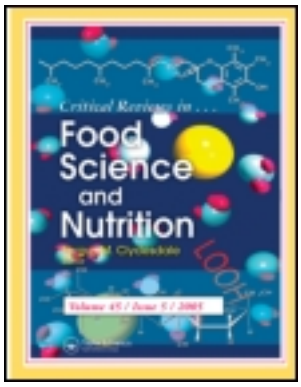

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Microemulsions: A Potential Delivery System for Bioactives in Food

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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. Microemulsions (so-called due to their small particle size; 5–100 nm) have found application in a wide variety of systems, such as pharmaceutical and oil recovery, but their application in food systems has been hindered by the types of surfactant permissible for use in food. The objective of this review is to provide an overview of the structures and phase behavior of microemulsions, methods of microemulsion formation, and techniques which may be used for characterization. A comprehensive review of previous work on both food-grade microemulsion systems, and non-food-grade systems of specific food interest is included. The application of microemulsions as reaction media, their ability to solubilize proteins and hence their use as a separation technique is also documented. In addition, attention is focused on the application of microemulsions as delivery systems for delivery of bioactive compounds, and the links between microemulsions and increased bioavailability. Future research, both applied and fundamental, should focus on surfactants which are not restricted for use in foods.

Keywords microemulsion, food applications, delivery systems, bioavailability, characterization, toxicity

INTRODUCTION

The conventional food industry approach to emulsion preparation has involved the application of energy (e.g. mechanical, sound) to a mixture of oil, water, and emulsifier. The emulsifier (such as synthetically formulated surfactants or amphiphilic proteins) acts to stabilize the interfacial layer between the dispersed and continuous phase which has been created through the addition of energy to the system. These emulsions, or macroemulsions, are turbid, have droplet sizes ranging from 0.2 to 10 μm and may remain stable for a considerable period of time; however, they are kinetically stable, albeit thermodynamically unstable.

Microemulsions, on the other hand, are thermodynamically stable, transparent isotropic solutions with particle sizes ranging from 5 to 100 nm, and arise from the spontaneous self-assembly of the hydrophobic or hydrophilic parts of surfactant molecules. Microemulsions have found numerous applications over a wide range of areas, including pharmaceutical, cosmetics, oil recovery, as models for biological membranes, and as reaction media, and new applications are constantly being reported. A very recent study investigated the possibility of using a polymerized microemulsion as stationary phase for capillary electrochromatography.[1]

However, the application of microemulsions in foods is limited by the types of surfactants which are used to facilitate microemulsion formation. Many surfactants are not permissible in foods; many more may only be added at low levels. More importantly, the solubilization of long-chain triglycerides (LCT), such as soybean oil, is more difficult to achieve than the solubilization of short- or medium-chain triglycerides. For these reasons, few publications discuss the properties of microemulsions in which LCTs are solubilized, and some of those that do utilize short-chain alcohols (such as C_3–C_5) which are not suitable for use in foods. However, many studies of non-food-grade microemulsion systems convey important information on the structures and characteristics of microemulsions, and provide fundamental knowledge of microemulsion behavior.

In the pharmaceutical sciences, extensive research has been conducted on a variety of drug delivery systems. In recent years, however, protein and peptide drugs have become more common, and research in delivery systems has shifted to address this new area. One delivery system which has attracted a considerable degree of attention for delivery of protein/peptides as therapeutic drugs is microemulsions. A number of studies in the pharmaceutical and cosmetic fields have reported enhanced solubilisation of poorly soluble compounds and improved bioavailability following incorporation into microemulsions.

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Meanwhile, in the food sciences, an increasing number of reports have detailed the bioactive capability of compounds isolated from varying food sources. Many of these compounds show significant health benefits when consumed in high concentrations. However, these bioactive compounds often exhibit poor solubility and low bioavailability, and food delivery systems are being developed to help overcome these problems. Microemulsions, as a food delivery system, may be a potential candidate to improve the solubility and increase the bioavailability of food-derived bioactive compounds.

DEFINITION OF MICROEMULSIONS

Oleopathic hydro-micelles were originally reported by Hoar and Schulman,[2] and were subsequently classified as "microemulsions."[3] Microemulsions are defined as systems which comprise of a mixture of water, hydrocarbons, and amphiphilic compounds which form thermodynamically stable, homogeneous (heterogeneous at molecular scale), optically isotropic solutions. The term amphiphiles makes the definition versatile for cosurfactants, which may or may not be required for microemulsion formation.[4]

While the definition of a microemulsion is essentially correct, microemulsion systems may be misrepresented by the title "microemulsion." This assertion is based on the definition of an emulsion, which includes the term "inherently unstable"; however, "microemulsions" are, by definition, inherently stable. Hoar and Schulman's definition primarily differentiated their systems from the (macro)emulsion systems which were well studied at that period. An alternate name, or moniker, which denotes a self-assembled, thermodynamically stable, isotropic system, would be superior to "microemulsion." However, the term "microemulsion" has been continuously used over a spectrum of scientific disciplines since its inception in 1959, and the introduction of a new term to describe "microemulsions" would be confusing in light of other terms (such as swollen micelles, solubilized micellar solutions) which have been used to describe self-assembled, thermodynamically stable, isotropic systems. In view of the above, it appears that, although technically incorrect, the term "microemulsion" is here to stay.

In addition to the conflict over the term "microemulsion," some workers have disputed the validity of describing systems which contain either low volume fractions of oil or water as microemulsions, suggesting that they should be referred to as swollen micelles, and reserving the name microemulsion for systems which have sufficient dispersed phase incorporated into the surfactant micelles, such that the micelle can be considered to have the properties of its bulk.[5] This is in contrast to the opinion that there is no difference between swollen micelles and microemulsions, and that the term micellar solution should be retained for self-assembled surfactant micelles in solution.[6] In view of the subjective nature of "sufficient dispersed phase," this author agrees with Malcolmson et al.,[7] that thermodynamically stable, isotropic systems, comprising of water, oil, and surfactant, should be termed microemulsions, and surfactant solutions should be called micelles or reverse micelles.

Theories of Microemulsion Formation and Stabilization

The three main theories of microemulsion formation and stabilization are discussed in brief here; the reader is directed to the works of Prince,[8] Schulman,[3] Friberg,[9] and Ruckenstein[10] for further information. In the mixed film theory, the interfacial film is considered as a duplux film, having different properties on the water and oil side of the interface.[3,8] The solubilization theory considers microemulsions as swollen micellar systems, i.e. solutions with solubilized water or solubilized hydrocarbon; in effect, one-phase systems.[9] The thermodynamic theory proposes that the free energy of formation of microemulsion, ΔG_m, consists of different terms, such as interfacial free energy, energy of interaction between the droplets, and an entropy term. When ΔG_m is of a very low or slightly negative value, microemulsion formation can be facilitated.[10] Irrespective of the mechanism of microemulsion stabilization, the reduction of the interfacial free energy to a very low value is critical in facilitating microemulsion formation.

Phase Behaviors

Single Phase Microemulsions

Thermodynamically stable, isotropic systems may form at specific concentrations of oil, water, and surfactant (and possibly co-surfactant). Generally, at low oil concentration (<30%) microemulsions are in the oil-in-water (o/w) form. Conversely, at low aqueous concentration, water-in-oil (w/o) microemulsions are formed. However, inside the specific o/w and w/o microemulsion areas, many different defined systems, such as micellar, reverse micellar, lamellar and bicontinuous phases may exist at distinct oil-water-surfactant concentrations. Micelles or reverse micelles may form through the spontaneous self-assembly of the surfactant molecules when there is no oil or water, respectively, present. All of these systems may exist, either alone, or in combination with other systems, as transparent one-phase microemulsions. However, the boundary between areas of microemulsion formation and areas which lie outside the area of one phase transparency may be quite indistinct. For example, Figure 1 shows the gradual change in the transparency on increasing oil concentration from 2 to 11% (w/w) in a polyoxyethylene non-ionic surfactant stabilized microemulsion system.

Multiple Phase Microemulsions

Microemulsions may also co-exist in equilibrium with excess oil or excess water phases. The appearance of phases generally occurs when there is insufficient surfactant in the system and transitions between phases may occur on addition of a new component, changes in composition of the microemulsion

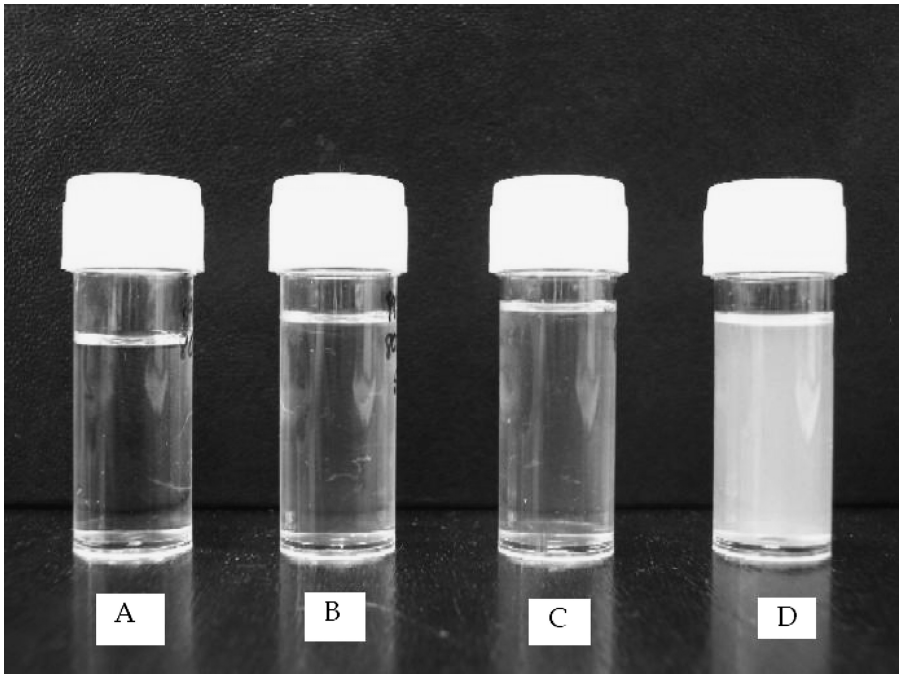

Figure 1 Photographs of 10-month old microemulsion samples containing 2, 5, 8 and 11% (w/w) soybean oil (a, b, c and d, respectively). Microemulsions were prepared using 20% (w/w) polyoxyethylene (POE) surfactant.

components, or changes in temperature. These phases can be described using the Winsor classification,[11] and are illustrated in Figure 2. A Winsor I system comprises of an o/w microemulsion system which is in equilibrium with an upper excess oil phase. Conversely, a Winsor II system consists of a w/o microemulsion system in equilibrium with a lower excess aqueous phase. When equal volumes of oil and water are present in the microemulsion system, a bicontinuous structure is formed, and this may coexist with an upper excess oil phase, and a lower excess aqueous phase; this is referred to as a Winsor III system. Finally, a Winsor IV system defines a macroscopically single-phase microemulsion system.

Nano-Emulsions

Some workers have also discussed the properties of nano-emulsions, as distinct from microemulsions. Nano-emulsions are reported to have particle sizes between that of conventional emulsions and microemulsions, ranging from 20 to 200 nm,[12] and the systems are described as "approaching thermodynamic stability,"[13] unlike microemulsions which are thermodynamically stable. These nano-emulsions have also been termed miniemulsions,[14,15] fine-disperse emulsions,[16] submicron emulsions,[17] unstable microemulsions[18] and translucent emulsions.[19] The systems described under the label

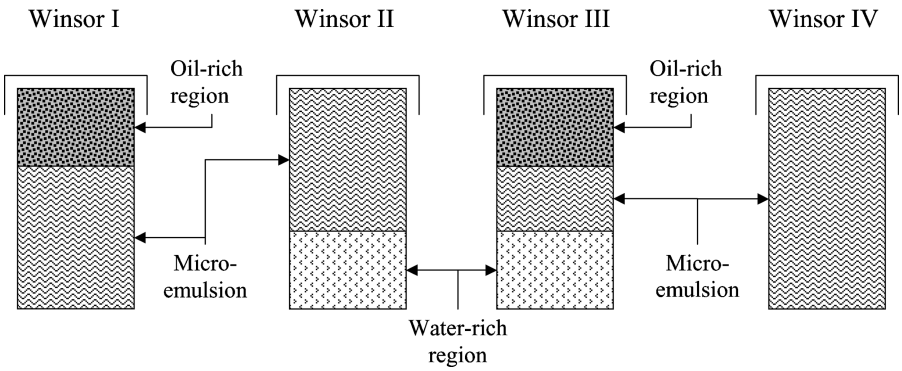

Figure 2 Winsor classification system, showing oil- and water-rich phases possible in microemulsion systems. Redrawn with permission [4]. Copyright (1997) CRC Press.

nano-emulsions seem to have, in some respects, similar attributes to previously described microemulsions (small particle sizes, and spontaneous formation or low energy methods required for formation), and are the subject of a recent book.[20] However, these systems appear to lie just outside the stable isotropic thermodynamic area in which "true" microemulsions form, in that they are not thermodynamically stable.

Of the microemulsion systems described above, single phase (Winsor IV) thermodynamically stable microemulsion systems are of primary interest to food technologists, and the remainder of this review will focus on these one-phase systems.

MICROEMULSION COMPONENTS

Surfactant

The surfactant, also called emulsifier or amphiphilic compound, plays an important role in microemulsion formation by reducing the interfacial tension. Reduced interfacial tension, in combination with the enthalpic contributions of reduced interactions between the hydrophobic tails of the surfactant and the polar solvent (and also including possible interactions of the solubilized compound) reduce the overall free energy of the system, thus facilitating microemulsion formation. In the dilute state, surfactants exists as monomers, but above a certain minimum concentration, termed the critical micelle concentration (CMC), the surfactant molecules spontaneously associate to form micelles. Formation of micelles is driven by either strong interactions of the hydrophobic tails of the surfactant, resulting in micelles, or by hydrophilic interactions of the polar head group of the surfactant, resulting in reverse micelles. The type of micelle formed is also influenced by the packing parameter of the surfactant in the micellar assembly. The critical packing parameter (CPP) can be calculated as follows:

$$\mathrm{CPP} = \upsilon/a.l$$

where υ is the partial molar volume of the hydrophobic portion of the surfactant; a is the optimal head group area; and l is the extended surfactant chain length. The critical length of the surfactant chain, l_c, may be substituted for l; l_c is generally assumed to be 70–80% of l.

The CPP is a measure of the preferred geometry adopted by the surfactant, and is therefore predictive of the type of micellar assembly which is likely to form. Different surfactant geometries and their effect on CPP are illustrated in Figure 3. When the

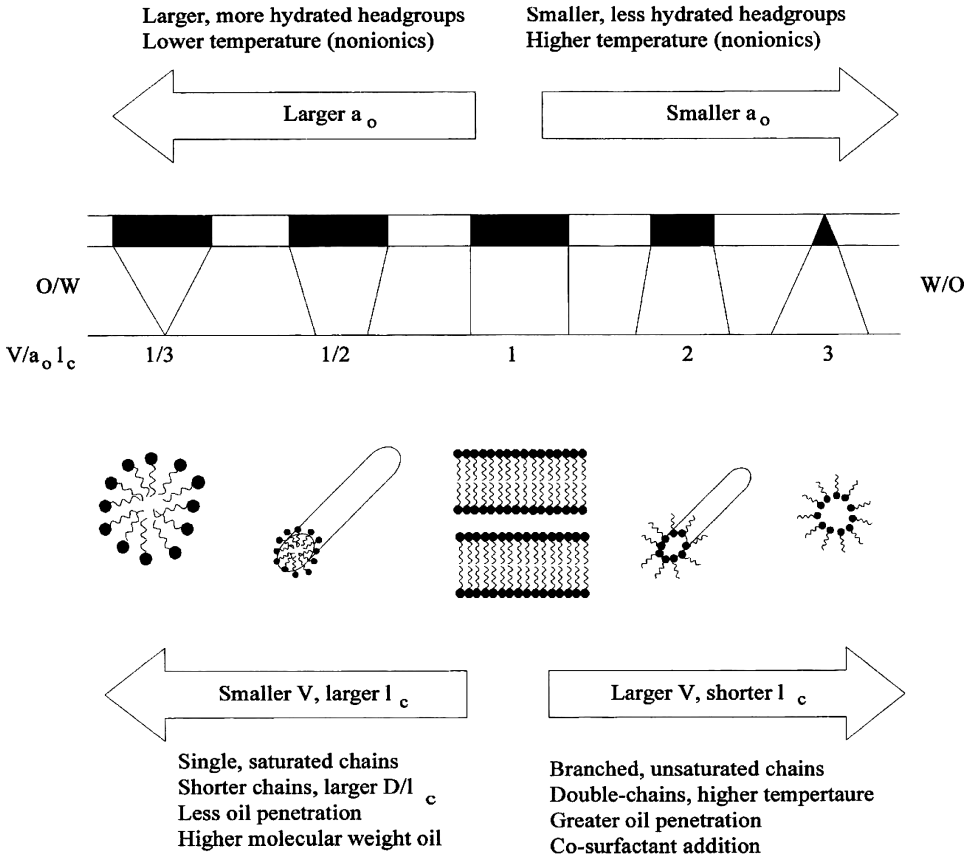

Figure 3 Effect of solution conditions on the headgroup size and resulting effect on the surfactant molecule shape and arrangement. Reprinted from Advanced Drug Delivery Reviews, Vol 45, Lawrence and Rees, Microemulsion-based media as novel drug delivery systems, Pages 89–121, Copyright (2000), with permission from Elsevier. Originally reprinted from Colloids and Surfaces A, Vol 91, Israelachvili, The science and application of emulsions—an overview, Pages 1–8, Copyright (1994), with permission from Elsevier.

headgroup of surfactants is of greater volume than its hydrophobic tail, o/w micelles will invariably form in the presence of oil, whereas surfactants with headgroups of smaller volume than their hydrophobic tails will form w/o micelles in the presence of oil. When the volumes of headgroup and surfactant chains are similar, bilayer structures are favored.

In addition, changes in microemulsion composition will also affect the geometry adopted by the surfactant, and hence the CPP of the surfactant. A decrease in the effective head group area of ionic surfactants will occur on increasing ionic strength, due to the shrinking of the double layer and the screening of the head groups. Similarly, the addition of hydrophilic molecules such as glycerol and sorbitol will influence optimal head group area by altering the solubility of the head group in the aqueous phase.

In contrast to CPP, the hydrophile-lipophile balance (HLB) takes into account the relative contribution of hydrophilic and hydrophobic parts of the surfactant molecule.[21] Generally, w/o microemulsion can be formed with surfactants with a low HLB range of 3–8, whereas o/w microemulsion can be formed with surfactants having a HLB range of 8–18. Furthermore, the phase volume ratio of oil: water will also influence the type of microemulsion formed.

Surfactants may be divided into four different classes: non-ionic, zwitterionic, cationic, or anionic. Non-ionic surfactants have been extensively used in pharmaceutical microemulsion preparation due to their relatively low toxicity or irritation potential.[22] Examples of non-ionic surfactants include sugar esters, such as sorbitan monooleate, and polyoxyethylene ether (POE) surfactants, such as Brij 96. Interestingly, although POE surfactants are not food grade, they have been studied extensively in pharmaceutical microemulsion systems, and occasionally in food-grade microemulsion systems, primarily due to the ease with which they form microemulsions. Phospholipids are an example of a zwitterionic surfactant, and have the greatest potential for use in microemulsions due to their designation as GRAS. Soybean and egg lecithin preparations are commercially available, and contain phosphatidylcholine as their major constituent. Few differences were found between the phase diagrams[23] or particle sizes[24] of microemulsions formed from soybean-derived lecithin, compared to egg-derived lecithin. However, a major disadvantage of lecithin is that cosurfactants are usually required for microemulsion formation.

It is also worth noting that most commercially available surfactant preparations are not homogenous; they may be quite heterogeneous in terms of either the fatty acid side chain length or degree of saturation, or in terms of the degree of fatty acid side chain modification (for example, degree of esterification). The United States Code of Federal Regulations number (US CFR no.), and the European Union number (EU no.) of surfactants which may be used in foods are detailed in Table 1.

Table 1 List of surfactants commonly used in foods

General Class	Examples	US 21 CFR[a]	EU no.
Lecithin and lecithin derivatives	Pure phospholipid (e.g. phosphatidyl choline) and mixed phospholipids	184.1400[b]	E322
	Hydroxylated phospholipids/lecithin	172.814[b]	E322
Lactylated Esters	Lactylic esters of fatty acids	172.848	
	Lactylated fatty acid esters of glycerol and propylene glycol	172.850	
	Calcium stearoyl-2-lactylate	172.844	E482
	Sodium stearoyl-2-lactylate,	172.846	E481
Glycerol fatty acid esters	Polyglycerol fatty acid esters	172.854	E475
	Polyglycerol polyricinoleate		E476
	Propylene glycol fatty acid esters (Propane-1, 2-diol esters of fatty acids)	172.856	E477
Partial glycerides and derivatives	Mono- and di-glycerides	184.1505[b]	E471
	Monosodium phosphate derivatives of mono- and diglycerides	184.1521[b]	
	Acetic acid esters of mono- and diglycerides	172.828	E472a
	Lactic acid esters of mono- and diglycerides	172.852	E472b
	Citric acid esters of mono- and diglycerides		E472c
	Stearyl citrate (mixture of mono-, di- and tri-stearyl esters of citric acid)	184.1851[b]	E484
	Diacetyl tartaric acid esters of mono- and di-glycerides (DATEMS)	184.1101[b]	
	Succinylated monoglycerides	172.830	E472g
	Ethoxylated mono- and di-glycerides	172.834	
Sucrose esters	Mono-, di-, and tri-esters of sucrose with fatty acids	172.859	E473
Sorbitan fatty acid esters	Sorbitan monostearate	172.842	E491
	Sorbitan tristearate		E492
	Sorbitan monolaurate		E493
	Sorbitan monooleate		E494
Polyoxyethylene sorbitan fatty acid esters	Polyoxyethylene (20) sorbitan monostearate (Polysorbate 60)	172.836	E435
	Polyoxyethylene (20) sorbitan tristearate (Polysorbate 65)	172.838	E436
	Polyoxyethylene (20) sorbitan monooleate (Polysorbate 80)	172.840	E433
Other	Ox bile extract	184.1560[b]	
	Propylene glycol	184.1666[b]	
	Sodium lauryl sulfate	172.822	

[a] United States Code of Federal Regulations, Title 21, Volume 3, Revised as of April 1, 2004, from the U.S. Government Printing Office. Accessed via http://www.gpoaccess.gov/

[b] Generally recognised as safe (GRAS).

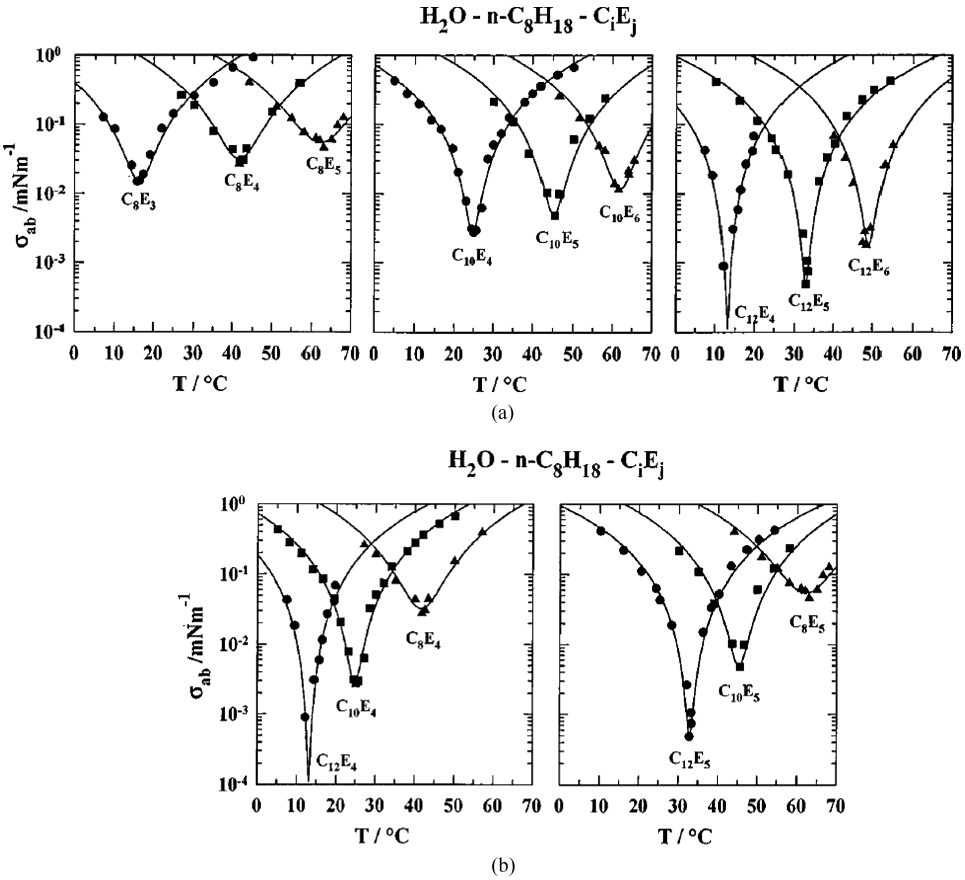

Figure 4 Effect of (a) polar headgroup size at fixed surfactant chain lengths; and (b) surfactant chain length at fixed headgroup size on the interfacial tension of octane/polyoxyethylene (POE) system. Reproduced with permission [26]. Copyright (1997) American Institute of Physics.

Microemulsions formulated with non-ionic surfactants may be susceptible to changes in temperature, as the solubility of non-ionic surfactants (especially the POE surfactants) decreases with increasing temperature.[25] As a result, microemulsion systems stabilized with non-ionic surfactants may have a phase inversion temperature (PIT) at which structural transitions occurs. The PIT of POE surfactants, and indeed the interfacial tension minimum, is affected by both the head group size and the surfactant chain length of the surfactant molecule, an effect clearly illustrated by Sottmann and Strey[26] who employed octane as the oil phase (Figure 4a, b). It is possible to alter the PIT of a microemulsion system by addition of an electrolyte; furthermore, the inclusion of other compounds in the microemulsion system can also alter the PIT.

At high surfactant concentrations, w/o microemulsion systems may form a thermoreversible gel, called an organogel due to the organic continuous phase. Like hydrogels, organogels are finding increasing applications in the pharmaceutical industry as a means of drug delivery. The applications of microemulsion-based organogels, including gels based on gelatin, lecithin, and water-in-sorbitan monostearate organogels have been reviewed in detail by Bagwe et al.[27] Gels with an optically isotropic gel phase and strong elastic properties may also be formed at high surfactant concentrations; these gels are remarkable for their resonating properties.[28,29] However, these gels have found few applications in the food or pharmaceutical industries as high surfactant concentrations (between 5 and 30%) are required for their formation.

Another approach to drug delivery has utilized oil-surfactant solutions with solubilised drugs which form microemulsion on dilution with water. These are called self-emulsifying drug-delivery systems (SEDDS), and have been reviewed by Constantinides.[30]

Toxicity of Surfactants

While all surfactants used in food systems would have been subjected to extensive toxicity studies, very little research has been carried out on the toxicity of surfactants in microemulsion form. However, some studies have suggested that the toxicities of surfactants may be altered when self-assembled in microemulsions.

The toxicities of two surfactants, POE surfactants $C_{18:1}E_{10}$ and $C_{12}E_{10}$, were determined alone and also in combination with a range of oils (ethyl esters and triglycerides) using the MTT assay.[31] The $C_{12}E_{10}$ surfactant was found to be toxic at

concentrations around or below its critical aggregation concentration, both alone and in the presence of oil. However, the $C_{18:1}E_{10}$ surfactant, either alone or in the presence of low molecular weight oils, was toxic above its critical aggregation concentration, and, in the presence of high molecular weight oils (Miglyol 812, soybean oil) the microemulsion systems were toxic only at concentrations significantly greater than their critical aggregation concentration. The authors proposed that the decreased toxicity of the high molecular weight oil microemulsions was due to the formation of a distinct oil core in the aggregates which reduced the ability of the system to solubilize components of the cell membrane.[31] The study of Warisnoicharoen highlights the importance of conducting toxicity studies on microemulsion systems (including microemulsions formulated with surfactants which have been designated GRAS status) as the toxicities of the surfactants may be altered when assembled in microemulsion structures. While toxicity evaluation is taken into consideration in some pharmaceutical studies,[32,33] the toxicities of surfactants in food-grade microemulsion systems does not appear to have been reported.

Cosurfactant

Microemulsion formation may also require the presence of a cosurfactant. Medium chain length alcohols, and to a lesser extent amines and acids, have been used as cosurfactants and perform a variety of functions to aid in microemulsion formation. The cosurfactant has the effect of further reducing the interfacial tension, whilst increasing the fluidity of the interface, thereby increasing the entropy of the system.[34,35] Cosurfactants may also adjust the curvature of the interfacial film by partitioning between the tails of the surfactant chains, allowing greater penetration of the oil between the surfactant tails. Furthermore, the presence of alcohol may influence the solubility properties of the oil and water phases, due to its partitioning between these phases.[21] Cosurfactants can also destabilize the lamellar liquid crystalline phase.[22]

The presence of a cosurfactant can often hinder the application of some microemulsion systems. If a microemulsion is to be delivered orally, the system will be diluted in the oral cavity and in the stomach. On dilution of a microemulsion containing cosurfactants, the cosurfactant may partition away from the interface into the continuous phase, resulting in the destabilization of the interface, and subsequent breakdown in the microemulsion structure. Microemulsions which may be subject to dilution require cosurfactants which will remain at the interface and maintain the low interfacial tension.

Toxicity of Cosurfactants

Not all cosurfactants are approved for human use, and short- or medium-chain alcohols, when employed as cosurfactants, can cause toxicity and irritation. The toxicities of 1-butanol, 2-butanol, ter-butanol have been reported.[34] As such, the only alcohol permissible for use in food is ethanol. However, not only have many studies used alcohols which are not suitable for use in foods, some of those that have, have used ethanol concentrations ranging from 10 to 20%, which would be perfectly acceptable if the microemulsion were to be incorporated into an alcoholic cocktail. Furthermore, alcohol is not permitted for consumption by certain religious denominations, thereby restricting the potential market for microemulsions which do require alcohol for formation.

Oil

As mentioned previously, hydrocarbon mineral oils have been the basis of many microemulsion studies, mainly due to ease of microemulsion formation and also probably due to the purity of the hydrocarbon systems. In contrast, formation of microemulsions with high molecular weight oils such as triglycerides is more difficult. Triglycerides contain long-chain fatty acids, are semi-polar compared to hydrocarbon oils, and are too bulky to penetrate the interfacial film to assist in the formation of an optimal curvature.[36] Because of the difficulty in formulating microemulsions with large triglycerides, researchers who are primarily interested in the characteristics and structures of microemulsion have studied hydrocarbon oils. Several researchers who have claimed to develop food-grade microemulsion have taken advantage of the increased ease of microemulsion formation with smaller molecular weight oils (for example, medium-chain length triglycerides such as the commercially available Miglyol 80, or essential oils such as limonene). Consequently, information available in the literature regarding microemulsion formulated with cheap, commercially available triglycerides, such as soybean oil, is noticeably lacking.

FORMATION OF MICROEMULSIONS

In theory, the arrangement of the emulsifier molecules (possibly aided by cosurfactant) occurs spontaneously. However, in some cases energy is provided to the system to speed up the rearrangement of the surfactant molecules, or to overcome a small kinetic energy barrier. There are three principle methods which may be used in microemulsion formation.

A. Low energy emulsification method

Microemulsion preparation can be achieved in three different low energy emulsification methods: dilution of an oil-surfactant mixture with water; dilution of a water-surfactant mixture with oil; and mixing all the components together in the final composition. As each of these methods involves the spontaneous formation of microemulsions, the order of ingredient addition may determine the formation of microemulsion.

Research in our laboratory has found that the order of ingredient addition played a significant role in the formation of microemulsions using ethoxylated mono- and di-glycerides

as surfactant, soybean oil as the oil phase, and 50% sucrose in 5% ethanol as the aqueous phase.[37] On dilution of solutions having different aqueous: surfactant ratios with oil, a one-phase transparent solution was only formed when the ratio of aqueous: surfactant was 60: 40 (Figure 5a). On dilution of solutions having different oil: surfactant ratios with the aqueous phase, one-phase transparent solutions were located at several locations within the psuedoternary phase diagram (Figure 5b). However, only one of these areas coincided with the one-phase area identified in Figure 5a. The importance of order of addition of ingredients seems to be quite important in some systems. Forgiarini et al.,[12] reported that in a water/decane/POE surfactant system, microemulsions could only be formed on dilution of an oil-surfactant mixture with water.

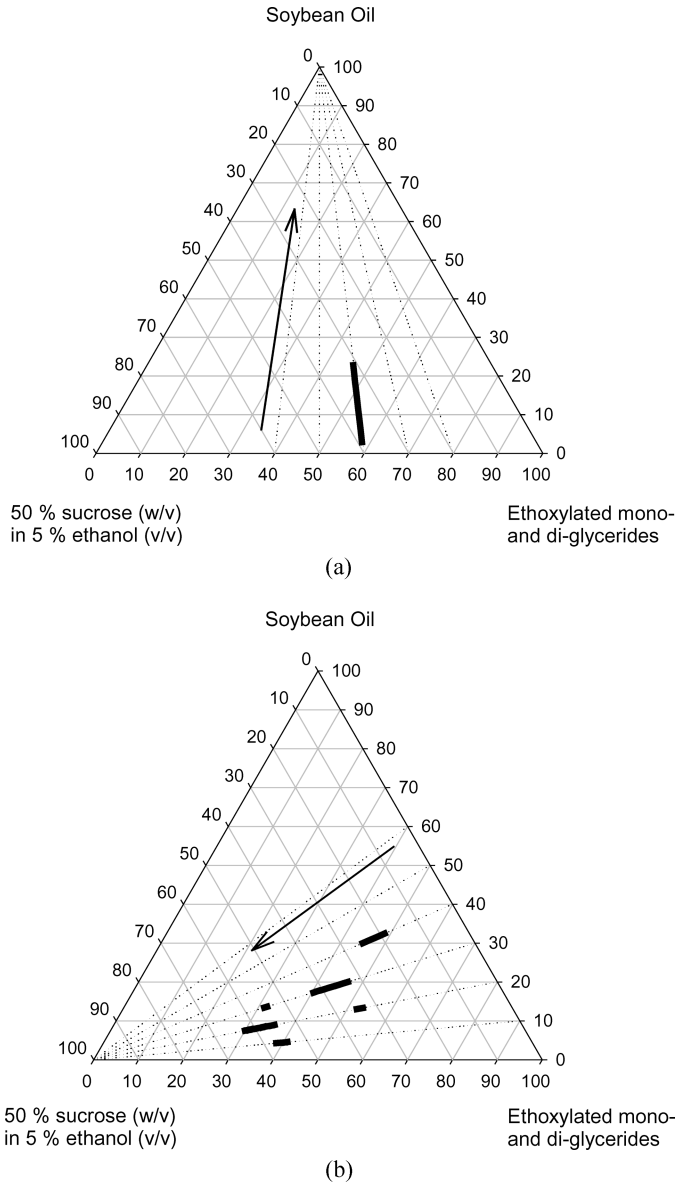

Figure 5 Pseudoternary phase diagrams of soybean oil, ethoxylated mono- and di-glycerides and sucrose/ethanol aqueous systems at 20°C. A—dilution of aqueous/surfactant solutions with oil. B—dilution of oil/surfactant solution with aqueous solution. Arrows show direction of dilution. One phase areas are indicated by heavy black line.

B. Phase inversion temperature (PIT) method

The PIT method for microemulsion formation is particularly useful when using ethoxylated non-ionic surfactants. When an o/w emulsion containing ethoxylated non-ionic surfactant is heated, the emulsion inverts to a w/o emulsion at a critical temperature, the PIT. At the PIT, the droplet size and the interfacial tension reach a minimum, and upon cooling while stirring, a stable o/w microemulsion forms.

C. High pressure homogenization

Homogenization may also be used to form microemulsions; however, the process of emulsification is generally inefficient (due to the dissipation of heat). In addition, the homogenization process may be extremely limited as the water/oil/surfactant mixture may be highly viscous prior to microemulsion formation.

CHARACTERIZATION OF MICROEMULSIONS

While microemulsions can be prepared quite easily, characterization of their microstructure is far from trivial. In practice, complementary methods are required in order to fully characterize microemulsions.

The first step in microemulsion characterization is the determination of where a clear isotropic region lies within the different concentrations of water, oil and surfactant. These variables can be plotted on a ternary phase diagram, where each corner, or vertex, of the diagram represents 100% of that particular component. Where four or more components are investigated, pseudo-ternary phase diagrams are used, with one corner of the phase diagram representing a defined mixture of 2 (or possibly more) components (Figure 6).

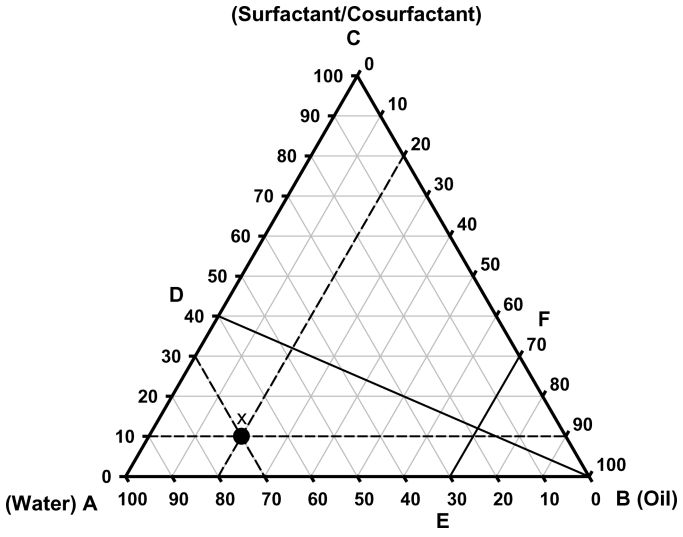

Figure 6 Plotting of pseudoternary phase diagrams. See text for further details.

The plotting of specific water/oil/surfactant concentrations may be explained using Figure 6. A point on the line connecting vertices A and C, for example, represents systems comprising of water and surfactant/cosurfactant only, with the absence of oil. Any line drawn from a vertex to a point on the line directly opposite the vertex will have a constant ratio of the 2 components. From Figure 6, any point on the line BD will have a constant ratio of water and surfactant/cosurfactant (60:40 in the current example). A line drawn parallel to any side of the triangle will have a constant proportion of one of the components, thus any point on the line EF will have 70% oil. Any point inside the triangle represents a mixture of water, oil, and surfactant/cosurfactant, and the exact composition can easily be calculated. Taking point x in Figure 6, the concentration of water in the system can be calculated by drawing a line through x, parallel to BC. As vertex A of the triangle represents 100% water, point x falls on the line representing 70% water. Applying the same rules to determine the concentration of oil, it can be calculated that point x represents 20% oil. The remaining 10% comprises of surfactant/cosurfactant.

Microemulsion characterization can be divided into 2 main areas, characterization at the macroscopic level and characterization at the microscopic level. Viscosity, conductivity, and dielectric measurements provide useful information at the macroscopic level. The presence of worm-like reverse micelles[38] and the transition between microemulsion structures[39] can be implied by changes in viscosity. The rheological properties of microemulsions has been recently reviewed by Gradzielski.[40] Conductivity measurements can be used to determine whether a microemulsion is oil-continuous or water-continuous, and may also be used to monitor percolation or phase inversion phenomena.[41–43] Dielectric measurements have been used to probe both the structural and dynamic features of microemulsions.[42,44,45] The optical clarity of microemulsions along with their isotropic nature makes their study by light-scattering methods quite straightforward; however, it is the minute particle sizes encountered in microemulsions which require special techniques. A variety of methods, such as freeze fracture electron microscopy,[46] and a range of light scattering methods,[47] such as small-angle X-ray scattering, small-angle neutron scattering, total intensity light scattering, photon correlation spectroscopy, may be used to determine the particle size of a microemulsion.

APPLICATION OF MICROEMULSIONS AS DELIVERY SYSTEMS

Since 1943, and the first description of a microemulsion system,[2] extensive research has been conducted on the formation, characterization, and potential applications of microemulsions. Unfortunately, from a food application point of view, most of the systems described are unsuitable for human consumption, mainly due to the ingredients used. The majority of research has involved microemulsion systems which comprised of short- to medium-chain length alcohols as cosurfactant, and hydrocarbon oils such as benzene and hexane as the oil phase, in conjunction with a variety of food- and non-food-grade surfactants. Documented examples of microemulsions which are acceptable for food applications are sparse. Studies which have described food-grade microemulsions utilizing triglycerides as the oil phase are extremely scarce. Due to the difficulty of solubilizing long-chain triglycerides, as outlined previously, medium chain triglycerides have more often than not been studied in food-grade microemulsions. Microemulsion systems which have utilized ingredients suitable for use in foods are reviewed in detail below; other microemulsion systems of interest are also discussed.

The research group at the Casali Institute of Applied Chemistry, Jerusalem, have been predominant in the formulation of food-grade microemulsions in the previous 10 years, and have conducted numerous studies on the effects of different food-grade surfactants on formation and characterization of microemulsions.

The application of non-ionic sucrose esters for the formation of microemulsions has been reviewed in detail by Garti et al.,[48] and a subsequent study compared the water solubilization properties of different sucrose esters using a range of short-chain alcohols, ranging from C_2 to C_5, at 37°C, with medium-chain (caprylic/capric) triglycerides (MCT) used as the oil phase.[49] Formation of w/o microemulsions using ethanol generally required between 4 and 6 parts surfactant to solubilise 1 part water; in most cases, the final microemulsion system was comprised of more than 25% ethanol. In addition, the use of ethanol as cosurfactant did not compare favorably to the use of butanol or *n*-propanol as cosurfactant in terms of concentration of water solubilized. In a subsequent study, Garti et al.,[50] compared the phase diagrams of microemulsions formed using sucrose monostearate (SMS) and different oil (MCT, soybean oil, limonene and oleic acid) and cosurfactant (butanol, propanol, and pentanol) components. However, soybean oil and limonene oil were only solubilized in the presence of butanol at a 3:1 butanol: oil ratio and the total areas of microemulsion formation on the phase diagrams were similar to that when MCT was substituted as the oil phase. The maximum concentration of water solubilized decreased from 59% for MCT with butanol as cosurfactant, compared to 33 and 36% water solubilized for soybean oil and limonene, respectively.[50] Other workers have conducted a fundamental study on the phase behaviour and effect of temperature on microemulsions formed with commercial grade sucrose dodecanoate and hydrocarbon oils.[51]

Garti et al.,[52] conducted an extensive study on the phase behavior of microemulsions based on five-component mixtures. Varying oil phases (R-(+)-limonene, MCT and soybean oil), short-chain alcohols (ethanol, propanol, and butanol), polyols (propylene glycol and glycerol) and surfactants (ethoxylated alcohols, ethoxylated sorbitan esters, polyglycerol esters, sucrose monolaurate, sucrose monostearate, and soybean phosphatidyl choline) were considered in a selection of combinations. The one-phase microemulsion area of an R-(+)-limonene, ethoxylated alcohol (Brij 96) and water increased from 13% to 30.3%

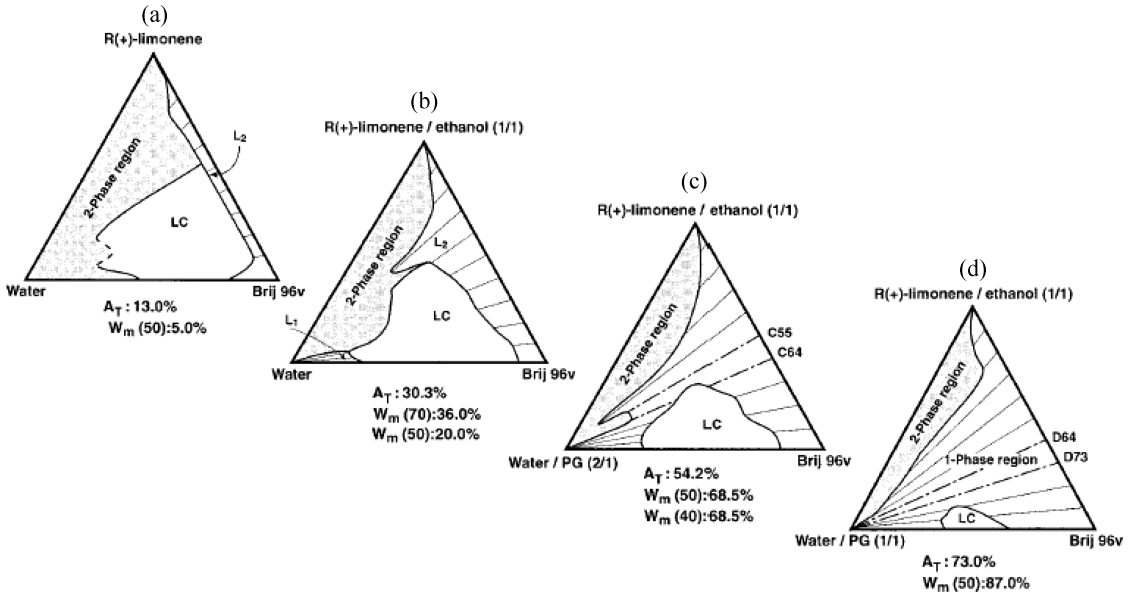

Figure 7 Pseudoternary phase diagrams of water/polyglycerol (PG)/R-(+)-limonene/EtOH/Brij 96v (POE) systems. L_1 denotes o/w microemulsion. L_2 denotes w/o microemulsion. LC denotes the liquid crystalline phase. A_T denotes the total one phase area as a percentage of the phase diagram. W_m denotes the maximum water concentration solubilised at a given surfactant concentration, in parenthesis. Reprinted with permission from [52]. Copyright (2001) American Chemical Society.

on addition of ethanol in a 1:1 proportion with R-(+)-limonene (Figure 7). Further increases in the one-phase area, 54.2 and 73%, were observed on addition of propylene glycol in a 1:2 and 1:1 proportion with water, respectively. These trends, increased one-phase area following increased concentrations of ethanol and propylene glycol, were also observed for the ethoxylated sorbitan ester Tween 60. Furthermore, when MCT were solubilized with ethoxylated sorbitan ester Tween 80, the one-phase area increased as concentrations of ethanol and propylene glycol or glycerol were increased. Replacing ethanol with propanol or butanol in the 5-component microemulsion system resulted in an increased one-phase area; however, pentanol or hexanol decreased the solubilization capacity of the system. Partial substitution of Tween 60 with phosphatidyl choline, even at high ethanol concentrations, did not increase the one-phase area.[52]

The solubilization capacity of lycopene (an essential carotenoid sensitive to light degradation) in w/o, bicontinuous and o/w microemulsions was determined using different individual surfactants (Tween 20, 40, 60, and 80, triglycerol monooleate, ethoxylated monodiglycerides, and sucrose esters) and combinations of these surfactants.[53] R-(+)-Limonene, an essential oil, was used as the oil phase. The solubilization of lycopene in a Tween 60 stabilized-microemulsion system was 4 times higher than the solubility of lycopene in limonene; lycopene is practically insoluble in water. Further increases in lycopene solubility were observed when mixtures of surfactants were used. Lycopene solubility increased from approximately 310 ppm with the use of a single surfactant (Tween 60), to approximately 450 ppm in the presence of three surfactants (Tween 60, ethoxylated monodiglycerides, and sucrose esters), indicating synergistic surfactant partitioning at the interface in surfactant mixtures.[53] In a subsequent study, the light stability of lycopene was found to be improved following solubilization of lycopene in Tween-based microemulsions compared to solubilization in acetone.[54] Garti et al.,[54] have also reported the encapsulation of a bromine-based bactericide in the presence of butyl lactate into microemulsions prepared with an ethoxylated surfactant, Brij 96. The authors claim that the microemulsion systems described to encapsulate bactericides could also be used to encapsulate pesticides.[54]

Up to 4% (w/w) lutein (a naturally occurring, poorly soluble, carotenoid associated with reduced risks of cataract development) was incorporated into the bicontinuous phase of microemulsions containing R-(+)-limonene, ethanol, glycerol, Tween 80, and water.[55] The presence of vegetable oil was found to detrimentally affect the concentration of lutein which could be encapsulated. In a subsequent study, Amar et al.,[39] determined the aqueous phase concentration at which the w/o → bicontinuous → o/w transitions occurred using viscosity, pulsed-gradient spin echo NMR and dynamic light scattering techniques.

The solubilization capacity of phytosterol (which has been shown to reduce the total plasma cholesterol and low-density lipoprotein (LDL) cholesterol levels in humans[56]) was increased 6-fold following incorporation in R-(+)-limonene, Tween 60 stabilized microemulsions, compared to the solubility of phytosterol in limonene.[57] The authors propose that it may be possible to lower cholesterol levels by consuming foods fortified with high concentrations of natural phytosterols concentrated in microemulsion form.

The research group at the Casali Institute of Applied Chemistry have also conducted extensive research on the microstructure of microemulsions. Pulsed-gradient spin-echo

nuclear magnetic resonance (PGSE-NMR) and viscosity measurements,[39,58] differential scanning calorimetry,[59,60] self-diffusion NMR,[57] small-angle neutron scattering,[61] small-angle X-ray scattering,[62] dynamic light scattering,[39,63] Fourier transform PGSE-NMR self-diffusion,[64] dielectric spectroscopy,[45] cryo-transmission electron microscopy,[65] and electrical conductivity (used in a number of studies), have been used to characterize microemulsion structures and elucidate transitions between microemulsion states.

A distinct series of studies concentrated solely on the solubilization of triglycerides in microemulsions using ethoxylated mono- and di-glycerides.[66–68] Phase diagrams of microemulsions formed with soybean oil, or hydrogenated soybean oil were generated, and the effect of adding sucrose and propylene glycol to the system was investigated.[67] The presence of sucrose enhanced the formation of o/w microemulsions but impeded the formation of w/o microemulsions. Ethanol was required in all cases for microemulsion formation. A subsequent study reported synergistic effects between sucrose and ethanol for the formation of microemulsions containing triglycerides.[68] High sucrose concentrations were found to favor the formation of hexagonal structures, while these structures could be destroyed on addition of ethanol. Up to 18% soybean oil could be incorporated into microemulsions when 10–12% sucrose was added; however, high surfactant (55–60%) and high ethanol (20%) concentrations were also required.[68]

Some isolated studies and patented works have reported on the formation and properties of microemulsions which may be of interest to food technologists. A limited study reported the solubilization and protection of Vitamin E against oxidation in microemulsions prepared with POE surfactants.[69] Microemulsions, prepared using Tween 60 and different cosurfactants, were used to solubilise a range of essential oils for application in flavored carbonated beverages; however, high alcohol concentrations (~10–15%) were required for microemulsion formation.[70] A patent application described the formulation of microemulsions for use in non-carbonated beverages.[71] Patents have also been filed for the protection of flavor or aroma[72,73] and vitamins[74] using microemulsion technology.

Microemulsions have also attracted interest due to their ability to increase efficiency of antioxidants. Water-in-oil microemulsions, formulated with soybean oil and monoglycerides as surfactant, were stable against oxidation after 70 days when 5% (w/w) ascorbic acid was incorporated into the water phase, compared to less than 20 days for similar microemulsions prepared without ascorbic acid.[75] A synergistic effect between ascorbic acid solubilized in the water phase, and α-tocopherol solubilised in the oil phase of the w/o microemulsions was also observed. Combinations of ascorbic acid and α-tocopherol to prevent oxidation have been studied in fish oil microemulsion systems as fish oil is extremely susceptible to oxidation due to the high concentration of unsaturated fatty acids. Jakobsson and Sivik[76] utilized microemulsions formulated using distilled monoglycerides, while Yi et al.,[77] utilized a reversed micellar system using phosphatidylcholine as surfactant; both studies reported that ascorbic acid and α-tocopherol acted synergistically to reduce lipid oxidation in fish oil.

Surprisingly, studies involving microemulsions which may be of interest to the food industry have been carried out by research groups whose interests are primarily in the pharmaceutical field. The research group of Prof M. J. Lawrence, Department of Pharmacy, King's College London, have conducted a series of studies on the utilization of POE non-ionic surfactants and phospholipids for the formation of microemulsions using soybean oil, among other pharmaceutically acceptable oils, as the oil phase.

In an initial study, Malcolmson and Lawrence found that the apparent aqueous solubility of lipophilic drugs such as testosterone and progesterone were increased following solubilization in microemulsions formulated with a POE non-ionic (Brij 96) surfactant and soybean oil.[78] In a further study, the ability of long- ($C_{18:1}E_{10}$) and short-chain ($C_{12}E_{10}$) POE non-ionic surfactants to form microemulsions using long- (soybean oil), medium- (Miglyol 812) and short-chain (ethyl esters) oils was examined.[79] The results showed that, in the systems studied, long chain surfactants complemented the solubilization of long-chain oils, and short-chain surfactants complemented the solubilization of short-chain oils in o/w microemulsions (Figure 8). Total intensity light scattering (TILS) and dynamic light scattering methods were also used to elucidate the effect of varying surfactant ($C_{18:1}E_{10}$) and soybean oil concentration on the particle sizes of microemulsions[80] and also of testosterone enanthate-containing microemulsions.[81] As the soybean oil concentration in a 14% (w/w) $C_{18:1}E_{10}$ stabilized microemulsion system increased from 0.5 to 5% (w/w), droplet radii, calculated from TILS increased from 50.8 Å to 72.0 Å,[81] indicating that the soybean oil was incorporated inside the microemulsion droplets. This is in contrast to the solubilization of low molecular volume oils (namely tributyrin, ethyl butyrate, or ethyl caprylate) where the hydrodynamic radius did not change much on increased oil addition, suggesting that the oils were preferentially located in the interfacial surfactant monolayer.[80]

The possibility of using lecithin to form pharmaceutically acceptable microemulsions was also reported by the research group at King's College. Due to the highly lipophilic properties of the long hydrocarbon chains of the lecithin molecule, cosurfactants, normally in the form of polar alcohols, were required for microemulsion formation. In a series of studies,[82–85] the authors examined the formation and characterization of phospholipid microemulsions using food-grade lecithin, obtained from soybean and egg, and a range of polar alcohols; however, these systems were unsuitable for food use due to the toxicity of the alcohol cosurfactant. Particle-size analysis of the phospholipid microemulsions was conducted using total intensity light scattering[86] and photon correlation spectroscopy.[24]

Other workers in the pharmaceutical field have also utilized lecithin/phospholipids as surfactant for microemulsion formation. As part of a doctoral study, von Corswant[87] described the phase behavior and microstructure of microemulsions formulated with soybean phosphatidyl choline and medium-chain and long-chain triglycerides.[88] The effects of adding hydrophilic

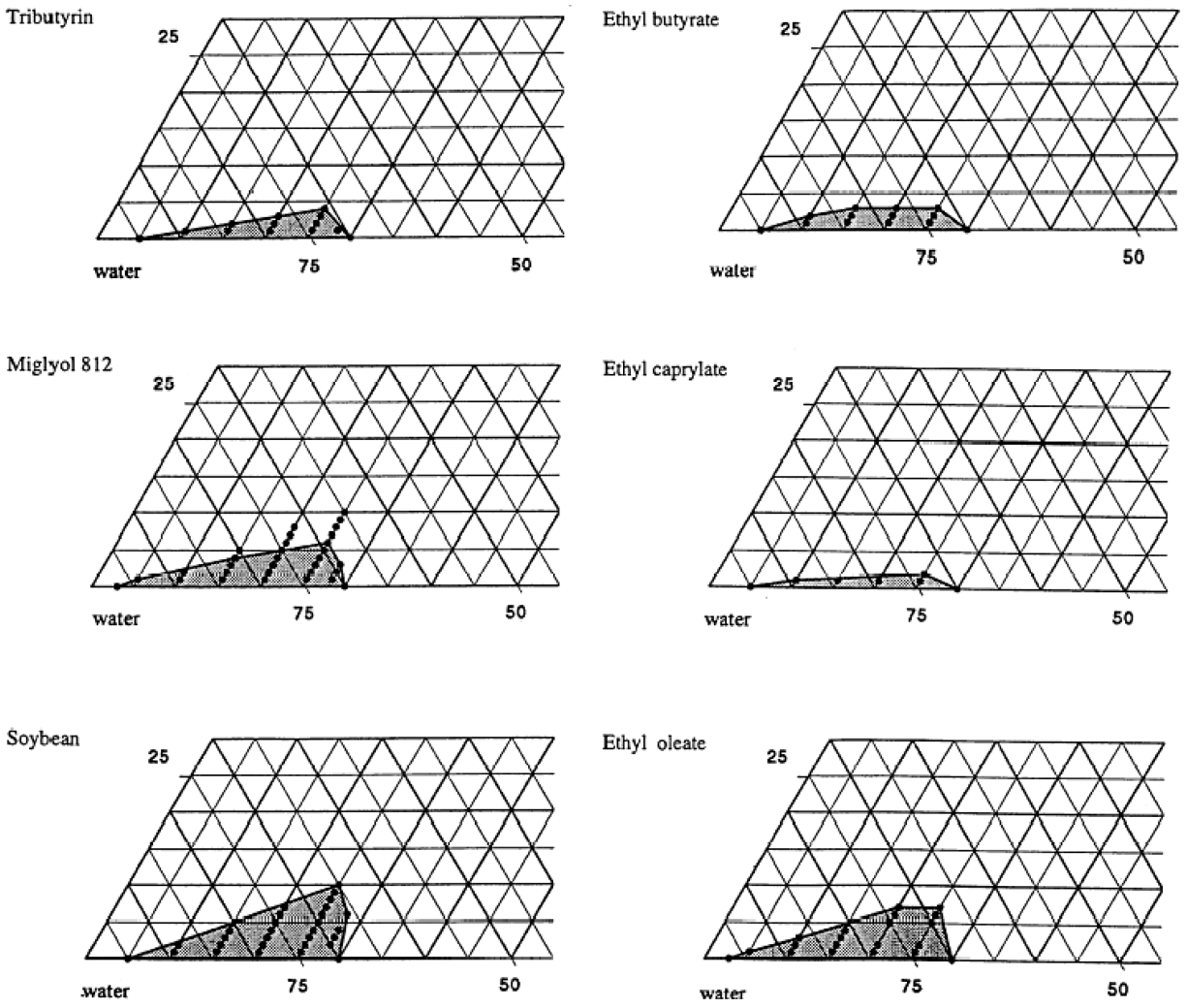

Figure 8 Partial triangular phase diagrams for o/w microemulsions formed with polyoxyethylene (POE) surfactant $C_{18:1}E_{10}$ and a range of oils at 25°C in water. Surfactant concentration increases from left to right, and oil concentration increases from bottom to top. Reprinted from International Journal of Pharmaceutics, 198, Warisnoicharoen et al., Nonionic oil-in-water microemulsions: the effect of oil type on phase behaviour, Pages 7–27, Copyright (2000), with permission from Elsevier.

amphiphiles (isopropyl myristate),[89] hydrophilic surfactants (such as sucrose monododecanoate)[90] and two sparingly soluble drugs[91] on the phase behavior and microstructure of the resulting microemulsions were examined. Subsequently, a pharmaceutically acceptable (and moreover food acceptable) microemulsion system, comprising of medium-chain triglycerides, a combination of soybean phosphatidyl choline and hydroxystearate polyethylene glycol as surfactants, and polyethylene glycol and ethanol as cosurfactants was developed.[92] The authors claimed that the microemulsion could be administered by intravenous infusion up to a dose of 0.5 mL/kg without producing any significant effect on the acid-base balance, blood gases, plasma electrolytes or heart rate of rats.

A further pharmaceutically targeted study also utilized food-grade components in the formation of lecithin-stabilized microemulsions. Moreno et al.,[32] described the phase diagrams, droplet size, viscosities, and particle sizes (by photon correlation spectroscopy) of microemulsion systems containing lecithin, polysorbate 80 and isopropyl myristate. In addition, toxicity and stability (temperature, freeze-thaw cycles, centrifugation) tests were conducted. An associated study found decreased toxicity levels in mice which were injected with a microemulsion system containing Amphotericin (a potent fungistatic and fungicidal antibiotic) compared to the commercially available mixed micellar dispersion of Amphotericin.[33]

Other studies conducted in the pharmaceutical field have also utilized lecithin for the formation of microemulsions;[93–100] however, these studies used cosurfactants, and in some cases hydrocarbon oils, which are not suitable for use in food.

An interesting recent development in the pharmaceutical field has been the application of organogels as delivery devices for hydrophobic and hydrophilic drugs and vaccines.[101–105] An important advantage of organogels over hydrogels is their ability to preserve the physical and chemical integrity of compounds solubilized within the microemulsion. In addition, microbial contamination of organogels is much less likely than contamination of

hydrogels due to the presence of an organic continuous medium; furthermore, the aqueous domains are in the nanometer range, and hence orders of magnitude smaller than typical bacteria.[27] In a recent review Bagwe et al.,[27] concluded that lecithin gels offer superior advantages over gelatin gels due to the biocompatibility of lecithin formulations and the relative ease with which they have been used. Further information on lecithin organogels has been provided by Shchipunov.[106] Organogels may find application in the food industry for delivery of poorly soluble and/or sensitive compounds and bioactives, particularly if the organogel could be added to foods in a mechanically sheared particulate form.

Microemulsions as Reaction Media

Despite the fact that chemical and enzymatic reactions are exploited in non-food microemulsion systems (see Capek[107] for a review on preparation of metal nanoparticles using w/o microemulsions, and Lopez-Quintela[108] for a recent review of more general aspects of microemulsion as organic reaction media), the use of microemulsions as microreactors in the food industry is still in the early stages of development. Microemulsions are ideal as reaction media because of their large interfacial area, and their ability to solubilise polar and non-polar reactants in a single homogeneous phase.[36] In a recent review by Garti,[109] the major advances in the use of microemulsions for the synthesis of food additives, such as emulsifiers and fats are discussed; however, most of the microemulsion systems described are not food grade. Studies which did utilize a food-grade microemulsion system examined the Maillard reaction in sugar-based w/o microemulsions and o/w microemulsions based on ethoxylated sorbitan esters.[110,111] Furfurylthiol, a unique product of Maillard browning, was produced at much higher concentrations in w/o microemulsions than in a water reference system. The initial reaction rate of the conversion of furfural to furfurylthiol could be increased by increasing the water proportion in the aqueous phase.[111] The authors state that microemulsions offer a new reaction medium to produce selective aroma compounds.

Microemulsions have also been used as microreactors to prepare new surfactants or known surfactants by alternate methods. However, as the end-uses of these products were for industrial or cosmetic applications, non-food-grade components were normally employed. In a series of studies, Holmberg used w/o microemulsions as reaction media to study fat oxidation, lipase catalysis and to synthesize monoglycerides.[112–115] Hayes[116] studied the selective formation of monoglycerides of fatty acids or triglycerides of fatty acids by esterification of saturated and unsaturated fatty acids with glycerol. The use of microemulsions as a reactive media for the reactions of lipase (to perform biocatalysis, esterification, transesterification, and hydrolysis reactions) has been reviewed by Carvalho and Cabral.[117] Morgado et al.,[118] prepared lysophospholipids and free fatty acids from lecithin by enzymatic hydrolysis using a porcine pancreatic phospholipase in a continuous reverse micellar microemulsion system. Additional advances in the use of microheterogeneous systems for reaction media were published in a special edition of Current Opinion in Colloid and Interface Science (Issue 2, Volume 8, 2004).

Protein Inclusion in Microemulsions

The inclusion of proteins in microemulsions is the basis for a number of research topics. While the delivery of proteins in pharmaceutical microemulsion applications has been extensively studied, delivery of proteins in food applications is only in the early stages of development. Proteins hosted in microemulsions may also find application in research into enzyme activity and protein separation; they can alter surfactant self-association and phase behaviour and can promote the formation of novel solvents and materials.[119] Microemulsions have been used as a model system to understand membrane transport behaviour.[120,121]

Microemulsions can also be used to separate and concentrate proteins, as the solubilization of individual proteins depends on the protein properties.[36] Goklen and Hatten[122] separated cytochrome *c* from lysozyme using an Aerosol-OT-based microemulsion system. The separation of xylose reductase[123] and lysozyme from egg white[124] have been reported using microemulsion technology, and the ability of reversed micellar systems to act as a bioseparation technique for isolation and purification of proteins has been reviewed by Pires et al.[125] A promising application of the ability of microemulsions to separate and concentrate proteins may be in the separation and purification of heterogeneous proteins, such as caseins and whey proteins, resulting in large-scale production of individual proteins with increased value.

Recently, the incorporation of immunoglobulin G[119] and α-lactalbumin[126] into microemulsion systems formulated with AOT, a non-food-grade anionic surfactant and isooctane has been reported. A significant increase in the volume of research conducted in the inclusion of proteins into microemulsion systems may be expected in the near future.

Increased Bioavailability Following Microemulsion Solubilization

It has been speculated that the absorption of poorly absorbed compounds in the body, such as bioactive peptides, vitamins, and drugs, may be enhanced using microemulsions. Indeed, as microemulsions have extremely small particle sizes, the total surface area of oil droplets in a 10% oil microemulsion for example, would be much larger that the total surface area of oil droplets in a normal emulsion. Thus, microemulsion, and their components, may theoretically be adsorbed much more rapidly from the intestine primarily due to the increased surface area, compared to normal emulsions.

Initial studies suggested that microemulsions could be used to enhance the oral bioavailability of drugs, including

peptides.[127–129] Subsequent studies substantiated this claim. Intraduodenal bioavailability of a water-soluble peptide antagonist was increased from 1% in saline solution to 29% in a food-grade microemulsion formulation.[30] Further studies on Calcein, a poorly absorbed molecular marker, showed similar results.[130] Lyons et al.,[131] found that, following intraduodenal administration to rats, the bioavailability of a muramyl dipeptide increased up to 10-fold when solubilized in w/o microemulsions, compared to aqueous solution. Increased bioavailability of β-carotene was observed when administered in a microemulsion form (using a mixture of sorbitan monostearate surfactant and its polyoxyethylene form), compared to a conventional dispersion.[132] Microemulsions have also been utilized for increasing dermal delivery of both hydrophobic and hydrophilic compounds.[133,134]

The enhancement of absorption activity observed in microemulsion-delivered compounds is dependent on the type of emulsifying agent, particle size of the dispersed phase (if absorption is dissolution rate limited), pH, solubility of the compound, and type of lipid phase used.[135] It was suggested.[129–131] that the presence of medium-chain triglycerides was the main reason for increased bioavailability as medium-chain triglycerides are known as absorption enhancing agents.[136] Although initial results appeared encouraging, little research has been conducted in the last five years on the bioavailability of poorly absorbed compounds following incorporation into microemulsions. However, the potential of using microemulsions to enhance absorption of peptides and protein drugs through peroral, parenteral and ocular routes are frequently discussed in drug delivery reviews[135,137–139]

FUTURE FOCUS

Primarily, difficulties in application of microemulsion as food delivery systems are two-fold: limitations in the choice of surfactant and poor solubilization of high molecular weight triglycerides. Both the type of surfactant and concentration of surfactant permissible for use in foods restrict the potential development of food-grade microemulsions. Few surfactants are suitable for use in foods, and of these few, many are only permissible at quite low levels. This would suggest that researchers should concentrate on surfactants which have been designated as GRAS for future fundamental and applied research. The production of new surfactants, either synthetically or isolated from natural sources may be critical in terms of expanding the applications of microemulsions. In addition, the development of dilutable microemulsion systems which could solubilise large quantities of triglycerides would enormously enhance the application of microemulsions as a delivery system for bioactives in foods, and indeed in all systems in which they are currently used. Organogels may also harbor potential for delivery of bioactives.

Increased solubilization of poorly soluble bioactive compounds has been demonstrated in a number of studies, and there is little doubt that microemulsion systems can perform this function. However, claims of increased bioavailability of bioactives need to be substantiated with *in vivo* animal and human trials. However, the toxicities of surfactants in microemulsion form should not be overlooked. Possible opportunities for collaboration between the pharmaceutical and food sciences need to be explored to make a concerted effort in the development of microemulsion delivery systems.

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