

# Nonionic oil-in-water microemulsions: the effect of oil type on phase behaviour

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Received 10 June 1999; accepted 2 September 1999

## Abstract

The formation of oil-in-water (o/w) microemulsions stabilized by the nonionic surfactants, polyoxyethylene-10-dodecyl ether, polyoxyethylene-10-oleyl ether, *N,N*-dimethyldodecylamine-*N*-oxide and *N,N*-dimethyloleylamine-*N*-oxide and containing a variety of pharmaceutically acceptable oils, namely ethyl butyrate, ethyl caprylate, ethyl oleate and the triglycerides, soybean oil, Miglyol 812 and tributyrin, has been examined at 298 K. The effect on microemulsion formation of replacing water with phosphate buffered saline (PBS) and complete PBS has been established. In addition, the effect of changing temperature (from 298 to 310 K) on the phase behaviour of microemulsions formulated using PBS as continuous phase has been determined. Although some small differences in phase behaviour were noted when altering the continuous phase, the greatest difference in phase behaviour was observed when changing the experimental temperature, particularly for microemulsions stabilized by polyoxyethylene-10-oleyl ether. Regardless of the temperature and aqueous phase used, however the larger molecular volume oils (soybean oil, Miglyol 812 and ethyl oleate) were solubilized to a lower extent than the smaller molecular volume oils (namely, ethyl butyrate and ethyl caprylate). The only exception to this rule was when polyoxyethylene-10-oleyl ether was used as surfactant, particularly at 298 K, where it was the larger molecular volume oils that were solubilized to the greatest extent. Cloud point/phase inversion temperature experiments suggested that the higher molecular volume oils were incorporated into the microemulsions prepared using the polyoxyethylene-based surfactants in a different way than the smaller molecular volume oils and suggest that the smaller molecular volume oils are acting in much the same way as a cosurfactant in that they interchelate with their hydrophilic group interspersed in the surfactant head group region. As *N,N*-dimethyldodecylamine-*N*-oxide does not exhibit a cloud point it was not possible to determine the mode of oil incorporation in microemulsions prepared with this surfactant. © 2000 Elsevier Science B.V. All rights reserved.

**Keywords:** Nonionic oil-in-water microemulsions; Polyoxyethylene ether surfactants; *N,N*-Dimethyl-alkylamine-*N*-oxide surfactants

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## 1. Introduction

Microemulsions are thermodynamically stable, transparent, low viscosity and isotropic dispersions consisting of oil and water stabilized by an interfacial film of surfactant molecules, typically in conjunction with a cosurfactant. Recently, microemulsions have attracted a considerable amount of interest as potential drug delivery vehicles largely due to their simplicity of preparation, their clarity and their abilities to be filtered and incorporate a wide range of drugs of varying lipophilicity (Attwood, 1994; Lawrence, 1994). Unfortunately, the widespread pharmaceutical use of microemulsions has been limited, partly by the requirement for pharmaceutically acceptable ingredients, and also by the need for cosurfactant that can lead to the destruction of the microemulsion upon dilution due to the partitioning of the cosurfactant out of the interfacial region into the continuous phase. This latter limitation can be overcome by the use of certain nonionic surfactants, such as the *n*-alkyl polyethylene oxides, which can frequently produce microemulsions without cosurfactant (Malcolmson and Lawrence 1995). One potentially serious limitation, however, with the use of such nonionic surfactants is that the microemulsions they form often exhibit a phase inversion temperature (PIT); that is at a certain temperature the previously water soluble surfactant becomes an oil soluble surfactant, thereby no longer supporting the formation of an oil-in-water microemulsion. If the PIT is close to the intended temperature of use, this may seriously limit the use of the microemulsion as a drug delivery vehicle. In this context it should be noted that the PIT of a microemulsion is sensitive to the presence of a range of additives, so that electrolytes such as  $\text{Na}^+$ ,  $\text{Cl}^-$  for example, frequently decrease the PIT (Schott and Han, 1975; Aveyard and Lawless, 1986).

The present study examines the formation of cosurfactant-free o/w microemulsions, prepared using nonionic surfactants from either the *n*-alkyl polyoxyethylene ether or the *n*-alkylamine-*N*-oxide series and containing a range of ethyl ester and triglyceride oils at 298 and 310 K, in water, PBS and complete PBS (cPBS), with a view to

their exploitation as drug delivery vehicles. The resulting microemulsions have been examined in order to determine the presence or absence of a phase inversion temperature in order to assess their temperature stability. The oils in the present study were chosen as they are generally considered to be pharmaceutically acceptable and are widely used in cosmetic and food preparations (Hamdam et al., 1996). The nonionic surfactants examined, namely, polyoxyethylene-10-dodecyl ether ( $\text{C}_{12}\text{E}_{10}$ ), polyoxyethylene-10-oleyl ether ( $\text{C}_{18:1}\text{E}_{10}$  or Brij 97), *N,N*-dimethyldodecylamine-*N*-oxide (DDAO) and *N,N*-dimethyloleylamine-*N*-oxide (DOAO), were chosen as they have been previously shown to form microemulsions without the need for a cosurfactant (Mehta et al., 1994; Malcolmson and Lawrence, 1995). Also, both classes of surfactant are currently used in a range of pharmaceutical and cosmetic formulations (Porter, 1991). In addition, the amine-*N*-oxides have been reported not to exhibit a cloud point (Gradzielski and Hoffmann, 1994) and should therefore produce microemulsions that exhibit a greater temperature stability than those prepared with polyoxyethylene surfactants.

## 2. Materials and methods

### 2.1. Materials

The polyoxyethylene surfactants, polyoxyethylene-10-oleyl ether,  $\text{C}_{18:1}\text{E}_{10}$  (or Brij 97) and polyoxyethylene-10-dodecyl ether ( $\text{C}_{12}\text{E}_{10}$ ), ethyl butyrate and soybean oil were supplied by Sigma Chemical Co. Ltd., (Dorset, UK). *N,N*-dimethyldodecylamine-*N*-oxide (DDAO,  $\text{C}_{12}\text{AO}$ ) in the form of a 30% w/w aqueous solution or powder (98% purity), *N,N*-dimethylstearylamine-*N*-oxide ( $\text{C}_{18}\text{AO}$ ), ethyl caprylate and tributyrin were purchased from Fluka Chemika Ltd., (Dorset, UK). *N,N*-dimethyloleylamine-*N*-oxide (DOAO,  $\text{C}_{18:1}\text{AO}$ ), a gift from Höchst, (Werk Gendorf, Germany), was supplied in the form of a 20% w/w solution, from which the surfactant in the form of a powder was obtained by freeze-drying (Laboratory Freeze Drier S.B.4, Chemlab Ltd., Essex, UK). Miglyol 812 was a gift from

Huls AG (Werk Witten, Germany) and ethyl oleate was supplied by the Aldrich Chemical Co. Ltd., (Dorset, UK). Phosphate buffered saline (PBS) (Dulbecoco and Vogt, 1954), pH 7.4, osmolality 285 mOsm/kg was used as continuous phase. For the formulation of complete PBS (Adam, 1980) PBS was supplemented with 0.905 mM calcium chloride·2H<sub>2</sub>O and 0.492 mM magnesium chloride·6H<sub>2</sub>O (BDH Ltd., Dorset, UK). All chemicals were of the highest grade available and were used as received. Distilled water obtained from a well-seasoned glass apparatus was used for all experiments.

The surfactants were examined for purity by TLC using precoated silica gel 60 F254 plates (Merck, Darmstadt, Germany) and a toluene/methanol/acetone (8:1:1, volume basis) mixture as mobile phase, together with either a solution of 5% w/v ammonium molybdate, 10% v/v concentrated sulphuric acid and 90% v/v water or a solution of 5% v/v *p*-anisaldehyde, 5% v/v concentrated sulphuric acid and 90% v/v ethanol for detection. The results showed that C<sub>18:1</sub>E<sub>10</sub> and DOAO contained a small but detectable amount of a lipophilic impurity assumed to be the hydrophobic residue from the manufacturing process. Such impurities were not found in either DDAO or C<sub>12</sub>E<sub>10</sub>. Both classes of surfactant were analyzed by <sup>1</sup>H NMR spectroscopy at 90 MHz (Perkin–Elmer R32, Bucks, UK) using either CDCl<sub>3</sub> or D<sub>2</sub>O as solvent. By assuming no variation of the protons in the alkyl chain, the number of the oxyethylene protons determined enabled an estimation of the number of oxyethylene oxide units present to be made after taking into consideration that two of the protons detected in this region of the spectrum arose from the first methylene group of the alkyl chain portion of the surfactant. The number of polyoxyethylene oxide units determined for C<sub>18:1</sub>E<sub>10</sub> and C<sub>12</sub>E<sub>10</sub> were 10.1 and 9.2 respectively. For the amine-*N*-oxide surfactants, the number and the environment of the protons obtained from <sup>1</sup>H NMR corresponded to their expected structures. The proton coupling constants for the double bonds present in C<sub>18:1</sub>E<sub>10</sub> and DOAO were 8–10 Hz indicating the presence of the *cis* isomer. The molecular mass of the alkylamine-*N*-oxide surfactants was determined

using fast atom bombardment mass spectroscopy or FAB-MS (VG ZAB-SE4F) and were in agreement with the predicted mass. The percent of the individual elements comprising the various surfactants has been determined using elemental analysis (Micro Analytical Laboratory, Department of Chemistry, The University of Manchester). While the percentage of the elements found for DDAO, C<sub>12</sub>E<sub>10</sub> and C<sub>18:1</sub>E<sub>10</sub> were within ± 0.3% of the expected values, the percentages determined for DOAO were in error by about 1.0%. DDAO and DOAO were examined by Fourier transform infrared (FTIR) spectroscopy (FTIR 1605, Bucks, UK) and the spectrum obtained was consistent with the expected chemical structure. Due to the hygroscopic nature of all the surfactants, they were stored in the dessicator when not in use.

## 2.2. Sample preparation and determination of phase diagrams

Samples were prepared individually by mixing the required weights of oil (were necessary), surfactant and water, PBS or complete PBS, heating to 343–353 K for 10 min and cooling to 298 K with vigorous stirring throughout. Note that the use of heat was essential for the production of microemulsions stabilized with the polyoxyethylene surfactants, while the application of heat was largely unnecessary for those systems prepared using the amine-*N*-oxide surfactants, with the exception of those samples near to the upper limit of the phase boundary. This difference may be due to the presence of a cloud point in the solutions prepared with the polyoxyethylene surfactants.

The area of microemulsion existence was determined by transferring the samples to a tightly sealed container and storing either at 298 or 310 K in a temperature-controlled dry incubator (LEEC, Nottinghamshire, UK). Extreme care was taken to ensure that none of the volatile oils, in particular ethyl butyrate, were lost during the preparation and storage of the samples. Samples that after 4 weeks remained completely clear, fluid and non-birefringent when observed through crossed Polaroids were classified as microemulsions. Stable microemulsion formulations were

plotted on the triangular phase diagram as being within the area of microemulsion existence. Determination of the area of microemulsion existence was performed at least in duplicate to ensure an accuracy of better than  $\pm 1\%$  w/w oil for each system.

### 2.3. Determination of phase inversion temperature and cloud point

The phase inversion temperature (PIT) of each microemulsion and the cloud point (CP) of the corresponding micellar solution were determined by repeatedly heating and cooling the samples to 373 and 298 K, at a rate of  $1 \text{ K min}^{-1}$  with stirring throughout. The temperature at the onset of turbidity (upon heating) and the appearance of clarity (when cooling) were noted. The PIT or cloud point quoted is the average of four determinations.

## 3. Results

If microemulsions are to be used as drug delivery systems, there is a need to determine the conditions under which they are formed, in particular which surfactants form microemulsions with which oils. All surfactants tested formed oil-in-water microemulsions at 298 K with the various oils used with the exception of  $C_{18}\text{AO}$ . It should be noted that, in the present study, no distinction has been made between a microemulsion and swollen micelle, with an oil-in-water microemulsion being considered to have been produced if  $>1\%$  w/w oil was incorporated into the aqueous micellar solution. For the purposes of brevity and to allow a comparison between the various systems studied, in some instances in preference to the partial phase diagram, only the maximum level of oil incorporation and the surfactant concentration at which this occurs is recorded. Note also that although the oils were added on a weight basis, because of the similarities in density within a series of oils, similar volumes were added. The only exception to this generalization was the oil, tributyrin, which was significantly more dense than soybean and Miglyol 812.

### 3.1. Phase behaviour of polyoxyethylene surfactants

The area of microemulsion existence obtained using  $C_{12}\text{E}_{10}$  is shown in Fig. 1, with the shaded region highlighting the one phase o/w microemulsion region.  $C_{12}\text{E}_{10}$  was found to stabilize o/w microemulsions containing all ethyl ester and triglyceride oils investigated, at 298 K, although with varying levels of oil incorporation. As expected however the structure and in part the molecular volume of the oils used (Table 1) influenced the amount of oil incorporated within the microemulsion. Within the triglyceride series of oils, tributyrin, the smallest molecular volume oil could be incorporated up to 3% w/w at a surfactant concentration of 35% w/w, while the larger molecular volume oils could only be solubilized to 2 and 1% w/w at approximately 30% w/w and between 10 and 35% w/w  $C_{12}\text{E}_{10}$  for Miglyol 812 and soybean oil respectively. Similarly for the ethyl esters, the smallest molecular volume oil, ethyl butyrate was solubilized to the greatest extent, namely 16% w/w at a surfactant concentration of 30% w/w, whereas the larger molecular volume oils, ethyl caprylate and ethyl oleate were incorporated at levels of 7 and 3% w/w, respectively, at surfactant concentrations of 25% w/w. These results suggest that the solubilization of a single-chain oil (ethyl ester of fatty acid oil) is easier than the three-chain (triglyceride) oil and, within the same series of oil, the oil with shorter alkyl chain length is solubilized to a greater extent than the longer chain length oils. These results were as expected and follow the trend recorded in the literature (Chaiko et al., 1984; Malcolmson and Lawrence, 1995; Miñana-Pérez et al., 1995a, Monduzzi et al., 1997).

Surprisingly, however, this trend was reversed in o/w microemulsions produced using the longer unsaturated alkyl chain surfactant,  $C_{18:1}\text{E}_{10}$ , where the largest molecular volume oils were solubilized to the greatest extent (Fig. 2). For example, of the triglyceride oils studied, the largest molecular volume oil, soybean oil, produced the greatest region of microemulsion existence with 10% w/w of the oil being incorporated at a surfactant concentration of 25% w/w, while only 6 and

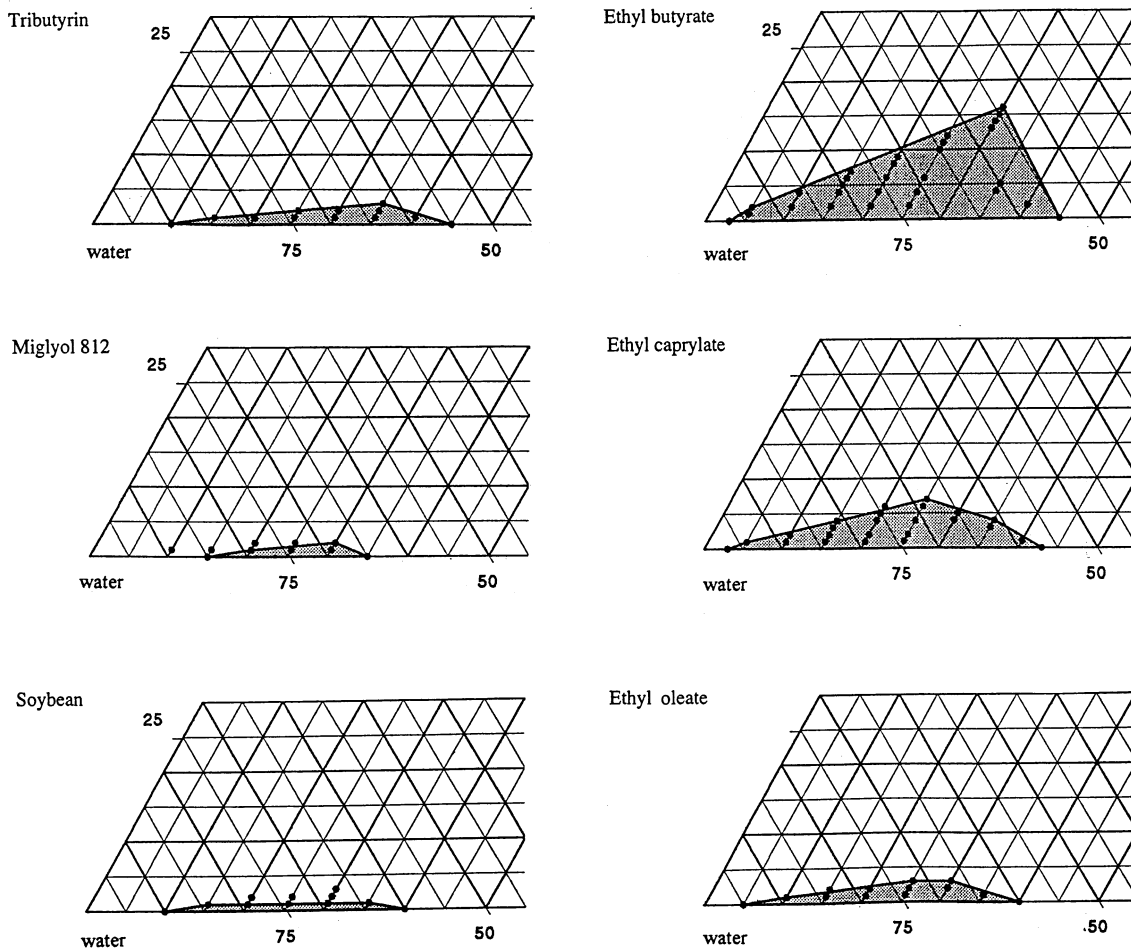


Fig. 1. Partial triangular phase diagrams of the o/w microemulsions formed with  $C_{12}E_{10}$ , water and oils at 298 K. On the abscissa, surfactant concentration (in weight%) is increasing from left to right while on the ordinate, oil concentration (in weight%) is increasing from bottom to top.

Table 1  
Physical chemistry properties of oils investigated

Oil	Molecular weight ( $\text{g mol}^{-1}$ )	Density <sup>a</sup> at $298 \pm 5 \text{ K}$ ( $\text{g cm}^{-3}$ )	Molecular volume <sup>b</sup> at $298 \pm 5 \text{ K}$ ( $\text{\AA}^3$ )
Soybean oil	881	0.9191	1592
Miglyol 812	554	0.903	1019
Tributyrin	302	1.032	486
Ethyl oleate	310	0.870	592
Ethyl caprylate	172	0.878	326
Ethyl butyrate	116	0.878	220

<sup>a</sup> Manufacturers label.

<sup>b</sup> Malcolmson et al. (1998).

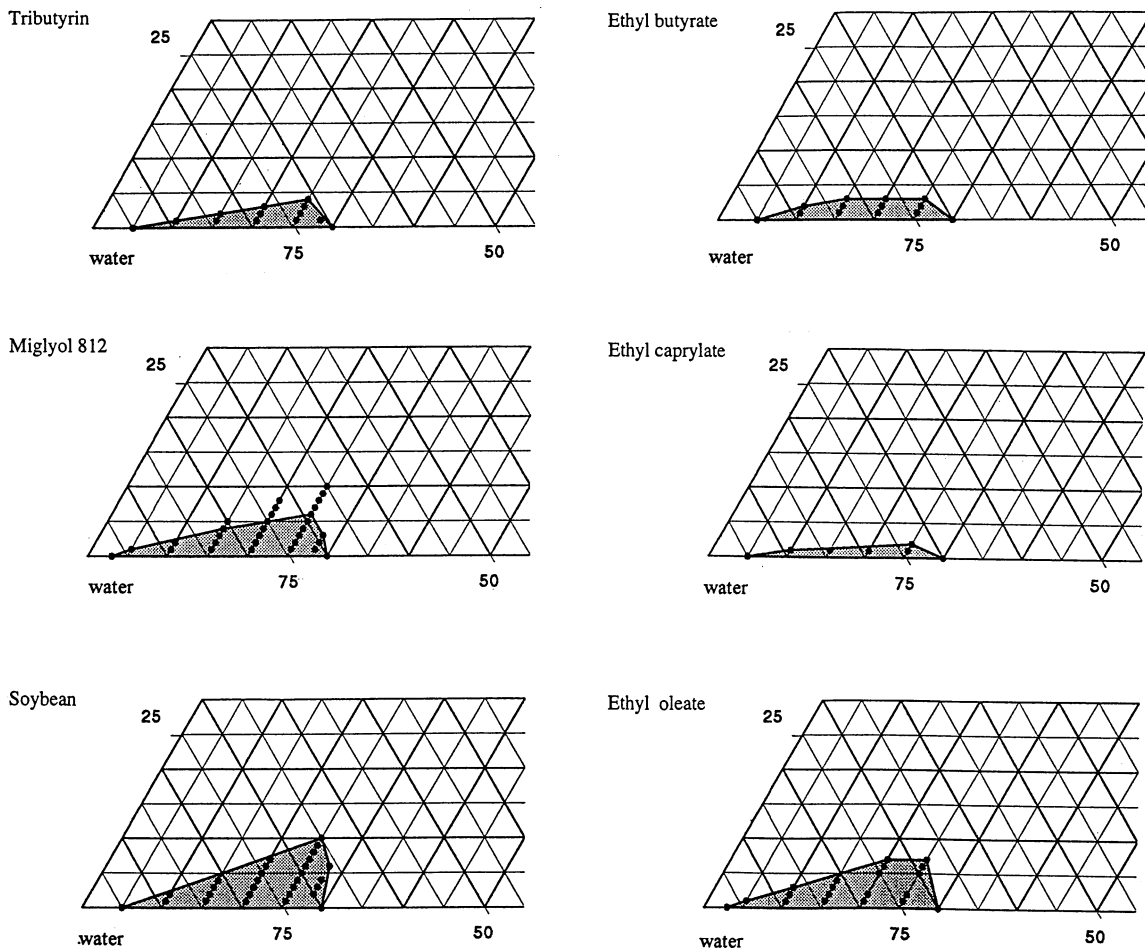


Fig. 2. Partial triangular phase diagrams for the o/w microemulsions formed with  $C_{18:1}E_{10}$  and a range of oils at 298 K in water. Abscissa and ordinate as Fig. 1.

4% w/w of the smaller molecular volume oils, Miglyol 812 and tributyrin respectively, could be solubilized at the same surfactant concentration. Similarly for ethyl esters, the largest molecular volume oil, ethyl oleate was taken up to the greatest extent with 7% w/w of ethyl oleate being incorporated at a surfactant concentration of 20–25% w/w in comparison to only 2% w/w of ethyl caprylate and 3% w/w of ethyl butyrate at the same surfactant concentration.

Malcolmson et al. (1998) have previously reported the formation of o/w microemulsions using the same triglycerides and ethyl esters and notionally the same surfactant as studied here, namely,

$C_{18:1}E_{10}$  (Brij 96). It should be noted that Brij 96 is the 'older' version of Brij 97 ( $C_{18:1}E_{10}$ ) and is no longer produced by the manufacturer. While in our hands the two versions of  $C_{18:1}E_{10}$  exhibited the same behaviour when examined by elemental analysis, TLC, IR and NMR spectroscopy, the CP of an aqueous Brij 96 solution was about 20 K higher than the corresponding Brij 97 solution suggesting that Brij 96 was the more hydrophilic surfactant and/or the presence of inorganic contaminants within the sample. In this respect it is worth noting that the commercially purchased polyoxyethylene surfactants contain a polydisperse head group that can result in a variation of

the cloud point between different batches of surfactant, however the differences in cloud point recorded here between Brij 96 and Brij 97 are greater than that generally seen with batch to batch variation. Interestingly the cloud point obtained for the sample of Brij 97 used in the present study is in close agreement with that reported by Schott (1969) for  $C_{18:1}E_{10}$ .

Of particular interest in the present study was that the microemulsion phase behaviour obtained with the two versions of  $C_{18:1}E_{10}$  was slightly different; this was particularly noticeable when comparing the levels of incorporation of the ethyl ester oils. For the triglyceride oils, however, the microemulsions prepared using Brij 96 in water (Malcolmson et al., 1998) gave the same trend of increasing oil incorporation with increasing molecular volume as that seen with formulations prepared with Brij 97, although the microemulsions prepared using Brij 97 solubilized a slightly greater amount of soybean oil. In the present study, however, Brij 96 exhibited a greater capacity to solubilize the ester of fatty acid oils than Brij 97 in that 8, 9 and 6% w/w ethyl butyrate, ethyl caprylate and ethyl oleate, respectively, were incorporated at a level of 20% w/w Brij 96. Note that when Brij 96 was used as the surfactant the smaller molecular volume ethyl ester oil, ethyl butyrate, was solubilized to the greatest extent while ethyl oleate was incorporated the least.

With the exception of Brij 96 (Malcolmson et al., 1998) the reversal in the trend of decreasing oil incorporation with increasing molecular volume has not been previously recorded for the type of surfactant under study, although a similar trend has been observed when using 'extended' surfactants that contain a short (semi-polar) polypropylene oxide (PO) chain inserted between a hydrophobic dodecyl chain and a hydrophilic ether sulphate head group (Miñana-Pérez et al., 1995a,b). It is not clear at present why the trend of increasing oil solubilization with decreasing oil molecular volume is reversed in systems prepared with the long hydrophobic chain surfactants, but it may be due to the relative differences in length of the oil and the hydrophobic chains of the surfactant, which in the case of ethyl butyrate and  $C_{18:1}E_{10}$  are very different.

### 3.2. Phase behaviour of alkylamine-*N*-oxide surfactants

In contrast to the wide spread use of the polyoxyethylene surfactants to produce microemulsions, the formulation of microemulsions using the alkylamine-*N*-oxide surfactants is rarely, if ever, reported, although Mehta et al. (1994) have used the amine-*N*-oxide surfactants to produce oil-in-water lecithin-based microemulsions. In the present study DDAO produced microemulsions over the surfactant concentration range of 5–35% w/w with all the oils studied, with the exception of soybean oil that could not be incorporated into a microemulsion at any surfactant concentration (Fig. 3). Microemulsions prepared by DDAO, in common with those made using  $C_{12}E_{10}$ , exhibited a greater solubilization capacity for the smaller molecular volume oils.

The results of the phase study with DOAO are shown in Fig. 4. When using this surfactant a maximum of 2% w/w soybean oil and tributyrin were found to be solubilized, while only 1% w/w of Miglyol 812, ethyl caprylate and ethyl oleate were incorporated. The smallest molecular volume oil, ethyl butyrate exhibited the greatest level of incorporation, namely, 4% w/w oil at a surfactant concentration in the range of 20–25% w/w. Interestingly, a few days after preparation, all the microemulsions regardless of the type of oil incorporated, appeared to become considerably less viscous than the micellar solutions from which the microemulsions were prepared, suggesting that the rod-shaped micelles formed by DOAO (Hoffman et al., 1992) became more spherical upon addition of very small amounts of oil. Interestingly the solubilization of a high amount of hydrocarbon in DOAO micelles has been reported to induce a similar shape transformation (Hoffmann and Gradzielski, 1994). Systems containing soybean oil just outside the microemulsion region became less cloudy after a week, an observation that substantiates the hypothesis that microemulsions may require a period of time to reach thermodynamic equilibrium with the rate of solubilization of smaller molecular volume oils being higher than that of the larger molecular volume oils (Murthy, 1993).

It is worth mentioning at this point that the saturated version of DOAO, namely octadecyltrimethylamine-*N*-oxide, did not form microemulsions at 298 K with the oils investigated. A similar result has previously been reported for  $C_{18}E_{10}$  at the same experimental temperature and has been attributed to the fact that the hydrophobic chain has a melting point greater than 298 K; here increasing the experimental temperature for this surfactant to 310 K restored chain fluidity and allowed the formation of a microemulsion (Malcolmson and Lawrence, 1995). In this study however, the effect of increasing temperature on

the formation of a microemulsion using the  $C_{18}$  chain amine-*N*-oxide surfactant was not examined, but it is considered likely that if a higher experimental temperature had been used, a microemulsion would have been produced.

When compared to microemulsions produced from  $C_{12}E_{10}$  and DDAO, ethyl oleate, soybean oil and Miglyol 812 were incorporated to higher levels in microemulsions stabilized by  $C_{18:1}E_{10}$ ; only ethyl butyrate and ethyl caprylate were solubilized to a greater extent in the DDAO and  $C_{12}E_{10}$  stabilized systems, with the systems prepared with DDAO demonstrating the greatest solubilization

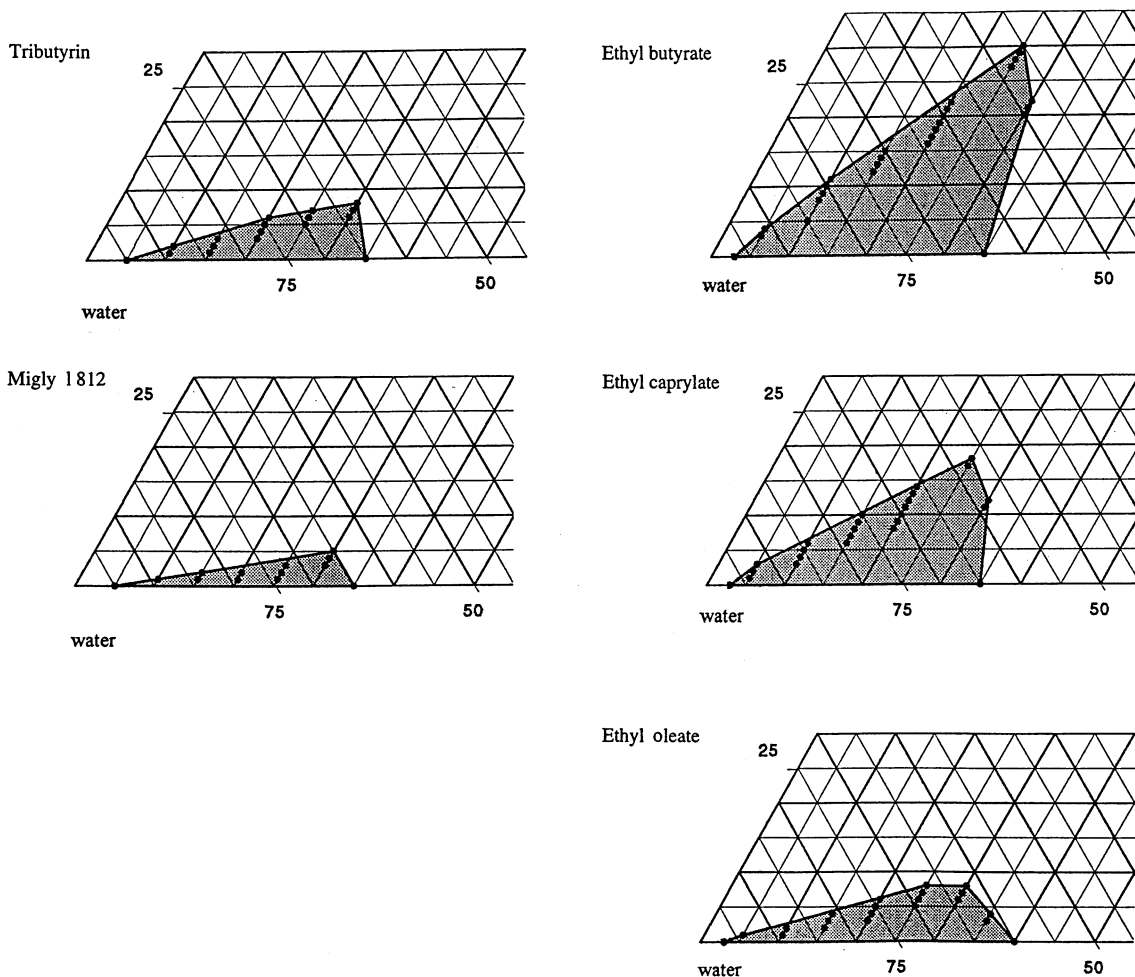


Fig. 3. Partial phase diagrams for the o/w microemulsions formed with DDAO and a range of oils at 298 K in water. Abscissa and ordinate as Fig. 1.



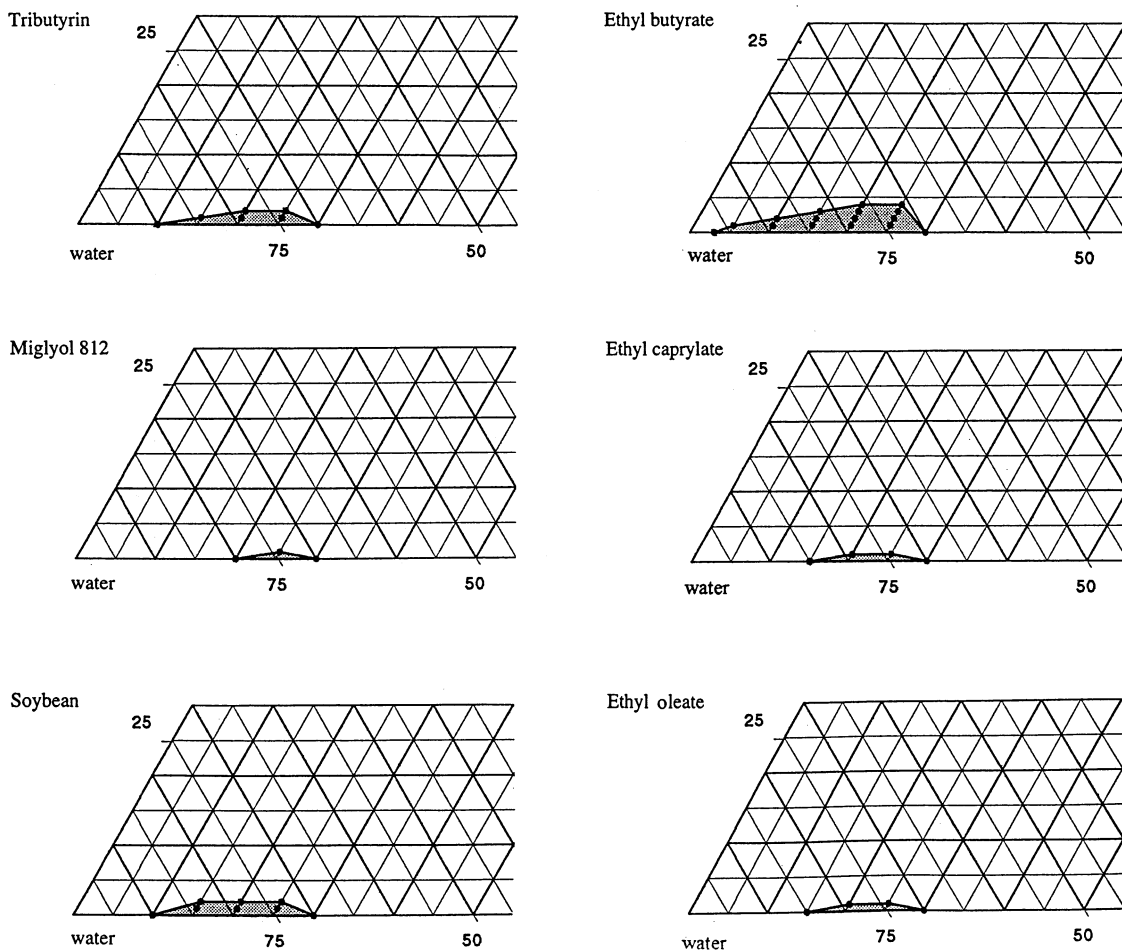


Fig. 4. Partial triangular phase diagrams for the o/w microemulsions formed with DOAO and a range of oils at 298 K in water. Abscissa and ordinate as Fig. 1.

capacity for the ethyl esters, ethyl butyrate and ethyl caprylate and tributyrin. It is worth commenting that the greater capacity for oil solubilization of the alkylamine-*N*-oxide surfactants than the more widely used polyoxyethylene surfactants may have some important implications for their pharmaceutical use, as it is well established that amine-*N*-oxide surfactants are good 'solubilizers' of drug (Lawrence and Devinsky, 1988; Tolle et al., 2000). Consequently therefore the existence of an extra locus for drug incorporation in a microemulsion, namely a core of oil in the centre of the aggregate, should further increase drug solubilization. Indeed, a greater

amount of the hydrophobic drug, testosterone propionate, can be incorporated into microemulsions formulated with DDAO (Satra et al., 1995) than those prepared using Brij 96 (Malcolmson and Lawrence, 1993).

### 3.3. The effect of electrolytes on the area of microemulsion existence

The presence of electrolytes in an aqueous micellar or microemulsion formulation has been known to affect the level of hydration of the polyoxyethylene head group and consequently may alter the area of microemulsion existence

seen with these surfactants, typically the presence of electrolytes reduces head group hydration (Schott and Royce, 1984). With the amine-*N*-oxide surfactants, the presence of electrolyte is known to reduce head group repulsions thereby allowing a growth in aggregate size (Imae and Ikeda, 1984) and most likely a change in the area of microemulsion existence, although there are no

reports in the literature examining this. In the present study the effect of electrolyte on the level of oil uptake in microemulsions at 298 K was examined by the use of PBS and cPBS in place of water; both PBS and cPBS are commonly used in pharmaceutical preparations. The results of this study are given in Tables 2–5, where the maximum level of oil incorporation and the surfactant

Table 2  
Incorporation of oil in microemulsions stabilized by C<sub>12</sub>E<sub>10</sub> at 298 K

Oil	Surfactant concentration range over which microemulsion formed (%w/w)			Maximum amount of oil solubilized (at surfactant concentration) (%w/w)		
	Water	PBS	CPBS	Water	PBS	CPBS
Soybean oil	10–40	5–40	10–40	1 (15–35)	1 (15–35)	1 (15–35)
Miglyol 812	15–35	10–35	10–40	2 (30)	2 (30)	2 (30)
Tributyrin	10–45	10–45	10–40	3 (35)	3 (35)	3 (35)
Ethyl oleate	5–40	5–40	10–40	3 (25–30)	3 (25–30)	4 (25–30)
Ethyl caprylate	5–45	5–45	5–40	7 (25)	7 (25)	6 (25)
Ethyl butyrate	5–45	5–45	5–40	16 (30)	17 (30)	17 (30)

Table 3  
Incorporation of oil in microemulsions stabilized by C<sub>18:1</sub>E<sub>10</sub> at 298 K

Oil	Surfactant concentration range over which microemulsion formed (%w/w)			Maximum amount of oil solubilized (at surfactant concentration) (%w/w)		
	Water	PBS	CPBS	Water	PBS	CPBS
Soybean oil	5–30	5–30	10–30	10 (25)	10 (25)	7 (25)
Miglyol 812	5–30	5–30	10–30	6 (25)	7 (25)	7 (25)
Tributyrin	5–30	5–30	5–30	4 (25)	4 (25)	4 (25)
Ethyl oleate	5–30	5–30	5–30	7 (20–25)	7 (25)	7 (20)
Ethyl caprylate	5–30	5–30	10–30	2 (25)	1 (15–25)	1 (15–25)
Ethyl butyrate	5–30	5–30	5–30	3 (15–25)	3 (20–25)	3 (25)

Table 4  
Incorporation of oil in microemulsions stabilized by DDAO at 298 K

Oil	Surfactant concentration range over which microemulsion formed (%w/w)			Maximum amount of oil solubilized (at surfactant concentration) (%w/w)		
	Water	PBS	CPBS	Water	PBS	CPBS
Miglyol 812	5–35	5–35	5–35	5 (30)	5 (30)	4 (25–30)
Tributyrin	5–35	5–35	5–35	8 (30)	8 (30)	7 (25–30)
Ethyl oleate	5–40	5–40	5–40	8 (25–30)	8 (30)	6 (25–30)
Ethyl caprylate	5–35	5–35	5–35	18 (25)	18 (25)	19 (25)
Ethyl butyrate	5–35	5–35	5–35	30 (25)	30 (25)	30 (25)

Table 5  
Incorporation of oil in microemulsions stabilized by DOAO at 298 K

Oil	Surfactant concentration range over which microemulsion formed (%w/w)			Maximum amount of oil solubilized (at surfactant concentration) (%w/w)		
	Water	PBS	CPBS	Water	PBS	CPBS
Soybean oil	10–30	15–30	0	2 (15–25)	1 (20–25)	0
Miglyol 812	20–30	0	0	1 (25)	0	0
Tributylin	10–30	15–30	10–30	2 (20–25)	2 (25)	2 (25)
Ethyl oleate	15–30	15–30	10–30	1 (20–25)	1 (20–25)	1 (15–25)
Ethyl caprylate	15–30	15–30	10–30	1 (20–25)	1 (20–25)	1 (15–25)
Ethyl butyrate	5–30	5–30	5–30	4 (20–25)	4 (20–25)	4 (20)

concentration at which this occurs is reported. It should be noted that the micellar solutions and microemulsions prepared from the alkylamine-*N*-oxide surfactants, DDAO and DOAO, in cPBS were very slightly cloudy, possibly due to the divalent cations present in cPBS reducing head group interactions and inducing the formation of very large aggregates in much the same way as seen when salt is added to a solution of ionic micelles. As a consequence, the first sample that appeared extremely turbid was considered to be outside the microemulsion phase boundary.

As can be seen in Tables 2–4, the phase behaviour of microemulsions prepared from C<sub>12</sub>E<sub>10</sub>, C<sub>18:1</sub>E<sub>10</sub> and DDAO in PBS and cPBS at 298 K was, within experimental error, very similar to that seen in water, suggesting that at this temperature, the phase behaviour of the surfactants is not very sensitive to the presence of electrolyte. Although the same is also true for microemulsions prepared using DOAO and containing the ethyl ester oils (Table 5), when the triglyceride oils were used with DOAO there was a difference in the extent of the uptake of oil when water was replaced by PBS or cPBS, with the extent of microemulsion formation tending to decrease suggesting that the presence of electrolyte had a detrimental effect on microemulsion formation. For example, soybean oil was incorporated to a level of 2% w/w in aqueous-based microemulsions, whereas a maximum of only 1% w/w could be incorporated in PBS based-systems, and no microemulsion was formed when cPBS was used as continuous phase.

### 3.4. The effect of temperature of the area of microemulsion existence

If o/w microemulsions are to be considered as delivery vehicles for lipophilic drugs, a knowledge of their stability at body temperature is essential, especially as increasing temperature is known to decrease the level of hydration of the polyoxyethylene chains, causing the surfactant to change its behaviour from water-soluble to oil-soluble and causes an inversion in microemulsion phase behaviour. From the much more limited amount of work investigating the effect of temperature on amine-*N*-oxide surfactants it would appear that these surfactants are far less temperature sensitive than their polyoxyethylene counterparts. Tables 6 and 7 gives the maximum level of oil incorporation for microemulsions formed with the polyoxyethylene and alkylamine-*N*-oxide surfactants respectively at 298 and 310 K.

In general, the results show that all nonionic surfactants tested, with the exception of DDAO with soybean oil and DOAO with Miglyol 812, which did not form microemulsions at either temperature, were able to form stable o/w microemulsions in PBS at 298 and 310 K with all the oils examined. Increasing the temperature from 298 to 310 K led to a slightly higher level of oil incorporation in systems prepared using C<sub>12</sub>E<sub>10</sub>; the only exceptions to this were the ethyl butyrate containing microemulsions, where the maximum amount of oil incorporated at 310 K (10% w/w) was less than that obtained at 298 K (17% w/w). In addition, the area of microemulsion existence seen

Table 6

Incorporation of oil in microemulsions formed with polyoxyethylene surfactants and PBS at 298 and 310 K

Oils	Surfactant concentration range over which microemulsion formed (%w/w)				Maximum amount of oil solubilized (at surfactant concentration) (%w/w)			
	C <sub>12</sub> E <sub>10</sub>		C <sub>18:1</sub> E <sub>10</sub>		C <sub>12</sub> E <sub>10</sub>		C <sub>18:1</sub> E <sub>20</sub>	
	298 K	310 K	298 K	310 K	298 K	310 K	298 K	310 K
Soybean oil	5–40	20–50	5–30	5–35	1 (15–35)	2 (35–40)	10 (25)	6 (30)
Miglyol 812	10–35	10–45	5–30	5–35	2 (30)	4 (35)	7 (25)	7 (25)
Tributyrin	10–45	5–45	5–30	15–40	3 (35)	4 (35)	4 (25)	1 (20–35)
Ethyl oleate	5–40	5–40	5–30	15–35	3 (25–30)	7 (35)	7 (25)	1 (20–30)
Ethyl caprylate	5–45	5–40	5–30	25–35	7 (25)	13 (30)	1 (15–25)	1 (30)
Ethyl butyrate	5–45	5–40	5–30	5–35	17 (30)	10 (20)	3 (20–25)	2 (25–30)

with C<sub>12</sub>E<sub>10</sub> tended to shift to higher surfactant concentrations when compared to the area of existence obtained at 298 K and, in several cases, samples containing a high amount of surfactant that were gels at room temperature, were fluid at 310 K. In contrast to the results seen with C<sub>12</sub>E<sub>10</sub>, the areas of existence seen at 310 K using C<sub>18:1</sub>E<sub>10</sub>, were generally significantly smaller than those seen at 298 K; for example only 1% w/w of tributyrin and ethyl oleate could be incorporated at 310 K in comparison to 4 and 7% w/w respectively at 298 K. Although again, the area of microemulsion existence extended to higher surfactant concentrations, in this case up to 30–35% w/w surfactant.

In contrast the microemulsions prepared using DDAO surfactant showed the least temperature

sensitivity with little or no alteration in the area of microemulsion existence being observed. A few of the DOAO stabilized systems did however exhibit a temperature sensitivity leading to a reduction in the area of microemulsion existence; for example the amount of ethyl butyrate decreased from 4 to 2% w/w in changing the temperature from 298 to 310 K.

The results suggest that the microemulsions prepared using DDAO and some of the microemulsions formulated with C<sub>12</sub>E<sub>10</sub> are suitable for use at 310 K. However, as a number of the microemulsion systems prepared with C<sub>18:1</sub>E<sub>10</sub> and DODO, for example, soybean oil/C<sub>18:1</sub>E<sub>10</sub>/PBS, ethyl oleate/C<sub>18:1</sub>E<sub>10</sub>/PBS and EB/DOAO, showed a decrease in oil incorporation upon increasing temperature, these systems may be of little use for

Table 7

Incorporation of oil in microemulsions formed with alkylamine-*N*-oxide surfactants and PBS at 298 and 310 K

Oils	Surfactant concentration range over which microemulsion formed (%w/w)				Maximum amount of oil solubilized (at surfactant concentration) (%w/w)			
	DDAO		DOAO		DDAO		DOAO	
	298 K	310 K	298 K	310 K	298 K	310 K	298 K	310 K
Soybean oil	0	0	15–30	15–30	0	0	1 (20–25)	1 (20–25)
Miglyol 812	5–35	5–35	0	0	5 (30)	6 (25)	0	0
Tributyrin	5–35	5–35	15–30	15–30	8 (30)	9 (25)	2 (25)	1 (20–25)
Ethyl oleate	5–40	5–40	15–30	15–30	8 (30)	8 (25)	1 (20–25)	1 (20–25)
Ethyl caprylate	5–35	5–35	15–30	20–30	18 (25)	18 (25)	1 (20–25)	1 (25)
Ethyl butyrate	5–35	5–35	5–30	10–30	30 (25)	32 (25)	4 (20–25)	2 (15–25)

Table 8  
Phase inversion temperature of systems prepared using DOAO

Oil	%w/w oil	20% w/w surfactant <sup>a</sup>	
		Water	PBS
–	–	NS	93.0
Soybean oil	1	71.0	57.6
Miglyol 812	1	–	–
Tributylin	1	63.1	51.8
Ethyl oleate	1	54.4	40.2
Ethyl caprylate	1	43.2	34.3
Ethyl butyrate	1	87.1	73.4

<sup>a</sup> Mean of  $n = 4$ ; S.D.  $\pm 1.0$ , NS, none seen

drug delivery, especially as the presence of drug may further reduce the phase transition temperature (Malcolmson, 1992). The reduction of the area of microemulsion existence observed with systems stabilized with  $C_{18:1}E_{10}$  is due to the surfactant possessing a low CP, which is further reduced in the presence of some oils (see below). In contrast the higher CP recorded for  $C_{12}E_{10}$  means that it is the least temperature sensitive of the two polyoxyethylene-based surfactants studied. As DDAO did not possess a CP in the temperature range investigated in the present study, it is not surprising therefore the DDAO stabilized microemulsions did not exhibit any temperature sensitivity.

### 3.5. Dilutability of nonionic o/w microemulsions

The ability of a microemulsion to be diluted is essential for its use as a drug delivery vehicle since, after administration, it will almost certainly be diluted by body fluids. From an examination of the area of existence, it may be expected that it would not be possible to dilute the microemulsions under study, because at low surfactant concentration the system can not form microemulsions. The results from the present study indicate however that the nonionic microemulsions prepared in water and PBS could be diluted from a 20–25% w/w surfactant concentration to an extremely low surfactant concentration (i.e.  $< 10^{-4}$  % w/w surfactant), and that the very dilute systems remained stable for at least a

month at 298 K (note that the microemulsions prepared with cPBS were not tested, although there is no reason to suspect that they would behave any differently). The only exceptions to this observation were microemulsions prepared at compositions very near the upper oil phase boundary, for example, microemulsions containing either 6% w/w ethyl caprylate and 20% w/w  $C_{12}E_{10}$ , or 6% w/w tributyrin and 20% w/w DDAO, as these could not be diluted. This ability to readily dilute the microemulsions under study contrasts starkly with the case of microemulsions containing cosurfactants that can not be diluted without at least altering, and in many cases, destroying the microemulsion (Attwood, 1994).

The corresponding micellar solutions all exhibited the ability to be infinitely diluted, with the exception of DOAO in PBS, which appeared extremely turbid after dilution, which may be a result of the fact that this surfactant is known to form large rod-shaped micelles (Imae and Ikeda, 1984).

### 3.6. Phase inversion temperature

The possession of a PIT by a microemulsion within the temperature range 293–323 K suggests that there may be problems with the use of the system for the purposes of drug delivery. The degree of temperature sensitivity of a microemulsion depends on the nature of the polyoxyethylene surfactant and oil used in the formulation. In line with earlier work, in the current work, micellar (and microemulsion) solutions of DDAO did not exhibit a cloud point (or PIT) at any surfactant (or oil) concentration, neither did a micellar solution of DOAO in water at a level of 20% w/w surfactant (no other concentrations were tested) (Table 8). As increasing the alkyl chain length of a polyoxyethylene surfactant is well known to decrease the cloud point (Gu and Sjöblom, 1992; Schuber and Kaler, 1996), it is not surprising therefore that the microemulsion formulations utilizing a short saturated alkyl chain surfactant,  $C_{12}E_{10}$  (Table 9) have a higher PIT than those formulated using a longer unsaturated one,  $C_{18:1}E_{10}$  (Table 10). This observation also explains why DOAO and not DDAO exhibits a CP/PIT.

Table 9  
Phase inversion temperature of systems prepared using C<sub>12</sub>E<sub>10</sub>

Oil	%w/w oil	% w/w surfactant <sup>a</sup>											
		10			15			20			25		
		Water	PBS	CPBS	Water	PBS	CPBS	Water	PBS	CPBS	Water	PBS	CPBS
–	–	88.5	81.5	80.2	90.0	83.0	81.6	91.9	86.4	83.2	93.9	87.7	85.3
Soybean oil	1	–	78.5	77.3	84.3	80.7	79.7	87.7	82.5	82.4	90.0	83.5	84.3
	2	–	–	–	–	–	–	91.9	87.2	83.7	93.0	89.0	84.7
Miglyol 812	1	–	–	–	–	75.7	76.3	85.1	79.2	78.7	87.7	80.7	80.2
	2	–	–	–	84.6	79.5	77.0	85.6	81.6	78.3	88.2	84.0	80.1
Tributylin	1	–	–	–	77.4	71.7	70.3	82.9	76.2	74.4	86.1	80.5	77.7
	2	–	–	–	–	–	–	–	–	–	81.5	78.0	72.2
Ethyl oleate	1	76.2	71.3	71.4	80.6	75.1	73.7	84.2	78.7	78.0	86.4	81.2	80.4
	2	–	–	–	76.6	72.5	70.5	79.6	75.5	74.1	83.4	78.7	77.3
Ethyl caprylate	1	68.4	64.6	62.4	75.9	70.7	68.6	79.9	75.6	74.1	84.2	78.5	78.0
	2	57.0	54.6	52.6	67.5	64.2	61.2	71.7	68.9	66.7	76.9	72.9	71.2
	4	–	–	–	53.0	50.0	47.9	57.4	50.4	50.5	63.7	60.4	57.7
	6	–	–	–	–	–	–	51.2	48.3	45.3	51.5	48.7	46.1
Ethyl butyrate	1	74.2	69.1	68.2	80.4	76.0	73.2	83.50	77.7	75.7	85.7	80.4	79.0
	2	63.6	61.4	61.6	71.9	67.1	66.6	77.6	71.3	66.4	80.6	76.4	73.3
	4	45.5	42.6	41.0	56.9	54.6	53.1	64.6	61.5	62.2	70.6	65.9	66.0
	6	–	–	–	44.2	41.5	38.9	50.0	48.7	45.1	61.7	55.0	54.0
	8	–	–	–	–	–	–	42.0	40.0	37.7	45.5	40.5	39.6

<sup>a</sup> Mean of  $n = 4$ ; S.D.  $\pm 1.0$ .

Table 10  
Phase inversion temperature of systems prepared using C<sub>18:1</sub>E<sub>10</sub><sup>a</sup>

Oil	%w/w oil	% w/w surfactant											
		10			15			20			25		
		Water	PBS	CPBS	Water	PBS	CPBS	Water	PBS	CPBS	Water	PBS	CPBS
–	–	58.1	53.7	53.4	60.1	56.7	56.7	61.5	58.0	57.0	60.0	58.4	57.9
Soybean oil	1	57.6	51.5	–	55.7	50.9	50.2	53.2	47.7	48.0	53.50	47.7	48.5
	2	66.4	57.6	56.5	59.7	51.7	54.0	55.6	49.0	50.6	52.5	47.9	46.4
	4	–	–	–	69.2	57.0	58.0	65.0	55.4	56.9	59.0	50.0	52.4
	6	–	–	–	–	–	–	74.0	69.5	68.7	70.2	65.7	64.3
Miglyol 812	1	55.2	48.0	48.7	46.5	45.0	43.5	44.7	42.0	41.8	43.5	40.0	40.8
	2	56.2	55.0	–	53.2	49.7	48.1	47.2	44.5	43.8	43.7	41.6	40.4
	4	–	–	–	59.2	55.6	54.7	53.0	51.0	49.6	49.4	46.5	46.0
	6	–	–	–	–	–	–	60.5	59.5	57.0	56.0	52.2	50.7
Tributyrin	1	36.0	34.0	33.0	33.5	32.5	31.5	35.2	34.0	35.0	39.0	37.0	33.6
	2	–	–	–	32.2	31.2	31.2	30.0	28.0	28.5	30.0	28.2	28.7
	3	–	–	–	–	–	–	31.25	29.5	29.5	29.0	26.5	25.1
Ethyl oleate	1	43.0	43.5	38.7	40.0	37.0	35.1	39.5	38.5	35.0	39.5	36.5	36.8
	2	42.5	43.0	42.5	38.5	37.7	38.2	35.5	32.5	30.9	33.0	32.2	30.2
	4	–	–	–	44.5	44.5	43.7	37.0	35.2	33.2	31.0	29.7	29.6
	6	–	–	–	–	–	–	44.5	40.2	40.2	38.5	34.5	31.7
Ethyl caprylate	1	–	–	–	34.0	30.0	29.5	32.0	29.0	28.5	32.0	27.0	28.0
Ethyl butyrate	1	40.0	36.5	35.5	45.7	43.5	42.5	49.2	44.5	44.0	49.5	47.2	45.5
	2	32.0	28.0	27.5	32.0	28.0	28.0	32.0	28.0	27.2	31.5	28.0	28.0

<sup>a</sup> Mean of  $n = 4$ ; S.D.  $\pm 1.0$ .

### 3.7. The effect of electrolytes on the phase inversion temperature

The presence of electrolytes frequently affects the PIT and consequently the area of microemulsion existence. Most of the salts present in PBS are known to 'salt out' (i.e. reduce the head group hydration of) polyoxyethylene surfactants,

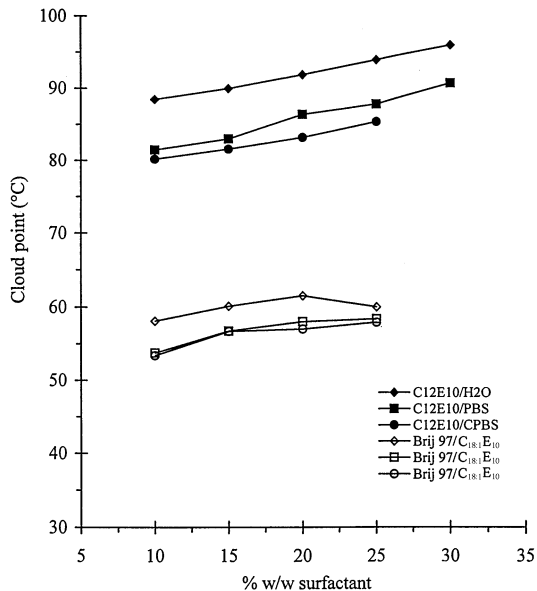


Fig. 5. Variation in the cloud points as a function of  $C_{12}E_{10}$  and  $C_{18:1}E_{10}$  concentration in water, PBS and complete PBS (cPBS).

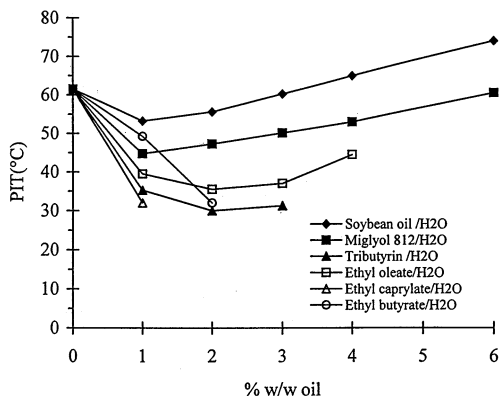


Fig. 6. Phase inversion temperature of microemulsions containing 20% w/w  $C_{18:1}E_{10}$  in water as a function of the amount of oil.

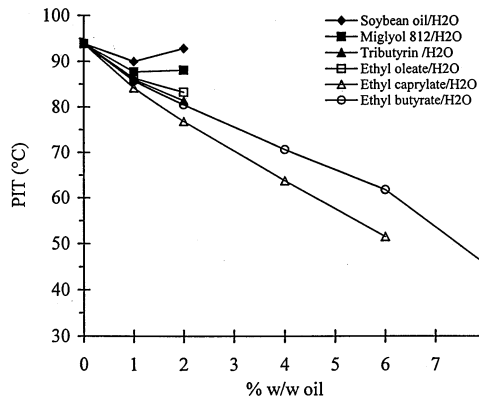


Fig. 7. Phase inversion temperature of microemulsions containing 25% w/w  $C_{12}E_{10}$  in water as a function of the amount of oil.

thereby decreasing the PIT (Schott and Han, 1975; Schott and Royce, 1984). This indeed was the trend observed in the present study, where those microemulsions prepared using PBS as continuous phase exhibited a lowering of the cloud point and PIT compared to those systems prepared in water (Figs. 5–7 and Tables 9 and 10). The additional  $Ca^{2+}$  and  $Mg^{2+}$  ions present in cPBS are known to have a variable effect on the PIT tending to reduce it when present at low concentrations such as in the present study (Schott, 1973; Schott and Royce, 1984). Interestingly although the presence of electrolyte did not cause micellar solutions of DDAO to exhibit a cloud point, micellar solutions of DOAO at a concentration of 20%w/w in PBS did, supporting the hypothesis that the presence of electrolyte causes a growth in the micelle size of the amine-*N*-oxide surfactant, thereby producing a more lipophilic surfactant. (Note that cPBS was not tested as continuous phase.) This is the first time that a cloud point has been reported for an alkylamine-*N*-oxide surfactant, although Satra (1998) has reported that certain lecithin-based DDAO microemulsions that contained polar, low molecular volume oils exhibited a PIT.

### 3.8. The effect of oil on the phase inversion temperature

In addition to the effect of the chemical nature



of the amphiphile and the presence of inorganic salts on the PIT, the nature and amount of oil can also alter the PIT of a microemulsion (Aveyard and Lawless, 1986) with large molecular volume oils tending to raise the PIT while small molecular volume oils initially cause a decrease in the PIT, which may in some instances be followed by an increase. These results are typically explained in terms of the way in which the oils are solubilized into the microemulsion droplets and while this explanation is widely accepted, it must be realized that it is based purely on phenomenological observations (Malcolmson et al., 1998).

Large molecular volume oils are considered to form a core in the centre of the surfactant aggregate, and this can cause one of two effects depending upon the initial shape of the droplet. If the droplet was already spherical, the presence of oil hardly alters the head group area and the hydrophobic volume of the surfactant, thereby allowing the aggregate to still maintain nearly the same degree of curvature and consequently a similar PIT as the corresponding micellar solution (Aveyard and Lawless, 1986). If however the aggregate was originally asymmetric, the addition of oil to form a core encourages a change in aggregate shape from asymmetric to spherical, thereby increasing the PIT of the system. In contrast, at low concentrations, the smaller molecular oils act in much the same way as a cosurfactant, increasing the effective hydrophobe volume (and decreasing the effective length of the hydrophilic head group) of the surfactant, thereby encouraging the formation of more asymmetric aggregates with lower spontaneous curvature and higher intermolecular interactions, and consequently lowering the PIT (Monduzzi et al., 1997). At higher concentrations the small molecular volume oils are thought to go into the core of the droplet, where they have little effect on the hydrophobe volume of the surfactant, transforming the asymmetric droplets to more symmetrical aggregates and restoring the PIT.

Obviously the definition of a 'small' and 'large' molecular volume oil is relative to the surfactant being studied, although it is generally considered that a small oil is one that has a chain length less than that of the hydrophobe of the surfactant,

while oils with alkane chain lengths beyond that of the surfactant are considered to be large (Chen et al., 1986; Evans et al., 1986). Furthermore, it is worth commenting that very little work to date has examined the effect of polar oils, such as those used in the present study, on the PIT of a microemulsion and it is not known whether they act in a similar manner to that described above. However, a recent study suggested that the molecular volume of an oil appeared to be more important in determining phase behaviour than its polarity (Aboofazeli et al., 1995).

In the current study, the PIT of microemulsions prepared with  $C_{18:1}E_{10}$  and  $C_{12}E_{10}$  containing the larger molecular volume oils exhibited rather complex behaviour upon changing the amount of oil incorporated, regardless of the continuous phase used (see Figs. 6 and 7 and Tables 9 and 10). For example the PIT of Brij 97 microemulsions containing soybean oil, Miglyol 812 and ethyl oleate first decreased and then increased as the concentration of oil increased (Fig. 7 and Table 10), suggesting that the micellar aggregates were originally slightly asymmetric, a fact confirmed in a light scattering study (Warisnoicharoen, 1998). The same trend was also observed for microemulsions prepared using  $C_{12}E_{10}$  (Fig. 6 and Table 9) although the effects were not so pronounced in this case due to the limited amount of the larger oils incorporated in these systems. In contrast, however, regardless of the surfactant used the smaller molecular volume oils, tributyrin, ethyl butyrate and ethyl caprylate caused only a decrease in the PIT of the microemulsions irrespective of the surfactant used and the amount of oil added. Also irrespective of the oil incorporated and the surfactant examined the microemulsions prepared using water always exhibited a higher PIT than those prepared in PBS, which in turn were higher than those prepared with cPBS when compared at an equivalent surfactant and oil concentration. Note that although microemulsions prepared with DOAO exhibited a PIT it was difficult to draw definite conclusions about the nature of inclusion of the oils in such a limited study. Interestingly, with both  $C_{18:1}E_{10}$  and DOAO, ethyl caprylate and not ethyl bu-

tyrate exhibited the lowest cloud point. These results suggest that the large molecular volume oils were incorporated into the microemulsions in very different ways than the smaller molecular volume oils.

#### 4. Discussion

Despite a number of attempts (using for example the hydrophile-lipophile balance (HLB) number and critical packing parameter (CPP) of a surfactant) it is not yet possible to predict successfully which combinations of surfactant, (co-surfactant) and oil will produce a microemulsion, although such knowledge would be extremely valuable when formulating pharmaceutical microemulsions. The shortcomings of the presently available prediction methods are well-illustrated in the following example, where  $C_{18:1}AO$  and its saturated counterpart possess virtually identical CPP and HLB numbers, yet only  $C_{18:1}AO$  is capable of forming microemulsions at 298 K. A similar observation has been reported by Malcolmson and Lawrence (1995) for  $C_{18:1}E_{10}$  and its saturated counterpart. This result is undoubtedly due to the high melting point of the long saturated hydrophobe hindering the formation of any surfactant aggregates.

Although there are problems encountered in predicting microemulsion formation it is clear from the results of the present study that certain generalizations can be made about the factors influencing their production. In particular the relative lengths/molecular volumes of the hydrophobic chains of the surfactant and oil are undoubtedly extremely important in determining whether the small or larger molecular volume oils are solubilized to the greater extent. For example when a surfactant containing a C12 hydrophobe is used, the smaller molecular volume oils tend to be solubilized to the greatest extent; a result in agreement with that obtained by other workers. In contrast, however, when a longer alkyl chain surfactant (C18:1) is used, this trend was reversed for microemulsions prepared using  $C_{18:1}E_{10}$  where the larger molecular volume oils were solubilized to the greater extent. This latter observation has

not been previously reported, possibly because surfactants with such long hydrophobes are not routinely used for microemulsion formation. Note that because of the low levels of oil solubilization seen with systems prepared using DOAO it is hard to draw any conclusions about the effect of hydrophobe chain length here, although from the limited data available it appears that, similar to the shorter hydrophobic chain surfactants, the smaller molecular volume oils are solubilized to the greater extent, illustrating the influence of the head group on microemulsion formation.

It is also noteworthy that under some circumstances the oil with the intermediate molecular volume was solubilized to the greatest extent, see for example ethyl caprylate in  $C_{12}E_{10}$  microemulsions at 310 K and Miglyol 812 in  $C_{18:1}E_{10}$  microemulsions also at 310 K. Interestingly McFann and Johnston (1993) have also observed the same trend for a homologous series of alkane oils incorporated into the nonionic nonylphenol ethoxylate surfactant (Igepal CO-520) where the oil hexane was incorporated to the greatest extent, in spite of alkanes with carbon numbers from 3 to 11 being examined. In this case the PIT of the systems they studied varied in a non-linear manner in that the systems containing hexane (C6) exhibited the lowest PIT. A similar trend was also seen in the present study when the PIT were compared for systems prepared with either  $C_{18:1}E_{10}$  or  $C_{12}E_{10}$  and containing ethyl butyrate and ethyl caprylate, and to a more limited extent DOAO microemulsions incorporating the same oils in that the PIT recorded for ethyl caprylate tended to be lower than that seen with ethyl butyrate. From the results of the present study it appears that oils possessing a very small chain length compared to the surfactant hydrophobe may be incorporated into the microemulsion droplet in a different manner to that originally thought, in other words an oil may be too small to act as a cosurfactant, possibly preferring to locate more towards the centre of the aggregate.

Clearly for surfactants containing the same hydrophobic chain length, the level of oil solubilization also depends upon the nature of the head group, for example DDAO microemulsions solubilized more oil than  $C_{12}E_{10}$  microemulsions. It

should be noted that to date, not much work has systematically investigated the effect of changing the nature of the head group on the extent of microemulsion formation because in most cases the systems are complicated by the requirement for a cosurfactant, the two classes of surfactant used in the present study are unusual in not requiring a cosurfactant for microemulsion formation. Although at present it is not possible to explain the reason for the higher oil solubilizing capacity of DDAO, the reason undoubtedly resides in the nature of the interaction of the oil with the different head groups. Indeed recent neutron reflection studies at the air/water interface suggest that the ethyl ester oils, ethyl caprylate and ethyl hexadecanoate, penetrate the interfacial surfactant monolayer produced by DDAO to a greater extent than that of a C12 polyoxyethylene surfactant (Warisnoicharoen, 1998; Lu et al., 1999). Why this differential penetration aids microemulsion formation is not yet understood.

In addition, the differential levels of oil solubilization may also be related to the shape of the aggregate formed by the surfactant prior to incorporation of oil that, for a constant hydrophobic chain surfactant, is obviously related to the nature of the interactions between the head groups. Table 11 shows the aggregation numbers obtained for micelles of each of the surfactants obtained from a total intensity light scattering study (Warisnoicharoen, 1998). Simple geometric considerations demonstrate that, in fact, none of the surfactants are likely to form spherical micelles as their aggregation numbers are too high to be

accommodated within a sphere (Tanford, 1974). However it is not unreasonable to consider the aggregates formed by the C12 surfactants as approximating to spheres, as their aggregation numbers are not much greater than those required for spherical aggregates. In contrast, because of the much higher aggregation numbers, the aggregates formed by the C18:1 hydrophobic chain surfactants are likely to be asymmetric. Note that while light scattering studies supported the presence of asymmetric aggregates for DOAO, no asymmetry was detected for C<sub>18:1</sub>E<sub>10</sub>, undoubtedly because the aggregates were too small to be detected by this method. Consideration of the PIT temperature studies however supports the fact that C<sub>18:1</sub>E<sub>10</sub> does indeed form asymmetric aggregates as the PIT temperature increases when a significant amount of the larger molecular volume oils are incorporated, taken as a sign that the oil is causing a transformation to a spherical droplet. Although interestingly these oils first cause a decrease in the PIT suggesting that initially they penetrate the surfactant monolayer. This transformation is obviously due to the fact that the C<sub>18:1</sub>E<sub>10</sub> aggregates are able to swell thereby accommodating the oil with the ability to swell being related to the mutual solubility of hydrocarbon and oxyethylene. The fact that the aggregates formed by DOAO can not incorporate much oil may be due to the different interaction of the oil with the surfactant head groups together with the fact that the surfactant behaves as a much more hydrophobic molecule than C<sub>18:1</sub>E<sub>10</sub>.

Interestingly while the spherical micelles formed by the C12 surfactants can accommodate relatively large amounts of the lower molecular weight oils that are thought to act more as cosurfactants, they can not readily 'swell' to incorporate the large molecular volume oils such as soybean and Miglyol 812, most probably because they already exist in a spherical form.

Table 11

Total intensity light scattering results for C<sub>12</sub>E<sub>10</sub>, DDAO, C<sub>18:1</sub>E<sub>10</sub> and DOAO in water and PBS at 298 K

Surfactant	Aggregation number	
	Water	PBS
C <sub>12</sub> E <sub>10</sub>	59	65
C <sub>18:1</sub> E <sub>10</sub>	307	352
DDAO	51	54
DODA <sup>a</sup>	1167–3249	N/D

<sup>a</sup> From reference Imae and Ikeda (1984).

## 5. Conclusion

The findings of the present study suggest that it is possible to formulate cosurfactant-free o/w microemulsions suitable for use as drug delivery

vehicles using either polyoxyethylene surfactants or amine-*N*-oxide surfactants. These microemulsions have several advantages over the more widely examined microemulsions for pharmaceutical purposes, in particular because they possess an ability to be diluted without destroying their integrity. Unfortunately however both classes of surfactant are sensitive to the presence of electrolyte, this is especially true if a more hydrophobic surfactant is used to produce the microemulsion for example DOAO and C<sub>18:1</sub>E<sub>10</sub> are more sensitive to electrolyte at the experimental temperature examined.

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