8, respectively. The change in viscosity indicates that the PF became more viscous in succeeding days following surgery. This matches with observations made surgeons when a patient has to be reentered in the days following surgery and the PF is found to be notably more jelly-like.

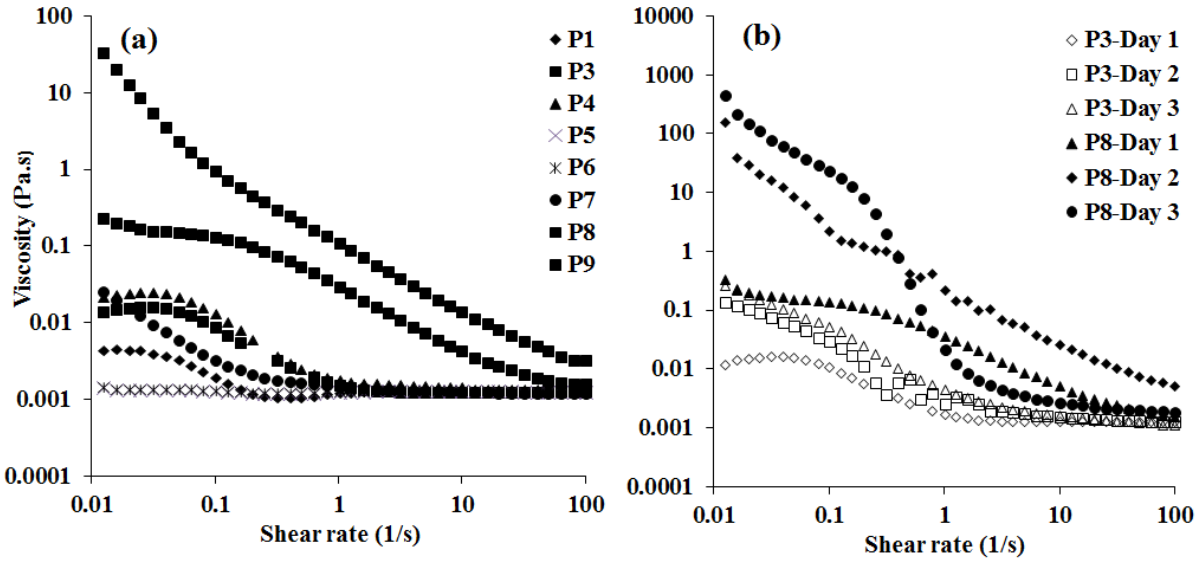

Figure 5.7. (a) Viscosity of human peritoneal fluid collected from different patients at day one post-surgery. (b) Time course of viscosity of human peritoneal fluid three days post-surgery for two of the patients.

Variations seen in the flow rheology of PF samples between different patients indicate the inability to simulate the hydrodynamics present in the intra-peritoneal environment by a single shear rate while performing the release testing at *in-vitro* settings. As predicted by the Noyes Whitney equation, the dissolution rate of drug is decreased when the viscosity of media is increased (224). Following surgery, patients typically have limited mobility. Therefore, the shear rate of PF in the pertioenal cavity is likely to be low, although exact values of the PF shear rate have never been measured or predicted. The release rate of susceptible medicines may differ between individual patients due to variations in the viscosity of PF. As the viscosity of PF increases over time, decreased drug release rates from medicines placed in the peritoneal cavity can be expected.

The composition and key physicochemical properties of PF varied between patients and for each patient over time. More importantly in relation to the performance of drug delivery systems, the key physicochemical properties of PF were significantly different when compared to PBS. As presented in earlier sections, the differences in physicochemical parameters have great potential to influence the drug solubility and the release performance of drug delivery systems. However, it is not known whether these differences in key physicochemical parameters will result in measureable alterations in solubility and drug release profiles. Moreover, it is difficult to predict which factor might be dominant in influencing the solubility and the release performance of our proposed sustained release formulations due to the multiple variations existing between PF and PBS.

5.6 Conclusions

Significant inter-patient and inter-day variability existed in the composition, physicochemical properties and rheological parameters of PF. Significant differences in physicochemical properties were demonstrated between PF and PBS, with higher pH and buffer capacity, alongside lower surface tension, evident in PF. Overall, the viscoelastic behaviour of PF obtained from the patients exhibited the characteristics of entangled networks. Viscosity, while varying between individual patients, increased over time. Due to the massive differences found in composition and physicochemical properties between PF and PBS, these data question the routine use of PBS as simulated release media for *in vitro* evaluation of intra-peritoneal drug delivery systems. The differences described between PF and PBS are further explored in Chapter 6, where lidocaine solubility and the release profiles of lidocaine from the EVA based formulation were determined.

CHAPTER 6. AN INVESTIGATION OF SOLUBILITY AND SUSTAINED RELEASE OF LIDOCAINE INTO PHOSPHATE BUFFERED SALINE AND PERITONEAL FLUID

6.1 Background

The release performance of any drug delivery system should be determined in a medium which closely resembles the environment at the intended administration site. For intra-peritoneal drug delivery systems, phosphate buffered saline is typically used to evaluate the *in vitro* release performance of intra-peritoneal drug delivery systems (IPDDS) (20). However, the investigations described in Chapter 5 demonstrated notable differences between PBS and peritoneal fluid (PF) in terms of composition and physicochemical properties. As highlighted in Chapter 2, differences in physicochemical properties including pH, buffer capacity and surface tension can greatly influence the release performance of drug delivery systems (155). However, it is not known how these differences in physicochemical parameters may result in measurable differences in the solubility and release performance of IPDDS. Accordingly, the drug solubility and release of lidocaine from the EVA based formulation were explored in both PBS and PF.

Local anaesthetics are commonly used drugs to relieve post-operative pain following abdominal surgery (225). Local anaesthetics are typically given to reduce pain and limit post-operative fatigue, while decreasing opioid consumption following abdominal surgery (3, 226). Lidocaine possesses a pK_a of 7.6 hence the difference in physicochemical properteis may alter solubility. Finally, the release data obtained in PF will be helpful in predicting the release performance of the developed sustained release formulation following implantation *in vivo* conditions.

Clinical data show that release of local anaesthetic into the peritoneal cavity over three days improves surgical recovery (105). It is hypothesised that prolonging release further into the post-operative period will further improve recovery after surgery. An EVA based formulation containing lidocaine was developed in the form of melt extrudates, and its ability to release lidocaine over a period of 10 days tested in PF and PBS. Retaining the original physicochemical properties of PF for 10 days was challenging due to possible microbial growth in PF. It was therefore necessary to add anti-microbial agents to the PF to inhibit microbial growth while maintaining its physicochemical properties throughout the study. A few antimicrobial agents such as chloramphenicol and sodium azide have been studied for their prevention of microbial growth in bio-relevant media and media comprising biological fluids (20, 227, 228).

Generally, the release performance of drug delivery systems is measured under sink conditions, where media drug concentration is maintained below the 10% saturated solubility of drug at all times (229). However for some drug delivery systems which are formulated with poorly water soluble drugs and/or are to be administered in body sites where a small volume of body fluid is available, determining release under non-sink conditions may be more clinically relevant. Lidocaine is a poorly water soluble drug and the EVA based formulation is intended for administraion into the peritoneal cavity, where there is little fluid volume available. Therefore, release testing should be performed under both sink and non-sink conditions in PF and PBS to better understand the release performance of the formulation.

6.2 Aim and objectives

The overall aim of the work described in this chapter was to compare solubility and release performance of lidocaine from an EVA based melt extrudate in both PF and PBS. The specific objectives were to:

- Select antimicrobial agents to prevent microbial growth
- Determine the solubility of lidocaine in PBS and PF
- Determine the release performance of lidocaine containing EVA melt extrudate in PBS and PF under both sink and non-sink conditions.

6.3 Materials

Human peritoneal fluid was obtained from patients at Middlemore Hospital, Auckland under the Health and Disability Ethics Committee (HDEC, Ministry of Health Wellington, New Zealand) approval 15/CEN/30. Phosphate buffered saline (PBS) tablets were bought from Sigma and dissolved in water obtained by reverse osmosis (MilliQ unit, Millipore) to produce 0.01 M PBS of pH 7.4. Lidocaine (base) was purchased from Sigma Aldrich (Dorset, UK). Poly(ethylene-co-vinyl acetate) (EVA) polymer, Ateva® 3325AC (33% VA content) was purchased from Celanese (Edmonton, Alberta Canada). Myristic acid, ≥98% was sourced from Fluka Analytical. Sodium azide and chloramphenicol powder were purchased from Sigma (Auckland, New Zealand). All reagents used were of analytical grade.

6.4 Methods

6.4.1 Selection of antimicrobial agents

First, stock solutions of chloramphenicol (1 mM) and sodium azide (200 mM) were individually prepared in water. Both PBS and PF were individually spiked with the stock solutions to prepare samples with either 0.02 mM of chloramphenicol or 6 mM of sodium azide. The respective samples were stored in Falcon tubes with a sealed cap in an oven at 37 °C for 10 days. The pH was monitored over the 10 days of storage.

6.4.2 Solubility of lidocaine

The solubility of the local anaesthetic lidocaine was compared in both PF and PBS. Approximately, 100 mg of lidocaine powder was taken in 10 mL of each medium and agitated in a water bath at 37 °C at 40 oscillations per minute. Samples of 0.5 mL were taken from each tube at 1, 2, 4, 6, 8, 24 and 48 h, and centrifuged at 5844 g for 10 min before analysis by HPLC. Concentrations increased up to 24 h after which a plateau was reached which continued out to 48 h, indicating the solubility limit had been achieved.

6.4.3 EVA melt extrudate

The EVA melt extrudates used for this Chapter were prepared by our collaborators at Queen's University, Belfast. A physical blend of EVA (33% VA), lidocaine and myristic acid was initially prepared using a mortar and pestle. Myristic acid was selected as an additive because of its ability to inhibit recrystallisation of lidocaine in EVA matrices, as previously described in Section 4.5.2 of Chapter 4. The extrudates were prepared using a co-rotating, intermeshing twin-screw extruder with a 2 mm cylindrical die (10 mm Twin Screw Extruder Microlab, Rondol, Strasburg, France). The mixtures were manually fed into the hopper and passed through a heated barrel to form the extrudate. The processing conditions in different zones of the extruder are shown in Table 6.1. The final extrudate had a diameter of around 2.3 mm and had 20% w/w of lidocaine loaded into matrices made up of 60% w/w of EVA and 20% w/w of myristic acid. The melt extrudate appeared transparent with no recrystallisation of lidocaine evident (Figure 6.1).

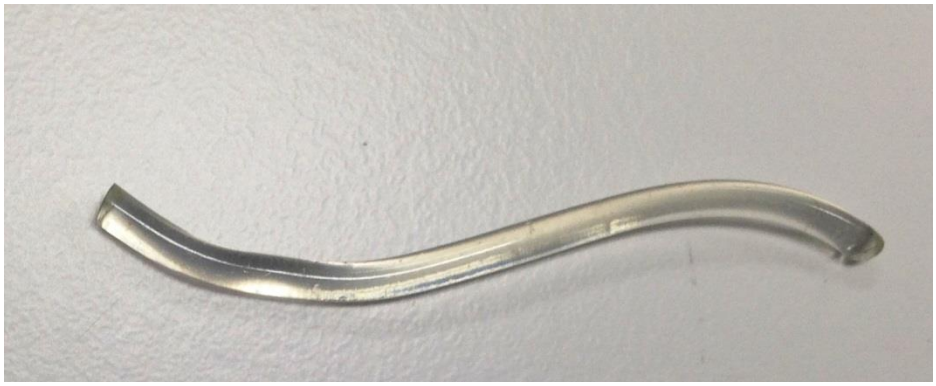

Figure 6.1. Photograph of melt extrudate containing 20% lidocaine, 20% myristic acid and 60% EVA (33% VA)

Table 6.1. Hot melt extrusion processing conditions

Temperature (°C)					Screw Speed (RPM)
Die	Zone 3	Zone 2	Zone 1	Feed Zone	
65	70	80	80	50	20

6.4.3.1 Content uniformity of EVA melt extrudates

The content uniformity of lidocaine in the melt extrudates was determined by the method described in Section 3.4.4.1 of Chapter 3. Ten pieces of EVA melt extrudate from different locations were cut, weighed and individually extracted and analysed by HPLC. Approximately 50 mg of melt extrudate was taken and lidocaine was extracted from the samples using DCM as a solvent. The samples were added to 50 mL of DCM and sonicated for 40 min. One mL of the resulting DCM solution was withdrawn with a glass pipette and dried at 60 °C for an hour in a volumetric flask. Methanol was added to the resulting drug film and sonicated for 30 min. The methanolic solutions containing lidocaine were centrifuged at 5844 g for 4 min and injected into HPLC for quantification. The analysis of samples was carried out against the standard solution prepared in methanol.

6.4.3.2 Release of lidocaine from EVA melt extrudate

Release of lidocaine from the melt extrudate was determined both in PBS and PF media. PF obtained from patient P3 at day three was examined for the study, as this sample had the largest volume. The release tests were performed by a similar method to that described in Section 3.4.4.1 of Chapter 3, where lidocaine release from the melt extrudate was tested. Release of lidocaine from the optimised melt extrudate was tested both in PBS and PF media containing 6 mM of sodium azide. Falcon tubes containing melt extrudate with PF and PBS were sealed with a cap and agitated at 40 oscillations per minute in a water bath maintained at 37 °C. Samples were withdrawn at pre-determined time intervals and centrifuged. The supernatant was extracted before analysis by HPLC. The quantification method used for the estimation of dissolved lidocaine in PF and PBS is described in detail in later sections.

The release study was conducted under both sink and non-sink conditions. For sink conditions, the sample was subjected in such a way that the final concentration of lidocaine in a medium remained below 10% of the saturated solubility (0.4 mg/mL) if all the lidocaine from the melt extrudate was released, whereas for non-sink condition it would reach over 40 % of saturated solubility (2.1 mg/mL) when all lidocaine was released. Approximately 40 mg and 200 mg of the melt extrudate were used for the release test under sink and non-sink conditions, respectively. The melt extrudate was sliced by a surgical knife and wrapped with stainless steel mesh to ensure that the extrudate remained submerged in the media at all times during release testing.

The data obtained were then studied through different mathematical models to understand the mechanism of lidocaine release. To fit the zero order model, the cumulative amount of released lidocaine was plotted against time. Meanwhile for the first order, release data were converted into log values of cumulative lidocaine remaining in the melt extrudate before plotting them against the time (230). For the Korsmeyer-Peppas model, the initial 60% of lidocaine release data was taken and the log values of cumulative percentage release plotted against the log time (231). The release mechanisms were interpreted based on 'n' values, where: $n<0.45$ indicated Fickian diffusion, $0.5<n<1$ indicated anomalous non-Fickian diffusion and $n<1$ indicated zero order release.

6.4.4 HPLC quantification of lidocaine

The quantification of the lidocaine involved the extraction followed by injection into HPLC. The extraction was performed by the method described in Section 3.4.4.3 of Chapter 3, where 800 µl of acetonitrile and 100 µl of water were added to 100 µl of the supernatant from the centrifuged sample obtained during the release test. The resulting solutions were vortexed and allowed to stand overnight at refrigerating conditions. The next day the samples were centrifuged at 5844 g for 10 min. Ten mL of the supernatant was then injected onto the HPLC.

An Agilent Series 1260 system (Agilent Corporation, Germany) consisting of a quaternary pump, an auto sampler and photo-diode array (PDA) detector were used, with data acquisition by Chemstation software (Agilent Corporation, Germany). Chromatographic separation for lidocaine consisted of a C18 column with a mobile phase comprising acetonitrile and potassium di-hydrogen phosphate buffer (pH 5.5; 0.02 M) in the ratio of 26:74 at a flow rate of 1 mL min^{-1} with detection at 230 nm. The chromatographic conditions had been presented in detail in section 3.5.1 of Chapter 3.

6.5 Results and Discussions

6.5.1 Selection of antimicrobial agents

Chloramphenicol and sodium azide were studied as potential anti-microbial agents for preventing microbial growth in peritoneal fluid. Without antimicrobials, the PF had visible solid lumps developing over 10 days. In previous reports, 0.02 mM of chloramphenicol or 6 mM of sodium azide have been used to preserve biological fluids (227, 232, 233). The antimicrobial used should not change the pH of biological fluids as the solubility of ionisable drugs may be altered. The measured pH of PBS and PF with and without anti-microbial agents at different time intervals is displayed in (Table 6.2). When chloramphenicol was used as an antimicrobial agent, the pH remained unchanged initially but then changed from 8.30 to 7.36 when measured after 10 days of storage at 37 °C. The cause of this dramatic decrease in pH after 10 days of storage is not known, although some reports have suggested the degradation of chloramphenicol to produce strong di-chloroacetic acid (234). When 6 mM of sodium

azide was used as the anti-microbial agent in PBS and PF, the pH remained unchanged even after 10 days of storage. Sodium azide was therefore selected as the antimicrobial agent.

Table 6.2. pH of PBS and PF with and without anti-microbial agents

Time	PBS			PF		
(days)	Control	0.02 mM CHL	6 mM SA	Control	0.02 mM CHL	6 mM SA
0	7.40 ± 0.00	7.50 ± 0.05	7.42 ± 0.01	8.30 ± 0.02	8.27 ± 0.05	8.32 ± 0.01
10	7.43 ± 0.02	7.46 ± 0.02	7.40 ± 0.01	8.40 ± 0.03	7.36±0.04	8.30 ± 0.01

Phosphate buffered saline-PBS, peritoneal fluid-PF (P8-Day 3), Chloramphenicol-CHL and Sodium azide-SA. Control means no antimicrobial agents were added.

6.5.2 Solubility of lidocaine

Local anesthetics are commonly administered intra-peritoneally to alleviate pain following abdominal surgery. The information available from the solubility of lidocaine in PF will be crucial to understand *in-vivo* performance of our developed EVA formualtion. The reported solubility of lidocaine in water is about 4.1 mg/mL (235). The estimated solubility of lidocaine in PBS was similar to water but was significantly higher in PF ($p<0.05$) (Table 6.3). Lidocaine is a weakly basic drug and has the pK_a value of 7.6. Relatively, higher solubility of lidocaine in PBS can be predicted when compared to PF obtained from P8 (pH 8.1), as solubility of local anaesthetics decreases over higher pH values (236). Similarly, the solubility of lidocaine should have been higher in PF obtained from P6 (pH 7.5) when compared to P8 (pH 8.1). Surprisingly, this was not the case as solubility of lidocaine was higher in PF when compared to PBS and the solubility was similar in PF obtaiend from P6 and P8. This indicates the effect of pH difference has a little infleucne over the solubility of lidocaine. Biological fluids contain naturally occurring surfactants which can facilitate micellar solubilisation (233).The presence of the surfactant component in PF may have dominated over other physicochemical properties, resulting in having higher solubility of lidocaine in PF. The value of surface tension described in Section 5.5.2 of Chapter 5 also support this argument as the average value of surface tension for PF was almost 15 mNm^{-1} lower than that of PBS. In addition, lidocaine strongly binds with protein (125) which could lead to increased apparent solubility. Previous studies also demonstated higher solubility of drugs in biolgocial fluids when compared to aquesous buffer (233). Recently, Dressman et al. reported higher solubility of the three poorly water soluble drugs ketoconazole, danazol and felodipine in human colonic fluids (HCF) compared to simple buffers (237).

Local anesthetics are commonly administered drugs to alleviate pain and reduce inflammation following abdominal surgery. In the future, the solubility data for a range of drugs in PF should be obtained. In addition, dissolution and solubility should be investigated over a range of shear rates to gain an

understanding of the effect of the different rheological properties evident in PF, both immediately after surgery and over time.

Table 6.3. Solubility of the local anaesthetic lidocaine in PBS compared with PF.

Solubility (mg/mL)		
PBS	PF (P6)	PF (P8)
4.3 ± 0.2	5.3 ± 0.5	5.2 ± 0.5

Phosphate-buffer saline-PBS and peritoneal fluid-PF

6.5.3 EVA melt extrudate

A solid implant has the ability to release drug locally in the peritoneal cavity and EVA based melt extrudates were explored for this purpose. The widespread use of EVA and the safety data available on its intra-peritoneal use in animal models made the use of this polymeric material suitable for our study (113). Melt extrusion is a convenient process for preparing implants and is easy to scale up. The melting points of lidocaine, EVA and myristic acid are all below 100 ºC, making them excellent candidates for melt extrusion. The addition of myristic acid resulted in the formation of transparent rods (Figure 6.1), with no evidence of lidocaine re-crystallisation. Although, the melt extrudate was clear and no visible sign of lidocaine re-crystallization was observed, a quantification method was necessary to confirm the uniform dispersion of lidocaine over the polymer matrix.

6.5.3.1 Content uniformity

Content uniformity testing was done in order to confirm the uniform dispersion of lidocaine in melt extrudate before drug release testing was performed. The content uniformity of lidocaine in EVA melt extrudate was determined by the method described in Section 3.5.4.1 of Chapter 3. As shown in Table 6.4, the estimated values ranged from 90.9 to 100.0%. The average value of 96.4 ± 2.9 with RSD value of 3.0% indicated uniform dispersion of lidocaine within the polymer matrices.

Table 6.4. Content uniformity of lidocaine in melt extrudate

Weight of ME (mg)	Theoretical amount of lidocaine (mg)	Amount of lidocaine recovered (mg)	Lidocaine recovered (%)	Recovery (%) Mean ± SD	RSD (%)
39.1	7.8	7.5	95.4		
36.5	7.3	7.3	100.0		
37.6	7.5	6.8	90.9		
37.0	7.4	6.9	93.1		
39.6	7.9	7.7	97.1		
40.2	8.0	7.7	95.6		
36.8	7.4	7.4	100.3		
38.0	7.6	7.3	95.4		
36.3	7.3	7.1	97.8		
40.2	8.0	7.9	98.1		

6.5.3.2 Release of lidocaine from EVA melt extrudate

Release of lidocaine from the EVA melt extrudate was performed under both sink and non-sink conditions in PBS and PF media. Sink conditions in the dissolution media are maintained when the media drug concentration does not exceed 10% of the saturated solubility of the drug (229). The solubility data obtained for lidocaine in both PF (5.2 mg/ml) and PBS (4.2 mg/ml) was useful for determining the amount of melt extrudate required to maintain both sink and non-sink conditions in both media. The release profiles of lidocaine from the melt extrudate are shown in (Figure 6.2).

As shown in Figure 6.2.a, the release of lidocaine occurred at a faster rate into PF than PBS. During the first 3 h the release profiles were similar, with about 11 and 12% of lidocaine being released into PF and PBS, respectively. However, the release rate of lidocaine into PF continued to increase rapidly after 3 h. The exact reason behind the measurable differences in release profiles would be complicated to identify, as multiple variations exist between PF and PBS in terms of pH, buffer capacity and surface tension. Considering the pH of the two media used, the release rate of lidocaine should have been lower in PF (pH 8.38) compared to PBS (pH 7.4). Similarly, the release rate was expected to be slower in PF as release rate decreases when the viscosity of the release media is increased (155). However, a higher release rate of lidocaine was seen in PF compared with PBS. As seen with the solubility data, the pH difference had a little more effect on lidocaine solubility over the other physicochemical properties, resulting in higher solubility of lidocaine in PF when compared to PBS (Section 6.5.2). Thus, the higher

solubility of lidocaine in PF may have contributed partially to the higher release of lidocaine in PF when compared to PBS. In addition to this, the surface tension of PF was found to be 10 mN/m lower than for PBS. This would result in better wetting and media penetration into melt extrudate in PF compared to PBS (151). Better wetting would then result in the faster diffusion of lidocaine from the melt extrudate, resulting in higher release of lidocaine from the EVA melt extrudate in PF compared to PBS. The EVA melt extrudate consisted mainly of hydrophobic EVA and myristic acid, hence wetting behaviour and media penetration into EVA melt extrudate may be dramatically different in PBS. Meanwhile, the extent of media penetration and wetting of the melt extrudate is expected to be much better in PF due to the lower surface tension value. A combination of all these factors may have contributed to a higher release of lidocaine in PF when compared to PBS.

The normal volume of peritoneal fluid present in the intra-peritoneal cavity is around 5- 20 mL although it varies widely with physiological conditions (238). It is therefore likely that following implantation, the melt extrudate may not be in sink conditions at all times. An understanding of lidocaine release under non-sink condition is equally important, in order to simulate the true release performance of implants *in vivo*. The release of lidocaine from the EVA melt extrudate under non-sink condition is displayed in Figure 6.2.b. The release profile generated under non-sink condition showed a similar trend, with higher release rates of lidocaine observed in PF compared to PBS.

The release rate of lidocaine from the melt extrudate decreased under non-sink conditions compared to sink conditions in both PF and PBS. The release rates were similar until 8 h, however slower release rates were observed into non-sink conditions when compared to sink conditions. Initially there was no boundary layer formation in the vicinity of the melt extrudate, hence lidocaine release occured at the same rate under both sink and non-sink conditions. As time progressed, sufficient formation of boundary layer occurred around the melt extrudate under non-sink conditions, resulting in a decreased release rate. Meanwhile, the boundary layer was continuously removed under sink conditions, hence resulting in higher lidocaine release even after a longer period of time (187).

It is not clear why the apparent difference between the release rates under sink and non-sink conditions was higher for PF compared to PBS. One of the reasons could be the non-Newtonian behaviour of PF at lower shear rates, as described in Section 5.5.3.2. Due to the higher viscosity of PF when compared to PBS, it is clear that it will be more difficult to disturb the boundary layer formed in PF compared to PBS. Thus, the effect of boundary layer becomes more pronounced in PF when compared to PBS, resulting in apparent differences in their release rates under sink and non-sink conditions.

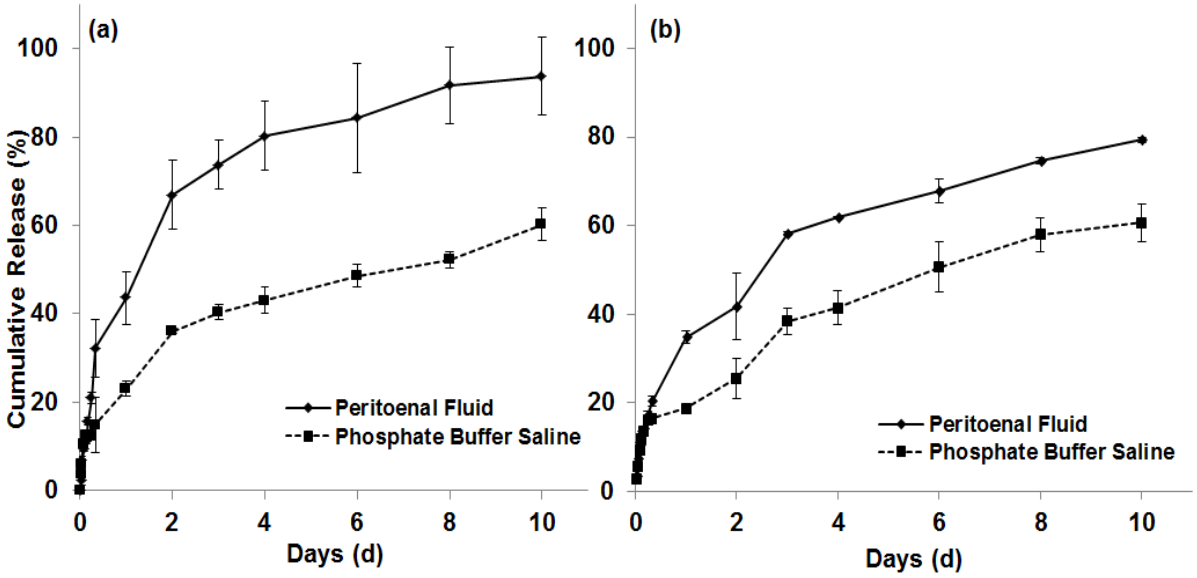

Figure 6.2. Release of lidocaine from the melt extrudate containing 20% lidocaine, 20% myristic acid and 60% EVA (33% VA), under (a) sink and (b) non-sink conditions. Sink condition represented the setting where the equilibrium concentration of lidocaine in media < 0.43 µg/mL even if all lidocaine was released. Meanwhile, non-sink condition represented the setting where the equilibrium concentration of lidocaine in media would > 2.12 mg/mL when all the lidocaine was released from the melt extrudate.

The data generated in PF and PBS under sink and non-sink conditions were fitted into mathematical models. The *r* values obtained indicated the best fit to a particular model and were used to explain the lidocaine release mechanism (239) (Table 6.5). Relatively lower *r* values were found for the zero order model (r < 0.93), while higher *r* values were found with the first order model (r> 0.93), indicating that the release of lidocaine were dependent upon the concentration of lidocaine in the extrudates. For PF, the n values obtained from the Korsmeyer-Peppas equation were in the range 0.52–0.73, indicating a non-Fickian diffusion mechanism of lidocaine release. Meanwhile, n values were lower than 0.45 under both conditions for PBS, which indicates a Fickian diffusion mechanism was involved in lidocaine release in PBS.

The underlying reasons behind the different lidocaine release mechanisms in PF and PBS is difficult to identify due to presence of multiple variations between the two media. The differences in solubility of myristic acid in PBS and PF could be one of the reasons behind this phenomenon. Usually, the solubility of myristic acid is very low in aqueous medium (240), hence it is likely that the solubility of myristic acid will be lower in PBS. Meanwhile, the solubility of myristic acid in PF (pH 8.3) could be higher when compared to PBS, as the solubility of fatty acids generally increases with high pH (211). Thus, the combined effect of pH, micellar solubilisation and increased media penetration could have resulted in minor erosion of myristic acid into the PF from the melt extrudates. In the future, the solubility of myristic acid should be determined in PF and PBS in order to understand the difference in lidocaine release behaviour between these media.

Table 6.5. Mathematical model-based interpretation of lidocaine release from the melt extrudates under various conditions

		Zero order	First Order	Korsmeyer -Peppas		
		r	***r***	***r***	**n**	**k**
PF	**Sink**	0.783	0.961	0.951	0.737	1.753
	Non-Sink	0.861	0.955	0.972	0.520	1.532
PBS	**Sink**	0.875	0.931	0.963	0.399	1.364
	Non-Sink	0.924	0.968	0.930	0.409	1.352

The EVA implant prepared by melt extrusion was able to release lidocaine in a sustained manner in both the media tested. After an initial burst of release into both PF and PBS, release slowed over time as the concentration of lidocaine in the melt extrudate decreased. As lidocaine was released in a sustained and controlled manner in PF obtained from patients following abdominal surgery, a similar release profile of lidocaine from the melt extrudate can be expected *in vivo* following its implantation. However the release rates of lidocaine in PF were dramatically higher when compared to PBS, hence the findings suggest that the difference in physicochemical properties between PBS and PF has an ability to influence the release performance of our developed sustained release formulation. Further, it also questions the routine use of PBS as a simulated release medium for *in vitro* testing of intra-peritoneal drug delivery systems. In the future, bio-relevant media closely resembling the physicochemical properties of PF should be developed in order to closely reflect the PF environement.

6.6 Conclusions

The effect of differences in key physicochemical properties of PF and PBS on lidocaine solubility and release from a sustained release EVA formulation was examined. The solubility of lidocaine was found to be higher in PF when compared to PBS. The EVA based formulation prepared by melt extrusion was transparent, with no visible signs of lidocaine precipitation. The content uniformity test confirmed the uniform dispersion of lidocaine in the EVA matrix. Lidocaine release occurred at a faster rate in PF compared to PBS. Release tests conducted under non-sink conditions also showed a similar trend, however the differences in release between PF and PBS were lower when compared for sink conditions. The release rate of lidocaine was slower under non-sink conditions in both media, when compared to the release rate under sink conditions. The differences observed in the solubility of the drugs and the release rate of lidocaine from melt extrudate raise obvious questions about the regular use of PBS as a simulated release medium for *in vitro* evaluation of intra-peritoneal drug delivery systems.

CHAPTER 7. GENERAL DISCUSSION, FUTURE DIRECTIONS AND CONCLUSIONS

7.1 General discussion

To manage post-operative pain and fatigue uninterrupted delivery of local anaesthetic into the intra-peritoneal region is required over a period of several days (3, 105). Contemporary approaches have relied on the use of infusion pumps and elastomeric devices, which are associated with risks of infection and catheter dislodgement. Developing a solid implant using poly(ethylene-co-vinyl acetate) (EVA) was seen as a suitable way to deliver local anaesthetic in a controlled manner, as this polymer has been previously used to release drugs in a controlled fashion for a prolonged period of time. Further, the non-biodegradable nature and non-swelling behaviour of EVA (241) provide advantages in terms of retention of the EVA formulation in peritoneal cavity and in facilitatating recovery in the event of local anaesthetic related toxicity, or at the completion of therapy. This thesis has described a formulation development process for an EVA formualtion containing lidocaine as a model local anaesthetic for the treatment of post-operative pain and fatigue following abdominal surgery. The formulation development process for the controlled release system consisted of multiple steps.

The development of a reliable analytical method was the first step before going into the preformulation stage. Previously described analytical methods for lidocaine were specific to a particular analytical purpose and demonstrated narrow application. Chapter 3 described the development and validation procedure of an HPLC method for lidocaine. The wide applicability of the developed method was demonstrated in meeting the analytical requirements for developing EVA formulation containing lidocaine for intra-peritoenal administration. The analytical method described was able to extract, resolve and quantify lidocaine from the forced degradation samples, EVA matrices and biological fluids. The analytical method was then employed in later stages of the thesis to quantify lidocaine from a range of samples and so to determine the stability of lidocaine with EVA, the solubility of lidocaine in phosphate buffered saline (PBS) and peritoneal fluid (PF), and to quantify the release of lidocaine from EVA formualtion in both PBS and PF.

Second, an understanding of the physical and chemical properties of lidocaine and EVA was necessary for the rational development of the formulation. Further, it was essential to ensure that the lidocaine remained compatible with EVA as there are no previous reports confirming the stability of local anaesthetics with this polymeric carrier. Compatibility studies between lidocaine and EVA with different VA compositions were described in Chapter 4, where binary samples of EVA and lidocaine were investigated using various analytical tools including TGA, DSC, NMR, FTIR and HPLC. The combined data from those analytical tools confirmed the compatibility of lidocaine with EVA, regardless of VA composition. Additionally, FTIR suggested an interaction between lidocaine which was pronounced with EVA having 33 and 40% VA composition. The interaction was further explored with solvent casting, as it favours better interaction because the drug and polymer are molecularly dispersed in the solvent. The absorbance spectra of various solvent cast samples were fitted using a combination of Gaussian-Lorentzian function to probe the shifting of the N-H peak of lidocaine. The position of the central wavenumber of the the fitted curve indicated that maximum shifting of the N-H peak occurred when

myristic acid was included with EVA and lidocaine. Interestingly, only the film prepared using the combination of EVA, myristic acid and lidocaine was clear with no visible sign of lidocaine recrystallisation, which indicated the ability of myristic acid to stabilise the formulation. Cryogenic grinding of lidocaine and EVA resulted in decreased crystallinity of lidocaine, indicating the potential application of this approach to disperse drug uniformly in EVA matrices.

While the majority of investigations during formulation development of controlled release systems are focused towards the formulation design, it is important to note that the performance of any drug delivery system depends not only on formulation design, but also on the environment at the administration site (155). An understanding of the composition and physicochemical properties of the microenvironment at the administration site for particular physiological conditions is equally important when designing controlled release system for novel application. Although the fluids present in the GI tract, intra-articular region and eye have been widely studied with relevance to the release performance of drug delivery systems, few investigations have been reported for PF. In addition to this, it is unknown whether the peritoneal fluid (PF) changes at different times post-surgery. A detailed investigation of human PF was therefore necessary. The data presented in Chapter 5 demonstrated that inter-patient and inter-day variations existed in PF in terms of composition, physicochemical properties and rheological parameters ($p<0.05$). Electrolyte and lipid levels varied between patients and for each patient over time. Statistically significant inter-patient variation was observed with regard to the following physicochemical properties: pH ($p<0.001$), buffer capacity ($p<0.05$), osmolality ($p<0.001$) and surface tension ($p<0.05$). The results of rheological examination of PF from the eight patients tested showed non-Newtonian behaviour followed by a Newtonian plateau at higher shear rates. Inter-patient and inter-day variability in the viscosity of PF was observed, and this was more pronounced at lower shear rates. For the PF obtained from six of the eight patients examined at day one,it behaved like an entangled network. The PF from the remaining two patients behaved as weak gels. Additionally, the key physiochemical properties of PF were compared with PBS of pH 7.4. PBS is a synthetic media typically used for *in vitro* evaluation of the release performance of intra-peritoneal drug delivery systems. However, all the key physicochemical parameters of PF were found to be significantly different ($p<0.001$) when compared to PBS. While Chapter 5 demonstrated the notable differences between PF and PBS, it was not certain how significantly these differences in properties might influence the solubility of drugs and the release behaviour of the developed EVA formualtion

The effect of the differences in physicochemical properties and rheological parameters between PBS and PF was further explored in Chapter 6 by determining the solubility of lidocaine and the release performance of melt extrudate. A higher solubility of lidocaine was noted in PF (5.3 mg/ml) when compared to PBS (4.3 mg/ml). The higher solubility of lidocaine in PF was attributed to the presence of natural surfactants in PF (232, 233). It was thought that the natural surfactants would favour micellar solubilisation, so increasing the solubility of drugs in PF when compared to PBS. The prepared extrudate showed no visible precipitation of lidocaine on the surface, thus confirming the stability of the formulation. The content uniformity and low RSD value of lidocaine in the melt extrudate further confirmed the uniform dispersion of lidocaine in the final preparation. The release of lidocaine from the extrudate occurred at a

faster rate in PF than in PBS. Better wetting due to lower surface tension of PF may have contributed to better penetration of media into the melt extrudate, resulting in faster release rate of lidocaine into PF (151). Ideally, the melt extrudate would contain sufficient lidocaine to release 250 mg of drug per day for 10 days following its implantation. It is therefore likely that the media concentration of lidocaine *in vivo* will deviate away from sink conditions due to the small volume of PF available *in vivo*. Hence, release data were also generated under non-sink conditions in order to predict the true release performance of the melt extrudate folliwng implantation *in vivo*. Release of lidocaine occurred at a slower rate in non-sink conditions compared to sink conditions, and this was more pronounced in PF than PBS. The slower release rate under non-sink compared to sink conditions was attributed to the formation of a boundary layer at the vicinity of the implant during the release test (187). However, the EVA implant released lidocaine in a sustained manner for a period of 10 days in both of the media. As the release data generated for the PF showed a sustained release profile of lidocaine over a period of 10 days, a similar trend of release behaviour from the developed formualtion can be expected *in vivo* conditions.

7.2 Limitations and future directions

The present work provides a solid foundation toward the development of EVA melt extrudate to provide sustained release of lidocaine to treat post-operative pain and fatigue following abdominal surgery. The data and analysis presented in this thesis have also revealed some exciting opportunities to explore in the future.

A review of the literature presented in Chapter 2 indicated that gels also have the potential to localize drugs into the peritoneal cavity and prevent premature leakage of drug, a problem generally encountered with particulate controlled release delivery systems (110). As no current application has been described for gels in delivering drugs into the intra-peritoneal region for the treatment of post-operative ailments, gels are one possibility for future exploration.

The analytical method described in Chapter 3 was found suitable for the quantification of lidocaine for *in vitro* purposes. However the method may not be suitable for *in vivo* experiments, when levels in the blood would need to be determined. Following implantation it is possible that the systemic concentration of lidocaine could fall below the described limit of quantification (LOQ). In this case, the extraction procedure described in Chapter 3 could be modified in order to moderately improve the sensitivity by evaporating and further re-constituting the extracted samples in a smaller volume before injection into HPLC (242). Alternatively, using a relatively more sensitive detector such as LC/MS could be explored (182). The optimised implant consisted of myristic acid in addition to EVA and lidocaine. Although myristic has relatively poor aqueous solubility (240), it will be essential to monitor the continuous release of myristic acid at some stage as described for lidocaine. However myristic acid has a poor chromophore, hence alternative quantitative methods like LC/MS (243) or GC/MS (244) should be developed.

The interactions between EVA and lidocaine were evident from the FTIR data described in Chapter 4. FTIR was able to probe the possible hydrogen bonding indicated by the shifting of the N-H peak of

lidocaine. The curve fitting was specific to the N-H group of lidocaine; however the carbonyl functional group of EVA and the carboxylic acid functional group of myristic acid involved in the interaction could be elucidated in further studies to have a broader understanding of interactions between lidocaine, EVA and myristic acid. Different combinations of lidocaine, myristic acid and EVA could be tailored in order to observe the changes in interaction behaviours with different lidocaine and myristic acid loading. Alternative tools including ^{1}H NMR (245) and ^{15}N NMR (246) could be used to detect interactions due to changes in electron densities around the hydrogen and nitrogen atoms of lidocaine involved in hydrogen bonding (247). Alternatively, nuclear Overhauser effect spectroscopy (NOES) could be used to investigate the strength of interaction between lidocaine, EVA and myristic acid (248).

The comprehensive investigation of human peritoneal fluid described in Chapter 5 provided useful information about PF with relevance to drug solubility and the performance of intra-peritoneal drug delivery systems. The data from this investigation could be used to develop a bio-relevant media for intra-peritoneal drug delivery systems in the future, as has been done for oral dosage forms (237).

Due to the limited sample volume, only the pH of peritoneal fluid could be monitored while selecting antimicrobial agents to prevent microbial growth in PF. In the future, composition along with other physicochemical properties could be monitored while selecting the antimicrobial agents (153). In addition to this, various measures could be taken in order to maintain the original composition of PF. Different cocktails could be included in the PF to retain the original composition of PF (153). While challenging, future work could seek to collect PF from a larger number of patients such that a variety of studies could be conducted.

This study determined the solubility of local anaesthetic lidocaine in PF and PBS. In the future, the solubility of a range of drugs including non-steroidal anti-inflammatory drugs (NSAIDs) and chemotherapeutic agents with different pK_a and solubility could be tested. The release testing described in Chapter 6 provided an understanding of release behaviour in PF and PBS, where a trend of burst release followed by sustained release was seen. The entire release test was conducted at the same agitation speed. Release testing at various agitation speeds could be conducted in order to observe the effect of PF viscosity on the release rate of lidocaine. Flow-through cells could be used in order to tailor different flow rates such that different shear rates could be precisely controlled (249).

It is important to note that the formulation carries enough lidocaine to cause toxicity if released all at once under physiological conditions. Therefore, the develoed formualation should be studied in animals to confirm the safety and toxicity profile before taking it into clinical trials (250). Animal models such as goats have been explored to study the safety and toxicity profile of controlled release systems conatining local anaesthetics for the treatment of post-operative pain (251). Larger animal models could be selected to study potential systemic and local toxicity, as they offer the advantage of multiple blood sampling. Systemic toxicity could be studied by the continuous monitoring of the lidocaine concentration in plasma, while local toxicity could be assessed by the histological examination of peritoneal tissue at the site of implantation (251). Sterilisation of the extrudate would be essential before implanting it into the animals.

However, an EVA based implant can only be sterilised though a gas sterilisation process (113). Hence, assurance will be needed that the sterilisation process doesn't alter the original properties of the melt extrudate. The physical and chemical stability of lidocaine could be confirmed by analysing the sterilised sample with various tools including DSC, FTIR and HPLC.

In the future, the developed system could also be explored as a way of delivering antimicrobial agents, analgesics, non-steroidal anti-inflammatory agents (NSAIDS), steroids and chemotherapeutic agents into the intra-peritoneal region to address a variety of clinical scenarios.

7.3 Thesis conclusion

In conclusion, this thesis has described a development process for a lidocaine containing EVA formulation with the ability to release lidocaine in a sustained manner over a period of 10 days. A simple, rapid and economical HPLC method was developed for the quantification of lidocaine from stressed samples, EVA matrices and biological fluids. Alongside this, a method for extracting lidocaine from EVA and biological fluids was developed which could be employed later for the quantification of other drugs formulated with EVA. The preformulation studies conducted confirmed the stability of lidocaine with EVA. Additionally, FTIR suggested the presence of interaction between EVA and lidocaine, possibly in the form of hydrogen bonding. The addition of myristic acid resulted in the formation of clear film, demonstrating its better ability to prevent lidocaine recrystallisation in EVA matrices. The interaction could be further explored in the future by studying the different combinations of EVA, lidocaine and myristic acid and then analysing them with FTIR and ^{1}H NMR. The comprehensive investigation of human peritoneal fluid collected from patients following abdominal surgery has provided detailed information with relevance to drug solubility and drug release performance for the first time. Inter-patient and inter-day variability was found to exist in the composition, physicochemical properties and rheological parameters of PF. Significant differences in physicochemical properties were demonstrated between PF and PBS, with higher pH and buffer capacity alongside lower surface tension evident in PF. The differences between PF and PBS were further studied by determining solubility and release of lidocaine from a melt extrudate formulation. The solubility of lidocaine was found to be higher in PF compared to PBS. A faster rate of lidocaine release in PF was observed when compared to PBS. Release of lidocaine under non-sink conditions showed a similar trend whilst the release rate was slower than sink condition. The differences observed in the solubility and release rate of lidocaine from melt extrudate suggest PBS is not an appropriate media for *in vitro* evaluation of intra-peritoneal drug delivery systems. Finally, the data presented in this thesis have demonstrated the potential for an EVA based formulation to be used as an intra-peritoneal implant to release lidocaine locally over 10 days following abdominal surgery.

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