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Phase transition of a microemulsion upon addition of cyclodextrin – applications in drug delivery

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Abstract

This study reports on the impact of cyclodextrin addition on the phase behavior of microemulsion systems. Three distinct oil-in-water microemulsions were formulated and subjected to increasing concentrations of various cyclodextrins. The prepared formulations underwent visual, textural and microscopic characterization followed by evaluation of their *in vitro* drug release and *ex vivo* tissue retention behavior. Combining microemulsions with cyclodextrins resulted in either phase separation or transition into a liquid crystalline state depending the concentration and type of cyclodextrin utilized. Formulations combined with α-cyclodextrin consistently demonstrated transition into a liquid crystalline state as confirmed by polarized light and cryo-scanning electron microscopy. In these cases, cyclodextrin addition was also positively correlated with an increase in formulation hardness, adhesiveness and turbidity. Release and clearance studies revealed that drug diffusion from the microemulsions could be slowed and tissue retention prolonged by increasing the cyclodextrin content. These findings pave the way for the development of novel cyclodextrin-microemulsion based liquid crystalline formulations in a variety of sustained drug delivery applications.

Keywords

Cyclodextrin; microemulsion; phase transition; liquid crystal; sustained drug release; drug delivery

Abbreviations

αCD	α-cyclodextrin
βCD	β-cyclodextrin
γCD	γ-cyclodextrin
AUC	Area under the curve
CD	Cyclodextrin
FTIR	Fourier transform infrared spectroscopy
HPβCD	Hydroxypropyl-β-cyclodextrin
IPM	Isopropyl myristate
IPP	Isopropyl palmitate
ME	Microemulsion
NMR	Nuclear magnetic resonance spectroscopy
PHCl	Pilocarpine hydrochloride
PBS	Phosphate buffered saline
SBEβCD	Sulfobutylether-β-cyclodextrin
SE	Standard error of the mean
SEM	Scanning electron microscopy

1. Introduction

The ability to sustain the delivery of drugs to the body remains of great interest, this being irrespective of the intended route of therapeutic administration. Sustained drug release is typically achieved through the use of viscous semi-solid systems such as gels and ointments, with such formulations effectively slowing therapeutic efflux while also demonstrating exceptional occlusive properties, which allow them to be retained at their target site for prolonged periods. We have developed a formulation combining cyclodextrins (Moya-Ortega et al. 2012, Moya-Ortega et al. 2013) and microemulsions (Chen et al. 2006, Wu et al. 2014) both of which demonstrate highly desirable safety and drug solubilization properties, as a platform for a broader range of drug delivery applications.

Microemulsions (ME) are isotropic mixtures of oil, water, surfactant and often a co-surfactant. Owing to the ultra-low interfacial tension between the water and oil phases, these systems demonstrate exceptional thermodynamic stability (Ruckenstein and Chi 1975, Wang et al. 2016). ME are routinely used in drug delivery due to their desirable wetting properties, ease of instillation and the ability to solubilize both hydrophilic and lipophilic drugs within the system (Lawrence and Rees 2000, Vandamme 2002). Formulations have been successfully administered via numerous routes including dermal (Butani et al. 2014), topical ocular (Fialho and Silva-Cunha 2004, Habib et al. 2011), oral (Li et al. 2016) and intravenous (Ma et al. 2012), demonstrating the versatility of this delivery system. Although not highly viscous in their native state, previous studies have demonstrated that ME may undergo transition into a liquid crystalline phase upon interaction with aqueous biological environments. Such systems, while demonstrating some liquid-like flow behavior, also possess an ordered

microstructure. In this instance, the increased structural order can also enhance retention of the formulation and thereby prolong therapeutic availability of the contained drug (Chan et al. 2007, Ren et al. 2012, Wu et al. 2014).

Cyclodextrins (CD) are ring-shaped oligosaccharides consisting six or more (α1-4-)-linked α-D-glucopyranose units (Duchêne and Bochot 2016, Thakur et al. 2015). These molecules possess a polar exterior and relatively apolar interior which allows them to harbor hydrophobic compounds in an aqueous environment. Variants of the six (αCD), seven (βCD), and eight (γCD) sugar-containing members of the family have seen routine use in drug delivery as solubilizing agents (Goodchild et al. 2015, Jóhannsdóttir et al. 2015, Sigurdsson et al. 2005), permeability enhancers (Másson et al. 1999, Rachmawati et al. 2013) and for their mucoadhesive properties (Ijaz et al. 2016, Moya-Ortega et al. 2013). Owing to these characteristics, CD serve as key excipients in various presently marketed pharmaceutical formulations (e.g. Yaz®, Abilify®, Voltaren Ophthalmic®).

Preliminary studies have demonstrated that combining CD with o/w ME results in the mixture adopting a liquid crystalline state and demonstrating a substantial increase in viscosity. Consequently, a two-component system of this nature may offer superior tissue residence while also prolonging drug release. In this study, we aimed to better understand and characterize the resulting product. Visual, textural and microscopic observations were complemented with *in vitro* drug release and *ex vivo* tissue retention studies to assess the utility of the formulation for sustained drug delivery.

2. Materials and Methods

2.1. Materials

Isopropyl myristate (IPM) and isopropyl palmitate (IPP) were gifted by Chemcolour Industries (Auckland, New Zealand). Span 20, Tween 20 and Tween 80 were gifted by Croda (Wetherill Park, Australia). Glycerol, Kolliphor® EL, βCD, hydroxypropyl-βCD (HPβCD), sodium fluorescein and pilocarpine hydrochloride (PHCl) were purchased from Sigma-Aldrich (St Louis, MO, USA). Sulfobutylether-βCD (SBEβCD) and γCD were purchased from Wacker Chemie AG (Munich, Germany). αCD was a gift from Manuka Health New Zealand, Ltd. Propylene glycol was purchased from Midwest Pharmaceutics, Ltd. (Hawke's Bay, New Zealand). 1-Butanol was purchased from Scharlau GmbH (Hamburg, Germany).

2.2. ME preparation

Three unique o/w ME formulations (listed in Table 1) were prepared using identical protocols. The investigative formulations were adapted from successful o/w ME identified in literature (Fialho and Silva-Cunha 2004, Habib et al. 2011, Shen et al. 2014). Preparation was commenced by weighing and mixing the surfactant and co-surfactant for 5 min. In the event that two surfactants were used (i.e. ME-3), these were thoroughly mixed prior to addition of the co-surfactant. The oily phase was subsequently added and this was followed by stirring for another 5 min. Finally, the aqueous phase was added drop wise to the mixture under continuous stirring at low speed and the formulation was stirred for an additional 10 min to achieve the final ME. All steps were carried out at room temperature.

Table 1. Compositions of investigated microemulsion systems.

	ME-1 (o/w) (Habib et al. 2011)		ME-2 (o/w) (Fialho and Silva-Cunha 2004)		ME-3 (o/w) (Shen et al. 2014)	
	Component	**%w/w**	**Component**	**%w/w**	**Component**	**%w/w**
Surfactant	Tween80	25	Kolliphor EL	25	Span20	13.65
Surfactant					Tween20	13.65
Co-surfactant	Glycerol	25	Propylene glycol	15	1-Butanol	9.1
Oily phase	IPM	5	IPM	5	IPP	3.6
Aqueous phase	PBS	45	PBS	55	PBS	60

2.3. Addition of CD

Five CD (αCD, βCD, γCD, SBEβCD and HPβCD) were investigated in this study. Each variety was added to the three ME at final concentrations of 0.05, 0.1, 0.2, 0.3 and 0.4 grams per gram of ME (listed hereafter as g/g). CD was added slowly to the final ME under vigorous shaking to ensure thorough incorporation occurred. Formulations were stored overnight at room temperature prior to visual evaluation and further characterization.

2.4. Polarized light microscopy

Formulation transition was characterized using a Leica DMR microscope (Leica GmbH, Germany). Samples were viewed under a cross-polarizer (40x magnification) to check for birefringence, which indicated transition of the ME into an anisotropic liquid crystalline state.

2.5. Fourier transform infrared spectroscopy

Complexes were prepared by dissolving or dispersing CD and each individual ME constituent (1:1 molar ratio) in deionized water and stirring the mixture at 80 °C for 2 h. The mixture was allowed to cool to room temperature before the water was evaporated. The precipitate was characterized using a Bruker Optics Tensor 37 Fourier transform infrared spectrometer (FTIR, Bruker, Billerica, MA, USA) equipped with a

diamond ATR module. The constituents were characterized individually and in a physical mixture with CD.

2.6. Texture analysis

Mechanical properties of the formulations prior to and following CD addition were evaluated using a TA.XT Plus texture analyzer (Stable Micro Systems, Godalming, England) with a 5 kg load cell. Samples were compressed once to a depth of 5 mm. The derived data was used to determine formulation hardness and peak negative adhesive force.

2.7. Cryo-SEM

Formulation microstructure was viewed using an FEI/Philips XL30 S-FEG scanning electron microscope (Hillsboro, OR, USA) with a Gatan Alto cryo chamber (Abingdon, England). Semi-solid formulations were loaded directly onto the sample holder whereas less viscous samples were dropped into small wells and sandwiched with a brass rivet. Samples were flash frozen in liquid nitrogen, fractured and subsequently sublimed at −90 °C for 30 min. Following sublimation, samples were sputter coated with platinum (240 s) and transferred to the viewing chamber where they were viewed at −185 °C using a 5 kV acceleration voltage.

2.8. In vitro drug release

PHCl was used as the model hydrophilic drug for *in vitro* release studies owing to its existence primarily in the aqueous phase of the formulation. As a result, it was expected that in-system phase transitions would not impact incorporation efficiency of the drug into the delivery system. The molecule was dissolved in PBS prior to addition to the other ME components to yield a final in-formulation concentration of 2% w/w. Drug release was evaluated using a Franz cell setup (receiver volume = 12 mL, diffusion surface = 0.77 cm^2). Briefly, 2 g of each formulation was loaded into the donor

chamber and separated from the receptor chamber by a 12,000-14,000 molecular weight cut-off cellulose dialysis membrane that had been presoaked for 24 h in release medium (PBS, pH 7.4). Samples (0.5 mL) were withdrawn at established time points (0, 0.5, 1, 2, 4 and 8 h) and replaced with fresh buffer to maintain sink conditions. Drug release was monitored over 8 h with PHCl concentrations being evaluated at 215 nm using UV-Vis spectroscopy.

2.9. Formulation clearance from mucous model tissue

The cornea was used as a model tissue for a mucous surface. The transparency of this tissue allowed formulation residence to be readily visualized using the available microscopic technique. An established *ex vivo* set up was utilized for tissue retention experiments (Liu and Wang 2009). Fresh porcine eyes were collected from a local abattoir. The entire cornea was excised, placed in a petri dish and subjected to a constant 5.73 µL/min flow of PBS using an Ismatec® pump (Cole-Parmer GmbH, Wertheim, Germany). The center of the tissue was then visualized at 5x magnification under a fluorescent microscope (Leica DMRA, Leica Microsystems, Heidelberg, Germany).

A weight of 10 mg of formulation pre-loaded with sodium fluorescein (1% w/w) was evenly applied to the tissue and formulation retention was monitored by quantifying the fluorescence intensity in the imaging zone (Nikon Digital Sight DS-U1 camera) using the 'ROI statistics → mean intensity' levels generated by the NIS Elements software (Version 2.10).

2.10. Statistical analysis

Data was analyzed using two-way ANOVA followed by Tukey's multiple comparisons test. A returned p-value < 0.05 was considered statistically significant. All samples were measured in triplicate and are displayed as mean ± standard error of the mean (SE), unless stated otherwise.

3. Results and Discussion

3.1. Visual observations

A variety of ME and CD combinations were assessed to better understand the interplay between the two systems. While all three ME were initially clear and colorless (Fig. 1A), subsequent addition of CD had varying effects on the formulations. When considering the three native CD, it was observed that αCD and γCD yielded liquid crystalline systems after mixing with ME-1 (Fig. 1B and Fig. 1D) with formulation viscosity positively correlating with the utilized CD concentration. Conversely, addition of βCD showed no such interaction and instead yielded a non-suspendable sediment (Fig. 1C). Much is known about the differences between the native CD varieties, including the formation of strong intermolecular interactions between βCD molecules, which result in this molecule having substantially lower aqueous solubility than αCD or γCD (Sabadini et al. 2006). Moreover, due to their cavity size differences, the three CD have very distinct and unrelated complexation and solubilization properties. Hence, the discrepancy observed here was unsurprising.

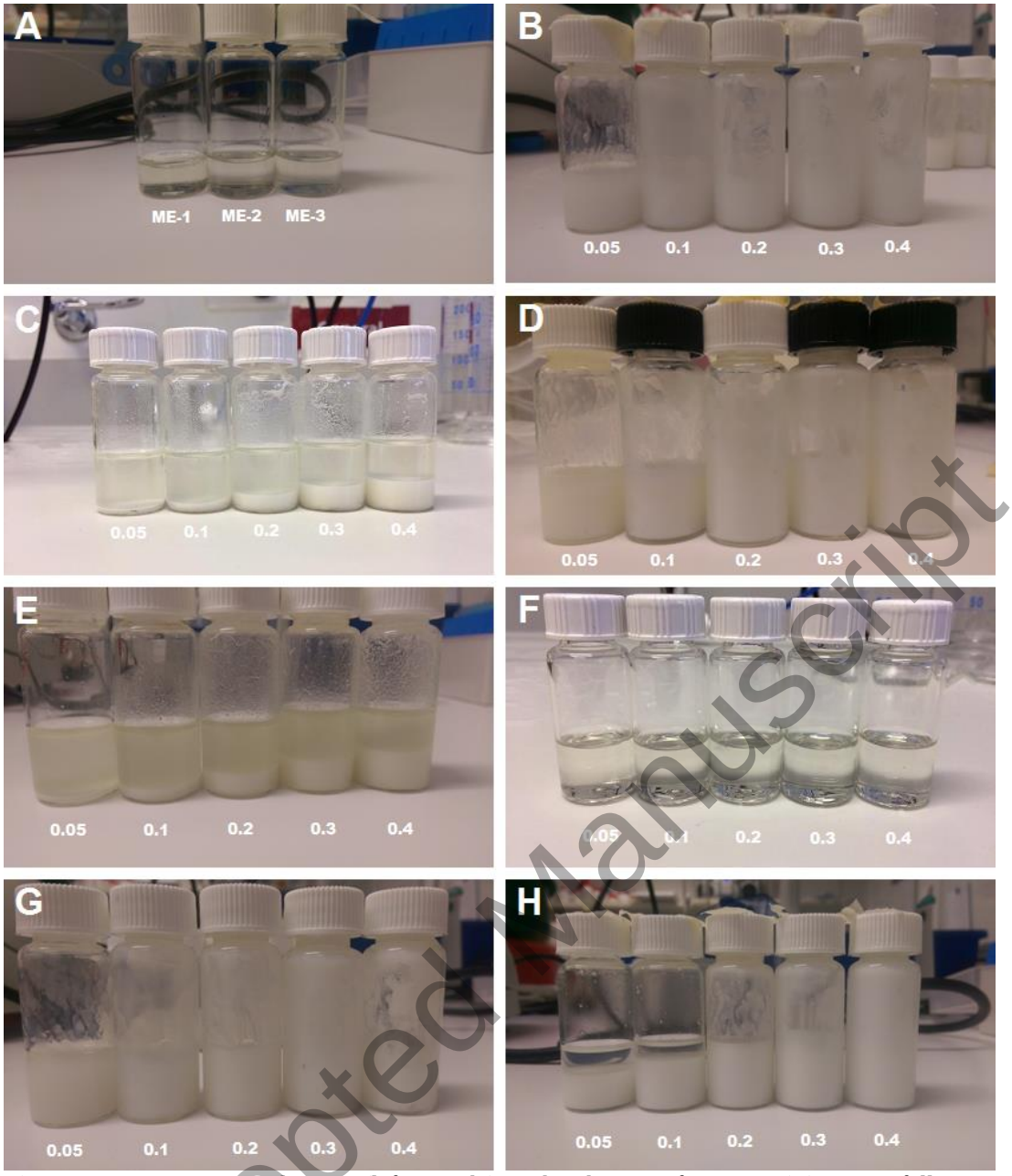

Figure 1. ME-1, ME-2 and ME-3 (left to right) in the absence of any CD (A). ME-1 following the addition of various concentrations of αCD (B), βCD (C), γCD (D), SBEβCD (E) and HPβCD (F). ME-2 following the addition of various concentrations of αCD (G). ME-3 following the addition of various concentrations of αCD (H). In (B-G), CD was added to the ME at concentrations of 0.05, 0.1, 0.2, 0.3 and 0.4 g/g (left to right, as illustrated in each figure).

To further expand on these initial observations, structural analogues of βCD were evaluated in combination with ME-1. Addition of SBEβCD (Fig. 1E) resulted in sedimentation in a similar fashion to βCD (Fig. 1C). On the other hand, addition of HPβCD caused only an increase in formulation viscosity while not grossly influencing transparency (Fig. 1F). While pure βCD is only sparingly used in the pharmaceutical

industry, its functionalized variants see routine applications given their superior aqueous solubility and biocompatibility when compared to the parent molecule (Frömming and Szejtli 1994). It is well-known that functionalization of CD impacts their in-formulation behavior and thus the observed differences between the βCD variants were foreseeable (Loftsson and Masson 1998). Both HPβCD and SBEβCD have previously been investigated as drug solubilizing agents within ME formulations while dissolved in the aqueous phase (Nandi et al. 2003). The findings made in our study are in line with those reported previously where addition of SBEβCD prevented ME formation, while HPβCD had a less pronounced effect on the system. Our findings suggest that the observed phase transition is not a function of CD solubility, but rather is related to the complexation behavior of these molecules with other in-formulation constituents.

αCD visibly formed the strongest interactions with ME-1 and was therefore investigated further. Addition of αCD to ME-2 (Fig. 1G) demonstrated comparable emulsification behavior to that of ME-1 (Fig. 1B) whereas the oligosaccharide had a less marked interaction with ME-3, showing some phase separation at lower αCD concentrations and a more homogenous product with lotion-like consistency at higher concentrations (Fig. 1H). The formed systems did not show any visible deterioration even after several months of storage (ambient conditions, away from light); suggesting that they were yielding a structure that is distinct from other reported CD-oil systems. Contrasting to our results, a previous study investigated the emulsification of IPM using αCD or γCD and found that the combination formed transient w/o emulsions which were not stable beyond a few days (Mathapa and Paunov 2013).

3.2. Polarized light microscopy

Liquid crystalline systems are anisotropic and refract monochromatic light (Wu 1986). This phenomenon is known as birefringence and allows the formulations to be visualized using a cross-polarized filter. Birefringence could be observed in all tested samples containing αCD (Fig. 2). Increasing CD concentration markedly increased the degree of birefringence and this characteristic was again seen to occur more extensively and at far lower αCD concentrations for ME-1 and ME-2 compared to ME-3 (Fig. 2).

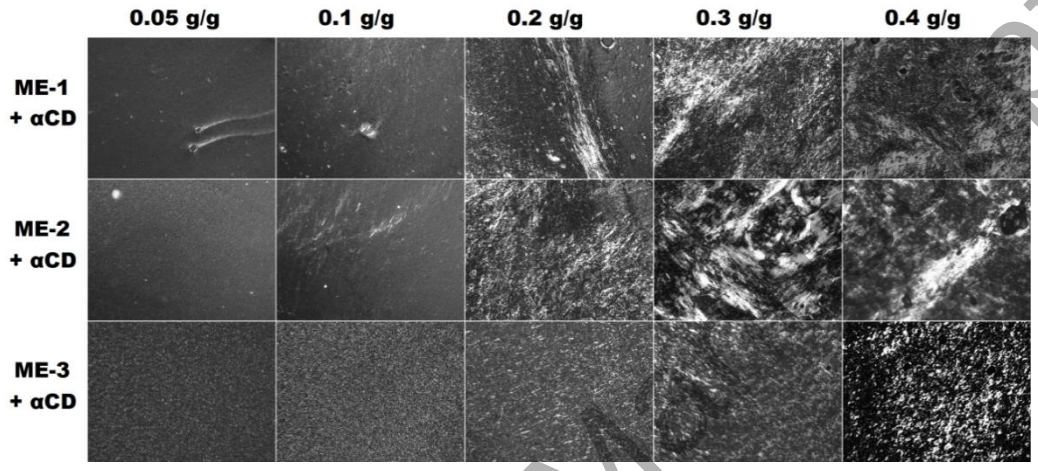

Figure 2. Polarized light microscopy images (reproduced in grayscale) of ME formulations following the addition of varying concentrations of αCD.

3.3. Fourier transform infrared spectroscopic evaluations

Qualitative analyses were performed to assess the interactions between αCD and the three individual ME-1 constituents. FTIR demonstrated that each constituent experienced some degree of complexation with the CD (Fig. 3). In the case of Tween 80, a sharp peak was observed in the fingerprint region of the complex corresponding to interactions with the ether functionalities found on the hydrophilic region of the molecule. No notable changes were observed on the alkyl region of the spectrum (2950-2900 cm^{-1}). In contrast, multiple peak intensity drops were identified for both IPM and glycerol, suggesting more uniform incorporation of these molecules into the CD cavity. All observed changes have been marked with arrows in Fig 3.

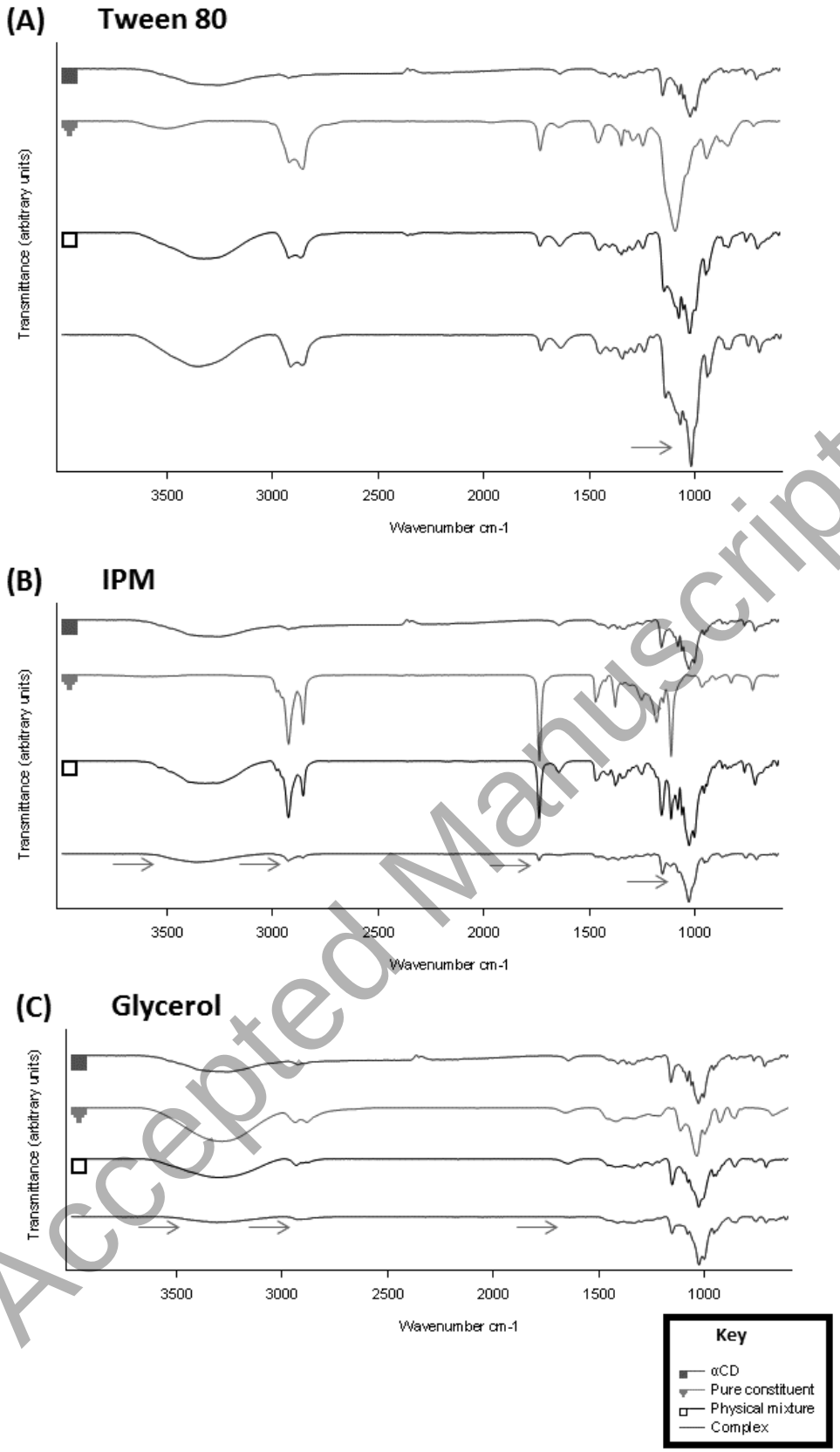

Figure 3. FTIR-ATR transmittance spectrum of Tween 80 (A), IPM (B), and glycerol (C) alone and in combination with αCD. Four spectra have been provided in each case, these being pure αCD, pure constituent, αCD-constituent physical mixture, and αCD-constituent complex. Arrows qualitatively highlight intensity changes observed in the spectra of the complexes indicating interactions between αCD and the respective constituent.

CD molecules are capable of interacting or forming complexes with many of the utilized in-formulation constituents including 1-butanol, glycerol (Buvari et al. 1983), Tween 80 (Chadha et al. 2011), and IPM (Mathapa and Paunov 2013). This is reinforced by our findings, and highlights the difficulties one may encounter in identifying the precise mechanism that is driving the phase transition.

In spite of this, ternary phase diagrams of the three ME may offer further insight into our observations. Namely, when we varied constituent ratios in the ME-1 system (Habib et al. 2011), we discovered that lowering the amount of surfactant and co-surfactant in-formulation yielded liquid crystalline systems (see supplementary data). Therefore, in our studies phase transition may have occurred due to complex formation between CD and glycerol and/or Tween 80 reducing the free concentration of these two components and thereby shifting the ME-1 system into the liquid crystalline region. Further studies, including docking evaluations that quantify binding affinity of the constituents to αCD, will be necessary to identify the precise interactions driving phase transition.

Liquid crystalline phase transitions are complex and non-uniform, with the molecules involved adopting many distinct conformations as ratios are varied. In the case of monoglycerides, the systems may adopt cubic, hexagonal, and lamellar conformations among others (Larsson et al. 1980). While X-ray and spectroscopic techniques can help determine the precise conformation present, it is known that birefringence only occurs when the system is anisotropic as is the case with hexagonal or lamellar phases (Alexandridis et al. 1998). Thus it can be postulated that the systems formed here possess at least one of these phases.

3.4. Textural properties

The hardness and peak negative adhesive force of all formulations improved by increasing the αCD content (Fig. 4). Both parameters were impacted at lower concentrations of αCD for ME-1 and ME-2 compared to ME-3, which followed earlier microscopic observations (Fig. 2). While ME-1 and ME-2 formulations demonstrated comparable hardness and peak negative adhesive forces up to 0.2 g/g of added αCD, further αCD addition had a more pronounced effect on ME-2, with this formulation demonstrating the greatest hardness and peak negative adhesive force ($p<0.0001$ in both cases) following the addition of 0.4 g/g αCD. This was in line with the observations made under polarized light (Fig. 2, middle row) where ME-2-αCD systems continued to increase in birefringence to a greater extent than ME-1-αCD formulations.

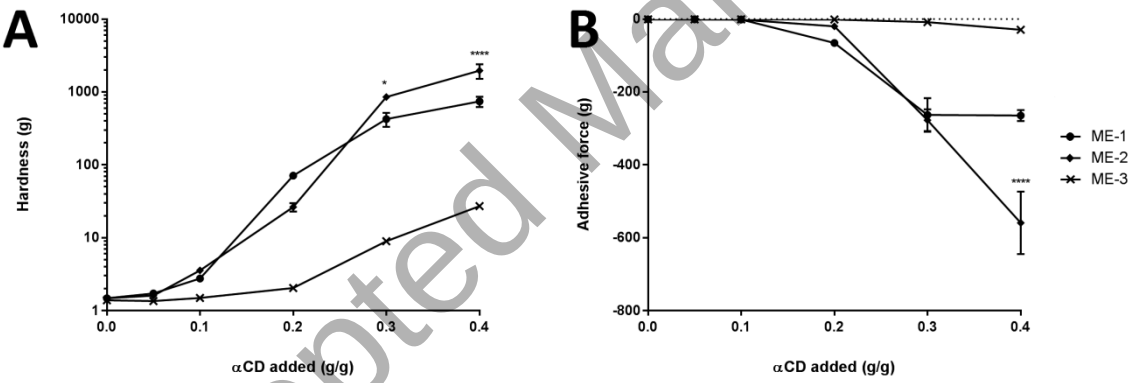

Figure 4. Hardness (A) and peak negative adhesive force (B) of ME formulations following incremental additions of αCD. Upon addition of 0.3 g/g αCD, ME-2 demonstrated significantly greater hardness than the other formulations (* $p<0.05$), while at 0.4 g/g αCD, ME-2 also displayed significantly greater hardness and peak negative adhesive force than ME-1 and ME-3 (**** $p<0.0001$). Results represent mean values ± SE, $n = 3$.

Textural properties of semi-solids have a substantial impact on their performance at the site of action. In the case of ointments, high hardness as well as negative adhesive forces ensure their occlusive nature (Akhtar et al. 2014, Bhagurkar et al. 2016, Tai et al. 2014). Cream formulations have comparatively moderate textural properties (Estanqueiro et al. 2016), whereas gels are the least firm of the three (Langasco et al.

2016, Pawar et al. 2017, Tai et al. 2014). Our results demonstrate ME-1 and ME-2 to possess viscous cream-like properties following and beyond addition of 0.3 g/g αCD, whereas ME-3 demonstrated properties comparable to a gel at all tested concentrations of αCD.

In spite of ME-2 formulations demonstrating the most pronounced textural properties, ME-1 preparations were more straightforward to formulate and exhibited superior homogeneity and reproducibility following 0.4 g/g αCD addition (Fig. 4), therefore this system was selected for further characterization.

3.5. Cryo-SEM

Transition of the ME into a liquid crystalline state was further confirmed by imaging its microstructure. While the blank ME-1 formulation could only be observed as nanoscale cavities (Fig. 5A), ME-1-αCD systems possessed highly ordered flow-line features even after addition of the smallest investigated quantity (0.05 g/g) of the oligosaccharide (Fig. 5B). Furthermore, an increase in structural order could be seen as the αCD concentration was increased to 0.4 g/g (Fig. 5C). The structure was in close correspondence to previously reported liquid crystalline systems (Ren et al. 2012).

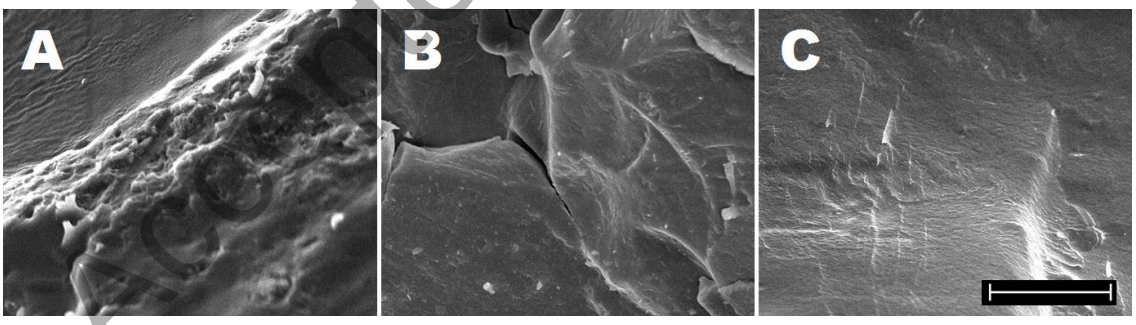

Figure 5. Representative cryo-SEM micrographs of ME-1 (A), ME-1 + 0.05 g/g αCD (B) and ME-1 + 0.4 g/g αCD (C). Scale bar = 5 μm.

3.6. In vitro drug release

PHCl was released from ME-1 at a slower rate than drug from PBS solution pH 7.4 (Fig. 6) due to the higher viscosity and thus slowed diffusion out of the ME system. Subsequent addition of αCD to the ME formulation further slowed drug release, this

again correlating with the amount of αCD added. After 8 h, less than 0.3% of the administered PHCl dose had been released from the ME-1 + 0.4 g/g αCD system (Table 2), equating to approximately 18% of the amount released from the native ME-1 formulation (96.52 µg ± 8.75 vs. 536.78 ± 28.29 µg).

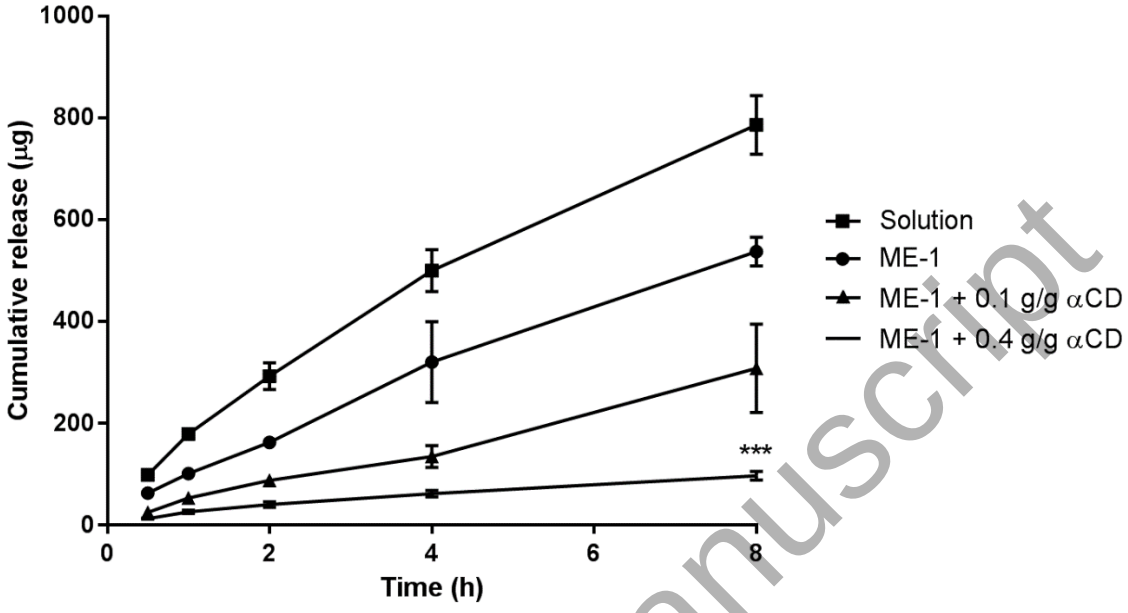

Figure 6. PHCl release from various formulations over 8 h as assessed using a Franz cell set-up. A mass of 2 g of each formulation was placed in the donor compartment while the receptor compartment contained 12 mL of PBS (pH 7.4). The two compartments were separated by a 12,000-14,000 molecular weight cut off dialysis membrane pre-soaked in PBS. Experiments were performed at 37 ± 1 ºC. At 8 h, ME-1 + 0.4 g/g αCD had released significantly less PHCl than all other studied formulations (*** $p<0.001$). Results represent mean values ± SE, $n = 3$.

Table 2. Amount permeated (µg), area under the 'concentration-versus-time' curve ($AUC_{0\text{-}8h}$), apparent permeation coefficient ($cm \cdot h^{-1}$) and Higuchi rate constant (k_H, $\%h^{-½}$) for the release of PHCl from the different formulations (results represent mean values ± SE, $n = 3$).

Formulation	Amount permeated after 8 h (µg)	$AUC_{0\text{-}8h}$	Higuchi k_H ($\%h^{-½}$)	R^2
Solution	786.19 ± 57.76	307.78 ± 18.25	0.72 ± 0.05	0.9728
ME-1	536.78 ± 28.29	198.73 ± 25.72	0.35 ± 0.09	0.9495
ME-1 + 0.1 g/g αCD	308.07 ± 87.38	100.28 ± 21.22	0.27 ± 0.07	0.9025
ME-1 + 0.4 g/g αCD	96.52 ± 8.75	38.60 ± 4.00	0.09 ± 0.01	0.9826

While this is not a common phenomenon, CD complexation with drug may seldom contribute to slowing its release (Stella et al. 1999). Moreover, in spite of being highly water soluble, PHCl is known to complex with many CD including αCD (Keipert et al. 1996) and as such, we evaluated if this phenomenon impacted its release rate. Free and αCD-complexed PHCl solutions showed no apparent differences in their *in vitro* release profiles (data not shown). Given the low log P of PHCl and negligible solubility in the non-aqueous ME constituents, it may be concluded that the sustained release characteristics were primarily the result of the system adopting a more viscous liquid crystalline conformation. These observations follow Fick's first law wherein the diffusion flux of a molecule from a medium is inversely proportional to its viscosity.

3.7. Formulation clearance from mucous model tissue

ME-1 formulation was subsequently promoted to *ex vivo* clearance studies. ME-1 on its own demonstrated significantly greater retention than the simple solution, with the latter rapidly draining off the tissue surface (Fig. 7). Ten minutes following instillation 17.11 ± 0.95% of the original fluorescent signal from ME-1 remained on the tissue as opposed to 4.69 ± 0.94% from the fluorescein solution ($p<0.01$). This finding is unsurprising as it is understandable that increased viscosity would prolong formulation residence. Previous reports have demonstrated that o/w emulsions and w/o ME could maximize the AUC of retention but did not prolong the residence of sodium pertechnetate (^{99m}Tc) in a similar *in vivo* rabbit model (Alany et al. 2006). In our case, both retention AUC and amount significantly increased. Possible reasons for the discrepancy here may relate to the differences in constituents employed to prepare the formulations.

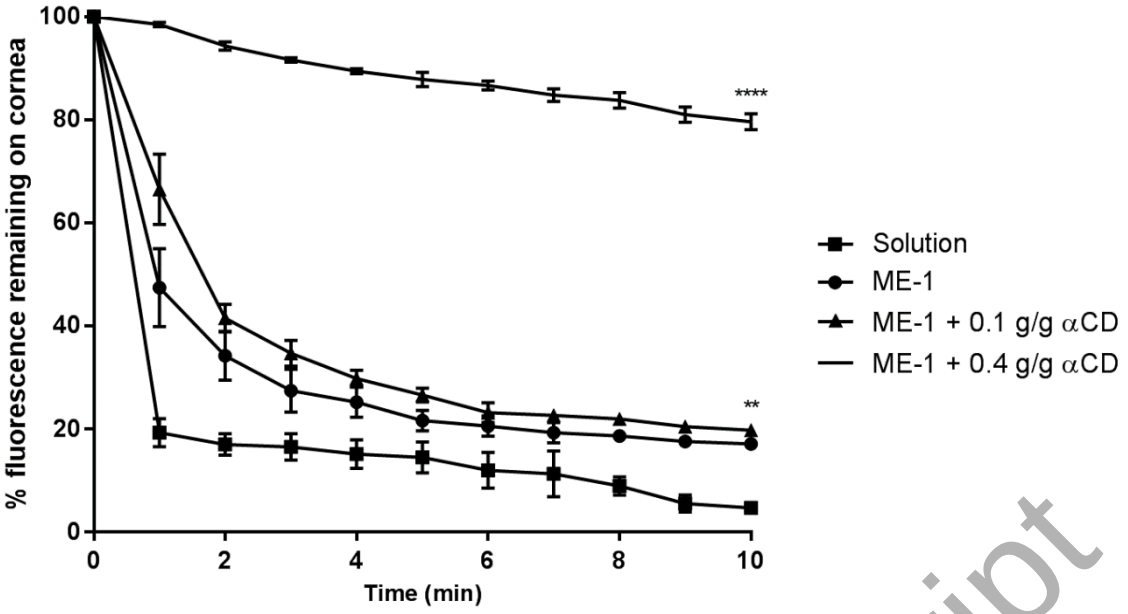

Figure 7. Sodium fluorescein signal on porcine cornea over 10 min when incorporated into the different formulations. The initial dye content was kept constant at 1% w/w and the apparatus was subjected to a steady 5.73 µL/min flow of PBS. Ten min following instillation, ME-1 + 0.4 g/g αCD demonstrated significantly greater fluorescence retention than all other tested formulations ($p<0.0001$). ME-1 and ME-1 + 0.1 g/g αCD also demonstrated significantly higher retention when compared to simple solution ($p<0.05$). Results represent mean values ± SE, $n = 3$.

Although texture analysis demonstrated that addition of 0.1 g/g αCD both enhanced ME-1 viscosity and adhesiveness (Fig. 4), this did not translate into greater tissue retention (Fig. 7). Here 19.72 ± 1.27% of the fluorescein signal was visible after 10 min, which was not significantly different to ME-1 alone. In this state, the formulation was seen to readily spread around and be cleared off the tissue. In stark contrast, ME-1 containing 0.4 g/g αCD had substantially greater residence on the tissue with 79.61 ± 1.57% of the initial fluorescence retained after 10 min, which was significantly greater than all other tested formulations ($p<0.0001$ in all cases). Owing to its more pronounced semi-solid properties, the 0.4 g/g αCD containing formulation demonstrated a retention profile distinct from ME formulations and more comparable to that observed with ointments (Greaves et al. 1993) or *in situ* gel formulations (Liu et al. 2006).

The ME-1 + 0.4 g/g αCD formulation holds potential as a semisolid preparation owing to its ability to achieve prolonged retention on tissue while sustaining drug release. Added to this are the benefits offered by in formulation constituents, such as CD improving formulation mucoadhesion (Moya-Ortega et al. 2013), drug solubility (Jóhannsdóttir et al. 2015, Sigurdsson et al. 2005) and assisting drug penetration (Másson et al. 1999, Rachmawati et al. 2013). Taken together, these properties make the formulation amenable to a variety of applications beyond those of conventional semisolids. Possible investigative approaches could include administration via the dermal and topical ocular routes, as well as administration to other mucosal membranes of the body (e.g. intranasal). Future studies will look to validate and utilize these properties to improve drug delivery in a relevant *in vivo* model through appropriate evaluations of formulation safety and efficacy.

4. Conclusion

Novel systems comprising o/w ME and CD were prepared and characterized. Mixing αCD with these three distinct ME resulted in rapid semi-solid phase transition, with the formed systems demonstrating birefringence indicative of liquid crystal formation. αCD-containing ME-1 and ME-2 formulations exhibited higher viscosities, hardness and adhesiveness compared to the ME-3 systems, with ME-1-αCD showing the greatest reproducibility. Increasing αCD slowed PHCl release from ME-1 formulations while also offering resistance to their clearance from *ex vivo* tissue which was seen to primarily be a function of the increased formulation viscosity. These properties render the ME-1-αCD combination attractive for use as a prolonged release semisolid for drug delivery applications.

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Disclosure of interest

The authors report no conflicts of interest.

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