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Formulation and physical characterization of water-in-oil microemulsions containing long- versus medium-chain glycerides

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Abstract

Stable self-emulsifying water-in-oil (w/o) microemulsions of extremely small particle size (5–30 nm) and consisting of an oil, a blend of a low and high HLB surfactants and an aqueous phase, have been developed using commercially available and pharmaceutically acceptable components. Their formation was monitored by the corresponding pseudo-ternary phase diagram. The oil phase contained long- or medium-chain triglycerides, and mono-/diglycerides or sorbitan esters (low HLB surfactants). Polysorbate 80 (Tween 80) was used as a high HLB surfactant. Microemulsions were readily prepared by admixing appropriate quantities of the various components with gentle hand-mixing or stirring to ensure thorough mixing. In the case of microemulsions incorporating long-chain glycerides and/or sorbitan esters, high temperature (40–60°C) was used to reduce viscosity and solubilize all components during the formation of microemulsions. Limited levels of aqueous phase ($< 10\%$, w/w) can be solubilized within w/o microemulsions incorporating long-chain glycerides and/or sorbitan esters. Microemulsions containing medium-chain glycerides (mono-/di-/triglycerides) can be formulated at ambient temperature and can solubilize aqueous phase up to 40% (w/w). The conductance, viscosity, refractive index, density and mean particle diameter of a typical w/o microemulsion incorporating medium-chain glycerides (Captex 355/Capmul MCM/Tween 80/saline, 65/22/10/3, % w/w), were: 0.540 μmhos/cm, 56.7 cP, 1.449, 0.9677, and 15.2 ± 4.1 nm (polydispersity of 0.153), respectively. The corresponding values of a w/o microemulsion incorporating long-chain triglycerides and monoglycerides (Soybean oil/Arlacel 186/Tween 80/saline, 65/22/10/3, % w/w) were: 0.177 μmhos/cm, 125.1 cP, 1.471, 0.9010, and 10.3 ± 2.5 nm (polydispersity of 0.114), respectively. Several water-soluble molecules/peptides of different molecular size and charge have been formulated in these w/o microemulsions at pharmacological relevant levels. These systems are discussed in terms of their drug delivery potential. © 1997 Elsevier Science B.V.

Abbreviations: A 80, Arlacel 80 (sorbitan oleate); A 83, Arlacel 83 (sorbitan sesquioleate); A 186, Arlacel 186 (glycerol monooleate and propylene glycol); DDME, drug delivery microemulsions; HLB, hydrophilic-lipophilic balance; MVRL, Myverol 18–99; W/O, water-in-oil.

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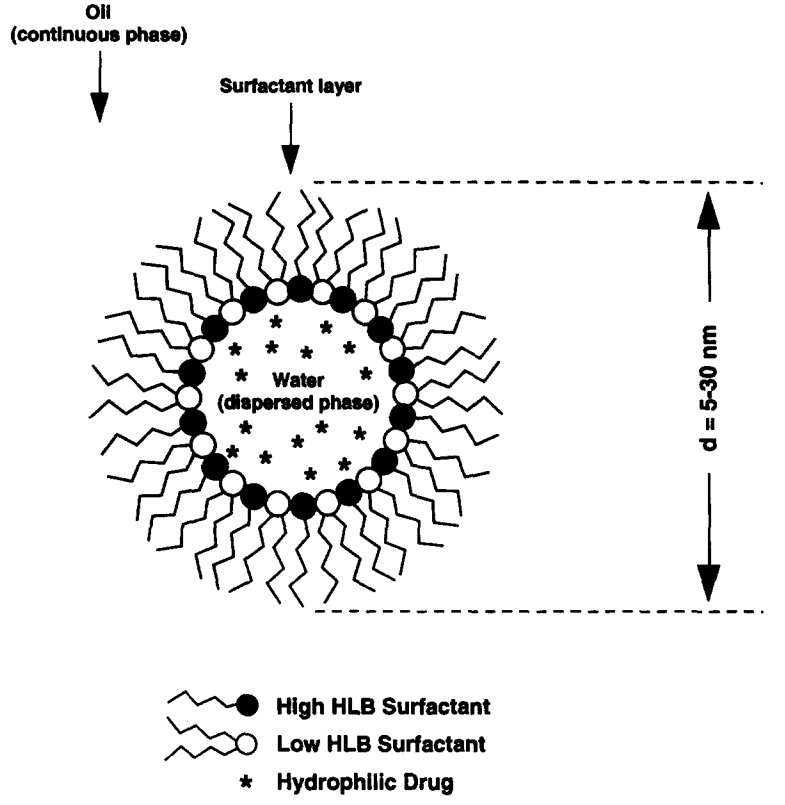
1. Introduction

Microemulsions within the scope of the present work (Scheme 1) can be defined according to Danielson and Lindman (1981) as a system of water with or without electrolyte, oil and non-ionic surfactant(s) which are single isotropic and thermodynamically stable liquid solutions. The formation of microemulsions usually involves a combination of three to five components, namely, oil, water, surfactant, cosurfactant and electrolyte. The tendency toward a water-in-oil (w/o) or an oil-in-water (o/w) microemulsion is dependent on the properties of the oil and the surfactant, the water-to-oil ratio and the temperature. Non-ionic surfactants are conveniently classified on an empirical scale known as hydrophilic-lipophilic balance (HLB) which runs from 1 to 20. In general, w/o microemulsions are formed using surfactants which have an HLB in the range of about 3–6 whilst o/w microemulsions are formed using surfactants which have an HLB value in the range of about 8–18. The role of the cosurfactant, usually a short-chain alcohol, is to increase the interfacial fluidity by penetrating into the surfactant film and consequently creating a disordered film due to the void space among surfactant molecules (Leung and Shah, 1989). However, the use of cosurfactant in microemulsions is not mandatory and alcohol-free self-emulsifying microemulsion systems have been described in the literature (Osborne et al., 1988).



Scheme 1.

Reverse micelles for the solubilization of small molecules and proteins are well known in the literature (Luisi et al., 1988). Engstrom (1990) described reverse micelles or the so-called L2-phase, in the system Soybean oil/Sunflower oil monoglycerides/water that can solubilize up to 10% (w/w) water (Engstrom, 1990, and references therein). This reverse micellar phase can also be defined as water-in-oil microemulsion according to Danielson and Lindman (1981). In these systems, however, high ratios of the monoglyceride to triglyceride were employed, ranging from 1/1 to 3/1, and there is no mention of the additional inclusion of the high HLB surfactant. The existence of an L2 phase has also been described earlier in the context of medium-chain glycerides for the system Tricaprylin/Monocaprylin/water (Friberg and Mandell, 1970). Again, there is no mention of the presence of a high HLB surfactant. High HLB surfactants, such as, polyoxyethylene (20) sorbitan monooleate (Polysorbate 80, HLB of 15.0) and polyoxyethylene (40) stearate (HLB = 17.8) are frequently used in microemulsion formulation, which, in combination with low HLB emulsifiers (monoglycerides), provide the conditions necessary for the formation of a stable w/o microemulsion (Osborne et al., 1988). The present report is focused on the formulation and physicochemical characterization of self-emulsifying w/o microemulsions incorporating long- versus medium-chain glycerides and several physical properties are compared. In addition, several water-soluble molecules/peptides were incorporated into w/o microemulsions at pharmacological relevant levels.

2. Materials and methods

2.1. Materials

Arlacel 80 (sorbitan oleate, HLB = 4.3), Arlacel 83 (sorbitan sesquioleate, HLB = 3.7) and Arlacel 186 (monoolein:propylene glycol, 9/1, HLB = 2.8) were provided by ICI Americas, Wilmington, DE). Captex 355 (C_8/C_{10} triglycerides) and Capmul MCM (C_8/C_{10} mono-/diglycerides, HLB = 5.5–6.0) were supplied by Abitec Corporation (formerly Karlshamns Lipid Specialties, Columbus, OH). The fatty acid distribution in Captex 355 according to the manufacturer is: 55% caprylic (C_8), 42% capric (C_{10}), and 2% caproic (C_6). Capmul MCM is approximately a 1:1 mixture of C_8/C_{10} mono-/diglycerides with 2% free glycerol and it has the following fatty acid distribution: 67% caprylic (C_8), 30% capric (C_{10}), 3.2% caproic (C_6), and $< 1\%$ palmitic (C_{16}). Myverol 18–99 (HLB = 3.7), a distilled monoglyceride from rapeseed oil was obtained from Eastman Kodak Chemicals, Kingsport, TN. As specified by the manufacturer, the primary fatty acids found in Myverol 18–99 are: 61% oleic ($C_{18:1}$), 21% linoleic ($C_{18:2}$), 9% linolenic ($C_{18:3}$), and 4% palmitic (C_{16}). Super refined soybean oil was purchased from Croda, Mill Hall, PA. According to Croda the fatty acid pattern of soybean oil is: 54% linoleic ($C_{18:2}$), 25% oleic ($C_{18:1}$), 6% linolenic ($C_{18:3}$), 4% stearic (C_{18}), and 11% palmitic (C_{16}). Tween 80 (polyoxyethylene sorbitan monooleate) was purchased from Sigma, St. Louis, MO. High purity Calcein (5(6)-carboxyfluorescein, MW = 623) was obtained from Molecular Probes, Eugene, OR. Bovine insulin (MW of about 6000), salmon calcitonin (MW = 3432) and [Val-Asp]-[Arg^8]-vasopressin (MW = 1299) were purchased from ICN Biochemicals, Cleveland, OH. The acetate salts of the RGD peptide SK&F 106760 (MW = 634) and the GHRP peptide SK&F 110679 (MW = 873) were provided by the Medicinal Chemistry and Pharmaceutical Technologies Department, respectively, SmithKline Beecham Pharmaceuticals, King of Prussia, PA. All drug supplies were stored at $-20°C$ in a desiccator.

2.2. Microemulsion formulation/phase diagrams

The existence of the microemulsion field was monitored by the corresponding phase diagram. The microemulsion phase was identified as the area in the phase diagram where clear and transparent formulations are obtained based on visual inspection of many samples. For an initial identification of the various phases within the entire phase diagram, 50–100 samples were prepared and once the microemulsion phase was present

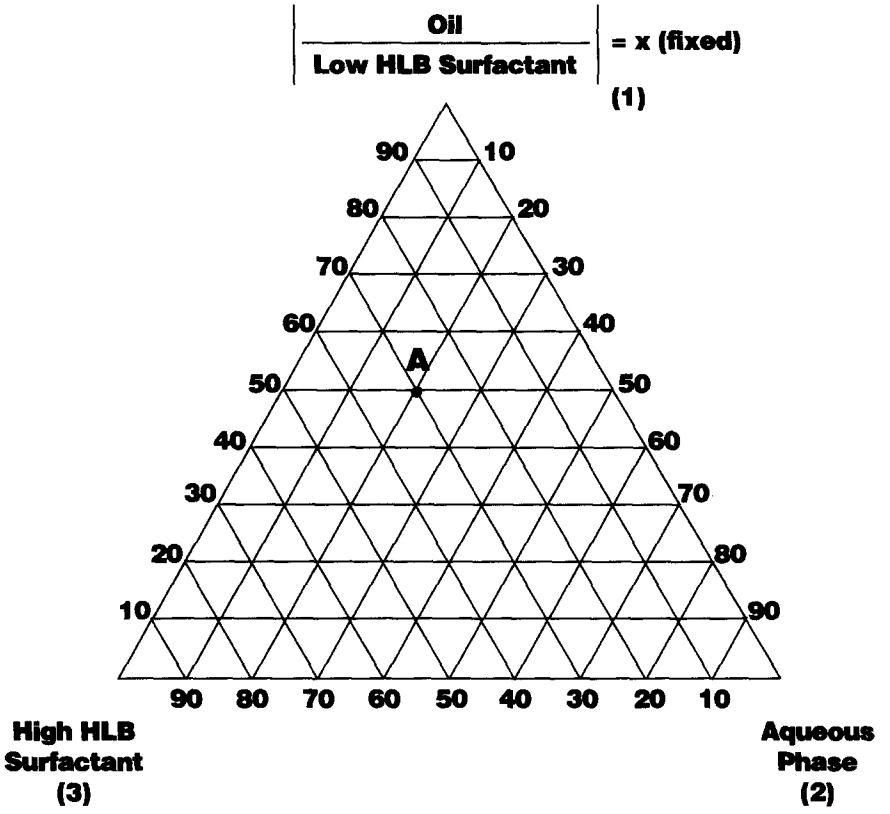


Fig. 1. A pseudo-ternary phase diagram reading. Point A stands for a mixture of 50% oil containing the low HLB surfactant at a fixed ratio x (w/w), 20% aqueous phase and 30% high HLB surfactant.

additional samples were prepared to determine boundary regions. Samples were allowed to equilibrate to the formulation temperature for 15 min before the phases formed were assessed. That temperature was ambient or 40–50°C for formulations incorporating medium- or long-chain glycerides, respectively. The o/w microemulsion phase and several other non-microemulsion phases are not shown in the phase diagrams since they are beyond the scope of the present work. Simple tests such as dilutability with oil, non-dispersability of a water-soluble fluorescent dye (Calcein) and extremely low conductance (< 1 μmhos/cm) were employed to verify that the microemulsions formed were of the w/o type. Their isotropic and thus non-birefringent behavior was confirmed by examination under polarizing light.

A pseudo-ternary phase diagram is illustrated in Fig. 1. Such phase diagram considers two of these components as a single one, in this case the mixture of the oil and low HLB surfactant which is shown at the top of the phase diagram. The high HLB surfactant and the aqueous phase are shown on the bottom left and right corner, respectively. For the construction of the phase diagram the weight ratio of the oil to the low HLB surfactant is kept constant with varying amounts of the high HLB surfactant and aqueous phase. In the example shown in Fig. 1, the mixture A is composed of 50% of the mixture oil plus low HLB surfactant, 20% aqueous phase and 30% of high HLB surfactant (all percentages are given per weight). All components were weighed out on analytical balance (Mettler AT 201) and mixed into a screw-cap scintillation vial using a magnetic bar on a stirring plate. In the case of microemulsions incorporating long-chain glycerides, the various components were added and mixed via a

magnetic bar at a slow speed at temperatures between 40 and 50°C, using a stirring plate (Fisher Thermix stirring hot plate model 210T). For components which are solid at room temperature premelting at the appropriate temperature was necessary using a water bath (Exacal, Neslab Insts). For these systems, further equilibration of the resulting microemulsion at 40–50°C for about 24 h was found to improve stability. Microemulsions incorporating medium-chain glycerides can be formulated at room temperature. However, depending on the viscosity of the components used, warming at temperatures between 37 and 50°C is helpful in order to assure complete mixing. Since the formation of microemulsion is thermodynamically favored, the order of addition of the components should not have any effect on the final size and stability of the particle.

2.3. Drug incorporation

For the preparation of the drug-containing microemulsion the following procedure was employed: the desired amount of the drug was first weighed out and then dissolved in the appropriate amount of the aqueous phase (saline). To ensure complete mixing and solubilization, the drug solution was gently vortexed. The aqueous phase containing the drug/peptide was subsequently added to the right amounts (per weight) of the oil plus the low HLB surfactant mixture that also contained the high HLB surfactant. Alternatively, the aqueous phase containing the drug was added to the high HLB surfactant and then upon complete mixing slowly added to the oil plus low HLB surfactant mixture.

2.4. Physicochemical characterization

2.4.1. Viscosity and refractive index

The viscosity of microemulsions (Ktistis, 1990) was monitored by a Cannon-Manning Semi-micro viscometer size 200 with a constant of 0.0984 mm^2/S^2 or cSt/s. The kinematic viscosity in centistokes (cSt) was calculated by multiplying the efflux time in seconds (s) by the viscometer constant. Multiplying the resulting value by the density of the sample gives the viscosity in centipoise (cP). The viscometer was calibrated with liquids of known viscosity, including oleic acid. For measuring refractive index, a Milton Roy refractometer was used. The instrument was calibrated with deionized water and oleic acid. Both measurements were conducted at ambient temperature.

2.4.2. Particle size

A Malvern Photon Correlation Spectrometer model 4700 equipped with an argon laser model 2000 from Spectra Physics was employed to monitor the particle size of microemulsions (Cabazat et al., 1980). The predetermined viscosity and refractive index of microemulsions were incorporated into the computer software which calculates the mean particle size and polydispersity from intensity, mass and number distribution. Light scattering was monitored at a 90° angle and 25°C with polystyrene beads used to check instrument's performance.

2.4.3. Conductance

The conductance of microemulsions (Latreille and Paquin, 1990) was determined at ambient temperature using a YSI model 32 (Yellow Spring Instruments) conductivity meter coupled to a YSI B3403 cell having a constant of 1.0/cm. Deionized water and saline were used to calibrate the instrument.

2.4.4. Polarized light microscopy

An Optiphot-Pol (NIKON, model 144850) microscope equipped with a camera was employed to examine the various fields (phases) of the phase diagram at ambient temperature and to verify the isotropic behavior of microemulsions (Leung and Shah, 1989). A drop of sample was placed between a coverslip and a glass slide and then examined under polarized light. Pictures were taken at 10 000 and 20 000 magnification.

2.4.5. Stability evaluation

Shelf-life stability of microemulsions, both as a function of time and storage temperature was routinely evaluated by visual inspection of the samples initially on a daily and later on a weekly basis. Stable systems were identified as those free of any physical change, such as, phase separation,

flocculation and/or precipitation. Particle size of the microemulsions upon storage was also determined to assess microemulsion stability in terms of drastic changes in the mean droplet diameter due to droplet coalescence and/or aggregation. Stability was monitored at 4°C, ambient temperature, 37 and 50°C.

3. Results and discussion

3.1. Microemulsion formulation/phase diagrams

Fig. 2 represents a partial pseudo-ternary phase diagram of soybean oil + Myverol 18–99 at a fixed ratio of 3/1, Tween 80 and water. Among the various phases formed by these four components, a field with clear and transparent liquid w/o microemulsions (shaded area in Fig. 2) has been identified. At low ratios of oil to low HLB surfactant (< 7/3), unstable systems were produced shortly after preparation while, at very high ratios two-phase systems were obtained. Replacement of water by physiological saline produced the phase diagram shown in Fig. 3. It is clear from these partial pseudo-ternary phase diagrams showing the field of interest in an expanded form that, w/o microemulsions in this system are formed with less than 10% water, between 0 and 25% Tween 80, and more than 70% (soybean oil + Myverol 18–99). Furthermore, by comparing Fig. 2 and Fig. 3, it appears that replacing water with saline leaves the microemulsion field largely unchanged with only small changes at the boundary regions. This is not surprising, however, since the various components of the microemulsion are non-ionic and thus unaffected by the ionic strength of the dispersed aqueous phase. Since Myverol 18–99 is solid at room temperature, premelting and subsequent formulation at high temperature (50°C) was necessary to ensure complete solubilization and mixing. Additional equilibration at this temperature was found to improve the stability of Myverol-containing microemulsions.

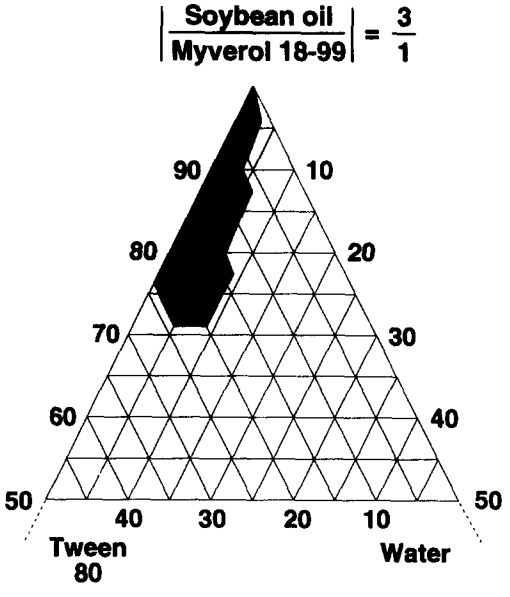


Fig. 2. A partial pseudo-ternary phase diagram of the system soybean oil/Myverol 18–99/Tween 80/water. The shaded area represents the w/o microemulsion existence field where stable clear and transparent formulations are produced. Other phases produced by this system are not shown.

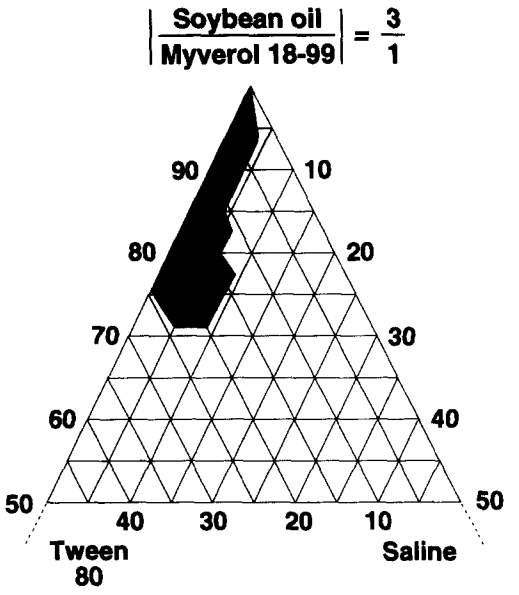


Fig. 3. A partial pseudo-ternary phase diagram of the system soybean oil/Myverol 18–99/Tween 80/saline. See legend to Fig. 2 for other details.

The partial pseudo-ternary phase diagram of a system containing Arlacel 186 as the low HLB surfactant is shown in Fig. 4. Arlacel 186, unlike Myverol 18–99, contains, in addition to glycerol

monooleate about 10% propylene glycol as specified by the manufacturer. The presence of propylene glycol lowers the melting point of this monoglyceride product and renders it liquid at room temperature which can be advantageous from a formulation perspective. Formulation of this system however, was carried out at about 40°C, in order to ensure complete mixing with the more viscous components, particularly Tween 80. Not surprising, a narrower microemulsion field was obtained with Arlacel 186 as compared to that of Myverol 18–99 (Fig. 2), most likely due to the HLB modification and decrease in surfactant concentration by the presence of propylene glycol. From Fig. 4 it appears that stable w/o microemulsions can be formed with less than 10% water, 5–15% Tween 80 and 80–95% (soybean oil + Arlacel 186) at a ratio of 3 to 1.

The phase diagram shown in Fig. 5 has been constructed with sorbitan sesquioleate (Arlacel 83). This sorbitan ester along with sorbitan oleate (Arlacel 80) are frequently employed in the formulation of w/o microemulsions and creams. It was, therefore, of a particular interest to compare them to monoglycerides (Myverol 18–99 and Arlacel 186) from a formulation perspective. As can be seen from Fig. 5, the microemulsion existence field produced by sorbitan monooleate was more extended compared to the one produced by Arlacel 186 (Fig. 4). Stable w/o microemulsions solubilize less than 10% water and contain 5–25% Tween 80 and 75–95% of a 3/1 mixture of soybean oil and sorbitan sesquioleate.

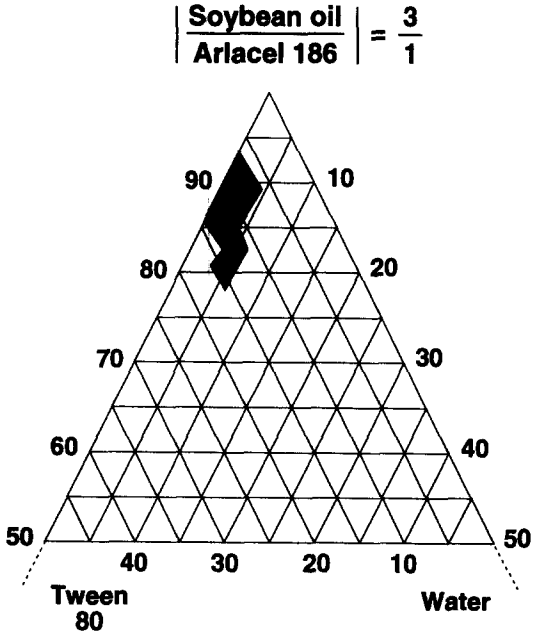


Fig. 4. A partial pseudo-ternary phase diagram of the system soybean oil/Arlacel 186/Tween 80/water. Arlacel 186 is a 9:1 mixture of glycerol monooleate and propylene glycol. W/O microemulsions formed by this system are represented by the shaded area of the phase diagram.

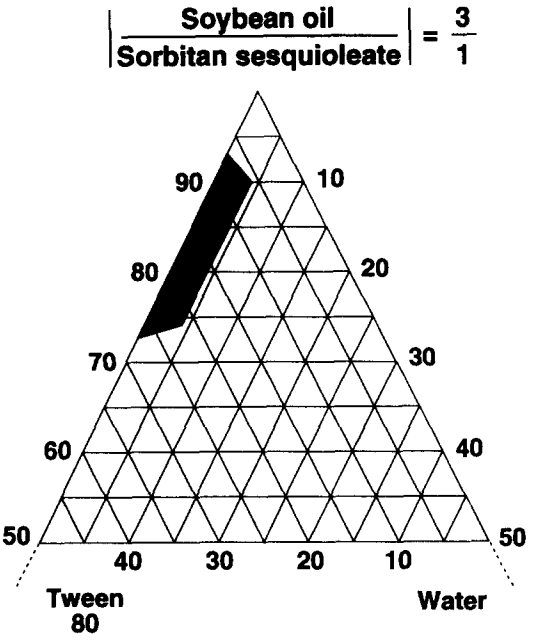


Fig. 5. A partial pseudo-ternary phase diagram of the system soybean oil/sorbitan sesquioleate/Tween 80/water. The ratio of soybean oil to sorbitan sesquioleate is kept constant at 3/1 (w/w).

Fig. 6 and Fig. 7 illustrate partial phase diagrams obtained with sorbitan monooleate (Arlacel 80) at a ratio of soybean oil to sorbitan monoleate of 3/1 and 4/1, respectively. It is evident that a narrower microemulsion field was present when the ratio of oil to sorbitan monooleate was increased or the absolute amount of this low HLB surfactant decreased. However, the observed differences in water solubilization between the systems shown in Fig. 6 and Fig. 7 were small.

A representative pseudo-ternary phase diagram of a system containing: C_8/C_{10} triglycerides (Cap-

tex 355 oil), Capmul MCM (C_8/C_{10} mono-/diglycerides) as a low HLB surfactant, a high HLB surfactant (Tween 80) and water is shown as Fig. 8. This system produced at ambient temperature, a wide range of clear and transparent microemulsions which are shown in the phase diagram as the microemulsion existence field, which field may usefully be sub-divided into regions (A), (B) and (C). The sub-division is based primarily on differences in conductance, viscosity and dilutability in the presence of excess water which is the dispersed or internal phase. Both the viscosity and conductance increase from region (A) to (C), with major changes between (B) and (C). Thus, with an aqueous phase of saline at 3% (w/w), the conductance of microemulsions within region (A), (B) and (C) varied between 0.5 and 4.0 μmhos/cm and the viscosity from 50–150 cP. In the presence of excess water or saline (100-fold) microemulsions of regions (A) and (B) are inverted to turbid emulsions (o/w) whereas microemulsions of region (C) remained clear indicative of a conversion to an o/w microemulsion. The calculated final HLB values for the blend of low and high HLB

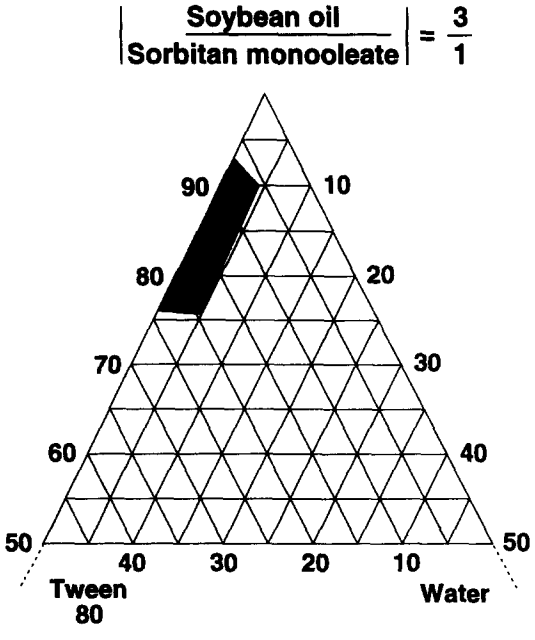


Fig. 6. A partial pseudo-ternary phase diagram of the system soybean oil/sorbitan monooleate/Tween 80/water at a fixed ratio (3/1) of soybean oil to sorbitan monoleate.

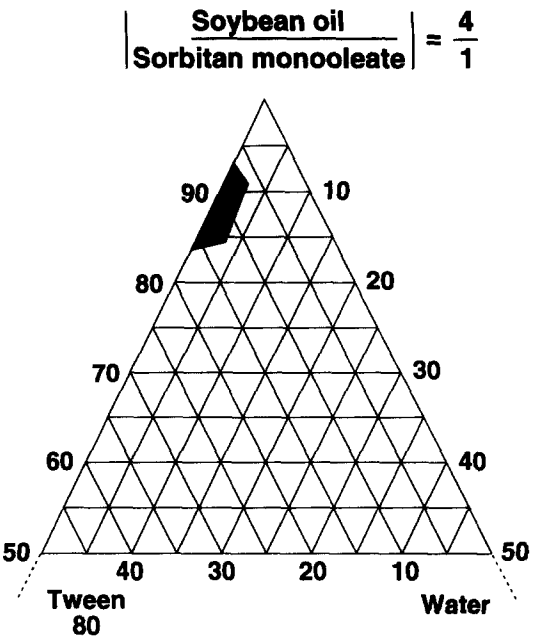


Fig. 7. A partial pseudo-ternary phase diagram of the system soybean oil/sorbitan monooleate/Tween 80/water at a fixed ratio (4/1) of soybean oil to sorbitan monoleate.

surfactants in the regions (A), (B), and (C) are 7–11, 11–13, and 13–15, respectively. Pseudo-ternary phase diagrams similar to the one shown in Fig. 8 have also be constructed at other oil to low HLB ratios (unpublished data).

The advantages of microemulsions over conventional emulsions or other lipid carriers is both improved stability and solubilization characteristics (Leung and Shah, 1989). The microemulsions of the present study form spontaneously when their components are brought into contact, that is without the application of high energy or the inclusion of short-chain alcohols that are known to cause tissue irritation (Osborne et al., 1988). The order of mixing of various components is not expected to influence the formation of microemulsions if the system is indeed thermodynamically stable (path independent). It is the low interfacial tension of microemulsions that favors the formation of a thermodynamically stable dispersion (Leung and Shah, 1989). Formulation at ambient temperature using medium-chain glycerides is particularly advantageous for thermolabile drugs,

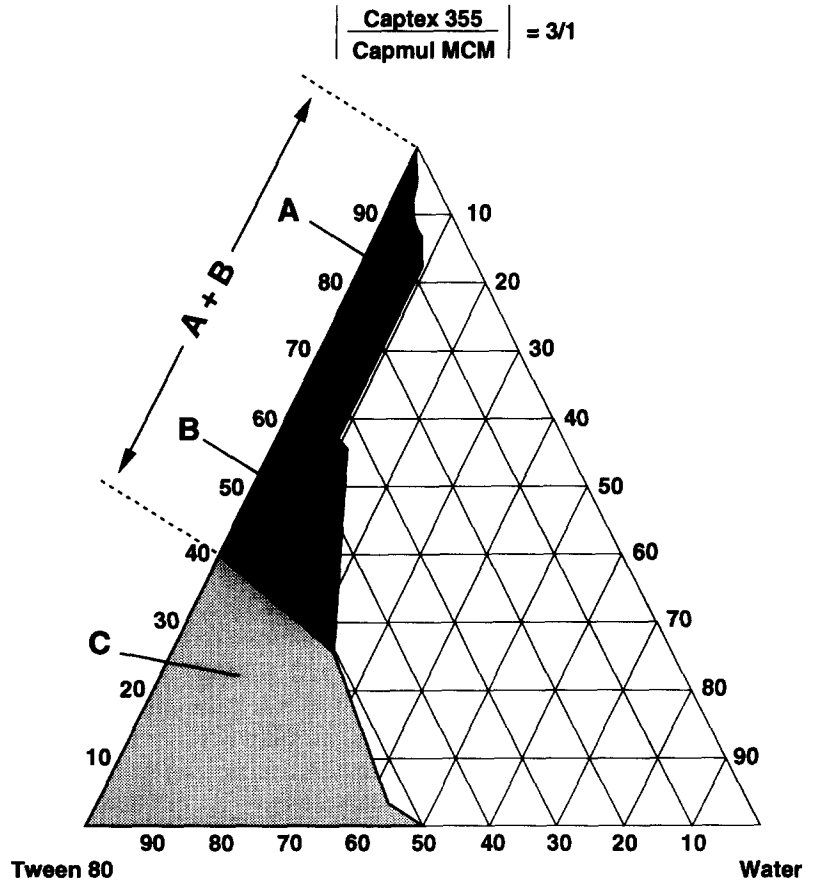


Fig. 8. A partial pseudo-ternary phase diagram of the system Captex 355/Capmul MCM/Tween 80/water. The w/o microemulsion existence field at ambient temperature extends through regions, A, B, and C of the phase diagram. The calculated HLB values of the surfactant blends in regions A, B, and C are 7–11, 11–13, and 13–15, respectively. The viscosity of the formulations at ambient temperature increases from A to C with major differences between B and C. Other phases produced by this system are not shown.

particularly peptides. The formation and stability of the present w/o microemulsions consisting of non-ionic components (oil plus surfactants) is not expected to be affected by the pH and/or ionic strength of the aqueous phase in the pH range between 3 and 10. This property can be beneficial for drug and other molecules exhibiting higher solubility and/or stability at low or high pH.

3.2. Drug incorporation

Molecules solubilized within the aqueous phase of the w/o microemulsions as described under Section 2 are shown in Table 1. It appears that water-soluble molecules having different physicochemical characteristics, such as, molecular size and charge, can be solubilized within these microemulsions. The aqueous phase of the w/o microemulsion for each of the incorporated molecule (Table 1) was selected to provide optimum solubility of the corresponding molecule/peptide. For non-ionic w/o microemulsions in general, the pH and/or ionic strength of the aqueous phase can be adjusted over a wide range without affecting their stability. Since as the level of the aqueous phase increases, both the level of the high HLB surfactant and viscosity of the formulation increase, the solubilized aqueous phase was maintained at 3% (w/w). A w/o microemulsion containing medium-chain glycerides was

Table 1
Molecules solubilized in self-emulsifying water-in-oil microemulsions[a]

Molecule	MW	Aqueous phase
Calcein (5(6)-carboxyfluorescein)	623	Isotonic 0.010 M Tris, pH 7.4
RGD peptide (Ac-Cys-(Nme)Arg-Gly-Asp-Pen-NH_2	634	Saline (USP), pH 6.0
GHRP His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2	873	Isotonic water for injection (USP), pH 5.0
(val-asp)-(arg^8)-Vasopressin Val-Asp-Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH_2	1300	0.040 M Acetate pH 5.0
Calcitonin (salmon)	3432	Saline (USP), pH 6.0
Insulin (bovine)	6000	Phosphate-buffered saline, pH 7.2

[a]Microemulsions containing long- or medium-chain glycerides were employed; 3% (w/w) aqueous phase incorporating the water-soluble molecule was solubilized by these microemulsions.

independently evaluated for absorption enhancement of Calcein and the RGD peptide (SK&F 106760) in the rat upon intraduodenal administration (Constantinides et al., 1995, 1994). We have reported significant absorption enhancement of both molecules from this microemulsion when compared to a saline solution of these molecules, most likely due to membrane permeability changes induced by microemulsion components, particularly by C_8/C_{10} mono-/diglycerides (Constantinides et al., 1995, 1994).

3.3. Physicochemical characterization

Table 2 summarizes the viscosity, refractive index and conductance of w/o microemulsions incorporating long-chain glycerides. These microemulsions are quite viscous at ambient temperature as indicated from the high viscosity values which are in the range of about 100–150 cP (Table 2). The extremely low conductance of these formulations (< 0.2 μmhos/cm) is characteristic of a water-in-oil particle. Interestingly, saline alone had a conductance of 13400 μmhos/cm (Table 2).

Table 3 compares the physical properties of water-in-oil microemulsions incorporating long- versus medium-chain glycerides. The microemulsion incorporating medium-chain glycerides corresponds to region (a) of Fig. 8, and contains a 3/1 mixture of Captex 355 and Capmul MCM (87%), Tween 80 (10%) and saline (3%). Major differences in density, refractive index and viscosity were observed whereas, similar conductance (< 1 μmhos/cm) and particle size were obtained (Table 3). Calcein- or the RGD peptide SK&F 106760-incorporating microemulsions exhibited physical properties similar to those produced by drug-free formulations (data not shown). The lower viscosity and formulation at ambient temperature of w/o microemulsions comprising C_8/C_{10} glycerides are attractive features for drug delivery and pharmaceutical development. From an absorption enhancement perspective, the bioavailability of Calcein upon intraduodenal administration in the rat was found to be significantly higher from w/o microemulsions incorporating medium-chain glycerides than from w/o microemulsions containing primarily long-chain glycerides (Constantinides et al., 1996).

Detailed particle sizing data using laser light scattering of w/o microemulsions containing long-chain glycerides or sorbitan esters and incorporating the RGD peptide SK&F 106760 are summarized in Table 4. The light scattering data was expressed as intensity, mass and number results and similar mean droplet diameter and polydispersity values were obtained in all three cases (data not shown). For clarity however and consistency with the data in Table 3 only the particle number data is shown. With all low HLB surfactants employed, extremely small particle size was obtained with a mean droplet diameter in the range of 5–30 nm. In addition, the low polydispersity index (< 0.2) is indicative of monodispersed (homogeneous) particles. Neither

Table 2
Physical properties of water-in-oil microemulsions incorporating long-chain glycerides at ambient temperature

DDME[a] (low HLB defined)	Viscosity[b] (cP)	Refractive index[b]	Conductance[c] (μmhos/cm)
DDME-Myverol 18–99	110.7	1.471	0.131
DDME-Arlacel 80	129.3	1.471	0.130
DDME-Arlacel 83	145.3	1.472	0.130
DDME-Arlacel 186	125.1	1.469	0.177
Oleic acid	23.4 (25.6)[d]	1.458 (1.458)[d]	ND
Deionized water	0.890[e]	1.333[e]	2.67
Saline	ND	ND	13 000

[a]Drug delivery microemulsions (DDME); these microemulsions contained 65% soybean oil, 10% Tween 80, 3% water or saline and 22% of the indicated low HLB surfactant (refer to Table 4 for specific composition).
[b]Corresponding microemulsions contained water as aqueous phase.
[c]Saline was the aqueous phase in conductance measurements.
[d]The viscosity and refractive index values shown in parentheses are from the CRC Handbook of Chemistry and Physics, 60th Edition.
[e]Literature values (CRC Handbook of Chemistry and Physics, 60th Edition).

particle size or polydispersity were significantly affected by the presence of the RGD peptide at 1 mg/ml of microemulsion (Table 4). These microemulsions can be stored at 4°, 30°, and 40°C for several months, without any phase separation and/or precipitation.

Drug development issues with microemulsions, in general, regarding their use to improve drug dissolution and oral absorption, are discussed in a recent review article (Constantinides, 1995). In the present work, the emphasis was mainly on drug formulation and physical aspects of water-in-oil microemulsions since the pharmaceutical industry shows increasing interest in these systems for drug delivery and absorption improvement of water-soluble drugs/peptides (Constantinides, 1995). We have shown that thermodynamically stable self-emulsifying water-in-oil microemulsions of a mean droplet diameter of 5–30 nm incorporating long- or medium-chain glycerides can be developed using commercially available and pharmaceutically acceptable excipients. Furthermore, it has been possible to solubilize several water-soluble peptides of different molecular structure, size and charge in w/o microemulsions containing long- or medium-chain glycerides. Clearly, a better understanding of the physical chemistry of these systems and their interaction with mucosal membranes, is necessary in order to optimize their drug transport and delivery potential and elucidate the underlying mechanism(s) of absorption enhancement.

Table 3
Comparison of the physical properties of water-in-oil microemulsions incorporating long- vs. medium-chain glycerides

Physical property[a]	Long-chain[b]	Medium-chain[c]
Density (g/cm^3)	0.9010	0.9677
Refractive index	1.471	1.449
Viscosity (cP)	125.1	56.7
Conductance (μmhos/cm)	0.177	0.540
Mean droplet diameter[d] $\pm$ S.D. (nm)	10.3 ± 2.5	15.2 ± 4.1
Polydispersity[d]	0.114	0.153

[a]At ambient temperature.
[b]Soybean oil/Arlacel 186/Tween 80/Saline (65/22/10/3, % w/w).
[c]Captex 355/Capmul MCM/Tween 80/Saline (65/22/10/3, % w/w).
[d]Both expressed as particle number results; polystyrene beads with a labeled mean diameter of 63 nm generated a mean particle diameter of 64.2 ± 15.1 nm and polydispersity of 0.031 (see Table 4).

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Table 4
Particle size of water-in-oil microemulsions incorporating long-chain glycerides

DDME (low HLB defined)	Composition (% w/w)	Droplet diameter[a], mean ± SD, nm (polydispersity)[a]
DDME-MVRL-C	Soybean oil/Myverol 18–99/Tween 80/saline (65/22/10/3)	3.9 ± 1.0 (0.145)
DDME-MVRL-P	Soybean oil/Myverol 18–99/Tween 80/saline + RGD peptide[b] (65/22/10/3)	4.0 ± 0.9 (0.118)
DDME-A 80-C	Soybean oil/Arlacel 80/Tween 80/saline (65/22/10/3)	7.2 ± 1.7 (0.185)
DDME-A 80-P	Soybean oil/Arlacel 80/Tween 80/saline + RGD peptide[b] (65/22/10/3)	11.8 ± 2.9 (0.121)
DDME-A 83-C	Soybean oil/Arlacel 83/Tween 80/saline (65/22/10/3)	9.9 ± 2.2 (0.166)
DDME-A 83-P	Soybean oil/Arlacel 83/Tween 80/saline + RGD peptide[b] (65/22/10/3)	9.9 ± 2.5 (0.153)
DDME-A 186-C	Soybean oil/Arlacel 186/Tween 80/saline (65/22/10/3)	10.3 ± 2.5 (0.114)
DDME-A 186-P	Soybean oil/Arlacel 186/Tween 80/saline + RGD peptide[b] (65/22/10/3)	13.1 ± 3.1 (0.052)
Polystyrene beads, 63 nm	—	64.2 ± 15.1 (0.031)

[a]Both expressed as particle number results.
[b]At 1 mg/ml of microemulsion.

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