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## Characterisation of microemulsions containing orange oil with water and propylene glycol as hydrophilic components

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The current study aims to investigate the effect of incorporation of orange oil, mainly consisting of the cyclic mono-terpene linolene, a known skin penetration enhancer, as oil component on microemulsion formation both in water and propylene glycol containing systems. Phase diagrams of pseudoternary mixtures containing orange oil, ethyloleate or a 1:1 mixture (w/w) of orange oil and ethyloleate as oil components, a 6:4 (w/w) mixture of polyoxyethylene 20 sorbitan monooleate and sorbitan monolaurate as surfactant components and water or propylene glycol as hydrophilic components were investigated. Smaller microemulsion regions were observed when orange oil was used as a substitute for ethyloleate in both water and propylene glycol containing systems. Polarising light microscopy, viscosity measurements, electrical conductivity measurements and cryo-field emission scanning electron microscopy were used to identify structural features of the microemulsions. Solution-type, w/o droplet-type microemulsions and microemulsion areas containing liquid crystals were found in varying areas in the phase diagrams of water containing systems. Liquid crystals formation occurs when the water concentration reaches 20%–22.5% (w/w). Only solution-type microemulsions were observed in propylene glycol containing systems. The dimension of solution-type microemulsion areas in the phase diagrams is likely to depend on the miscibility of components and larger microemulsion areas were found when ethyloleate was used instead of orange oil and propylene glycol was used instead of water. W/o droplet-type microemulsions of systems containing orange oil and ethyloleate as oil components appear in different areas of the phase diagrams. Incorporation of orange oil as a penetration enhancer into a topical microemulsion affects its physical characteristics. This in turn may lead to instability of the microemulsion and/or can influence the release patterns of drugs from these microemulsions when applied as topical formulations.

### 1. Introduction

Transdermal drug delivery offers many attractions compared to oral administration, including a high potential for controlled drug release and increased bioavailability of drugs with low systemic absorption or undergoing extensive hepatic first-pass metabolism (Kreilgaard 2002). Microemulsions are optically isotropic, spontaneously forming colloidal systems consisting of appropriate combinations of oil, water and surfactant/cosurfactant mixtures (Tenjarla 1999; Lawrence and Rees 2000). They have become of increased pharmaceutical and commercial interest as promising candidates in transdermal drug delivery (Thevenin et al. 1996; Delgado-Charro et al. 1997; Paolino et al. 2002; Peltola et al. 2003) as they provide several advantages over conventional topical formulations such as creams, ointments and gels. Firstly, the manufacturing of microemulsions is easy and the products are thermodynamically stable. Secondly, the flexibility in the composition enables microemulsions to solubilise either oil-soluble,

water-soluble, or amphiphilic materials, depending on the type of microemulsions used. Thirdly, the permeation rate of the drug from microemulsions may be controlled by changing type or ratios of the components (Kreilgaard 2002; Rhee et al. 2001).

The percutaneous absorption of many drugs however is low due to the barrier function of the stratum corneum of the skin. A penetration enhancer is usually required to increase drug diffusivity through the skin. Terpenes, derived from various essential oils, have low skin irritancy and low systemic toxicity (Williams and Barry 1992). They are, therefore, good candidates as skin permeation enhancers. A variety of terpenes offer good penetration enhancing activity for both hydrophilic (Williams and Barry 1991; Hori et al. 1991; Morimoto et al. 1993; Obata et al. 1990) and lipophilic drugs (Williams and Barry 1991; Okabe et al. 1989). Although microemulsions usually contain surfactants and cosurfactants which may reversibly reduce the diffusional barrier of the stratum corneum, the incorporation of terpenes into microemulsions is consid-

ered useful to increase drug diffusivity through the skin. Rhee et al. (2001) investigated the effect of addition of various terpenes, i.e. limonene, cineole, menthol and camphor into microemulsions, on the skin permeation of keto-profen. The results showed that limonene exhibited the strongest permeation enhancing property by increasing the flux 3-fold over the microemulsion containing no terpenes. Although the benefit of incorporation of terpenes as a skin penetration enhancer has been demonstrated, the effect of incorporation of these substances on the characteristics of microemulsions has not been fully evaluated.

In this study, microemulsions containing either orange oil, ethyloleate or a mixture of orange oil and ethyloleate as an oil phase were prepared and characterised by using various techniques to identify the microstructures of the microemulsions obtained. Orange oil was used to represent terpenes as it contains up to 96% of limonene (Steuer et al. 2001). The effect of orange oil on the characteristics of microemulsions containing propylene glycol as a hydrophilic component instead of water was also investigated. The synergistic penetration enhancing effects of propylene glycol with lipophilic skin penetration enhancers (Goodman and Barry 1988) and terpenes (Barry and Williams 1989) were reported previously. Microemulsions using

propylene glycol as substitute for water may prove especially useful for the development of topical delivery systems.

## 2. Investigations, results and discussion

### 2.1. Pseudoternary phase diagrams

The phase diagrams of pseudoternary mixtures containing orange oil, ethyloleate or a 1:1 mixture (w/w) of orange oil and ethyloleate as oil components, a 6:4 (w/w) mixture of polyoxyethylene 20 sorbitan monooleate and sorbitan monolaurate as surfactant components and water or propylene glycol as hydrophilic components are shown in Figs. 1a–f. Combinations providing optically clear, one-phase systems were identified by visual observation and defined as microemulsions. They were marked with different symbols depending on the type of microemulsion (see below) in all phase diagrams.

As shown in Fig. 1a, microemulsions containing pure orange oil could form up to a water concentration of 40%. At water concentration of 20%–40%, a high amount of surfactant-mix of at least 50% was required in the ternary systems to form microemulsions. Microemulsion formed at two separate areas when water was present at 10% con-

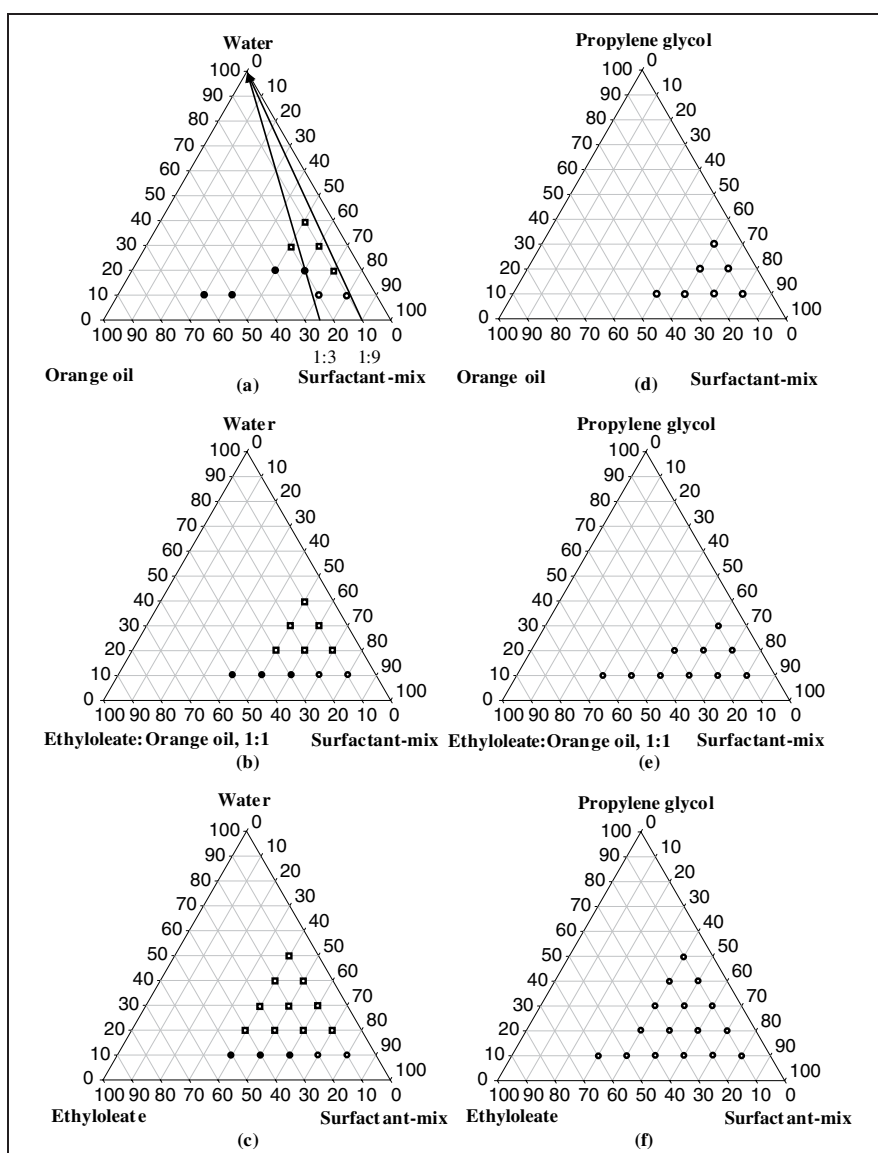


Fig. 1: Pseudoternary phase diagrams of (a) orange oil/water/surfactant-mix (polyoxyethylene 20 sorbitan monooleate:sorbitan monolaurate, 6:4); (b) orange oil:ethyloleate, 1:1/water/surfactant-mix; (c) ethyloleate/water/surfactant-mix; (d) orange oil/propylene glycol/surfactant-mix; (e) orange oil:ethyloleate, 1:1/propylene glycol/surfactant-mix; (f) ethyloleate/propylene glycol/surfactant-mix.  $\circ$  = solution type;  $\bullet$  = w/o droplet type;  $\square$  = microemulsion + liquid crystal

centration, namely at surfactant-mix concentrations of 10%–20% and 50%–60%. At surfactant-mix concentrations of 30% and 40% the mixtures looked clear initially but slightly opaque upon shaking indicating presence of two-phase systems.

The microemulsion areas of systems containing a 1:1 mixture (w/w) of orange oil and ethyloleate (Fig. 1b) at water concentrations equal or greater than 20% were identical to the systems containing pure orange oil, but a difference could be observed at a water concentration of 10% where all combinations with the surfactant-mix concentration of at least 40% showed microemulsion formation.

When ethyloleate was used as an oil component, a larger microemulsion region in the phase triangle was observed (Fig. 1c). The formation of microemulsions was found for all oil/water combinations when the surfactant-mix concentration was at least 40%. Most samples obtained outside the microemulsion areas in the water containing systems were opaque and characterised as coarse emulsions.

The pseudoternary phase diagram of orange oil/surfactant-mix/propylene glycol is shown in Fig. 1d. Microemulsions formed at any orange oil/propylene glycol combination which contained a surfactant-mix concentration of at least 60% and at least 50% at a propylene glycol concentration of 10%. The microemulsion region of systems containing a 1:1 mixture (w/w) of orange oil and ethyloleate (Fig. 1e) was larger than that consisting of pure orange oil, particularly at a propylene glycol concentration of 10% where microemulsions formed already at a surfactant-mix concentration of 30%. The largest microemulsion area was found when ethyloleate was used as an oil component (Fig. 1f). Microemulsions formed at all ethyloleate/propylene glycol combinations when the surfactant-mix concentration was at least 40%. The microemulsion region was slightly larger than in the comparable system using water as a hydrophilic component (Fig. 1c). Previously, the pseudoternary phase diagrams of ethyloleate/polyoxyethylene 20 sorbitan monooleate and sorbitan monolaurate/water or propylene glycol have been extensively investigated (Alany et al. 2001; Krauel et al. 2005). It was found that systems using propylene glycol showed larger microemulsion areas in the pseudoternary diagrams than the comparable systems using water and butanol as a cosurfactant due to the partial miscibility of propylene glycol with ethyloleate and the surfactant-mix. The smaller microemulsion regions in the phase diagrams of orange oil and 1:1 mixture of orange oil and ethyloleate containing systems thus might be explained in a similar fashion. As d-limonene is less polar than ethyloleate, orange oil is likely to be less miscible with propylene glycol than ethyloleate and therefore leads to decreased microemulsion regions in the phase diagram. Samples prepared outside the microemulsion areas in the phase diagrams of propylene glycol containing systems were unstable coarse emulsions. They separated into two layers soon after the preparation.

The polarising light microscope allows the differentiation between microemulsion and microemulsion containing liquid crystals. Microemulsions are isotropic showing no birefringence while liquid crystals are anisotropic (exception: cubic phases) thus showing unique textures such as maltose crosses and oily streaks for lamellar liquid crystals. All microemulsion samples identified by visual observation were then investigated under a polarising light microscope and combinations containing liquid crystals were marked as shown on the phase diagrams in Figs. 1a, 1b and 1c. Samples prepared along 1:3 and

1:9 oil:surfactant-mix (o:s) cuts were also observed under the polarising light microscope to investigate the concentrations at which liquid crystals formed. Birefringence appeared when the water concentration reached 20% in both cuts of all systems, except for the o:s 1:3 cut of systems containing pure orange oil which required a water concentration of at least 22.5% to form liquid crystals. No liquid crystal formation was found in propylene glycol containing systems.

## 2.2. Viscosity measurements

The viscosity of microemulsions prepared along the o:s 1:3 cuts of ethyloleate and 1:1 (w/w) orange oil and ethyloleate systems remained constant upon the addition of water from 0–5% but gradually increased with increasing water concentrations above 7.5–15% (Fig. 2a). For systems containing orange oil, the viscosity progressively increased with an increasing amount of water from 0–15%. Addition of water, having a lower viscosity than the mixture of surfactant-mix and oil, should not lead to an increase in viscosity for a true solution. The obtained results therefore indicated that the ternary systems in these regions were not solution-type microemulsions but likely to be w/o-droplet microemulsions wherein the increased viscosity upon addition of water is believed to be a result of an increased amount of water droplets in the w/o microemulsions (Alany et al. 2001). Viscosity abruptly increased when water concentrations reached 20.0% coinciding with the appearance of more rigid structures in the samples, which were determined to be liquid crystals in section 2.1. A large increase in viscosity was also observed in samples prepared along the o:s 1:9 cut at a water concentration of 20% (Fig. 2b). It can be seen that the viscosity at water concentration range of 20–25% was substantially higher than those of samples of the o:s 1:3 cuts, indicative of the formation of more liquid crystals due to the higher surfactant concentrations in the o:s 1:9 systems.

In propylene glycol containing systems, the viscosity of mixtures prepared along both o:s 1:3 and 1:9 cuts decreased with an increased concentration of propylene glycol (Figs. 2c–d). This decrease was more pronounced in the samples of the o:s 1:9 cut, probably due to the overall higher viscosity of these systems. The decreased viscosity upon addition of propylene glycol was likely to be due to the dilution of the systems with the lower viscosity component propylene glycol which is common for a true solution. The microemulsions containing propylene glycol as hydrophilic component were thus preliminary classified to be of solution type. Apart from being a hydrophilic component, propylene glycol also acts as a cosurfactant which might inhibit the formation of o/w droplets by interacting with the head group of the surfactant molecules (De Gennes and Taupin 1982). No liquid crystals were formed in propylene glycol containing systems as evident by the absence of immediate increases in viscosity and the absence of birefringence when observed under the polarising light microscope. This finding might be explained in terms of the cosurfactant properties of propylene glycol that lead to the increased fluidity of any surfactant interface, thus not favouring the formation of liquid crystals. The use of one technique, particularly viscosity measurements is however, not sufficient to identify the microstructure of microemulsions and the use of several techniques in conjunction is necessary to provide accurate results (Krauel et al. 2005).

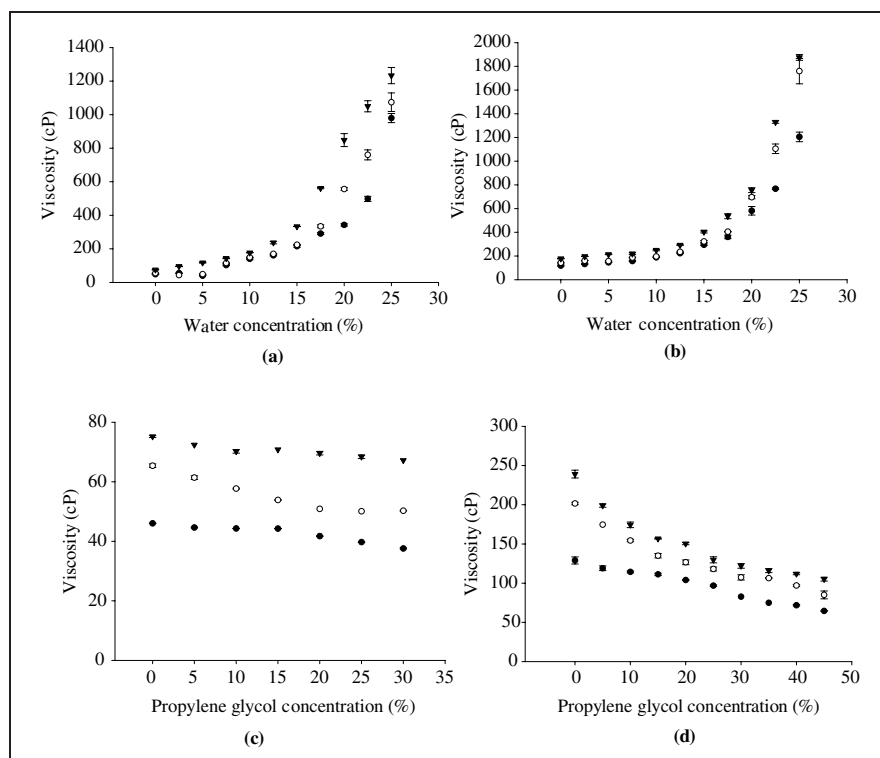


Fig. 2: Viscosity of microemulsions of varying oil:surfactant-mix ratios (a) 1:3, (b) 1:9 as a function of water concentrations of the systems; (c) 1:3, (d) 1:9 as a function of propylene glycol concentrations of the systems (values represent mean  $\pm$  S.D.,  $n = 3$ ) ● orange oil containing systems; ○ orange oil/ethyloleate containing systems; ▼ ethyloleate containing systems

### 2.3. Electrical conductivity measurements

Electrical conductivity measurements are a helpful method to differentiate w/o droplet-type from bicontinuous and solution-type microemulsions. In general, systems having low conductivity are classified as w/o droplet microemulsions since water is not present in the continuous phase but forms discrete droplets. Systems showing high conductivity are defined as bicontinuous or solution-type microemulsions as the presence of water in the continuous phase causes measurable conductivity. In this study, changes in the electrical conductivity were hence used to determine the type of microemulsions and monitor transitions of their microstructures.

Electrical conductivity of samples prepared along the o:s 1:3 cuts initially increased with increasing water concentration and reached a first maximum at a water concentration of 5% in systems containing orange oil and 1:1 (w/w) orange oil and ethyloleate. For the system containing ethyloleate conductivity reached a first maximum at a water concentration of 7.5% (Fig. 3a). A progressive decrease in electrical conductivity was observed at higher water concentrations up to a water concentration of 20%, followed by a steep increase in conductivity for the orange oil and orange oil:ethyloleate containing systems. Similar findings were reported previously by Alany et al. (2001). The increasing conductivity upon adding water at low water concentrations is believed to result from the hydration of ethylene oxide which is the hydrophilic headgroup of polyoxyethylene 20 sorbitan monooleate. At this stage, the microemulsions can be regarded as a solution-type in which the addition of water led to hydration of the surfactant headgroups in the continuous phase. The viscosity measurements were in agreement with the conductivity measurements for systems containing ethyloleate and 1:1 (w/w) orange oil and ethyloleate since their viscosity was relatively constant upon addition of water from 0–5% (Fig. 2a) and only showed an increasing viscosity at higher

water concentrations. This in turn was explained by formation of droplets in the systems. Conductivity measurements thus indicated that microemulsions were of solution-type rather than w/o droplet-type at very low water concentrations (0–7.5%). The decrease in conductivity as the water concentration was further increased suggested the microstructural transitions of the microemulsion from solution-type into swollen reverse micelles. As the hydrated monomeric surfactant molecules in the solution aggregate to form swollen reverse micelles, water was retained as confined droplets thereby resulting in the decreased electrical conductivity observed in the graphs (Alany et al. 2001).

Electrical conductivity of the mixtures containing orange oil was lower than for systems containing 1:1 (w/w) orange oil:ethyloleate and ethyloleate alone, respectively. The fact that the overall lowest electrical conductivity was found for systems containing just orange oil as the oil phase suggested that these systems show the highest ability to incorporate water inside swollen reverse micelles.

The conductivity of the pseudoternary mixtures in the ethyloleate and 1:1 (w/w) orange oil:ethyloleate system showed an increasing trend again at a water concentration above 20%. The formation of liquid crystals in the microemulsions of these systems is believed to contribute to the increase in conductivity (Alany et al. 2001).

Figs. 3b–3d show that electrical conductivity increased with the increasing concentration of water or propylene glycol in the o:s 1:9 cuts of water and propylene glycol containing systems and o:s 1:3 cut of propylene glycol containing system. These ternary mixtures are thus classified as solution-type microemulsions as the presence of more hydrophilic components in the continuous matrix led to higher electrical conductivity of the systems. The leveling off of the conductivity values in o:s 1:9 cuts of water containing systems at water concentration above 17.5% (Fig. 3b) coincides with the formation of liquid crystals.

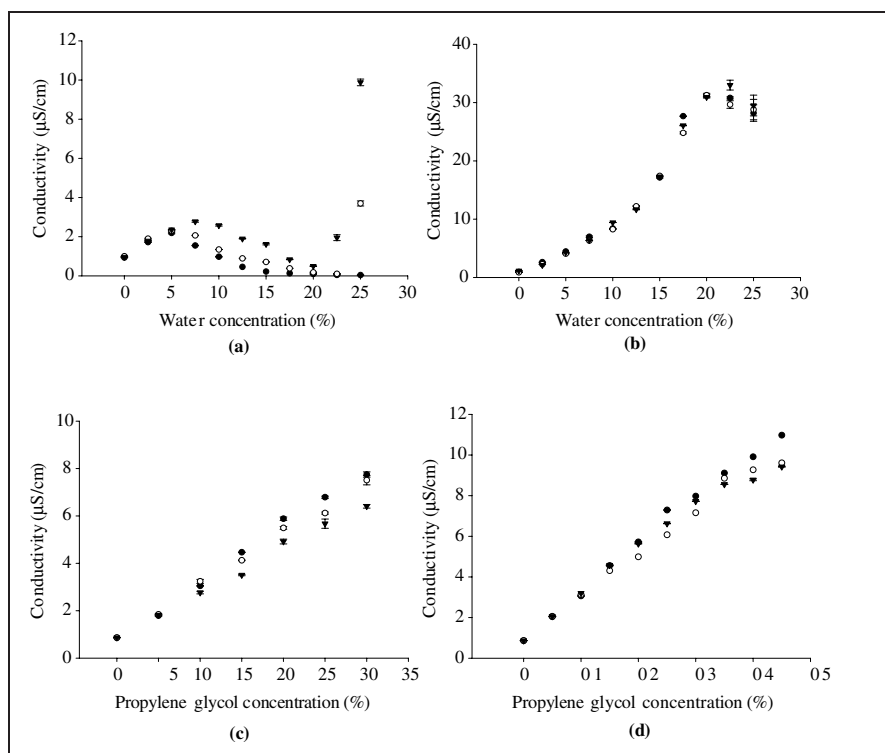


Fig. 3: Electrical conductivity of microemulsions of varying oil:surfactant-mix ratios (a) 1:3, (b) 1:9 as a function of water concentrations of the systems; (c) 1:3, (d) 1:9 as a function of propylene glycol concentrations of the systems (values represent mean  $\pm$  S.D.,  $n = 3$ )  
 ● orange oil containing systems; ○ orange oil/ethyloleate containing systems; ▼ ethyloleate containing systems

Conductivity measurements were also used to characterise the other samples in the microemulsion areas of all phase diagrams (Figs. 1a–1f). Generally, the solution type microemulsion regions of all systems appear at the right corners of the phase diagrams where surfactant-mix concentrations are high with their areas varying according to the miscibility of their components. Propylene glycol is more miscible with oils than water and therefore showed larger areas of solution-type microemulsions than the comparable systems using water as a hydrophilic phase. Systems containing the different oils exhibit w/o droplet type microemulsion at varying areas, obviously caused by different oil properties. In order to form structural microemulsion such as w/o droplet-type, a few essential conditions are required as described by Shulman et al. (1959). Firstly, a very low interfacial tension at the water and oil interface is necessary. Secondly, the molecules of the oil phase have to penetrate and associate with the interfacial surfactant film, and thirdly, a highly fluid interfacial surfactant film needs to be formed. The results, therefore, indicate that orange oil which mostly consists of the cyclic mono-terpene d-limonene exhibits interfacial properties different from the fatty acid ester ethyloleate. It is likely that incorporation of orange oil at high concentration into an oil phase or using it as the only oil component influences the properties of microemulsions formed. These changes might result in changes in the release patterns of drugs from microemulsions when applied as topical drug delivery formulations.

#### 2.4. Cryo-field emission scanning electron microscopy (Cryo FESEM)

Selected microemulsions from the phase triangles in Fig. 1 were investigated with cryo FESEM. Colloidal systems are conventionally visualised with freeze-fracture transmission electron microscopy, where a replica of the frozen sample material is prepared, which can then be

viewed under the microscope after mounting the replica onto a grid. The sample preparation via this method is time consuming and only a dry replica of the usually liquid sample can be observed. With cryo FESEM sample preparation is less time-consuming, usually <30 min, and the colloidal sample can be viewed close to its natural state as only a freezing step of the actual sample is involved, which can then be directly viewed in the frozen state.

As already shown by Krauel et al. (2005) cryo FESEM can be a helpful method in the determination of the structure type of microemulsions and droplet, bicontinuous or solution type systems can be identified. Fig. 4 shows examples of a w/o droplet type microemulsion with the composition 20% water, 60% surfactant-mix and 20% orange oil (Fig. 4a), a solution-type microemulsion with the composition 10% propylene glycol, 60% surfactant-mix and 30% oil (ethyloleate:orange oil) (Fig. 4b) and liquid crystalline structures in a system with the composition 30% water, 50% surfactant-mix and 20% oil (ethyloleate:orange oil) (Fig. 4c). The micrograph of the w/o droplet type microemulsion shows a granular fine structure, whereas the micrograph of the solution-type microemulsion is devoid of any discernable microstructure. Bicontinuous structures, which would have a sponge-like appearance were not found in the microemulsions of this study, but have been visualised previously (Krauel et al. 2005). The third micrograph shows regular layered structures, typical for lamellar liquid crystals.

The cryo FESEM investigation of the microemulsions in this study was only carried out with a few systems to show that visualisation of the microstructures is possible with the systems in this study. Determination of the type of microemulsion was possible however, with the more common techniques of polarising light microscopy, viscosity and conductivity, where especially conductivity measurements gave conclusive results on the type of microemulsion in the investigated samples.



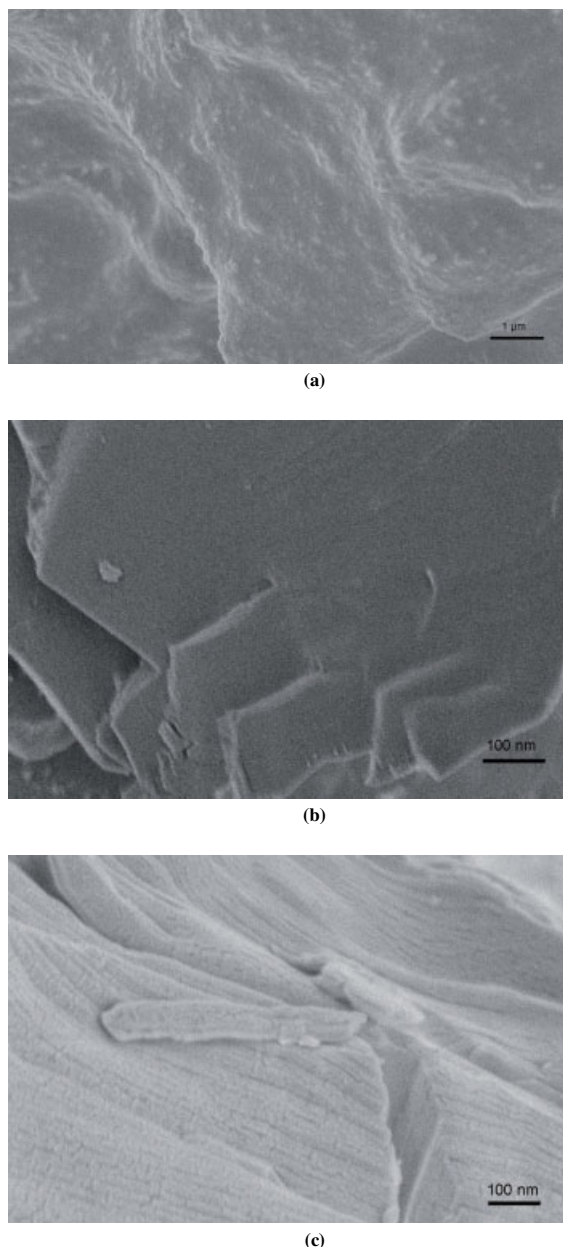


Fig. 4: Cryo-FESEM micrographs of (a) w/o droplet microemulsion (20% water, 60% surfactant-mix and 20% orange oil, magnification 17 000  $\times$ ) (b) solution-type microemulsion (10% propylene glycol, 60% surfactant-mix and 30% oil (ethyloleate:orange oil) magnification 30 000  $\times$ ) and (c) liquid crystalline structures (30% water, 50% surfactant-mix and 20% oil (ethyloleate:orange oil), magnification 30 000  $\times$ )

### 3. Experimental

#### 3.1. Materials

Orange oil [*Citrus sinensis* (L.), sweet] was obtained from Dolphin Clinic Limited (Auckland, NZ). Ethyloleate (Crodamol EO<sup>TM</sup>), sorbitan monolaurate (Crill 1<sup>TM</sup>) and polyoxyethylene 20 sorbitan monooleate (Crillet 4 super<sup>TM</sup>) were kindly donated by BTB chemicals (Auckland, NZ). Propylene glycol and sodium chloride were obtained from BDH Chemicals Ltd. (Poole, UK).

#### 3.2. Methods

##### 3.2.1. Construction of pseudoternary phase diagrams

Pseudoternary phase diagrams with three different oils, i.e. orange oil, ethyloleate and a 1:1 (w/w) mixture of orange oil and ethyloleate were constructed. Oil, a surfactant-mix with a ratio of 6:4 (w/w) polyoxyethylene 20 sorbitan monooleate:sorbitan monolaurate and water or propylene glycol at various weight ratios were mixed in glass vials using a vortex mixer and left overnight at room temperature to equilibrate.

Visual observation and polarising light microscopy (Nikon Optiphot PFX microscope, Tokyo, Japan) were used to identify the mixtures. Optically clear, one-phase systems were defined as microemulsions whereas systems showing birefringence under the polarizing light microscope were classified as microemulsions containing liquid crystals. Opaque systems having two-phases and showing no birefringence under the polarising light microscope were identified as coarse emulsions.

##### 3.2.2. Determination of microstructures of microemulsions

###### 3.2.2.1. Viscosity measurements

The viscosity of all mixtures as illustrated in the phase diagrams exhibiting one-phase systems were measured using a Brookfield DVIII viscometer (Brookfield Engineering Laboratories, Stroughton, MA) equipped with a CP-42 cone spindle. The viscosity measurements were performed in triplicate at 25 °C. More samples were prepared along the 1:3 and 1:9 oil:surfactant-mix (o:s) cuts by progressively adding water or propylene glycol at increments of 2.5–5% w/w as shown in Fig. 1a. Their viscosity was also measured to follow a change in phase behavior.

###### 3.2.2.2. Electrical conductivity

Electrical conductivity of samples was measured using a Riac CM/100 conductivity meter fitted with an YSI 3418 electrode (Yellow Springs Instruments Inc., Yellow Springs, OH). The cell constant for the electrode was 0.11 cm<sup>-1</sup>. To enable conductivity measurements, sodium chloride was added at a concentration of 1 μM to water and propylene glycol used for preparation of the ternary mixtures. Incorporation of NaCl at this concentration did not affect the phase diagram. Readings were taken after the conductivity values had been constant for at least 5 min. Measurements were carried out in triplicate at 25 °C.

###### 3.2.2.3. Cryo-FESEM

Microemulsion samples were loaded into brass rivets and plunge frozen in liquid propane with a Reichert KF 80 (Leica, Germany). Samples were then transferred under liquid nitrogen into an Alto 2500 cryo preparation chamber (Gatan, UK), fractured and transferred onto the cryo-stage of a JEOL 6700F field emission scanning electron microscope (JEOL, Japan). Samples were etched at -95 °C for approximately 4 min and then transferred back into the cryo chamber for sputter coating with platinum. Samples were then introduced back onto the cryo stage of the microscope and viewed at an accelerating voltage of 2.5 kV and a temperature of -140 °C.

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