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In-situ phase transition from microemulsion to liquid crystal with the potential of prolonged parenteral drug delivery

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ABSTRACT

This study is the first to investigate and demonstrate the potential of microemulsions (MEs) for sustained release parenteral drug delivery, due to phase transition behavior in aqueous environments. Phase diagrams were constructed with Miglyol 812N oil and a blend of (co)surfactants Solutol HS 15 and Span 80 with ethanol. Liquid crystal (LC) and coarse emulsion (CE) regions were found adjacent to the ME region in the water-rich corner of the phase diagram. Two formulations were selected, a LC-forming ME and a CE-forming ME and each were investigated with respect to their rheology, particle size, drug release profiles and particularly, the phase transition behavior. The spreadability in an aqueous environment was determined and release profiles from MEs were generated with gamma-scintigraphy. The CE-forming ME dispersed readily in an aqueous environment, whereas the LC-forming ME remained in a contracted region possibly due to the transition of ME to LC at the water/ME interface. Gamma-scintigraphy showed that the LC-forming ME had minimal spreadability and a slow release of ^{99m}Tc in the first-order manner, suggesting phase conversion at the interface. In conclusion, owing to the potential of phase transition, LC-forming MEs could be used as extravascular injectable drug delivery vehicles for prolonged drug release.

1. Introduction

It has been estimated that 40% of newly discovered drugs have solubility problems, either in oil or in water (Kini et al., 2011), which presents a challenge to formulation scientists particularly with parenteral injectable formulation development. Injectable formulations should usually be in a liquid form and optimized not only for solubility and stability, but also for injectability and tissue tolerability. Although complete solubilization of the drug is not a prerequisite for extravascular injections such as subcutaneous and intramuscular administrations, and suspensions and microparticles are commercially available, particle size control and physical stability of these particulate systems can be significant hurdles in formulation development particularly during large scale manufacture. A number of solubilization approaches have been investigated for parenteral formulations, including the use of organic co-solvents, pH adjustment or salt formation, addition of surfactant or formation of inclusion complexes with cyclodextrins (Strickley, 2004). The parenteral formulations resulting from these approaches are conventionally used to establish rapid onset of drug action. A frequent problem encountered with these conventional formulations is post-administration drug precipitation at the injection site, due to the rapid release of a poorly water soluble drug into the body's aqueous environment (Wu et al., 2010a,b). To this effect, sustained release formulations, modulating the drug concentration at the injection side, may provide a solution (Wu et al., 2010a). Recently, various in situ forming injectable gels emerged as controlled and sustained drug delivery systems due to the ease, and reduced frequency, of administration (Liu and Venkatraman, 2012; Nirmal et al., 2010). However these aqueous based systems have limited solubilizing capacity.

Microemulsions (MEs) are transparent, thermodynamically stable, colloidal systems that form spontaneously when suitable combinations of water, oil and surfactant with a co-surfactant are mixed (Gabriele et al., 2006; Talegaonkar et al., 2008). They are easy to prepare and have low viscosity with the capacity of solublizing both lipophilic and hydrophilic drugs (Alany et al., 2001). Hence MEs have evolved as novel parenteral delivery vehicles for both oil- and water-soluble drugs (Date and Nagarsenker, 2008; Gupta and Moulik, 2008). MEs as parenteral delivery systems for poorly soluble drugs, including a few anticancer drugs, have shown distinct advantages over solvent based formulations including; bioavailability improvement (Date and Nagarsenker, 2008), longer resident time in the blood circulation (Zhang et al., 2006) and a reduction in drug irritation (Lee et al., 2002).

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Liquid crystals (LCs) also consists of water, oil and surfactant(s), and have been explored as drug delivery systems due to their unique characteristics (Mueller-Goymann and Hamann, 1993). LCs are semisolids with crystalline structures combining properties of both solid and liquid states (Hans, 1980). The crystalline structures of LCs render the systems highly viscous; as a result the diffusion coefficient of a drug within a LC phase is about half the magnitude compared to that in solution (Mueller-Goymann and Frank, 1986). This feature has been exploited for sustained drug release following topical administration (Mueller-Goymann and Frank, 1986; Mueller-Goymann and Hamann, 1993). However, a limitation of the high viscosity is that LC systems cannot usually be injected.

Therefore, a system which transitions in an aqueous environment, such as tissue fluids, from a low viscosity ME to a high viscosity LC is envisioned to be highly promising for parenteral injections with the possibility of achieving prolonged drug release. This in situ phase transition concept was reported by Mueller, who used reverse micellar solutions as the medium vehicle which transitioned to a LC system after contact with water and subsequently demonstrated a sustained release rate (Mueller-Goymann and Hamann, 1993).

So far, little research has been carried out to investigate the phase transition behavior of ME formulations in contact with an aqueous environment, specifically the effects of formulation spreadability and drug release. The aim of the present study was to develop two different ME formulations, one with the potential to form liquid crystals and another with the potential to form a coarse emulsion upon contact with water. These formulations were characterized and compared with respect to spreadability and in vitro drug release following phase transition in an aqueous environment to simulate in vivo behavior.

2. Material and methods

2.1. Materials

Solutol HS 15 (Macrogol 15 Hydroxystearate, hydrophilic–lipophilic balance, HLB = 15) was provided by BASF, Fine Chemicals Division, Germany. Span 80 (Sorbitan monooleate, HLB = 4.3), Soybean oil, Nile Red, methylene blue and dialysis tubing cellulose membrane (M.W. 12,000 cut-off) were all purchased from Sigma–Aldrich NZ Ltd., New Zealand. Miglyol 812N, a mixture of medium chain triglycerides, was purchased from Sasol GmbH Oleochemicals, Witten, Germany. Milli-Q water was prepared using the Milli-Q water purification system (Millipore Corp., MA, USA). Absolute ethanol was purchased from ECP-Analytical Reagent ECP Ltd., NZ. Propylene glycol was purchased from Unilab, Ajax Laboratory Chemicals, Australia. Acetonitrile was supplied from Merck KGaA, Germany. Progesterone was purchased from Pfizer Inc. (NY, USA). The raw isotope Molybdenum (Mo-99) was supplied from ANSTO, Australia, to produce the radioactive agent sodium pertechnetate (^{99m}Tc), using a Gentech Technetium Generator (ANSTO, Sydney, Australia). All chemicals and reagents used were of analytical grade, without further purification.

2.2. Formulation development

2.2.1. Construction of pseudo-ternary phase diagrams

The pseudo-ternary systems were prepared using the titration method based on Miglyol 812N (oil), Milli-Q water and a mixture of Solutol HS 15 and Span 80 (surfactants) and ethanol (cosurfactant). The weight ratio between Solutol HS 15, Span 80 and ethanol was 3:1:0.5. The mixtures of oil:surfactant ranging at a constant ratio from 10:90 to 90:10 (w/w), were titrated with Milli-Q water under constant magnetic stirring at ambient temperature (20 °C) at 1% increments of water. Formulations were prepared in parallel at ambient temperature (approximately 20 °C), and were stored at either 20 °C or 37 °C for 24 h before observation was made for the construction of pseudo-ternary phase diagrams at each temperature.

2.2.2. Visual observation and polarized light microscopy

Visual observation and polarized light microscopy (DM RXP, Leica DMR, Germany) were used to identify LCs, MEs and CEs. LCs were identified as semisold systems exhibiting birefringence under cross-polarized light microscopy due to their double refraction property. Clear-transparent samples with an isotropic appearance under cross-polarized light microscopy were regarded as MEs. Samples with phase separation under phase-contrast microscopy and no birefringence under polarized light microscope were classified as coarse emulsions (CE).

2.2.3. ME formulation selection

From the pseudo-ternary systems two different ME formulations were selected; one with the potential to form liquid crystals (LC-forming ME), and another with the potential to form a coarse emulsion (CE-forming ME) upon contact with water. Consideration was given to the viscosity for injectability and the influence of temperature.

2.3. Characterization of MEs

Besides the selected ME formulations, samples were selected from dilution lines A and B (Fig. 1), for various characterization studies. These formulations contained 45:55 and 20:80 (w/w) oil: surfactant/cosurfactant combinations, respectively, possibly representing the changes to the selected MEs as a result of the diffusion of water into the formulations.

2.3.1. Rheological property

Viscosity was measured with a Brookfield DV-III cone and plate rheometer (Brookfield Engineering Laboratories Inc., USA) fitted with a CP-40 spindle. The sample cup was connected to the circulating water bath maintained at 20 °C or 37 °C. A sample volume of 500 μl was used. The measurements were made from 0–240 rpm in triplicate. Data analysis was performed by Rheocalc V3.1 operating software (Brookfield Engineering Laboratories Inc., USA). Viscosity values were recorded at 20 rpm.

2.3.2. Dynamic light scattering (DLS) measurements

Dynamic light scattering (DLS) was used to determine the droplet size of selected formulations distributed along the dilution line A and B. The measurements were made with a Malvern Zetasizer Nano ZS instrument (Malvern Instruments, U.K.). The instrument contained a 4 mW He–Ne laser operating at 633 nm and non-invasive backscattering optics. The measurements were made at a detection angle of 173° and the measurement position was automatically selected by the software. The viscosity of the formulations investigated was used as the viscosity of the dispersant. Each measurement was made in triplicate at 20 °C and 37 °C and subsequently the average droplet size (Z-Average) and Polydispersity Index (PDI) was calculated as a measure of homogeneity of the sample in size.

2.3.3. Transmission electron microscopy (TEM) analysis

Morphology and microstructure of the selected formulations were studied using freeze fracture Transmission electron microscopy (FF-TEM) at an acceleration voltage of 120 kV and typically viewed at a range of magnification of 25,000–88,000×. The

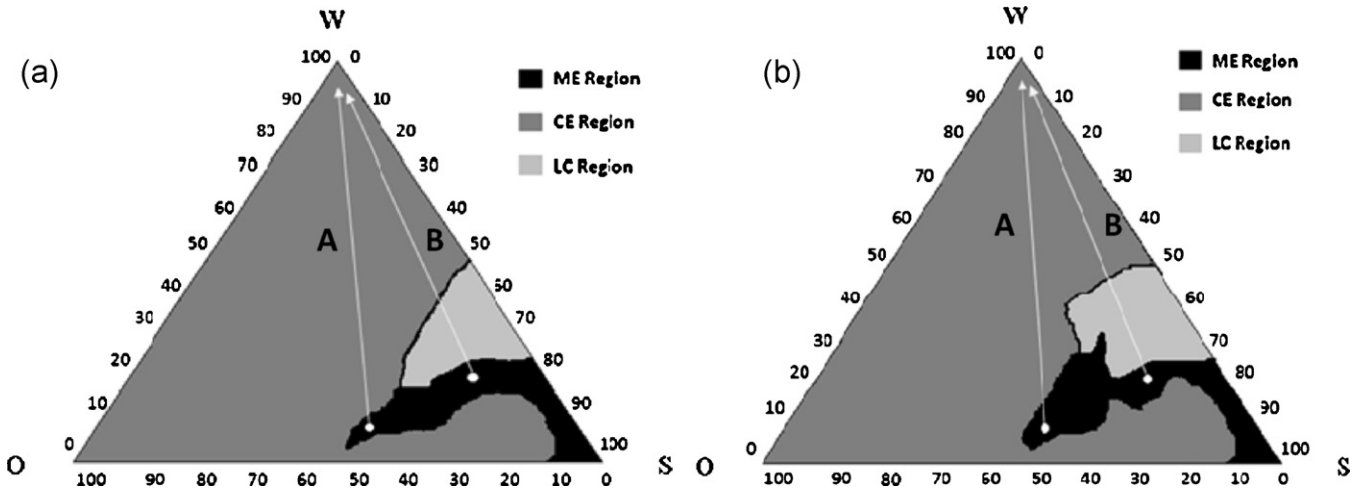

Fig. 1. Pseudo-ternary systems prepared at (a) 20 °C and (b) 37 °C; two sampling trends A and B were subsequently derived from the CE and LC forming ME indicated as two white dots.

size of the colloidal structures was determined using a Philips CM12 TEM (FEI Company, Eindhoven, Netherlands).

2.4. ME spreadability and phase transition in an aqueous environment

To simulate the behavior of the ME formulations at the injection site, the spreadability and phase transition in an aqueous environment were investigated using visual observation and light microscopy.

2.4.1. Visual observation on ME spreadability in aqueous media

Each ME formulation (100 μl), dyed with Nile Red, was injected in the center of a scaled Petri-dish pre-filled with 5 ml of water using a 20 gauge needle. The increase in the diameter of each formulation was monitored over time. Additionally, each ME formulation was injected into ~300 ml of water and the effect of the aqueous environment on each formulation was observed at different time points.

2.4.2. Microscopic observation of ME/water interface

The ME/water interface was observed using the method described by Wu et al. (2010a). Briefly, around 30 μl of each ME formulation was added onto a microscopic slide, under phase contrast microscope. One drop of water (~30 μl) was added beside the formulation drop. A clean sharp needle was used to bring the water drop to the formulation droplet. Once the two drops met, the phase conversion and the interface between the two drops were monitored under the light microscope. To enable unambiguous observations, the ME formulations and water were dyed with Nile Red (lipid soluble, 50 μg/ml) and methylene blue (water soluble, 0.1 mg/ml) respectively, and polarized microscope was used to confirm the formation of LC.

2.5. Gamma-scintigraphy studies

To monitor the phase transition and release profile of each ME formulation in aqueous medium, the formulations were radiolabeled with ^{99m}Tc. This radioactive material was also used to represent a water soluble drug. The LC-forming ME and CE-forming ME were spiked with a stock solution of ^{99m}Tc (1000 MBq/ml) in water. The final concentration of ^{99m}Tc in both MEs formulations was 20 MBq/ml. Mixing was ensured by gently inverting the glass vial upside down five times.

A set of polystyrene Petri-dishes (60 mm × 15 mm) prefilled with 5 ml of distilled water were placed under the radioactive scanner. Aliquots of 100 μl of each ME formulation were added into the distilled water at the center of the Petri-dish using a pipette. Static images were recorded every 1 min for the first 1 h and then every 5 min for the next two hours.

The Gamma-scintigraphy was performed on an Infinia Hawkeye 4 SPECT/CT scanner equipped with the Dual-head gamma camera (GE Healthcare, UK) connected to a commercial nuclear medicine PC, and controlled using an Infinia Functional Imaging Scanner software (Version 4; GE Healthcare, UK). The release kinetics for two radiolabled MEs was investigated based on the loss from the initial radioactivity over 3 h following the method reported by Bello et al. (1994). The initial radioactivity was defined in the area where radioactivity was concentrated before the MEs spread. The natural decay of ^{99m}Tc was corrected by the software.

2.6. Stability and release kinetics of drug loaded MEs

Progesterone, a poorly water soluble drug with a log P of 3.87 (Zargar-Shoshtari et al., 2008), was used as a model lipophilic drug in this study to assess the physical stability of the selected ME formulations. The final progesterone concentration of 1% (w/w) was selected based on Progesterone Injection (USP) and the literature (Biruss et al., 2007). Progesterone was dissolved into Miglyol 812N under constant stirring. To this sample Milli-Q water and the blend of surfactants/solvent were added. This was followed by stirring and sonication of the samples for a further 15 min.

The physical stability of MEs loaded with progesterone (1%, w/w) was assessed with different samples stored at 4 °C/60% RH, 25 °C/60% RH and 40 °C/75% RH for up to 3 months. The formulations were monitored for transparency, color change, phase separation and drug precipitation. Formulations remaining as single phase systems with no drug precipitation were considered stable.

Drug release was determined using standard Franz diffusion cells (Logan Instrument Corporation, NJ, USA) with a 15-mm-diameter orifice (providing a diffusion area of 1.77 cm^2), thermostated by a water jacket at 37 ± 0.5 °C. The receptor (12 ml) chambers were filled with release media which was stirred using magnetic bars. The donor chamber was charged with 2 ml of formulations which formed a 1.1 cm thick slab. Donor and receptor chambers were separated by a 12,400 molecular weight cut-off dialysis membrane (Sigma, USA), which was presoaked in the

release medium for 24 h. The release media (40% PG in water, v/v) was determined to be able to maintain sink conditions for the drug permeated through the dialysis membrane. Samples (1 ml) were withdrawn at predetermined time points, and replaced with an equal volume of the fresh release media. The samples were analyzed using the HPLC method as described previously (Zargar-Shoshtari, 2011). Progesterone dissolved in Migloyol 812N (1%, w/w) was used as a control solution. The experiments were done in triplicate. The release data was fitted to various kinetic release models including zero- and first-order, Higuchi (Higuchi, 1961) and Korsmeyer–Peppas (Ritger and Peppas, 1987) models using GraphPad Prism for Windows, Version 4.01 (GraphPad, San Diego, CA, USA).

$$\frac{M_t}{M_\infty} = kt^n$$

where M_t is defined as the mass of drug released at time t, M_∞ is the mass of drug released at time infinity and k is a parameter being the fraction released at unit time, and n is used to characterize the release mechanism from a cylindrical shaped matrix.

The release rate parameters of the formulations were compared using a one-way ANOVA followed by Tukey's pairwise comparison at a 95% confidence interval.

3. Results and discussion

3.1. Formulation development

In this study, subcutaneous injection was suggested as a parenteral administration route for future in vivo research of the formulations. Therefore, all materials chosen for the formulation must be biocompatible, injectable, sterilizable, available as non-pyrogenic grade, non-irritating to nerves and non-hemolytic (Date and Nagarsenker, 2008; Strickley, 2004).

The choice of the surfactant is not only critical for the safety of injections but also the formulation of microemulsions. In general, the surfactant concentration in parenteral microemulsions should be minimized as far as possible. Solutol® HS 15 has emerged as a safe surfactant as well as a solubilizer with good tolerance following parenteral administration. In some intravenous formulations, it is used at concentrations as high as 50% (i.e. Cryopharm, Mexico) (Strickley, 2004). Span 80, is a non-ionic ester of sorbitan oleate, and is present in the FDA approved products for intramuscular injection (Wade and Weller, 1994).

On the generated phase diagrams both CE and LC regions were found adjacent to the ME region at 20 °C and 37 °C when the specified ratio of lipophilic Span 80 (low HLB) and hydrophilic Solutol® HS 15 (high HLB) was used (Fig. 1). The use of a single surfactant Solutol® HS 15 did not form significant LC regions. Addition of ethanol and Span 80 was required to have the system arranged into long-range order by interacting with either the head or the tail-group of Solutol® HS 15 allowing for the formation of LC (Alany et al., 2001). In addition, ethanol was found to be useful to further reduce the viscosity of the ME systems.

To address the aims of this project, one ME formulation was selected along the line B with the potential to form a LC upon dilution with water, whereas another formulation with the ability to transform into a CE was selected along the dilution line A. The composition of the two selected ME formulations is listed in Table 1. MEs made of nonionic surfactants are known to be sensitive to temperature changes due to the change of solubility of the surfactant in water/oil hence the interfacial curvature of the surfactant (Wang et al., 2008). However, in this ternary system the temperature only slightly increased the area of ME region mainly in the oil rich region of the phase diagrams (Fig. 1). With increase in temperature the solubility of surfactant molecules may have changed from water soluble to more oil soluble, leading to formation of w/o MEs. To ensure that the effect of temperature does not interfere with the hypothesis of this study, both formulations selected are microemulsions at 20 °C (on shelf) and 37 °C (in vivo), and on addition of water they have the potential to transition into either a CE or a LC.

Table 1
The composition of CE-forming and LC-forming MEs (expressed as %, w/w) as prepared at the fixed ratio of Solutol HS 15:Span 80:ethanol (3:1:0.5). The formulations were selected from sampling path A and B, respectively and used for the characterization tests.

Formulations	Water	Oil	Solutol HS 15	Span 80	Ethanol
CE-forming ME	8	41	34	11.3	5.7
LC-forming ME	20	15	43.3	14.5	7.2

3.2. Formulation characterization

3.2.1. Rheological property

Both selected ME formulations exhibited Newtonian flow behavior and low viscosity whereas the LC and CE derivatives from the dilution lines A and B exhibited Non-Newtonian flow (slightly pseudo-plastic) and larger viscosity values at both 20 °C and 37 °C (Table 2). This may simulate the viscosity changes to the selected MEs caused by diffusion of water from the surrounding tissue fluids into the formulations. For the LC-forming ME without dilution (containing 20% water), the viscosities at both 20 °C and 37 °C increased 7–10 times with the water content increased to 23% and 30% as these samples fell in the LC region. The high viscosity may be explained by its semisolid crystalline structure of the LC formulation (Hans, 1980).

3.2.2. Dynamic light scattering (DLS) measurements and transmission electron microscopy (TEM)

As shown in Table 2 the CE sample had larger droplets (798.4 ± 74.6 nm) compared to the original CE-forming ME formulation (69.8 ± 1.6 nm). No droplets could be analyzed by DLS in the LC samples due to the lamellar crystalline structure as DLS measurement is based on the analysis of scattered light fluctuations caused by the movement of small particles. This information was confirmed by the FF-TEM micrographs shown in Fig. 2, where the crystalline structure of the LC is visible. The droplet size of ME formulations maintained relatively constant at 20 °C or 37 °C, whereas the CE showed an increase in droplet size when the temperature was increased. This could be attributed to the thermodynamic stability of MEs compared to CEs (Azeem et al., 2009).

3.3. ME spreadability and phase transition in an aqueous environment

Fig. 3 shows the different spreadability for both ME formulations in water. For the CE-forming ME formulation, after 1 min, the outer edge had extended and become miscible with water. After 30 min the CE-forming ME had completely mixed with water. In contrast, the LC-forming ME remained in a contracted region of a similar size and at the same position as it was added. The interface between the LC-forming ME and water remained clear after 1 min. The LC-forming ME sunk to the bottom and remained unmixed with water even after 1 h. Without shaking, the formulation took >24 h to fully mix with the water. This is possible due to the formation of a 'LC' at the ME/water interface, as observed under microscopy. This was further evidenced when the ME formulations, dyed with Nile Red, were injected into beakers containing water. The CE-forming ME immediately dispersed in the water phase and a milky emulsion was formed within a minute. In contrast, the LC-forming ME formed a 'depot' in the water phase and retained intact with a

Table 2
The effect of phase transition on viscosity and droplet size of the selected MEs and formulations selected from each dilution trend (Fig. 1) with specified water content ($n = 6$).

Dilution line	Formulation	Water content (%)	Viscosity (mPa s) ± SD		Droplet size (nm) ± SD (PDI)	
			20 °C	37 °C	20 °C	37 °C
A	CE-forming ME	8	57.2 ± 0.7 (Newtonian)	24.5 ± 0.6 (Newtonian)	69.8 ± 1.6 (0.1)	69.3 ± 1.5 (0.1)
	CE	30	421.5 ± 56.3 (Non-Newtonian)	278.2 ± 20.4 (Non-Newtonian)	798.4 ± 74.6 (0.6)	907.8 ± 89.6 (0.9)
B	LC-forming ME	20	92.5 ± 1.4 (Newtonian)	43.2 ± 2.1 (Newtonian)	41.8 ± 1.5 (0.1)	41.5 ± 1.3 (0.2)
	LC	23	698.5 ± 23.0 (Non-Newtonian)	254.0 ± 11.1 (Non-Newtonian)	N/A	N/A
	LC	30	823.5 ± 19.5 (Non-Newtonian)	357.2 ± 10.2 (Non-Newtonian)	N/A	N/A

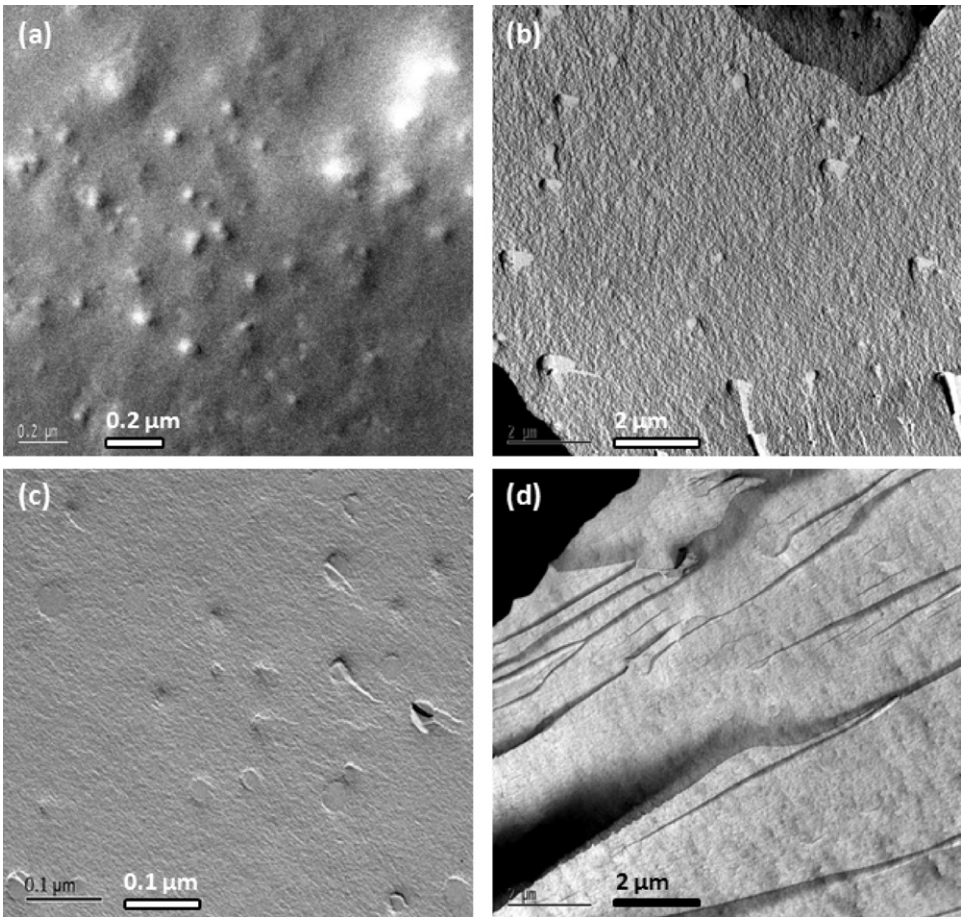

Fig. 2. FF-TEM micrographs for each colloidal sample at different magnifications; (a) CE-forming ME; (b) CE sample from sampling path A; (c) LC-forming ME; and (d) LC sample from sampling path B. Scale bars were redrawn beside the original ones to enable the visual observation.

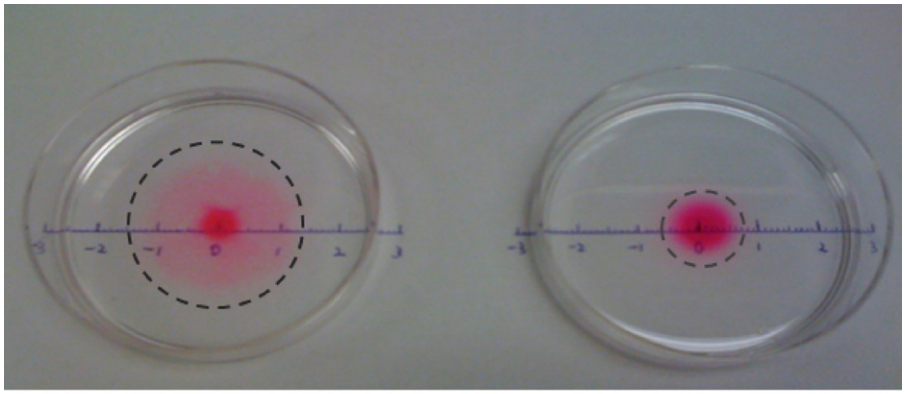

Fig. 3. Spreadability of each ME formulation after 1 min in water. Dashed lines show extent of formulation spread; (a) CE-forming ME; and (b) LC-forming ME.

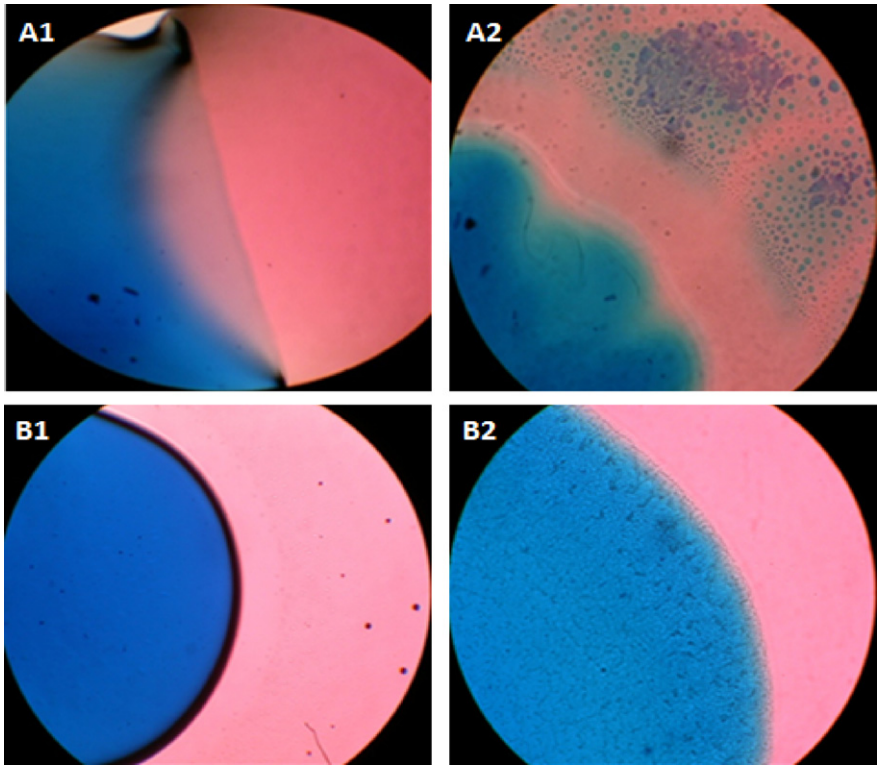

Fig. 4. Microscopic observation of the interface between water (containing methylene blue) and microemulsion (containing oil soluble dye Nile Red) for CE-forming ME at 5 s (A1), and at 30 s (A2); and for LC-forming ME at 5 s (B1), and at 30 s (B2).

clear boundary for at least 10 h. This strongly supported the phase transition of the LC-forming ME into a LC on contact with water.

As displayed under microscopy (Fig. 4), when the CE-forming ME contacted water the interface was disturbed with droplets forming rapidly, most likely O/W droplets, while the formulation spread into the water phase. The LC-forming ME retained a clear boundary and no droplet structures were immediately observed when the ME formulation was placed in contact with water. At the interface birefringence was observed suggesting liquid crystals were formed; this structured 'LC-shell' prevents further spreading of the formulation. In contrast to the CE-forming formulation, the LC-forming ME only formed emulsion droplets at the water/ME interface.

Based on these results it may be hypothesized that the LC-forming ME will have low spreadability upon administration into biological fluids. This 'LC-shell' restrains the formulation in a small area and hence gives rise to the possibility of slow release.

3.4. Gamma-scintigraphy studies

As shown in Fig. 5, the gamma camera images showed variations in the physical appearance on both MEs over time. Both formulations were initially imaged as a localized shiny spot. The CE-forming ME rapidly spread over a larger area matching the area available in the dish, indicating the faster spreading and release kinetics of radioactive material (^{99m}Tc) through the formulation. However, the LC-forming ME remained localized as indicated by a small intense area suggesting a small amount of release of ^{99m}Tc. Fig. 6 shows the decline profiles of ^{99m}Tc at the site of injection. The decline was due to spreading of the ME vehicle and/or the release of ^{99m}Tc across the defined area around the injection site. For the CE-forming ME formulation a faster decline rate of ^{99m}Tc was initially observed for up to 25 min, the rate then slowed with the curve becoming flat, which indicates an equilibrium has been reached between the ME and the medium, possibly as a result of the completion of formation of an O/W coarse emulsion. The overall first-order half lives were <10 min. Conversely, the LC-forming ME shows a slow loss of radioactivity from the injection site over time following a first-order manner with an overall half life >90 min. The static images obtained at 20 min showed a less concentrated 'cloudy' radioactivity layer around the LC-forming ME indicating the decay occurred predominately due to the release of ^{99m}Tc, rather than spreading of the vehicle. Overall, the results show the prolonged release kinetics for the LC-forming ME compared to the CE-forming ME and highlighted the effect of phase conversion from ME to LC at the ME/water interface.

3.5. Stability and release kinetics of drug loaded ME

Both ME formulations remained clear and transparent without phase separation or drug precipitation for up to 3 months in all storage conditions. The percentage of drug incorporated compared to time zero ranged from 99.7% to 100.2%.

Cumulative progesterone release profiles from the various systems are shown in Fig. 7. As expected, the slowest rate of release was observed from the oily solution due to the slow partitioning process of this lipophilic drug from the oil phase to the aqueous phase across the limited interfacial area. The LC-forming ME showed slower release ($P < 0.01$) than the CE-forming ME with only 8% (versus 12%) of the loaded drug released over 10 days. This suggests the effective delivery period may be greater than a month. However, the dialysis membrane used in the in vitro set-up might have prevented the surface erosion release that will be expected in vivo. Although it was not easy to observe in the Franz cells by eye, microscopic observation suggested both MEs will form emulsion droplets on contact with water at different rate, with an LC layer forming at the LC-forming ME/water interface. To be released from the MEs the drug must partition from the oil droplets to the surrounding aqueous phase of the donor compartment and then across the

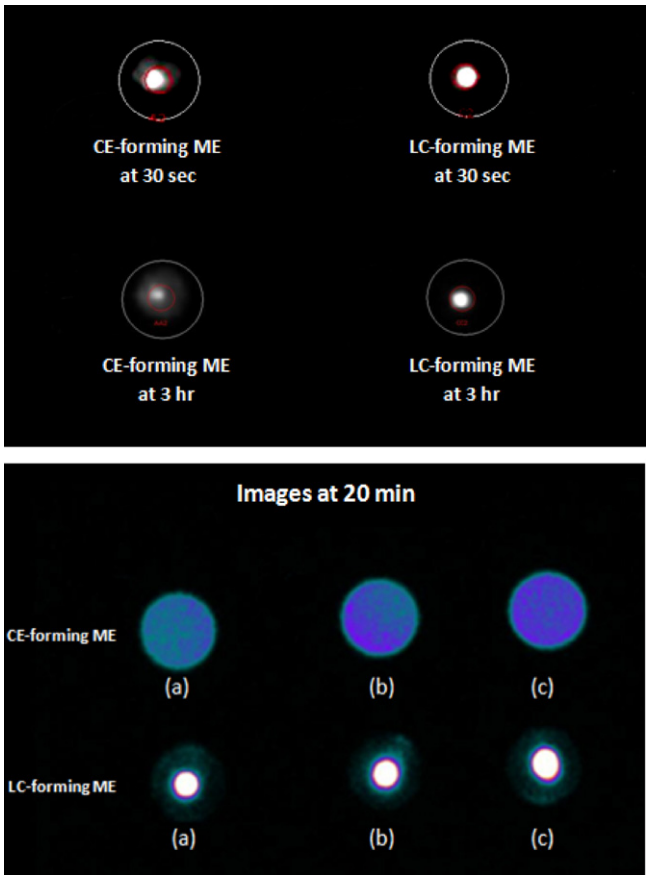

Fig. 5. (Top) Typical gamma-scintigraphic images for both ^{99m}Tc loaded MEs gained at 30 s and 3 h, and (bottom) a representative static image taken at 20 min of triplicate samples represented as (a), (b) and (c) for each of the two MEs showing different spreading in the aqueous medium between the CE-forming ME and LC-forming ME.

interposed dialysis membrane to reach the receptor compartment. The surface area available for drug to partition from the formulation is a vital parameter and is determined by the droplet number and size. While the CE-forming ME rapidly formed CE droplets, the LC-forming ME formed a 'LC shell' which slowed down further water absorption and only formed droplets gradually, therefore providing slower release. In addition, the high viscosity of the 'LC-shell' reduces the drug diffusion rate.

Curve fitting of the release profiles to various kinetic models showed that the best fit model was first-order ($R^2 \geq 0.999$,

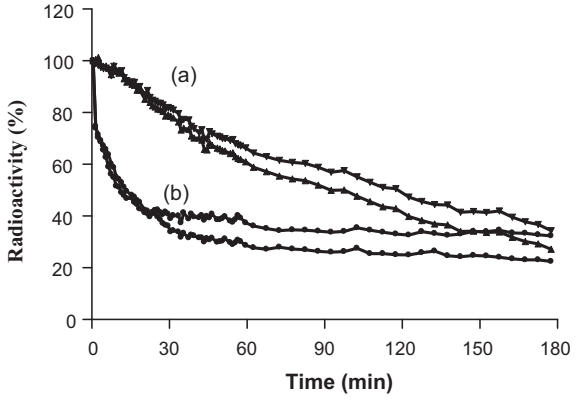

Fig. 6. First-order plot showing the percentage of radioactive isotope remaining at the site of injection for each ME formulation over 3 h ($n = 2$). Data series (a) represents the LC-forming formulation and (b) the CE-forming formulation.

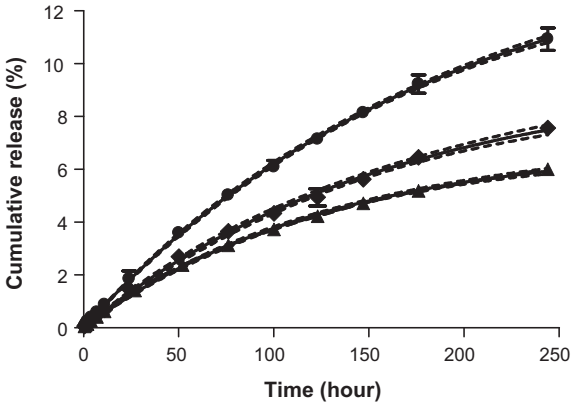

Fig. 7. Release profiles from various formulations. The solid lines are generated from nonlinear regression using a first-order model based on the observed values for CE-forming ME (●); LC-forming ME (♦) and oil solution (▲). (Data are means ± SD, $n = 3$). Dash lines are 95% confidence bands of the best fit curves.

Fig. 7) which describes a concentration dependent process. The R^2 values obtained from the Higuchi model which describes Fickian diffusional release are <0.96 in all cases. Improved fitting ($R^2 = 0.993–0.996$) was observed with Korsmeyer–Peppas model. The n values (0.647–0.727) indicate anomalous (non-Fickian) diffusion. The results may collectively demonstrate that multiple processes involved in the drug release while the mass transferring across the membrane was predominately concentration-dependent.

It is worthwhile to mention the fact that in this Franz cell in vitro release study, the effective surface area for drug release from the formulations was fixed and identical. It did not reflect the different spreading behavior the formulations would be expected to show in an in vivo environment. In an in vivo aqueous environment the LC-forming ME would be expected to show less spreading and therefore even slower release. Therefore, the in vivo performance of these MEs as 'depots' formed by phase transition would be better predicted by the gamma-scintigraphy study when the formulations were injected into the aqueous medium. However, in both release studies the LC-forming ME showed a slower first-order release rate than the CE-forming ME ($P < 0.01$).

4. Conclusions

LC-forming MEs and CE-forming MEs have been successfully formulated using Miglyol 812N oil, Milli-Q water, Solutol HS 15, Span 80 and ethanol. The LC and CE regions were found adjacent to the ME region in the water-rich areas of the pseudo-ternary phase diagram.

Both ME formulations loaded with the poorly water soluble (lipophilic) drug progesterone showed low viscosity and were stable for at least 3 months. The LC-forming ME showed a slower drug release profile from the Franz cells and was less spreadable than the CE-forming formulation upon contact with water. Gamma-scintigraphy studies, in which formulation spreadability was taken into consideration to predict the in vivo situation, also demonstrated the slower release profile of ^{99m}Tc from the LC-forming ME compared to the CE-forming ME. Therefore, it is hypothesized that the LC-forming ME has the potential to be used as a delivery vehicle for both water- and lipid-soluble drugs for extravascular injections. At the injection site the diffusion of water from the surrounding tissue into the ME may result in phase transition of ME and provide a sustained release profile. Future animal studies have been planned to investigate the in vivo relationship between spreadability and release profile at the subcutaneous injection site. In addition, it is our intention to investigate the hemolytic effect and the

pharmacokinetic impact of the two different types of formulations after intravenous administration.

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