Formulation and Physicochemical Characterization of Imwitor 308 Based Self Microemulsifying Drug Delivery Systems

Sara ZARGAR-SHOSHTARI,* Jingyuan WEN,[a] and Raid Ghassan ALANY[a]

Drug Delivery Research Unit (2DRU), School of Pharmacy, University of Auckland; Private Bag 92019, Auckland, New Zealand.

Received April 7, 2010; accepted July 6, 2010; published online July 7, 2010

Self Microemulsifying Drug Delivery Systems (SMEDDS) are a novel alternative to the conventional transdermal delivery systems. SMEDDS are water-free systems, made up of oils and surfactants that can readily form a microemulsion upon dilution within an aqueous medium. Before SMEDDS can be used as a drug delivery system it is necessary to investigate the internal microstructure of the resulting microemulsion. Novel Imwitor 308 based SMEDDS were prepared and investigated. Phase behaviour of the comprising components was investigated through the construction of pseudoternary phase diagrams. The formed systems were characterized using visual inspection, measurement of electrical conductivity, viscosity and droplet size. Amongst the pseudoternary systems investigated, IPM/Cremophor EL (50% w/w)/Imwitor (50% w/w) and Myritol 318/Tween 85 (64% w/w)/Transcutol P (20% w/w)/Imwitor (16% w/w) possessed the largest microemulsion area. Electrical conductivity and viscosity studies depict structural transitions from w/o microemulsion to bicontinuous or o/w microemulsion around 20—35% water. This was further supported by the droplet size and Fourier transform (FT)-IR measurements. The FT-IR data suggests that below the percolation threshold (φ_C) the water molecules are mainly bounded to the surfactant head group (bound water). Above this value, water molecule move to the outer phase of the microemulsion mainly interacting with each other though hydrogen bounding (free water). It was also found that pseudoternary systems with water content of less than 30% were stable at 32 °C. Such systems may form stable microemulsion upon contact with the skin. Absorption of water may also result in a supersaturated solution with enhanced transdermal flux.

Key words self microemulsifying drug delivery systems; structural characterization; phase behaviour

Microemulsions are thermodynamically stable, transparent, isotropic systems, consisting of oil, water and surfactant with or without a cosurfactant. They are considered ideal for drug delivery due to their low viscosity, ease of preparation, improved drug stability and solubility, small droplet size, surfactant provoked permeability and protection against enzymatic degradation.[1] The later characteristics are responsible for improved permeability of the drug across biological membranes including the skin. Although the oil and surfactant constituents are known to enhance the solubility of lipophilic drugs, at higher weight fraction of water (φ), their solubility may be greatly compromised. To overcome this effect microemulsions containing cyclodexterine have been investigated. Incorporation of cyclodexterines has been shown to improve the solubility of the model steroid drug progesterone in selected microemulsions by 3300 folds.[2] Self Microemulsifying Drug Delivery Systems (SMEDDS) can be considered a novel alternative to conventional transdermal delivery systems.[3] SMEDDS are preconcentrated (water-free) microemulsions, made up of oils and surfactants. Upon dilution with an aqueous medium and gentle agitation, these systems can form microemulsions.[4] Enhanced solubility and improved bioavailability are amongst the main advantages of SMEDDS.

Before SMEDDS can be used as drug delivery systems it is necessary to characterize the internal structure of the resulting microemulsion. This is highly critical as multiple colloidal and coarse dispersions may coexist. Amongst these are microemulsions, coarse emulsions, various liquid crystalline systems (hexagonal, reverse hexagonal, lamellar, cubic) and gels. These can form upon mixing oils, surfactants and water. Moreover microemulsions are known to have different microstructures namely oil in water (o/w), water in oil (w/o) and bicontinuous.[5] Elucidating the internal structure of microemulsions is of a great interest as the phase behaviour of these systems can influence drug solubility, stability and *in vitro* release. Phase behaviour of microemulsions can be monitored through visual inspection, measuring electrical conductivity, viscosity, droplets size, density determination, surface tension measurements, and diffusion coefficient measurements. Visual observation is one of the first and foremost techniques used to establish the phase boundaries and distinguish microemulsions from coarse emulsions, liquid crystals and gels. To further differentiate microemulsions form lamellar liquid crystals one can take advantage of other experimental techniques such as polarized light microscopy and viscosity measurements. In general microemulsions display Newtonian flow with low viscosity whilst liquid crystals exhibit non-Newtonian flow and are of relatively high viscosity.[5] Furthermore viscosity can also be used to study the microstructure of microemulsions.[6—8] Normally o/w microemulsions have a lower viscosity compared to w/o microemulsions in which the more viscous oil forms the external phase. Measurement of electrical conductivity is another invaluable technique for understanding the structural changes taking place within a microemulsion system with the water continues phase showing high electrical conductivity as compared to the insulating oil continuous system.[1,9,10]

Thermal stability or temperature sensitivity of microemulsions can be monitored through measurements of cloud point (T_{Cloud}), also known as Phase Inversion Temperature (PIT) or hydrophilic lipophilic balance (HLB) temperature. For o/w and w/o microemulsions, T_{Cloud} has been defined as the temperature at which the solubility of the surfactant molecule changes from water soluble to oil soluble or *vice versa*.[11] At this temperature the net interfacial curvature of the surfactant

---

* To whom correspondence should be addressed. e-mail: s.zargarshoshtari@auckland.ac.nz

molecule is changed from positive (o/w microemulsions) to negative (w/o microemulsions) by passing through a zero curvature (bicontinuous microemulsions). The T_{Cloud} has also been described as the lowest temperature at which the sample turns cloudy or separates out into two phases.[12,13] Dynamic light scattering (DLS) or photon correlation spectroscopy (PCS) is another invaluable technique which is used for measurement of particle size in the submicron range.

In the present investigation Imwitor 308 was used as the cosurfactant. The active constituent of Imwitor 308 is glycerylmonocaprylate, monoester (>80%), which is manufactured through esterification and molecular distillation of caprylic acid and glycerol. In pharmaceutical formulations, Imwitor 308 is frequently used as a solubilising agent, penetration enhancer, plasticizer and as a co-emulsifier.[14] In particular Imwitor 308 has been used successfully as a cosurfactant to construct pseudoternary phase diagrams with large microemulsion area.[15—17] Podgolar *et al.* has characterised the microstructure of Imwitor 308/Tween 40/Isopropyl Myristate (IPM) pseudoternary system.[15,18] However the selected microemulsions were only evaluated at a constant surfactant concentration. As such the effect of dilution of SMEDDS with an aqueous medium cannot be assessed. Moreover no attempts were made to identify other colloidal systems which may coexist within the phase diagram. Monitoring the of stability microemulsions with changes in temperature is also a critical step in evaluating colloidal systems, that has not been reported in the aforementioned studies.[15,18]

The aim of this study was to identify novel oil, surfactant/cosurfactant combinations, which can form a potential SMEDDS for transdermal delivery of the model steroid progesterone. The oils selected were IPM and Myritol 318. The choice of these oils was based on the saturated solubility of progesterone which was previously reported as 36 mg/ml (27 °C)[19] and 17 mg/ml (25 °C)[2] in Myritol 318 and IPM respectively. Various oil/surfactant/cosurfactant combinations were screened, in order to find a system capable of solubilising high percentage of water. This selection is based on the amount of water present in the stratum corneum which is about 15 to 20% of its dry weight.[20] The selected combinations were then used to construct pseudoternary phase diagrams. The gradual changes in the microemulsion microstructure were investigated by electrical conductivity, viscosity and droplet size measurement. Temperature stability of selected systems was analyzed through measurement of cloud point. FT-IR was used to study the state of the water within the microemulsion (bound or free water).

Experimental

Materials Isopropyl Myristat, Tween 85, Tween 80, Tween 40, Brij 30 and Span 80 were purchased from Sigma-Aldrich (U.S.A.). Myrtiol 318 was obtained from Cognis Care Chemicals (Germany), Labrasol and Transcutol P were kindly donated by Gattefossé (Saint Priest Cedex, France). Inwitor 308 was obtained from Sasol (Germany). Cremophor EL was supplied by BAFS (Germany). All samples were used without any further purification. Triple-distilled water was obtained in-house by reverse osmosis (MilliQ, Millipore, U.S.A.).

Method. Screening of Surfactants and Cosurfactants The aim of this study was to identify potential pseudoternary systems capable of forming a large microemulsion area. $W_{\max}$ represents the maximum amount of water that can be solubilised in the oil surfactant/cosurfactant mixture. This parameter was used to screen oil and surfactant combinations which can form the largest microemulsion area. The effect of different surfactants/cosurfactants and K_{m} (mass ratio of surfactant to cosurfactants) on $W_{\max}$ was investigated. $W_{\max}$ was measured using the titration method. Mixtures of surfactants and cosurfactants were formulated at different K_{m} ratios (4 : 1, 2 : 1, 1 : 1). The obtained mixtures were then mixed with the oil phase at a weight ratio of 1 : 1. To this mixture water was added drop-wise under constant magnetic stirring. $W_{\max}$ was determined as the point of transition from a clear, one-phase system to a two phase system. The surfactants used were Tween 40, Tween 80, Tween 85, Labrasol, Cremophor EL, Brij 30, and Span 80. Imwitor 308 was used as a cosurfactant.

Construction of Pseudoternary Phase Diagrams Pseudoternary phase diagrams were constructed using the equilibration method. Briefly, predetermined amounts of oil, surfactant/cosurfactant were mixed with water, at ambient temperature. Mixtures of surfactant/cosurfactant were formulated at different K_{m} ratios (4 : 1, 2 : 1, 1 : 1). The ratio of oil to the surfactant mixture was varied from 9 : 1 to 1 : 9 at 10% increments. Predetermined amount of this oily mixture was prepared (5—95% w/w), and to this water (95 to 5% w/w) was added drop-wise under constant magnetic stirring. After being equilibrated overnight, visual observation and polarized light microscopy (Leica DMR, GmbH, Germany) were used to identify microemulsions and differentiate them from various liquid crystalline systems and coarse emulsions. The contribution of each system to the total area of the phase diagrams was measured by the cut and weight method.[21] The phase diagrams were printed on an A4 paper sheet. The weight of each system and the total weight of the phase diagram were determined and were then used to calculate the percentage occupied by the different regions.

Electrical Conductivity Measurements The conductivity was measured at room temperature (22—23 °C) using a Seven Easy Mettler Toledo (Mettler, Switzerland) conductivity meter with a Mettler Toledo Incap® 730 electrode. All Measurements were autocorrected for the variation in the temperature. The range of application was between 0.01 to 1000 mS/cm with accuracy of ±0.5%. The preconcentrated formulations (water-free) were titrated with water. Initially 200 μl of water was added to the preconcentrated SMEDDS. The sample was then equilibrated for at least 1 min before a reading was taken. This was continued until a two phase system formed. All measurements were carried out at least in duplicate to ensure reproducibility of the results.

Viscosity Measurements The dynamic viscosities (η_{r}) of microemulsions were measured with Brookfield DV-III cone and plate rheometer (Brookfield Engineering Laboratories Inc., U.S.A.) fitted with a CP-40 spindle. The sample cup was connected to the circulating water bath maintained at 25±0.5 °C. A sample volume of 600 μl was used. The measurements were made from 75—500 rpm and in triplicate. Data analysis was performed by Rheocalc V3.1 operating software (Brookfield Engineering Laboratories Inc., U.S.A.). Viscosity values were recorded at 150 rpm.

Dynamic Light Scattering Measurements Dynamic Light Scattering (DLS) was used to determine the droplet size of selected microemulsions. 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 SMEDDS investigated was used as the viscosity of the dispersant. Each measurement was made in triplicate at 25 °C and subsequently the average droplet size (Z-Average) and Polydispersity Index (PDI) were calculated. The PDI measures homogeneity of the sample. A PDI value of 0.5 or higher indicates a sample of high heterogeneity/polydispersity.[22]

Measurement of Cloud Point (T_{Cloud}) The effect of temperature on the phase behaviour of microemulsions was evaluated through measurement of the T_{Cloud}. T_{Cloud} was measured using a Malvern Zetasizer Nano ZS instrument (Malvern Instruments, U.K.) temperature controlled chamber according to the conditions set above. The size-temperature trend analysis was made at a starting temperature of 18 to 20 °C and was increased by 1 °C increment. Each sample was equilibrated at the specified temperature for at least 2 min. At the end of each measurement the samples were visually assessed for optical transparency or sings of phase separation.

Fourier Transform Infrared Spectroscopy (FT-IR) The FT-IR spectrum of selected microemulsions were recorded on a Bruker Tensor 37 FT-IR spectrometer (Bruker Optik GmbH, Germany) using the OPUS software (OPUS 6.5, Germany). The Absorbance mode was used for all measurements. A clean diamond window was used to measure the background spectrum. The FT-IR spectrum of each sample was then recorded at 2 cm^{-1} spectral resolution with an average of 120 scans. The spectrum of water was resolved into bound water, trapped water and free water using the least squares curve fitting (OMNIC 7.3, Thermo Electron Corporation, U.S.A.). The area under the curve for each peak was then calculated using OMNIC software.

Results and Discussion

Screening of Surfactants and Cosurfactants Figure 1 shows the effect of K_m (expressed as the weight fraction of surfactant (% w/w) in the surfactant/Imwitor mixture) on W_{max} determined for systems comprising of IPM and Myritol 318. When IPM was used as the oil phase (Fig. 1A), Tween 40, and Tween 80 showed an increase in W_{max} of up to 95% (w/w) as the K_m and HLB reduced. W_{max} was similar for both surfactants at all K_m values. Tween 40 (HLB=15.5) and Tween 80 (HLB=15) are polysorbates, which are derived form pegylated sorbitin esterified with fatty acids. Tween 85 is a triolate derivative with three saturated hydrocarbon chains in its lipophilic tail and has a lower HLB value (HLB=11). Addition of Tween 85 at all investigated K_m values resulted in a lower W_{max} which was found to decrease from 40% (w/w) to 16.7% (w/w) as the K_m was reduced to a weight ratio of 1 : 1. At a K_m of 4 : 1 the Tween 85 system had an HLB value of 10 that reduced to 8.5 with increase in weight fraction of Imwitor 308. This corresponded to a decrease in W_{max}. The more hydrophilic surfactants, Tween 40 and 80, showed higher W_{max} as the HLB of the surfactant mixture approached 10.5. Similar results were observed with the Cremophor EL system which showed a W_{max} of 95% (w/w) when the HLB of its surfactant mixture reduced from 11.6 at a K_m of 4 : 1 to 10.5 at a K_m of 2 : 1. This trend was observed for all the surfactants investigated. The optimum HLB value at which up to 95% (w/w) water could be solubilised was about 10.5—10.8. When the HLB deviates out of this range a significant decrease in W_{max} was observed. The amount of water solubilised was also dependent on the HLB value of the individual surfactants. For example W_{max} was less than 5% for Span 80 which is highly lipophilic with an HLB of 4.3. The most effective systems were Tween 40 (K_m 1 : 1), Tween 80 (K_m 1 : 1) and Cremophor EL (K_m 2 : 1, 1 : 1).

Similar trend was observed when Myritol 318 was used as the oil phase (Fig. 1B). Maximum amount of water (70% w/w) was solubilised at an HLB of 10. For systems with HLB values ranging from 7.85 to 9.5, between 15 to 30% (w/w) water could be solubilised. The most effective surfactant was Tween 85 (K_m 4 : 1), which could solubilise 70% (w/w) water. The aforementioned trend was observed for all oils and surfactants investigated, with the exception of Labrasol, which could only solubilise less than 10% (w/w) water.

When IPM was used as the oil phase, Tween 40, Tween 80, Brij 30 and Cremophor EL formed liquid crystals. Furthermore, selected Myritol 318 systems were also capable of forming liquid crystals. The microscopic appearance of these liquid crystals was typical of lamellar liquid crystals with the characteristic oily streaks, maltese crosses and fan-shape textures (Fig. 2). Liquid crystals were formed at higher water and surfactant concentration and contributed to 10—30% of the total area of the phase triangle.

Phase Behaviour Studies In order to investigate the phase behaviour of the identified surfactant(s)/cosurfactant, oil(s) and water blends and identify the optimum microemulsion area, pseudoternary phase diagrams were constructed. Systems investigated comprised Myritol 318/Tween 85/Imwitor/water and IPM/Cremophor EL/Imwitor/water at different K_m ratios, as these combinations could solubilise up to 70—95% (w/w) water. Specifically Cremophor EL was preferred over the Tween 40 and Tween 80 surfactants due to the higher solubility of the model storied progesterone. The saturated solubility of progesterone in Cremophor EL is about 45±1.94 mg/ml (unpublished data) as compared to 11.9±2.3 mg/ml and 20.7±1.8 mg/ml for Tween 80 and Tween 40 respectively.[23] Figure 3 shows the differences observed in the

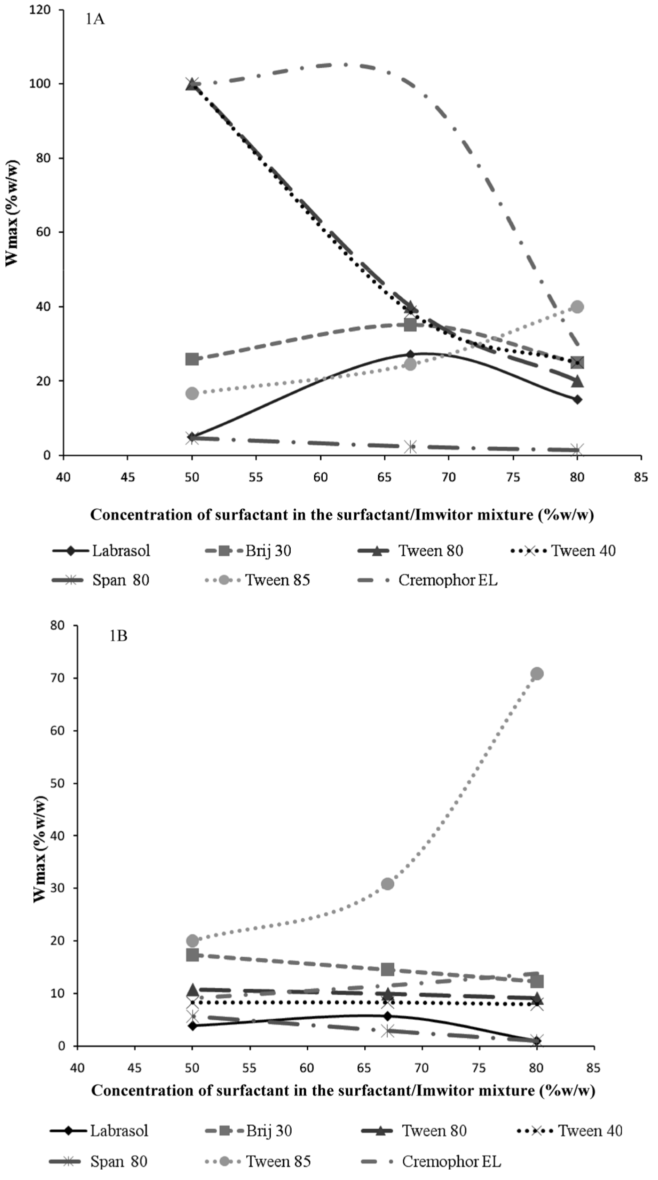

Fig. 1. The Effect of K_m (Expressed as Concentration of Surfactant in the Surfactant/Imwitor Mixture) on Water Solubilisation Capacity Measured for Selected Surfactants

(A) IPM is used as the oil phase, (B) Myritol 318 is used as the oil phase.

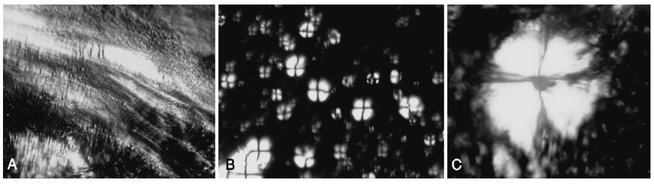

Fig. 2. Polarized Light Micrographs Showing (A) Lamellar Liquid Crystals with Characteristic Oily Streaks (IPM/Tween 40/Imwitor 308/Water) at 10× Magnification, (B) Maltese Crosses (IPM/Brij 30/Imwitor 308/Water) at 10× Magnification, (C) Maltese Crosses Outlying the Characteristic Fan-Shaped Texture (IPM/Brij 30/Imwitor 308/Water) at 40× Magnification

microemulsion area at different K_m ratios. For the Cremophor EL and Tween 85 systems, the optimum surfactant/cosurfactant ratio was at a K_m of 1 : 1 and 4 : 1 respectively. In both systems a large liquid crystalline area could also be observed with increase weight fraction of the cosurfactant. Imwitor 308 is a lipophilic cosurfactant with lesser tendency to dissolve into water. At lower concentrations (K_m 4 : 1) less Imwitor 308 is incorporated into the lipophilic tail of the surfactant and at the interface leading to the long range order and packing of the surfactant molecule and hence the formation of liquid crystals (Table 1). The high concentration of Imwitor (K_m 2 : 1) destroyed this surfactant arrangement, which consequently reduced the size of the liquid crystalline region. No liquid crystalline region was observed at a ratio of 1 : 1.

Despite the large W_{max} of the Tween 85 system (K_m 4 : 1), the microemulsion area was small. In an attempt to further optimize the phase boundary, the effect of a secondary cosurfactant, Transcutol P, was investigated. At a K_m of 4 : 1, Transcutol P significantly enlarged the microemulsion area without further affecting W_{max}. At higher concentrations however, W_{max} was reduced, consequently destroying the microemulsion and the liquid crystalline phases. Transcutol P (diethylene glycol monoethyl ether) has a smaller molecular volume (224.48 cm^3/mol)[24] compared to Imwitor 308 (613.73 cm^3/mol),[25] and thus can be easily incorporated into the interface, with its lipophilic tail perturbing the long range packing of the surfactant. In other words it can act as a typical cosurfactant. Although Transcutol P is highly lipophilic (HLB value of 4.2), some of it can escape from the interface into the aqueous phase due to its amphiphilic nature. Thus at a K_m of 1 : 1, high concentration of Transcutol P in the aqueous phase may minimize W_{max} and destroy the microemulsion area. This phenomenon might be highly important in the view point of SMEDDS and supersaturated systems. Within the aqueous phase Transcutol P may act as a cosolvent. When water is absorbed into the saturated SMEDDS, the extent of supersaturation can be minimized leading to a lower penetration rate across the skin. Systems comprising of IPM/Cremophor EL (50% w/w)/Imwitor (50% w/w) and Myritol 318/Tween 85 (64% w/w)/Transcutol P (20% w/w)/Imwitor (16% w/w) were selected for further characterization. Cremophor EL pseudoternary systems were characterized across the 7 : 3 and 5 : 5 dilution lines, whilst Tween 85 pseudoternary systems were characterized across the 4 : 6 and 2 : 8 dilution lines.

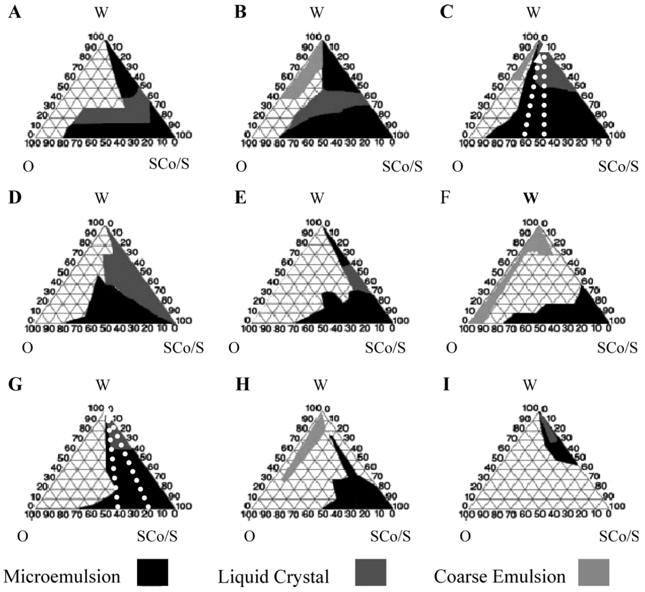

Fig. 3. Pseudo-Ternary Phase Diagram of Microemulsion Systems Comprising

IPM/water/Cremophor EL (0.80% w/w)/Imwitor 308 (0.20% w/w), (B) IPM/water/Cremophor EL (0.67% w/w)/Imwitor 308 (0.33% w/w), (C) IPM/water/Cremophor EL (0.50% w/w)/Imwitor 308 (0.50% w/w), (D) Myritol 318/water/Tween 85 (0.80% w/w)/Imwitor 308 (0.2% w/w), (E) Myritol 318/water/Tween 85 (0.67% w/w)/Imwitor 308 (0.33% w/w), (F) Myritol 318/water/Tween 85 (0.50% w/w)/Imwitor 308 (0.50% w/w), (G) Myritol 318/water/Tween 85 (0.64% w/w)/Imwitor 308 (0.16% w/w), Transcutol P (0.20% w/w), (H) Myritol 318/water/Tween 85 (0.54% w/w)/Imwitor 308 (0.13% w/w), Transcutol P (0.33% w/w), (I) Myritol 318/water/Tween 85 (0.40% w/w)/Imwitor 308 (0.10% w/w), Transcutol P (0.50% w/w), W: water, O: oil, SCo/S: surfactant/cosurfactant mixture. The arrows represent dilution lines investigated.

Table 1. Percentage of Different Phases as a Function of the Total Area of the Phase Diagram Presented in Fig. 3

System	Microemulsion area (% w/w)	Liquid crystal area (% w/w)	Coarse emulsion area (% w/w)
A	36.11	19.86	0
B	49.17	17.22	6.90
C	64.15	12.60	4.55
D	20.78	28.65	0
E	30.56	4.65	0
F	27.87	0	15.59
G	40.38	5.7	0
H	24.55	0	9.11
I	6.82	0	2.50

Conductivity Measurements Figure 4A is a representative electrical conductivity (σ) *versus* water weight fraction (φ) plot for the Cremophor EL system (7 : 3 dilution line). The curve can be divided into three distinct parts. Initially the electrical conductivity is very low (less than $5\,\mu$s/cm^{-1}) and increases slowly as the φ increases up to about 10% (w/w). This is followed by a steep increase in the conductivity up to 40% (w/w) water. Above this the conductivity of the system increases slightly and reaches a plateau. Figure 4B demonstrates that the maximum change in the conductivity ($d\sigma/d\varphi$) is observed around 23% (w/w) water. Such behaviour can be explained though the concept of percolation.[26,27] At a critical point in the system, known as the percolation threshold (φ_C) bicontinuous or o/w microemulsions are formed.[28]

In the present investigation φ_C was estimated as the maximum change ($d\sigma/d\varphi$) in the electrical conductivity ($d\sigma$) relative to changes in water weight fraction ($d\varphi$) (Fig. 4B). For the 7 : 3 dilution line of the Cremophor EL pseudoternary system φ_C was approximately 23% (w/w). This value increased to 31% (w/w) when the surfactant concentration was increased to 50% (5 : 5 dilution line). The Tween 85 microemulsions behaved similar to the Cremophor EL, with the system undergoing percolation transition around 21% (w/w) and 25% (w/w) water at 6 : 4 and 8 : 2 dilution lines respectively. Such increase in the φ_C may be attributed to greater interaction of water molecules with the ethylene oxide head groups (increase hydration of ethylene oxide groups) as the surfactant concentration is increased. In effect it has been found that φ_C is dependent on the interaction of water molecules with each other and the surfactant head group, which in turn will effect the probability of forming water clusters or

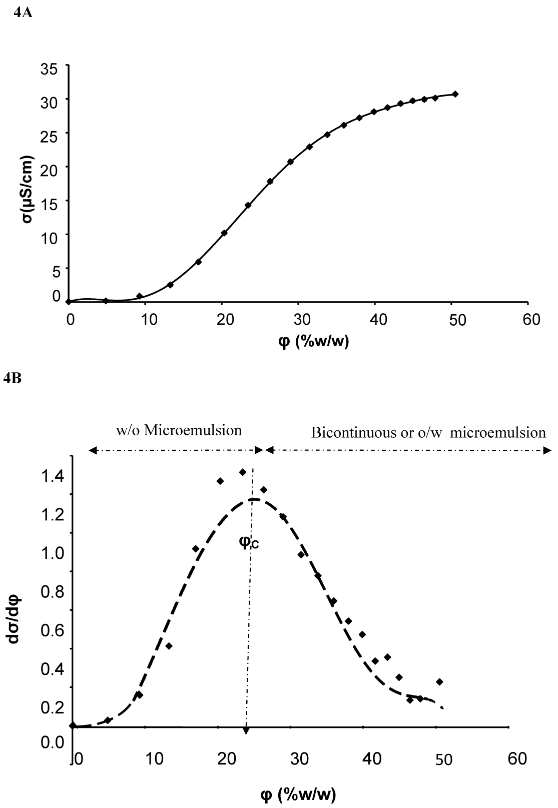

Fig. 4. Variation in, (A) Electrical Conductivity (σ), (B) $d\sigma/d\varphi$, with φ (Expressed as % w/w) along the 7 : 3 Dilution Line of Cremophor EL Pseudoternary System

Table 2. Viscosity Values of Pseudoternary Systems at 0% Water (SMEDD) and at φ_C (Microemulsion) for Selected Dilutions Lines

Dilution line	Viscosity (mPa · s)	
	0% water (SMEDDS)	φ_C (microemulsion)
Cremophor EL (7 : 3)	18.09±0.73	34.87±1.76
Cremophor EL (5 : 5)	36.93±0.03	61.48±0.01
Tween 85 (4 : 6)	68.01±0.01	104.72±3.60
Tween 85 (2 : 8)	80±0.01	104.20±1.57

continuous channels.[6)] Low values of φ_C can be the direct result of strong attractive interaction of water molecules in the system.[29)] Microemulsion systems based on ionic surfactant AOT and Tween 85 have been shown to undergo percolation transition around 4% water.[7)]

Viscosity Measurements The dynamic viscosity was measured for all systems as a function of φ. All microemulsion samples showed Newtonian flow behaviour. For the Cremophor EL pseudoternary system the viscosity ranged from 18—34 mPa · s across the 7 : 3 dilution line, and increased by a factor of 2 as the ratio of surfactant to oil increased (Table 2). Similar trends were observed for Tween 85 pseudoternary system, although the viscosities were much higher as compared to the Cremophor EL system.

A representative dynamic viscosity versus φ plot (Cremophor EL pseudoternary system) at the 7 : 3 dilution line is shown in Fig. 5. The plot can be divided into two parts. Initially the viscosity increases from 18 mPa · s to a maximum of 34 mPa · s as the weight of water rises to 20% (w/w). This is followed by a gradual decrease in the dynamic viscosity as the φ further increases. The existence of a maximum at 20% (w/w) water is indicative of the presence of two possible colloidal structures. Namely, the transition of oil continuous microemulsion to bicontinuous or water continuous form. The initial increase of viscosity indicates the presence of droplets within the core of the system. True colloids obey Einstein equation ($\eta = \eta \cdot (1 + 2.5\varphi)$).[5)] Below 20% (w/w) the attractive interaction and molecular reorganization of droplets at the interface will dominate. Above 20% (w/w) the viscosity changes are gradual, which is characteristic for bicontinuous systems.[6,10,30)]

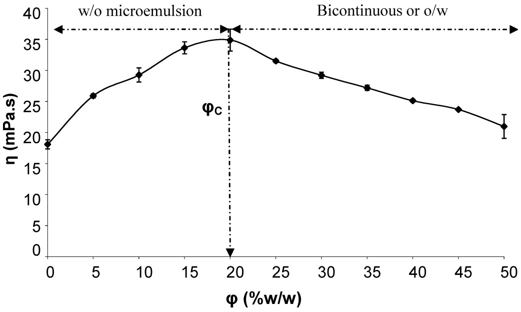

Fig. 5. Variation in Dynamic Viscosity (η) as Function of φ (Expressed as % w/w) across the 7 : 3 Dilution Line of Cremophor EL Pseudoternary System

Results are presented as mean values±S.D., $n=3$.

Table 3. Summary of the φ (Expressed as % w/w) at Which the Transition from w/o Microemulsion to Bicontinuous or o/w Microemulsion Was Observed

Surfactant	Dilution line	Electrical conductivity	Viscosity	DLS
Cremophor EL	7 : 3	23% w/w	20% w/w	15% w/w
Cremophor EL	5 : 5	31% w/w	30% w/w	30% w/w
Tween 85	4 : 6	21% w/w	15% w/w	15% w/w
Tween 85	2 : 8	24% w/w	25% w/w	35% w/w

The values were obtained by the following techniques; electrical conductivity, viscosity and DLS measurements.

The above changes in the viscosity were observed for 3 : 7, 5 : 5 dilution lines of the Cremophor EL pseudoternary system as well as 4 : 6 and 2 : 8 dilution lines of the Tween 85 pseudoternary system, and were in agreement with the conductivity data. The data are summarized in Table 2.

Dynamic Light Scattering (DLS) DLS was only effective for 7 : 3 dilution line of Cremophor EL and 4 : 6 dilution line of Tween 85 system, for which a single polydispersed peak was obtained. At higher surfactant concentrations, DLS showed polymodal distribution with polydispersity of >0.50. As these concentrated microemulsions were diluted, a single monodispersed peak started to emerge again. The observation of a monomodal distribution corresponded well with the onset of percolation observed in viscosity and conductivity measurements (Table 3). Overall clear changes in the average droplet size were observed for 7 : 3 dilution line of Cremophor EL system and 4 : 6 dilution line of Tween 85.

In general the droplet size was small, ranging from 1.5 to 12 nm. This is in line with previously published data.[31—33)] The Tween 85 system displayed the characteristic swelling profile observed when the φ of dispersed phase (water) is increased. The droplet size increased by 47% (w/w) to 3.5 nm as the φ increased to 15% (w/w). This was followed by a gradual decreased in size. Conversely for the Cremophor EL system a decrease in the droplet size was observed for up to 15% water after which the size remained fairly constant. With increase in water content larger globules or assembly of molecules may form that are very close to each other. This was the case for the Tween 85 system. For the Cremophor EL system, it is possible that some of the added water dissolved in the surfactant head group. A decrease in the particle size may also suggest an increased number of the droplet rather than their size with increased φ.[34]

The viscosity, conductivity, and DLS studies all suggest that the Cremophor EL (7 : 3 dilution line) and Tween 85 (4 : 6 dilution line) pseudoternary systems percolate around 15 to 25% water. Above these values bicontinuous or o/w microemulsions are expected to form. As the preconcentrated microemulsion is diluted, the droplet structure may transform from spherical to rod shape reverse micelles, lamellar liquid crystals and bicontinuous or o/w microemulsion.[35] Further dilution will eventually give rise to a coarse o/w emulsion.

Cloud Point (T_{Cloud}) Determination Temperature dependent phase behaviour is one of the major problems associated with microemulsions, especially when dealing with non ionic surfactants. An ideal formulation should remain as a one-phase transparent system at its storage temperature and at the temperature of its intended use.

T_{Cloud} was measured at a constant φ (30% (w/w) water) and at a constant weight ratio of oil to surfactant across the dilution lines listed in Table 3. In general T_{Cloud} ranged from 20 °C for pseudoternary systems with high percentage of water to >100 °C for pseudoternary systems with lower water content. T_{Cloud} was inversely related to water content of the sample, increasing exponentially as the φ decreased. Conversely T_{Cloud} did not change significantly at constant φ with the exception of pseudoternary systems containing high percentage of surfactant (>90%), where the T_{Cloud} increased by about 5—10 °C. Instability or phase separation of samples observed at T_{Cloud} can be related to the temperature dependent solubility of the surfactant molecules.[36,37] Increase in the temperature may result in a decrease in the solubility of the surfactant in water, the dehydration of the head group and phase separation.

The Tween 85 pseudoternary system (at the 4 : 6 dilution line) had a slightly higher T_{Cloud} compared to the Cremophor EL pseudoternary system. This is noticeable in w/o microemulsions at a lower water concentration. This can be attributed to the greater hydrophilicity (higher HLB) of the Tween 85 compared with the Cremophor EL. Moreover the amount and the molecular volume of the oil phase may contribute to the temperature sensitivity of the microemulsions. For example it was observed that larger molecular volume oils tend to have a higher T_{Cloud} compared to smaller volume oils for o/w microemulsions.[38] Smaller molecular oils may penetrate into the interface behaving as cosurfactant and thus resulting in a more asymmetrical structure with lower T_{Cloud}. Although this explanation was applied to o/w systems, the results of the current investigation shows that w/o Cremophor EL microemulsions containing the lower molecular volume oil (IPM) have also a lower T_{Cloud}. However it is acknowledged that the oil and surfactant composition of these two pseudoternary systems are variable and a direct comparison of the effect of the oil and surfactant phase is difficult.

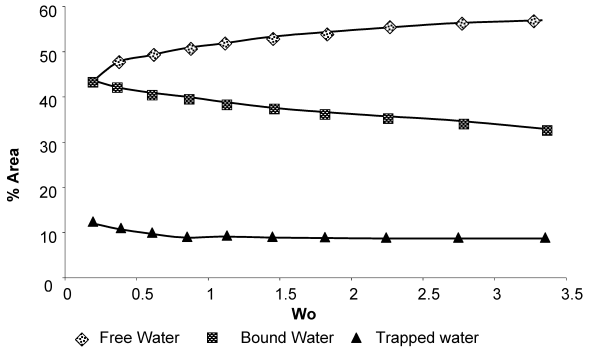

Fig. 6. Percentage Area of Free Water (◈), Bound Water (▩) and Trapped Water (▲) as a Function of Total Water Content (Wo) across the 7 : 3 Dilution Line of Cremophor EL Pseudoternary System

Free water represents water molecules interacting with each other, bound water represents the water molecules which are interacting with the surfactants head group and trapped water represents the non hydrogen bonded monomers trapped between the surfactant chains. The total water content is the sum of fee water, bound water and trapped water.

T_{Cloud} was also measured at a constant weight of water (30% w/w), whilst changing the ratio of oil to the surfactant mixture. For the Cremophor EL pseudoternary system, T_{Cloud} was constant for up to 75% surfactant above which a significant increase was observed. Conversely, the Tween 85 showed an initial decrease in T_{Cloud} for up to 80% surfactant, above which it increased linearly with the surfactant concentration. These findings may be due to the stronger interaction of water molecules with the surfactant head group at higher fraction of surfactant and possible changes in droplet shape. Overall Tween 85 and Cremophor EL pseudoternary systems containing up to 35% and 25% water respectively were stable at 32 °C.

FT-IR Spectroscopy Figure 6 shows the percentage of each type of water as a function of total water content within the microemulsion. Initially the percentage of bound water was large compared to free water or trapped water. These water molecules are likely to be bound to the head group of the surfactant. As the φ is increased, the surfactant head group starts to become saturated. This leads to the interaction of water molecules with each other, which can results in formation of aggregates and eventually bicontinuous water channels. This corresponds to an increase in percentage of free water and a decrease in percentage of bound water (Fig. 6). On the other hand the percentage of trapped water did not change with changes in microemulsion structure and contributed to only 10% of the total water content. This is in line with previously reported data.[42] At 50% water the contribution from each type of water is similar to that of free water with free water molecules dominating amongst the three types of water. Moreover a downshift in the OH stretching frequency of free water component was observed. Increasing the free water molecule in the system will minimizes the oscillation of the OH stretching (due to increase hydrogen bonding) leading to a downshift in OH frequency.

Conclusion

Novel Imwitor 308 based SMEDDS were investigated as potential transdermal delivery systems for the model steroid progesterone. Preliminary screening of oils and surfactants demonstrated that IPM/Cremophor EL (50% w/w)/Imwitor (50% w/w) and Myritol 318/Tween 85 (64% w/w)/Transcutol P (20% w/w)/Imwitor (16% w/w) SMEDDS were capable of forming microemulsions when diluted with water. The resulting pseudoternary systems were shown to undergo phase transition (from w/o to bicontinuous or o/w microemulsion) at about 15—35% water. This was further supported by droplet size and FT-IR measurements. FT-IR data showed that the percentage of bound water will dominate below φ_{C} where w/o microemulsions are present. Furthermore the selected SMEDDS were found to be stable at skin temperature (32 °C) when diluted with up to 30% water. When applied to the skin (under occultation) these systems have the potential to absorb water and form a microemulsion. Dilution with water will reduce the saturation solubility of the drug, leading to a supersaturated solution with higher thermodynamic activity. Such a formulation can remain stable upon storage, yet offer enhanced transdermal drug flux through the skin.

Acknowledgments The authors are grateful to the Foundation of Research, Science and Technology (FoRST) in New Zealand for offering a PhD scholarship to Sara Zargar-Shoshtari and Pharmaceutical Compounding New Zealand for their financial support.

References

1) Mehta S. K., Kaur G., Bhasin K. K., *Colloids Surf. B Biointerfaces*, **60**, 95—104 (2007).
2) Nandi I., Bari M., Joshi H., *AAPS PharmSciTech*, **4**, 71—79 (2003).
3) El Maghraby G. M., *Colloids Surf. B Biointerfaces*, **75**, 595—600 (2010).
4) Tang J.-L., Sun J., He Z.-G., *Curr. Drug Ther.*, **2**, 85—93 (2007).
5) Alany R. G., El Maghraby G. M., Krauel-Goellner K., Graf A., "Microemulsions: Properties and Applications," ed. by Fanun M., CRC Press, Boca Raton, 2006.
6) Fanun M., *J. Molec. Liquids*, **142**, 103—110 (2008).
7) Liu H., Li S., Wang Y., Han F., Dong Y., *Drug Devel. Ind. Pharm.*, **32**, 549—557 (2006).
8) Boonme P., Krauel K., Graf A., Rades T., Junyaprasert V. B., *Pharmazie*, **61**, 927—932 (2006).
9) Mehta S. K., Kaur G., Bhasin K. K., *Pharm. Res.*, **25**, 227—236 (2008).
10) Djordjevic L., Primorac M., Stupar M., Krajisnik D., *Int. J. Pharmaceut.*, **271**, 11—19 (2004).
11) Acosta E. J., *Colloids Surf. A Physicochem. Eng. Aspects*, **320**, 193—204 (2008).
12) Myakonkaya O., Eastoe J., Mutch K. J., Rogers S., Heenan R., Grillo I., *Langmuir*, **25**, 2743—2748 (2009).
13) Warisnoicharoen W., Lansley A. B., Lawrence M. J., *Int. J. Pharmaceut.*, **198**, 7—27 (2000).
14) Scherlund M., Brodin A., Malmsten M., *Int. J. Pharm.*, **211**, 37—49 (2000).
15) Podlogar F., Besìœter Rogacìœ M., Gasìœperlin M., *Int. J. Pharmaceut.*, **302**, 68—77 (2005).
16) Tomsìœicìœ M., Podlogar F., Gasìœperlin M., Besìœter-Rogacìœ M., Jamnik A., *Int. J. Pharmaceut.*, **327**, 170—177 (2006).
17) Podlogar F., Besìœter Rogacìœ M., Gasìœperlin M., *Eur. J. Pharm. Sci.*, **25** (Suppl. 1), S173—S175 (2005).
18) Podlogar F., Gasìœperlin M., Tomsìœicìœ M., Jamnik A., Rogacìœ M. B., *Int. J. Pharmaceut.*, **276**, 115—128 (2004).
19) Land L. M., Li P., Bummer P. M., *Pharm. Res.*, **22**, 784—788 (2005).
20) Benson H. A. E., *Curr. Drug Deliv.*, **2**, 23—33 (2005).
21) Taha M. O., Al-Ghazawi M., Abu-Amara H., Khalil E., *Eur. J. Pharm. Sci.*, **15**, 461—478 (2002).
22) Biruss B., Valenta C., *Int. J. Pharmaceut.*, **349**, 269—273 (2008).
23) Nandi I., Bari M., Joshi H., *AAPS PharmSciTech*, **4**, Article Number: 10 (2003).
24) European Commission, Scientific Committee on Consumer Product 2008 (1200). Available from: <http://ec.europa.eu/health/ph_risk/committee/04_sccp_o_082.pdf>.
25) "Glyceryl Monostearate, Material Safety Data Sheet," sciencelab.com, Inc. cited 2009; Available from: <www.sciencelab.com>.
26) Moha-Ouchane M., Peyrelasse J., Boned C., *Phys. Rev. A*, **35**, 3027 (1987).
27) Safran S. A., Webman I., Grest G. S., *Phys. Rev. A*, **32**, 506 (1985).
28) Wang F., Fang B., Zhang Z., Zhang S., *J. Dispers. Sci. Technol.*, **29**, 1166—1172 (2008).
29) Thevenin M. A., Grossiord J. L., Poelman M. C., *Int. J. Pharmaceut.*, **137**, 177—186 (1996).
30) Krauel K., Davies N. M., Hook S., Rades T., *J. Controlled Release*, **106**, 76—87 (2005).
31) Subramanian N., Ghosal S. K., Acharya A., Moulik S. P., *Chem. Pharm. Bull.*, **53**, 1530—1535 (2005).
32) Rojas O., Koetz J., Kosmella S., Tiersch B., Wacker P., Kramer M., *J. Colloid Int. Sci.*, **333**, 782—790 (2009).
33) Polizelli M. A., Telis V. R. N., Amaral L. Q., Feitosa E., *Colloids Surf. A Physicochem. Eng. Aspects*, **281**, 230—236 (2006).
34) Gao Y., Hilfert L., Voigt A., Sundmacher K., *J. Phys. Chem. B*, **112**, 3711—3719 (2008).
35) Goddeeris C., Cuppo F., Reynaers H., Bouwman W. G., Van Den Mooter G., *Int. J. Pharmaceut.*, **312**, 187—195 (2006).
36) Karlstroem G., *J. Phys. Chem.*, **89**, 4962—4964 (1985).
37) Kabir M. H., Aramaki K., Ishitobi M., Kunieda H., *Colloids Surf. A Physicochem. Eng. Aspects*, **216**, 65—74 (2003).
38) Aveyard R., Lawless T. A., *J. Chem. Soc., Faraday Trans. 1 Phys. Chem. Condensed Phases*, **82**, 2951—2963 (1986).
39) Dioumaev A. K., Lanyi J. K., *Photochem. Photobiol.*, **85**, 598—608 (2009).
40) Valero M., Sanchez F., Gomez-Herrera C., Lopez-Cornejo P., *Chem. Phys.*, **345**, 65—72 (2008).
41) Bey Temsamani M., Maeck M., El Hassani I., Hurwitz H. D., *J. Phys. Chem. B*, **102**, 3335—3340 (1998).
42) Brubach J. B., Mermet A., Filabozzi A., Gerschel A., Lairez D., Krafft M. P., Roy P., *J. Phys. Chem. B*, **105**, 430 (2000).