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Formulation of electrically conducting microemulsion-based organogels

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Abstract

Gelatin-containing, electrically conducting, rigid water-in-oil (w/o) microemulsion-based organogels (MBG), both with and without the presence of a model drug, have been prepared using pharmaceutically acceptable oils and surfactants. As a precursor to MBG formation, preliminary formulation work was carried out investigating the factors affecting the preparation of w/o microemulsions containing large amounts of dispersed aqueous phase. From these studies isopropyl myristate (IPM) was favoured as oil due to its ability to support w/o microemulsion formation over a wide range of compositions. The single most effective surfactant for stabilising the w/o microemulsions was found to be Aerosol-OT (AOT), although synergistic effects on the extent of w/o microemulsion formation were observed upon its combination with a variety of non-ionic surfactants. Upon addition of gelatin to the w/o microemulsion, MBG could be formed when using AOT as stabiliser with most of the oils investigated (with the exception of the medium and large triglyceride oils, Miglyol 812 and soybean oil, respectively) and with a number of AOT/non-ionic surfactant/IPM combinations (both in the presence and absence of model drugs such as sodium salicylate). MBG could not however be formed with non-ionic surfactants alone, or when used in combination with another non-ionic surfactant (regardless of the oil used). This latter observation was found to be not only a result of the inadequate level of water available for hydration of the surfactant head group and any gelatin present but also a consequence of the inability of these systems to form, upon heating, the percolated microstructures necessary to facilitate the supramolecular assembly of gelatin at the macroscopic level, a pre-requisite for MBG formation.

Keywords: Formulation; Microemulsion; Organogel; Phase behaviour; Transdermal drug delivery

1. Introduction

Microemulsion-based organogels (MBG) are clear, stable one phase systems consisting of water in oil dispersions, stabilised by a surfactant and which have been gelled using gelatin (Haering and Luisi, 1986; Quellet and Eicke, 1986). MBG are formed by the addition of solid gelatin or a concentrated aqueous gelatin solution to a water-in-oil (w/o) microemulsion or reverse micelle solution, respectively (Haering and Luisi, 1986;

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Quellet and Eicke, 1986). Although MBG have not been widely studied to date, they have been well characterised both in terms of their microstructure (Luisi et al., 1990; Atkinson et al., 1991; Quellet et al., 1991) and their bulk physicochemical properties (Atkinson et al., 1991; Rees et al., 1991; Jenta et al., 1997). While there are subtle differences in detail between the various MBG structures proposed, these apparent discrepancies are most likely to be a consequence of the different compositions studied by each research group. Common to each of the models however is the presence of an interconnected gelatin network which is hydrated and stabilised against direct contact with the continuous oil phase, by a monolayer of surfactant. One important consequence of this hydrated gelatin network is the formation of gel matrices of comparable viscosity to those observed with conventional hydrogels (Kantaria et al., 1999); another is the ability of MBG to conduct electricity. Both of these properties are unique among organogels and make MBG potential vehicles for the transdermal delivery of water-soluble drug using iontophoresis (Kantaria et al., 1999).

Other properties that make MBG attractive as iontophoretic drug delivery vehicles include their potential to increase the maximum loading of a water-soluble agent by an order of magnitude in comparison to their parent w/o microemulsion (Jenta et al., 1997). Although this enhancement is achieved principally because the volume fraction of the aqueous phase in MBG is typically much higher than in a conventional w/o microemulsion, it must be recognised that not all the additional water will be in its 'free' form due to the requirement for any gelatin present to be hydrated. Other advantages of MBG include their ability to tolerate relatively high concentrations of additives without undergoing the (unfavourable) phase transitions often seen in w/o microemulsions. Additional benefits relate to their general robustness and ease of use, and to the fact that the MBG provide an environment in which drug stability may be improved, especially in comparison to the use of hydrogels where the presence of an aqueous continuous phase may allow degradative processes to occur. Finally the use of a topical vehicle with an oily continuous phase should result in a reduced water loss from the skin, enhancing the iontophorectic delivery of drug.

The aim of the present study was to formulate MBG suitable for transdermal iontophoretic drug delivery. Almost without exception previous MBG formulations have been primarily based on hydrocarbon oils, and have virtually always employed salts (almost exclusively the sodium salt) of the anionic surfactant bis-2-(ethylhexyl) sulphosuccinate (AOT). As such materials are generally regarded as pharmaceutically unacceptable, we have endeavoured in the present study to replace both oil and surfactant with more suitable ingredients, such as ethyl ester oils and non-ionic surfactants.

One difficulty encountered when attempting to replace AOT when formulating a MBG is the requirement for the w/o microemulsion to percolate upon approaching the upper temperature phase boundary (UTPB; Atkinson et al., 1991). Percolation, which is a consequence of increasingly 'elastic' collisions between the individual w/o microemulsion droplets, results in individual microemulsion droplets coalescing to form aggregated clusters and channels. It is these electrically conducting, percolated structures that facilitate the formation of the gelatin network throughout the system. Provided that other factors are favourable, such as the percolation temperature being slightly higher than the gelling temperature of the gelatin (ca. 30 °C) then a MBG may form upon cooling the system to a temperature below the UTPB of the original w/o microemulsion. Unfortunately for the pharmaceutical applications of MBG, it has been established that non-ionic surfactants percolate upon cooling rather than heating (Lipgens et al., 1998) making the formation of a gelatin-stabilised MGB from a w/o microemulsion stabilised by a non-ionic surfactant impossible. Encouragingly however there has recently been a very limited amount of work in the literature that shows it is possible to form w/o microemulsions that percolate at the UTPB and in which some of AOT has been replaced by non-ionic surfactant (Lui et al., 1998). This observation suggests that it may be possible to form MBG from w/o systems stabilised by AOT in combination with non-ionic surfactant. To date, however, most work in the

literature examining the percolation of AOT containing w/o microemulsions has used low molecular weight aliphatic oils such as hexane and octane as the continuous phase. Yet for the purposes of pharmaceutical formulation it is large molecular volume polar oils such as soybean oil that are preferred. Unfortunately it has been suggested that an increase in the rigidity of the interfacial surfactant film reduces the tendency of the microemulsion to percolate (Lui et al., 1998) and it seems probable, therefore, that the large molecular volume oils favoured in the present study, would increase the rigidity of the interfacial surfactant film. Furthermore the presence of electrolyte, such as NaCl has been reported to increase the percolation temperature, with high electrolyte concentrations leading to the abolition of percolation (Lui et al., 1998). As most drugs are salts their presence in the w/o microemulsions may affect the latter's ability to percolate. All these factors need to be considered when attempting to formulate pharmaceutically acceptable MBG.

2. Materials and methods

2.1. Materials

Sodium salicylate and the sodium salt of AOT (HLB: 10.2, RMM 444.6) were supplied by BDH. Miglyol 812N, a medium chain triglyceride, was a gift from Hüls (Germany). Polyoxyethylene (20) sorbitan monolaurate (Tween 20, HLB: 16.7, RMM 1128), polyoxyethylene (4) sorbitan monolaurate (Tween 21, HLB: 13.3, RMM 523), polyoxyethylene (20) sorbitan monooleate (Tween 80, HLB: 15.0, RMM 1310), polyoxyethylene (5) sorbitan monooleate (Tween 81, HLB: 10.0, RMM 645), polyoxyethylene (20) sorbitan trioleate (Tween 85, HLB: 11.0, RMM 1839), sorbitan trioleate (Span 85, HLB: 1.8, RMM 958), sorbitan monooleate (Span 80, HLB: 4.3, RMM 429), sorbitan monolaurate (Span 20, HLB: 8.6, RMM 346), polyoxyethylene (4) lauryl ether ($C_{12}E_4$, HLB: 9.7, RMM 363), polyoxyethylene (10) lauryl ether ($C_{12}E_{10}$, HLB: 14.0, RMM 626), polyoxyethylene (10) oleyl ether ($C_{18:1}E_{10}$, HLB: 12.4, RMM 708) and polyoxyethylene (20) oleyl ether ($C_{18:1}E_{20}$, HLB: 15.3, RMM 1148) were purchased from Sigma (UK). Polyethoxyethylene (25) glycerol trioleate (Tagat TO, HLB: 11.3, RMM 1982) and polyoxyethylene (6) lauryl ether ($C_{12}E_6$, HLB: 10.6, RMM 451) were gifts from Th Goldschmidt AG (Germany) and Rewo Chemicals Ltd. (UK), respectively. The HLB of the various surfactant mixtures examined was determined using the weight fractions of the surfactants. For example the HLB of the 1:1 weight ratio mixture of Tween 85 and Span 20 was calculated as $0.5 \times 11.0 + 0.5 \times 8.6 = 9.8$. All surfactants were used as supplied without further purification. Triglyceride oils and fatty acid esters were supplied by Fluka (UK). Acid-hydrolysed pig-skin gelatin (Bloom Number 300) was obtained from Sigma (UK). Triply distilled water was used throughout.

2.2. Examination of phase behaviour

Partial ternary or pseudo-ternary phase diagrams were constructed from data obtained by titrating aliquots of the disperse phase, either water or aqueous drug solution, into mixtures of oil and surfactant and converting the volume of the added solution into weight using the density of the aqueous solution determined using pycnometry. After each addition of aqueous phase the mixture was stirred briefly and visually inspected. It should be noted that in some instances it was not possible to determine the phase behaviour of systems containing high concentrations of surfactant because of their gel-like behaviour. Each sample was checked for birefringence using a polarised light source. Clear, fluid, non-birefringent fluid systems were classed as microemulsions and were stable for at least 1 month. Systems exhibiting birefringence or streaming birefringence were classed as liquid crystalline, although no attempt was made to examine the long term stability of these phases. Furthermore no distinction was made between oil-in-water (o/w), w/o and bicontinuous microemulsions, although the one phase, clear, non-birefringent systems of interest seen in the water poor region of the phase diagram in the present study are most probably w/o microemulsions. The upper limit of water solubilisation of these systems was characterised by the

parameter $\omega_{o,max}$, where ω_o is the mole ratio of water to surfactant, i.e. $\omega_o = [H_2O]/[Surfactant]$. Note that unless otherwise stated, drug concentrations quoted in the text refer to concentrations in the aqueous phase.

2.3. MBG preparation

The MBGs were typically prepared by addition of 1.5 g powdered gelatin (Bloom number 300) to 10 g of a mixture comprising of 25% w/w surfactant, 20% w/w aqueous phase and 50% w/w oil at 25 °C, leading to a final sample composition of 13% w/w gelatin, 21.7% w/w surfactant, 17.4% water. Note that, in some instances, the composition of the initial mixture before the addition of gelatin, was outside the region of existence of a single phase w/o microemulsion. The temperature of the mixture was then increased to 55 °C with constant stirring until solubilisation of the gelatin was complete. Agitation was then stopped and the temperature of the sample returned to ambient. Formulations yielding clear, homogeneous, non-birefringent gels were examined for their ability to conduct electricity using an AC of 50 Hz (a DC caused electrolysis of the gels); the voltage was altered in 0.2 V steps over the range 0–1.2 V. Only gels that were electrically conducting were classified as MBG. Note however that all the clear, homogeneous, non-birefringent gels tested did conduct electricity.

All MBGs referred to in the present study were prepared as described above, however it is worth mentioning that a number of other systems were also tested for their ability to form MBGs, namely systems with initial surfactant concentrations in the range 10–25% w/w and initial aqueous phase concentrations in the range 5–20% w/w at 5% w/w intervals. In addition, varying amounts of gelatin was added for gelation purposes (final concentrations in the range 4.8–16.7% w/w). The conclusion from these studies was that as the surfactant concentration was reduced, the amount of water and gelatin required for MBG formation was also reduced. However it was found that if a system did not gel using the composition reported in this paper (namely an initial surfactant concentration of 25% w/w and 20% water) then it did not gel at the other compositions investigated but not reported in the study. It should be noted however that just because a system gelled under the conditions reported in the present study, it did not necessarily mean that it gelled at all or indeed any of the other compositions tested.

Furthermore in some case cloudy, slightly cloudy or birefringent gels were formed. By definition these systems were not MGB, however, cloudy gels could often be transformed into MBG by the addition of slightly more aqueous phase to the initial mixture. Interestingly both the cloudy and birefringent gels were also found to conduct electricity.

3. Results and discussion

3.1. W/o microemulsion phase behaviour

Prior to attempts to formulate MBG using the various pharmaceutically acceptable surfactants and oils, it was of interest to determine the extent of formation of a clear, one phase (microemulsion) region in the oil rich part of the phase diagram. This was especially of interest because of the work of Atkinson et al. (1991) which has shown that with AOT-stabilised MBG that there needs to be a minimum amount of water in the system to hydrate both the surfactant and the gelatin, as well as sufficient surfactant in the system to adequately stabilise the gelatin by preventing its intimate contact with the hydrophobic oil phase (Haering and Luisi, 1986; Atkinson et al., 1991). Significantly Atkinson et al. (1991) found that this minimum amount of water increased in the presence of electrolyte, most likely as a consequence of the electrolyte reducing surfactant headgroup repulsions and thereby increasing the lipophilicity of AOT. This observation has important consequences if charged drugs are to be added to MBG. In this context it may be expected that the replacement of some of the AOT in the w/o microemulsion by a non-ionic surfactant would be beneficial as it would reduce the sensitivity of the microemulsion to the presence of electrolyte (Hussain et al., 1997). Although it is acknowledged that high concentrations of electrolyte can also

increase, albeit to a lower extent, the effective hydrophobicity of non-ionic surfactants, possibly counteracting their beneficial effects (Warisnoicharoen et al., 2000).

Phase behaviour studies were undertaken using the anionic surfactant AOT and non-ionics belonging to the following classes: (i) polysorbates and sorbitan esters; (ii) glycerol polyethoxylates and (iii) *n*-alkyl polyethoxyethylene ethers, tested alone or in combination, with a variety of oil phases including hydrocarbons, liquid fatty acid esters and triglycerides. The aqueous phase for the most part was either distilled water or solutions of the model drug sodium salicylate at 5 or 10% w/w in the aqueous phase, although a small number of experiments were undertaken using alternative model drugs, namely aniline hydrochloride and benzamide. It is of note that virtually no data exists in the literature reporting the phase behaviour, in particular the formation of w/o microemulsions, with the components of interest, most work to date being performed on combinations of components unacceptable for pharmaceutical use. In particular there is little work describing the ability of pharmaceutically acceptable oils, which tend to be large and polar in nature, to support formation of w/o microemulsions.

Representative illustrations of the phase behaviour for fluid, one phase, clear oil rich (microemulsion) systems are shown as partial ternary phase diagrams in Figs. 1–4. Note that unless some further studies, such as light or neutron scattering, or NMR self-diffusion measurements are performed to determine the microstructure of the clear, fluid, one phase system, it is not possible to state unambiguously whether a microemulsion has been formed, although it is unlikely that any of the clear isotropic systems seen in the present study are cosolvent systems.

A summary of the data relevant to our stated aim of formulating new pharmaceutically acceptable MBGs obtained from the results of the phase behaviour studies is given in Tables 1–6. For simplification purposes, the water solubilisation capacity is only given for samples containing 25% w/w surfactant. This nominal surfactant concentration was selected on the basis that the level of water incorporation seen was reasonably representative of the ability of that system to form a w/o microemulsion. For example from the partial phase diagrams shown (Figs. 1–4), it can be seen that the use of this single surfactant concentration for characterising microemulsion formation is a good indicator of the ability of that system to form a w/o microemulsion over a wide range of compositions. Furthermore it was this surfactant concentration that was primarily tested for its ability to form MBG on the basis that such a surfactant concentration would be able to solubilise a sufficiently high amount of water whilst at the same time form spherical w/o microemulsion droplets.

With the exception of AOT, where a summary of the results of the studies obtained for all oils tested is given, only the data obtained with the oil isopropyl myristate (IPM) are quoted for the non-ionic surfactants and their mixtures (Tables 1–6). This is because assessment of the extent of the w/o microemulsion formation showed that, with relatively few exceptions, the general trend of the results obtained with AOT was repeated with the different non-ionic surfactants and combinations thereof (Kantaria, 1998). In addition, the biocompatibility of IPM led to its selection as the oil of choice for further detailed study. The use of IPM has the additional advantage of being a commonly employed non-toxic skin penetration enhancer (Kibbe, 2000).

It is clear from the results summarised in Tables 1–6 that the most effective surfactant used on its own with IPM as oil for microemulsification purposes at 25 °C was AOT. Furthermore, not only does AOT form w/o microemulsions with all the oils tested, but it also has the greatest water solubilisation capacity when the aqueous phase contains moderately high drug loadings (Tables 5 and 6). It is worth commenting that the synergism so often exhibited by surfactant mixtures in emulsion and microemulsion stabilisation was apparent in the behaviour of mixed surfactant systems, in particular when certain non-ionic surfactants were used in combination with AOT. Thus inspection of Tables 3 and 4 shows clearly that the water solubilisation capacity is frequently greater for the surfactant mixtures than it is for either surfactant alone, although there was a

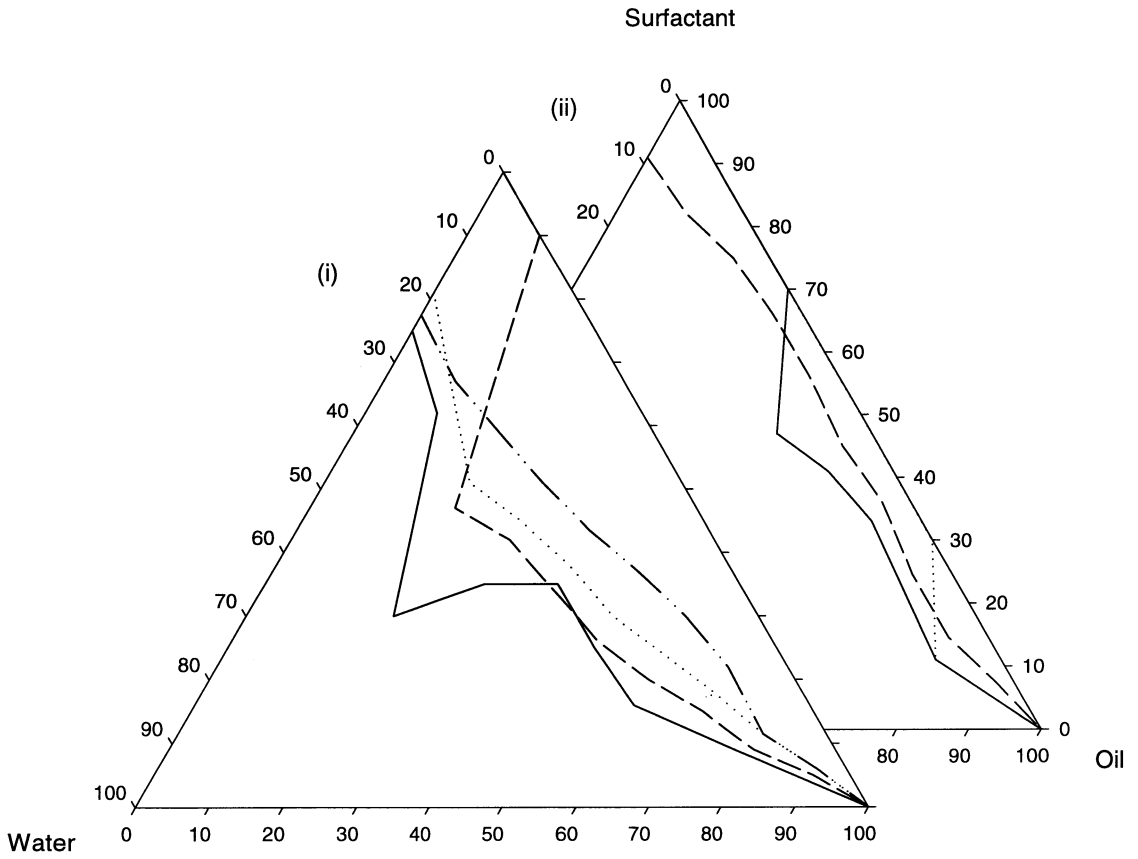

Fig. 1. The effect of oil selection on the partial ternary phase diagrams of AOT/oil/H_2O at 25 °C. Unless otherwise indicated compositions to the right of the phase boundary are optically clear, single phase microemulsions. Diagram (i) heptane (solid), ethyl butyrate (dash dot), ethyl caprylate (dot), IPM (dash); (ii) Miglyol 812 (solid), soybean oil (dot) and tributyrin (dash).

tendency for the area of microemulsion existence to decrease as the proportion of non-ionic surfactant increased. This surfactant synergy is also evident in the partial ternary phase diagrams shown (Fig. 4) for mixtures of Tween 85 and AOT with IPM/H_2O at 25 °C, suggesting that it may be possible to produce MBG from systems stabilised by combinations of AOT and non-ionic surfactant using IPM as oil. This synergistic behaviour was also observed using mixtures of non-ionics such as Tween 85 or Tagat TO in combination with Span 20 (Table 3), although the shifts in the position of the phase boundary were much less dramatic compared with those seen when AOT was used as one of the surfactants.

Interestingly, while the Hydrophile–Lipophile Balance (HLB) has long been used to classify surfactants and their ability to form emulsions, and more recently microemulsions, there was no obvious correlation found between the HLB of the surfactant and the extent of microemulsion formation in this particular study. This observation can be clearly seen by examination of Table 2 which lists both the HLB of the surfactant and the maximum level of water incorporation achieved using 25% w/w surfactant together with IPM as oil. It is obvious that there is no clear trend in the level of oil incorporation with HLB, although there was a slight tendency for surfactants possessing a HLB of around 10 (note that AOT possesses a HLB of 10.2) to exhibit the largest area of microemulsion existence. The poor correlation of HLB with microemulsion phase behaviour is, however, well known and means that it is not

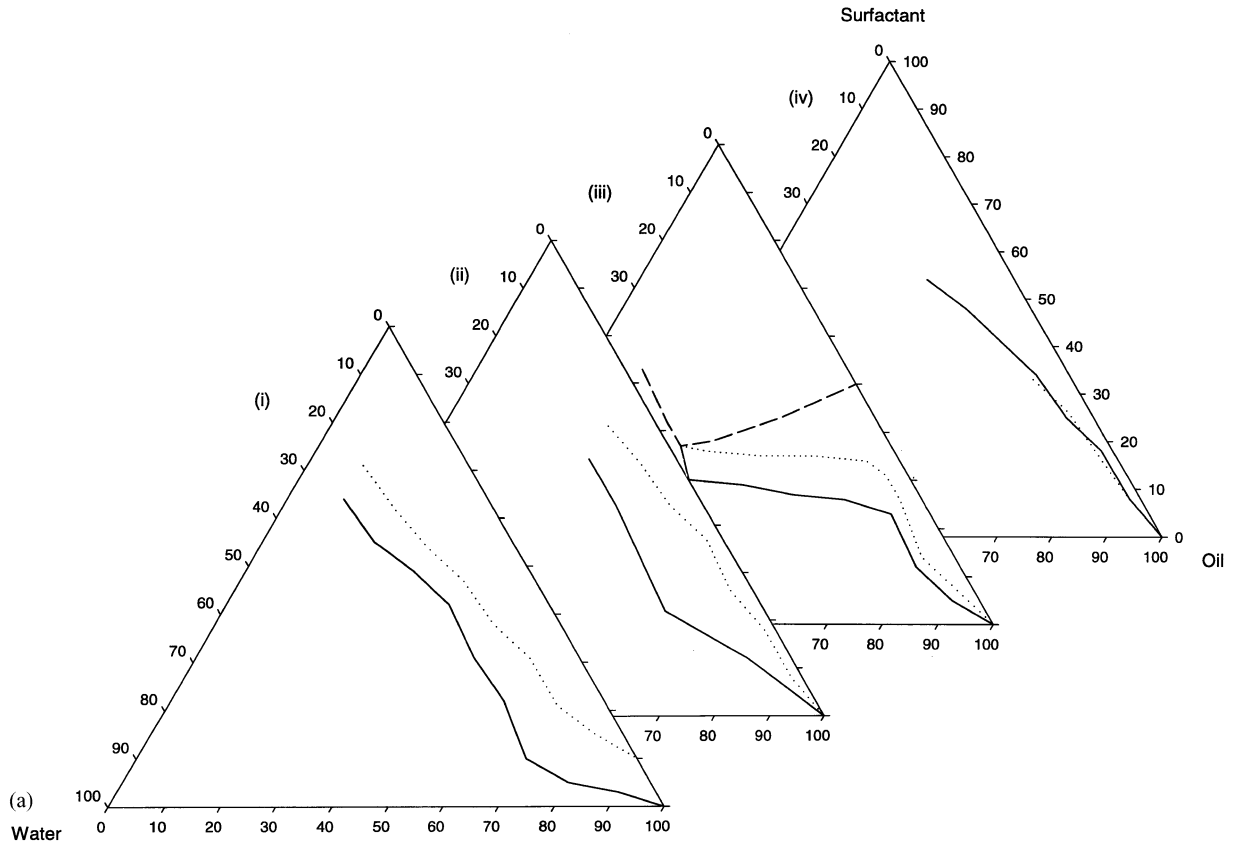

Fig. 2. The effect of oil selection on the partial ternary phase diagrams of Tween/oil/H_2O at 25 °C. Unless otherwise indicated compositions to the right of the phase boundary are optically clear, single phase microemulsions. Diagram (a) Tween 21 (i) heptane-upper water limit (solid) lower water limit (dot); (ii) ethyl caprylate-upper water limit (solid) lower water limit (dot); (iii) IPM-upper water limit (solid), lower water limit (dot) (note that the second small clear, microemulsion region is not marked), clear system formed within area marked by dashed lines; (iv) Miglyol 812 (solid), soybean oil (dot) (b) Tween 81 and (c) Tween 85 in *n*-heptane (solid), ethyl caprylate (dot), IPM (dash), Miglyol 812 (dash–dot–dot) and soybean oil (long dash).

possible readily to predict which surfactant or surfactant mixture is best to use for microemulsion formation and demonstrates the need for the type of study performed here.

For each of the surfactants examined (data only shown for AOT, Table 1) the extent of micro-emulsification depended on the choice of oil phase. On the basis of existing literature however it would be expected that the extent of the single phase region would shrink in response to increasing molecular weight and volume of the oil (McFann and Johnson, 1993.). While this trend was in part followed in the present study there are some noticeable exceptions. For example, in the large majority of surfactant systems studied, water incorporation decreased as the nature of the oil changed in the following order *n*-heptane > IPM > ethyl caprylate > Miglyol 812 and soybean oil, with, in many instances, IPM containing systems surprisingly solubilising comparable levels of water to those prepared with *n*-heptane. As the molecular volumes of these oils are 245, 573, 340, 925 and 1563 $Å^3$, respectively (Malcomson et al., 1998), these results show that, on the basis of molecular volume, microemulsions prepared with IPM unexpectedly solubilise more water than those prepared using ethyl caprylate. In addition when using AOT as sole surfactant (Table 1) it can be seen that a much smaller region of microemulsion existence was seen when hexadecane (mole-

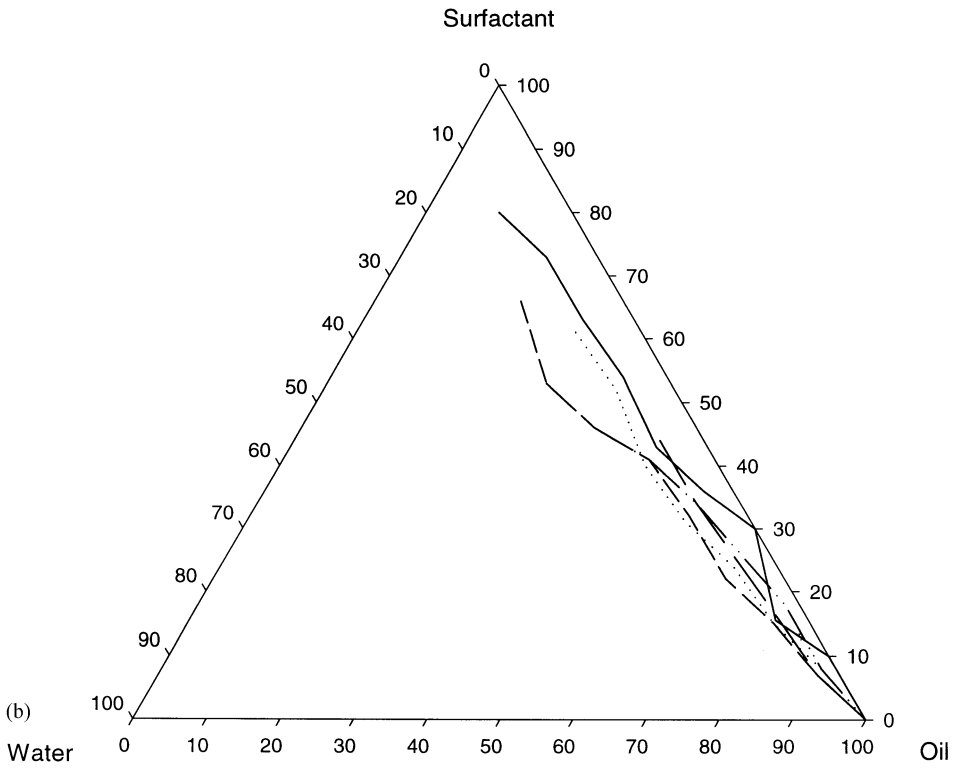

Fig. 2 (*Continued*)

cular volume 488 Å^3) as opposed to when IPM or IPP was used as oil. Furthermore, although ethyl butyrate (molecular volume 232 Å^3) was expected, on the basis of its molecular volume, to solubilise large amounts of water, it was in fact far poorer at supporting microemulsification than oils of a larger molecular volume such as ethyl caprylate or IPM. These results show that introducing polar moieties into the oil can have a significant effect on the extent of microemulsion formation, an effect hitherto not widely studied. To date there are very few reports in the literature determining microemulsion phase behaviour using very small molecular volume oils. Of these, the work of McFann and Johnson (1983) using (compressible) hydrocarbons such as butane demonstrates an opposite trend to that generally accepted, in that the extent of w/o microemulsion formation decreased with a decrease in the molecular volume of the oil. In this study optimal solubilisation was seen with oils of carbon chain length around C6-8.

The results obtained in the present study are indicative of the complex interplay of factors which affect the phase behaviour, although it is likely that the variation of water solubilisation capacity seen is largely the result of the differing extents of solvent penetration into the surfactant tail region. A reduced level of penetration would be expected to result in less favourable hydrophobic interactions, and a reduced entropic contribution to the Gibbs free energy of formation from the motionally restricted hydrocarbon tails. It must be remembered that the use of a wide range of surfactants with varying head group and hydrophobe, together with oils of varying sizes and polarity makes the detailed interpretation of the results difficult. Further complicating the interpretation is the use of a single surfactant concentration to compare the data.

It should be noted that incorporation of water-soluble model drugs, into the various formulations had a variable effect on the extent of microemul-

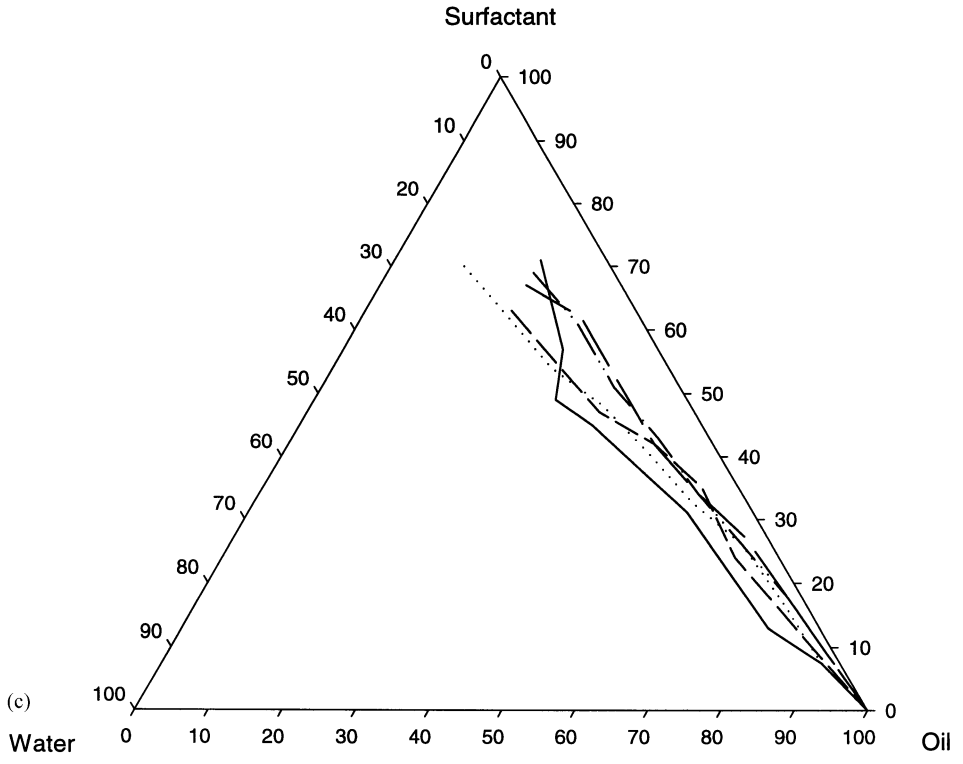

Fig. 2 (*Continued*)

sion formation (Tables 5 and 6). This can be clearly illustrated by the effect of the presence of drug on the extent of the area of microemulsion formation by the mixed surfactant systems. For example in systems containing AOT and either Tween 85, Tagot TO, $C_{12}E_4$, $C_{12}E_6$ or $C_{12}E_{10,}$ an increase in the extent of the area of microemulsion existence was seen, while slightly less solubilisation was seen in the systems containing Span 20, Tween 81 or Tween 21 and AOT. Interestingly the systems containing Tween 80 gave extremely variable results. The two systems worthy of note were those stabilised by Tween 85/AOT and $C_{12}E_4$/AOT, both of which were able to solubilise 50% more sodium salicylate than the comparable microemulsion stabilised by AOT alone. When considering these results it is worth commenting that sodium salicylate is itself a hydrotrope and consequence may not only reside in the disperse aqueous phase but may also be present in the interfacial surfactant monolayer. Indeed previous studies have shown that sodium salicylate is capable of penetrating into an interfacial surfactant layer (Ward and Osborne, 1988).

In conclusion the data, summarised in Tables 5 and 6, show that it was possible to formulate microemulsions containing moderate overall drug concentrations, typically at levels around 1–3% w/w of microemulsion and that the presence of drug may actually have a beneficial effect on the extent of microemulsion formation.

3.2. MBG phase behaviour and formulation

Included in Tables 1–6 are data relating to the ability of the systems tested to form MBG. Also listed is the number of moles of water per mole of surfactant at the composition used to test microemulsion formation. It can be seen that in many cases, the amount of water incorporated under such conditions is insufficient to satisfy the hydration of the surfactant(s) head group (assuming a

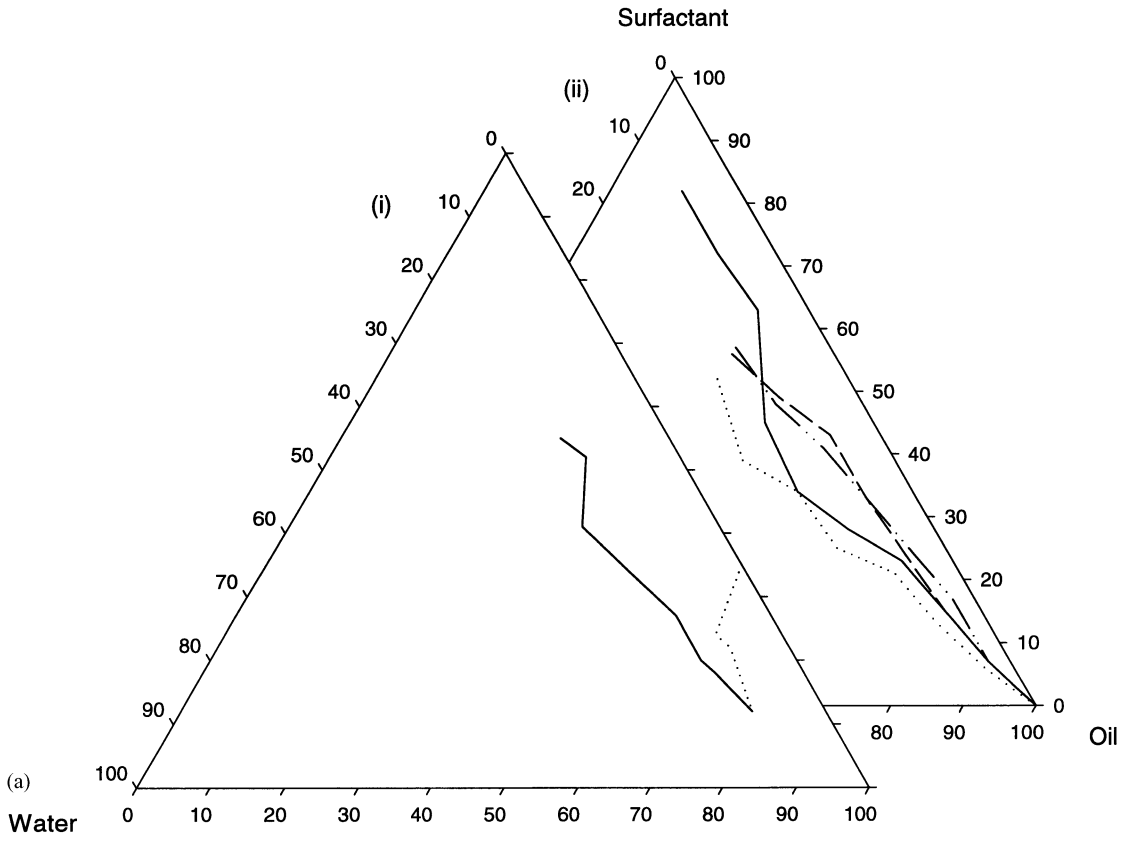

Fig. 3. The effect of oil selection on the partial ternary phase diagrams at 25 °C of oil/H_2O and (a) Tagat TO (i) heptane-upper water limit (solid), lower water limit (dot); (ii) ethyl caprylate (dot), IPM (dash), Miglyol 812 (dash-dot-dot) and soybean oil (long dash) or (b) $C_{12}E_4$ (i) heptane; (ii) IPM-two enclosed areas represent microemulsion region; (iii) ethyl caprylate (dot), Miglyol 812 (dash–dot–dot) and soybean oil (long dash). Unless otherwise indicated compositions to the right of the phase boundary are optically clear, single phase microemulsions.

hydration level of six water molecules per AOT head group (Aliotta et al., 1996) and two water molecules per ethylene oxide unit (Ravey and Buzier, 1984), let alone hydrate any gelatin present. As a consequence most systems under study should more correctly be considered as hydrated or partially hydrated reverse micelles, rather than w/o microemulsions. As such, the scope for solubilisation and solvation of other ionic species, including biopolymers such as gelatin, is probably limited. It is worth commenting that in many instances the composition tested for its ability to form MBG would, at the outset, have comprised more than one phase, suggesting the presence of a large excess of water. Interestingly the addition of gelatin to such multiphase mixtures in many cases caused the system to undergo a transition to a clear one phase system, undoubtedly as a consequence of the requirement for gelatin to be hydrated, thereby 'mopping up' any 'free' water available. Frequently high concentrations of gelatin were required to be present before the system became clear.

The data summarised in Tables 1–6 show that the MBGs in general were more difficult to formulate than the parent microemulsions, which was as expected. AOT once again proved the most versatile surfactant stabilising MBGs in six out of the nine tested oils as shown in Table 1. Notably MBG formation was least successful in oils characterised by the possession of a large molecular volume, namely the triglycerides. This was

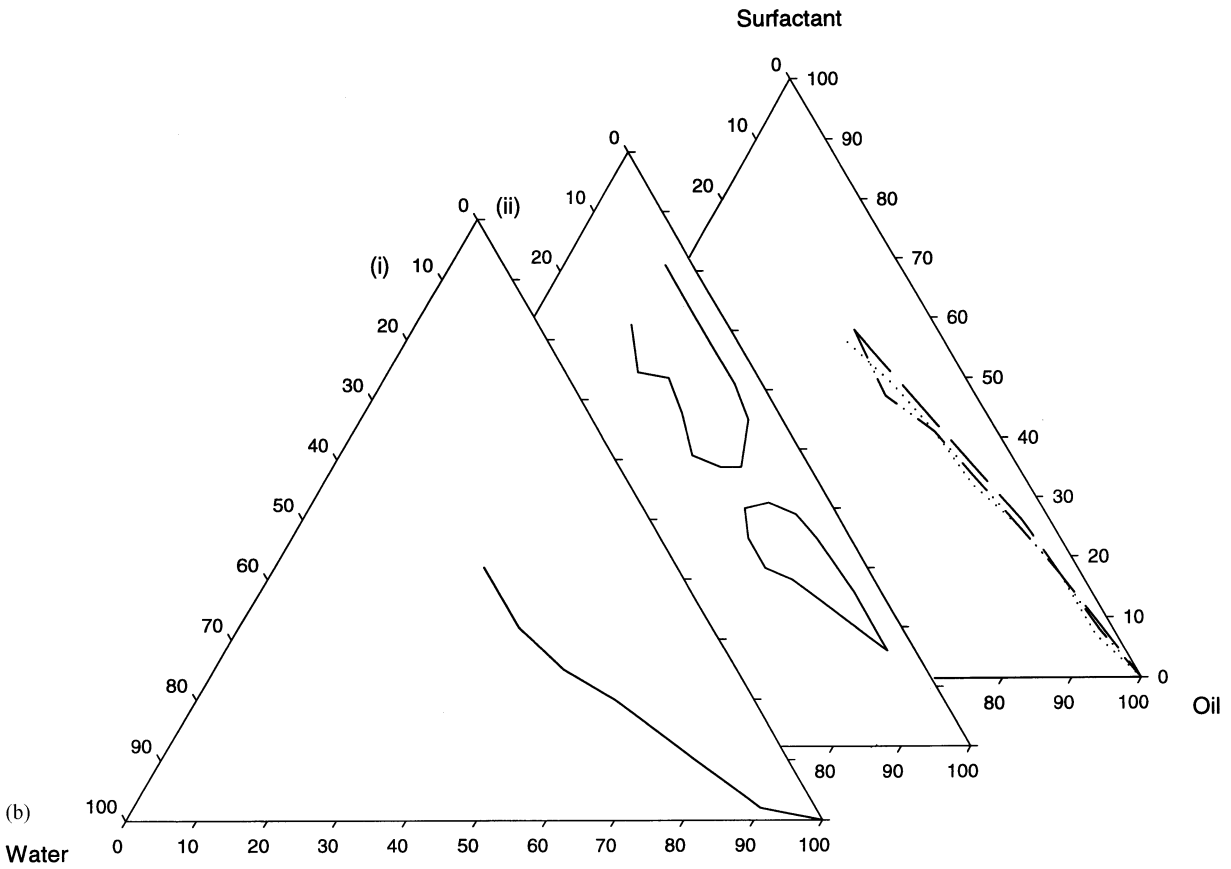

Fig. 3 (*Continued*)

probably due to the fact that the large oils did not penetrate the hydrophobic chain of the interfacial surfactant film and thereby increased the rigidity of the interfacial surfactant film. Furthermore the MBG formed using AOT and IPM were effective in solubilising a variety of different model drugs, in particular sodium salicylate, as indicated in Table 5. The non-ionics in contrast were unable to stabilise the formation of MBG in any of the selected oils when employed as the sole surfactant. This was the case even when the surfactants had exhibited water solubilisation capacities in the region of that seen with AOT, for example Span 85 in heptane (13%w/w water solubilisation at 25%w/w surfactant), $C_{12}E_4$ in *n*-heptane (namely 18% water solubilisation at 25%w/w surfactant) and $C_{18:1}E_{10}$ and Tagat TO in ethyl caprylate (namely 15% water solubilisation at 25%w/w surfactant). This observation was as expected and supports the fact that percolation at the UTPB is necessary for microemulsion formation.

Given that the combination of non-ionic surfactants with AOT had proved effective in increasing the water solubilisation capacity of microemulsions as described earlier, it was encouraging that (as shown in Table 4b and c) IPM-containing MBG could be formulated using binary mixtures of AOT and non-ionic surfactants (the only two non-ionic surfactants tested which could not be formulated with AOT to give an MBG being $C_{12}E_4$ and $C_{12}E_6$). Since non-ionics alone do not form MBG it is clear that there must be a minimum AOT requirement for successful MBG formation, the value of which would be expected to vary according to the exact nature of the non-ionic surfactant and was not determined in the

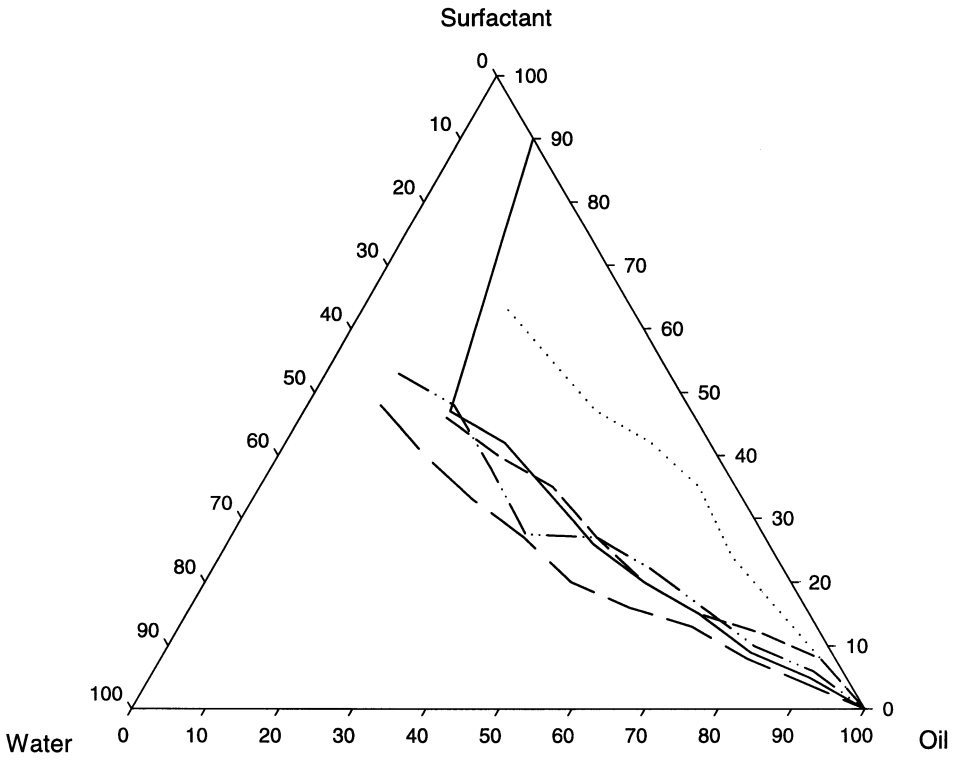

Fig. 4. Partial ternary phase diagram for Tween 85 (dot) and AOT (solid) alone, or as a surfactant mixture in combination with IPM and H_2O at 25 °C. The weight ratios of Tween 85:AOT employed were 2:1 (dash),1:1 (dash–dot–dot) and 1:2 (long dash).

present study. As anticipated it can be seen that in most cases increasing the non-ionic surfactant content tended to destroy MBG formation, although surprisingly in a few instances the presence of an increased amount of AOT destroyed the tendency of the mixed system to form MBG; for example both Tween 21:AOT and $C_{18:1}E_{10}$:AOT mixtures at 1:1 weight ratios formed

Table 1
Summary of formulation for AOT systems containing distilled water at 25 °C

Oil phase	% H_2O max at 25% w/w surfactant	$\omega_{o,max}$, 25% w/w surfactant	MBG formed
Heptane	26	8.3	Y
Hexadecane	8	2.6	Y
IPM	24	7.7	Y
IPP	13	4.2	Y
Ethyl butyrate	8	2.6	Y
Ethyl eaprylate	17	5.4	Y
Tributyrin	5	1.6	C
Miglyol 812	7	2.3	N
Soybean oil	3	1.0	N

Solubilisation capacity determined using 25% w/w surfactant in oil. For some systems a composition range (or ranges) over which a clear system is detected is recorded. MBG formation attempted by addition of 13% w/w gelatin (final concentration) to systems initially containing 25% w/w AOT and 25% w/w distilled water. Abbreviations: IPM, isopropyl myristate; IPP, isopropyl palmitate; Y, yes; N, no and C, cloudy.

Table 2
Summary of formulation for single surfactant systems based on IPM containing distilled water at 25 °C

Surfactant	HLB	% H_2O max at 25% w/w surfactant	$\omega_{o,max}$ at 25% w/w surfactant	ω_o at 25% w/w surfactant and 20% w/w H_2O
Span 85	1.8	2	1.4	13.8
Span 80	4.3	–	–	6.2
Span 20	8.6	3	0.7	4.7
$C_{12}E_4$	9.7	4–10*	1.0–2.6*	5.2
Tween 81	10.0	8	3.7	9.3
AOT	10.2	24	7.7	6.4
$C_{12}E_6$	10.6	11	3.6	6.5
Tween 85	11.0	6	7.9	26.5
Tagat TO	11.3	9	12.8	28.5
$C_{18:1}E_{10}$	12.4	–	–	10.1
Tween 21	13.3	4–11*, 16–20*	1.5–4.1*, 6.0–7.5*	9.6
$C_{12}E_{10}$	14.0	3	1.4	9.3
Tween 80	15.0	4–12*	3.8–11.3*	18.8
$C_{18:1}E_{20}$	15.3	–	–	16.4
Tween 20	16.7	6–9	4.9–7.3	16.5

* Samples only clear within ranges indicated.

Table 3
Summary of formulation for mixed non-ionic surfactant systems based on IPM containing distilled water at 25 °C

Surfactant weight ratio	% H_2O max at 25% w/w surfactant	$\omega_{o,max}$ at 25% w/w surfactant
Tween 85:Span 20		
1:2	6	3.6
1:1	16	12.6
2:1	2–5*	1.9–4.8*
Tagat TO:Span 20		
1:2	0.5–5*	0.32–3.2*
1:1	0.5–4*, 16–21*	0.42–3.4*, 13.6–17.6*
2:1	0.5–4*, 16–21*	0.52–4.1*, 16.4–21.5*

* Samples only clear within concentration ranges indicated.

MBG, while at 1:2 weight ratios they formed birefringent gels.

The behaviour of these mixed surfactant systems in the presence of different drug loadings is summarised in Table 6a–d. It proved possible to formulate MBG containing sodium salicylate as a model drug in the majority of systems studied, however Tween 80 and Span 20 were added to the list of unsuccessful surfactants mentioned earlier. Interestingly some of the $C_{12}E_6$:AOT systems which previously failed to form a gel in the absence of drug now formed a birefringent gel. From Table 6a–d there appears to be greater scope for increasing drug loading in the case of MBG formulations compared with their microemulsion counterparts, a property recently exploited in biotransformation applications (Jenta et al., 1997). In all of the systems in which MBG were successfully formulated, drug loadings of approximately 2.5% w/w of MBG were achieved without difficulty. One MBG formulation was attempted in which salicylate would have been incorporated at 5% w/w (Table 6a) but this was not successful. Intermediate drug loadings were not attempted. Interestingly, it was not possible to formulate the MBG in the presence of sodium chloride (Table 6a). This result is consistent with the findings of Atkinson et al. (1991) with regard to the destabilising effects of electrolytes.

Laser light scattering studies (total intensity light scattering and photon correlation spectroscopy) on selected systems showed that none of the w/o microemulsions tested exhibited percolation (i.e. a sudden and very large increase in size upon a small increase in temperature), surprisingly not even those prepared solely from the ionic surfactant AOT and IPM (Kantaria, 1998). However

Table 4

Surfactant weight ratio	% H_2O max at 25% w/w surfactant	$\omega_{o,max}$ at 25% w/w surfactant	MBG formed
(*a*) *Summary of formulation for mixed AOT:Tween 81, Tween 85, Tween 21, Tween 80 or Tween 20 systems based on isopropyl myristate (IPM) containing distilled water at 25 °C.*			
Tween 81:AOT			
1:2	40	14.1	Y
1:1	30	11.8	Y
2:1	20	8.3	Y
Tween 85:AOT			
1:2	32	20.8	Y
1:1	20	16.4	Y
2:1	23	22.8	Y
Tween 21:AOT			
1:2	38	12.9	B
1:1	34	11.8	Y
2:1	25	8.9	Y
Tween 80:AOT			
1:2	18	9.5	Y
1:1	12, 30–35*	7.6, 18.9–22.1*	Y
2:1	–	–	N
Tween 20:AOT			
1:2	20	9.6	Y
1:1	8–12*	4.5–6.8*	N
2:1	–	–	N
(*b*) *Summary of formulation for mixed non-ionic/ionic surfactant systems based on isopropyl myristate (IPM) containing distilled water at 25 °C.*			
Span 20:AOT			
1:2	33	9.8	N
1:1	18	5.1	Y
2:1	9	2.5	Y
Tagat TO:AOT			
1:2	33	35.0	Y
1:1	7	6.1	Y
2:1	5	3.4	B
$C_{18:1}E_{10}$:*AOT*			
1:2	21	8.0	B
1:1	12	5.0	Y
2.1	10	4.5	N
$C_{12}E_4$:*AOT*			
1:2	31	9.3	B
1:1	24	7.0	B
2:1	28	7.8	N
$C_{12}E_6$:*AOT*			
1:2	16	5.1	N
1:1	12	3.9	N
2:1	10	3.2	N
$C_{12}E_{10}$:*AOT*			
1:2	16	5.8	Y
1:1	12	4.6	C
2:1	9	3.7	C

Abbreviations: Y, yes; N, no; C, cloudy and B, birefringent.

* Samples clear within concentration range indicated.

when IPM was replaced with heptane in the AOT-stabilised microemulsion, the system could be made to percolate, illustrating the detrimental effect of the presence of a large molecular volume oil on percolation (Kantaria, 1998). Significantly however the MBG-forming systems studied here could be made to percolate when gelatin was present, presumably because gelatin was instrumental in altering the nature of the interactions between the microemulsion droplets to become attractive on increasing temperature. A similar requirement for the presence of gelatin to induce percolation has also been reported in some AOT systems (Aliotta et al., 1996; Schlicht et al., 1996).

Little has been said of the importance of ω_o in this study, other than to indicate its role as a rather crude measure of the 'hydration state' in w/o microemulsions. This estimation can only be made if the hydration requirements of the surfactant and the proportion of surfactant located at the oil/water interface are known. In the case of ionic surfactants it is a reasonable assumption that almost all of the surfactant will be located at the interface, however this is much less likely to be the case for microemulsions stabilised by non-ionic surfactants due to the polydisperse nature of their head group. In this case the shorter, more hydrophobic surfactant species may preferentially partition into the oil phase, thereby, altering the effective hydrophilicity of the surfactant associated with the microemulsion droplet (Wormuth and Geissler, 1991).

3.3. MBG stability studies

A primary aim of this study was to develop MBG for use in transdermal drug delivery. Four of the best formulations (as judged by stability and drug loading capacity) were, therefore, subjected to further study. In general, MBG are noted for their robustness and retain their integrity when contacted with a wide variety of hydrocarbon solvents (Rees et al., 1991), and even when contacted with a solution of the parent microemulsion (Atay-Guneyman et al., 1995; Jenta et al., 1997) There is, however, much less known about MBG stability in water miscible solvents. As a consequence, MBG formulated with 13% w/w gelatin, 17.4% H_2O and 21.7% w/w surfactant in IPM were contacted with a variety of solvents or solutions and monitored over a 7 h period. The observations regarding weight changes are summarised in Table 7. In the presence of distilled water, the gels swell, and become opaque, losing their integrity as water is absorbed and the gel structure disrupted. Weight gains were also observed when the MBG were contacted with drug solutions, although the degree of swelling and gel disruption was noticeably reduced possibly as a consequence of osmotic effects. In contrast, acetone and ethanol caused weight loss and shrinkage presumably because of water and solvent interdiffusion. The weight change was much more prominent in the case of acetone, even though the densities of acetone (0.791 g cm^{-3}) and

Table 5
Summary of formulation for AOT systems based on isopropyl myristate (IPM) containing different drug loadings at 25 °C

Aqueous phase	% H_2O max at 25% w/w surfactant	$\omega_{o,max}$ at 25% w/w surfactant	MBG formed
Na salicylate 1%	33	10.5	Y
Na salicylate 5%	25	8.0	Y
Na salicylate 10%	25	8.0	Y
Na benzoate 1%	27	8.7	Y
Na benzoate 5%	23	7.4	Y
Na benzoate 10%	23	7.4	Y
Aniline HC1 1%	32	10.6	Y
Aniline HCI 3.5%	14	4.5	C
Benzamide 1%	27	8.7	Y

$\omega_{o,max}$ calculated without taking into account any hydration of the incorporated drug. Details as Table 1.

Table 6

Surfactant weight ratio	Aqueous phase	%H_2O max at 25% w/w surfactant	$\omega_{o,max}$ at 25% w/w surfactant	MBG formed
(*a*) *Summary of formulation for mixed non-ionic/ionic surfactant systems based on isopropyl myristate (IPM) containing different drug loadings at 25 °C.*				
Tween 81:AOT				
1:2	Na salicylate 5%	18	6.3	Y
1:1	Na salicylate 5%	17	6.7	Y
2:1	Na salicylate 5%	9	3.7	Y
1:2	Na salicylate 10%	24	8.5	SB
1:1	Na salicylate 10%	21	8.3	Y
2:1	Na salicylate 10%	5, 18–23*	2.1, 7.5–9.5*	Y
Tween 85:AOT				
1:2	Na salicylate 5%	57	37.1	Y
1:1	Na salicylate 5%	45	36.9	Y
2:1	Na salicylate 5%	21	20.8	Y
1:2	Na salicylate 10%	30	19.5	Y
1:1	Na salicylate 10%	57	46.7	Y
2:1	Na salicylate 10%	27	26.8	C, B
2:1	Na salicylate 20%	16	15.9	N
2:1	NaCl 5%	18	17.8	N
2:1	NaCl 10%	7	2.5	N
(*b*) *Summary of formulation for mixed non-ionic/ionic surfactant systems based on isopropyl myristate (IPM) containing different drug loadings at 25 °C.*				
Tween 21:AOT				
1:2	Na salicylate 5%	22	7.5	Y
1:1	Na salicylate 5%	11	3.8	Y
2:1	Na salicylate 5%	20	7.1	Y
1:2	Na salicylate 10%	17	5.8	Y
1:1	Na salicylate 10%	9	3.1	Y
2:1	Na salicylate 10%	43	15.3	C
Tween 80:AOT				
1:2	Na salicylate 5%	45	21.6	N
1:1	Na salicylate 5%	1	5.7	N
2:1	Na salicylate 5%	1	0.7	N
1:2	Na salicylate 10%	15	7.2	N
1:1	Na salicylate 10%	13	7.4	N
2:1	Na salicylate 10%	2, 11–15*	1.5, 8.0–10.9*	N
(*c*) *Summary of formulation for mixed non-ionic/ionic surfactant systems based on isopropyl myristate (IPM) containing different drug loadings at 25 °C.*				
Span 20:AOT				
1:2	Na salicylate 5%	14	4.2	N
1:1	Na salicylate 5%	19	5.3	N
2:1	Na salicylate 5%	13	3.6	N
1:2	Na salicylate 10%	16	4.8	N
1:1	Na salicylate 10%	12	3.4	N
2:1	Na salicylate 10%	10	2.8	N
Tagat TO:AOT				
1:2	Na salicylate 5%	55	58.0	Y
1:1	Na salicylate 5%	18	15.6	SC
2;1	Na salicylate 5%	13	8.8	N
1:2	Na salicylate 10%	15, 24–65*	15.9, 25.4–68.9*	C, B
1:1	Na salicylate 10%	20	17.4	C, B
2:1	Na salicylate 10%	14–18*, 23–48*	9.5–12.2*, 15.6–32.6*	N

Table 6 (*Continued*)

Surfactant weight ratio	Aqueous phase	%H_2O max at 25% w/w surfactant	$\omega_{o,max}$ at 25% w/w surfactant	MBG formed
(*d*) *Summary of formulation for mixed non-ionic/ionic surfactant systems based on isopropyl myristate (IPM) containing different drug loadings at 25 °C.*				
$C_{12}E_4$:AOT				
1:2	Na salicylate 5%	52	15.6	B
1:1	Na salicylate 5%	30	8.8	B
2:1	Na salicylate 5%	17	12.8	N
1:2	Na salicylate 10%	59	17.7	N
1:1	Na salicylate 10%	43	12.5	N
2:1	Na salicylate 10%	11	3.1	N
$C_{12}E_6$:AOT				
1:2	Na salicylate 5%	30	9.6	B
1:1	Na salicylate 5%	23	7.5	B
2:1	Na salicylate 5%	6	1.9	C, B
1:2	Na salicylate 10%	20	6.4	N
1:1	Na salicylate 10%	23	7.5	N
2:1	Na salicylate 10%	13	4.2	C
$C_{12}E_{10}$:AOT				
1:2	Na salicylate 5%	30	10.9	Y
1:1	Na salicylate 5%	13	4.9	Y
2:1	Na salicylate 5%	6, 16–18*	2.5, 6.6–7.4*	N
1:2	Na salicylate 10%	20	7.3	Y
1:1	Na salicylate 10%	13	4.9	Y
2:1	Na salicylate 10%	14	5.8	N
$C_{18:1}E_4$:AOT				
1:2	Na salicylate 5%	23	8.8	Y
1:1	Na salicylate 5%	16	6.7	Y
2:1	Na salicylate 5%	5	2.3	B
1:2	Na salicylate 10%	42	16	Y
1:1	Na salicylate 10%	10	4.2	C, B
2:1	Na salicylate 10%	22	9.9	N

$\omega_{o,max}$ calculated without taking into account any hydration of the incorporated drug. Abbreviations: Y, yes; N, no; C, cloudy; SC, slightly cloudy; B, birefringent and SB, slightly birefringent.

* Samples clear within concentration range indicated.

ethanol (0.785 g cm^{-3}) are effectively the same (Lide, 1996). The MBG form hardened opaque masses after prolonged exposure to acetone or ethanol, and there was some evidence of internal fracture formation. The weight changes for MBGs contacted with normal saline (NS) were an order of magnitude smaller than those observed with distilled water or the drug solutions. This observation was of practical value in our subsequent drug delivery studies which employed NS in the receptor compartment of our iontophoretic cell (Kantaria et al., 1999).

The large majority of these experiments were conducted at 25 °C, but clinical application would require the use of the MBG formulations at temperatures approaching 37 °C. Temperature variations during transit or storage are also an issue. Consequently, simple visual observations were made at different temperatures for the different MBG formulations prepared in IPM. At 0 °C the formulations were white in colour owing to the solidification of the IPM. Storage at or below this temperature would presumably inhibit the curing phenomenon observed during

Table 7
Effect of solvent or sodium salicylate (NaSal) solutions on MBGs containing 13% w/w gelatin, 17.4 % H_2O and 27.4% w/w surfactant in IPM at 25 °C

Surfactant weight ratio	Distilled water	Normal saline	Acetone	Ethanol	5% w/w NaSal	10% w/w NaSal
AOT	109	23	−37	−9	157	180
Tween 8l:AOT	350	16	−49	−9	187	197
Tween 21:AOT	345	21	−31	−1	156	176
Tween 85:AOT	150	23	−43	−12	171	101
AOT	300	24	−51	−3	256	208
Tween 81:AOT	355	21	−46	−1	187	134
Tween 21:AOT	267	14	−35	−4	145	114
Tween 85:AOT	107	14	−47	−9	162	126

Data refers to % weight change of MBG after 7 h immersion. Upper set formulated using water, the lower set with 10% w/w sodium salicylate.

rheological monitoring at 25 °C (Kantaria et al., 1999). At physiologically relevant temperatures representing skin temperature (32 °C) and internal body temperature (37 °C), all the formulations remained in the gel state except for formulations containing only AOT as stabiliser, suggesting that the gelling temperature for microemulsions containing non-ionic surfactant is higher than for those containing AOT alone. These phase transitions appear to be fully reversible upon heating and cooling the gel for at least three cycles.

At fixed gelatin concentrations, the conductivity of the MBG is observed to decrease with an increase in salt or sodium salicylate concentration (data not shown). This is accompanied by changes in the rheological properties of the MBG as determined by dynamic oscillatory testing (Kantaria et al., 1999). The addition of salt and sodium salicylate appear to destabilise the gel structure resulting in a reduction in their elastic properties. This behaviour is most likely due to salt interactions which inhibit the gelatin coil-helix transition required for gel formation (von Hippel and Wong, 1964).

4. Conclusions

These organogel studies clearly support previous findings concerning a minimum water and surfactant requirement for MBG formation. The formulation of MBG in pharmaceutically acceptable oils has been demonstrated using AOT and a variety of binary mixtures of AOT and non-ionic surfactant. Formulating MBG with non-ionic surfactants alone proved unsuccessful. This failure is undoubtedly linked to the differing nature of the phase transitions in microemulsions stabilised by ionic and non-ionic surfactants. The standard protocol for an AOT-stabilised MBG formulation involves taking the system through the UTPB where the percolated structure may comprise connected networks of strongly interacting droplets, although it may become bicontinuous in which case the net curvature is effectively zero. In contrast, the interaction between droplets stabilised by non-ionic surfactants as the UTPB is approached is similar to those experienced in microemulsions stabilised by ionic surfactants at the lower temperature phase boundary (LTPB). The differences arise because the solubility of ionic surfactants increases as a function of temperature, whereas the solubility of non-ionic surfactants decreases. Microemulsion droplets stabilised by non-ionic surfactants are not strongly interacting at the UTPB. Consequently, highly percolated structures do not form (Schulz et al., 1994), and what appears to be an important mechanism facilitating development of a gelatin network is inhibited. This study has demonstrated for the first time that microemulsions and MBG can be successfully formulated using pharmaceutically acceptable oils and surfactants, and that these systems can be used to encapsulate model drugs. Although AOT is a very effective surfactant in its

own right, a synergistic effect was obtained when used in combination with some non-ionic surfactants, and had the beneficial effect of increasing the extent of the microemulsion region. MBG could not be formulated using non-ionic surfactants alone, but in combination with AOT a wide range of MBG were prepared with similar or improved properties to MBGs formulated with AOT alone. MBG containing sodium salicylate have previously been reported to be of a slightly lower gel strength than 'empty' MBG (Kantaria et al., 1999).

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