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Phospholipid-Based Microemulsions Suitable for Use in Foods

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The preparation of nonaqueous microemulsions using food-acceptable components is reported. The effect of oil on the formation of microemulsions stabilized by lecithin (Epikuron 200) and containing propylene glycol as immiscible solvent was investigated. When the triglycerides were used as oil, three types of phase behavior were noted, namely, a two-phase cloudy region (occurring at low lecithin concentrations), a liquid crystalline (LC) phase (occurring at high surfactant and low oil concentrations), and a clear monophasic microemulsion region. The extent of this clear one-phase region was found to be dependent upon the molecular volume of the oil being solubilized. Large molecular volume oils, such as soybean and sunflower oils, produced a small microemulsion region, whereas the smallest molecular volume triglyceride, tributyrin, produced a large, clear monophasic region. Use of the ethyl ester, ethyl oleate, as oil produced a clear, monophasic region of a size comparable to that seen with tributyrin. Substitution of some of the propylene glycol with water greatly reduced the extent of the clear one-phase region and increased the extent of the liquid crystalline region. In contrast, ethanol enhanced the clear, monophasic region by decreasing the LC phase. Replacement of some of the lecithin with the micelle-forming nonionic surfactant Tween 80 to produce mixed lecithin/Tween 80 mixtures of weight ratios (K_m) 1:2 and 1:3 did not significantly alter the phase behavior, although there was a marginal increase in the area of the two-phase, cloudy region of the phase diagram. The use of the lower phosphatidylcholine content lecithin, Epikuron 170, in place of Epikuron 200 resulted in a reduction in the LC region for all of the systems investigated. In conclusion, these studies show that it is possible to prepare one-phase, clear lecithin-based microemulsions over a wide range of compositions using components that are food-acceptable.

KEYWORDS: Phospholipids; food-acceptable microemulsions; triglycerides; ethyl esters

INTRODUCTION

Functional foods, nutraceuticals, pharmaconutrients, and dietary integrators are all terms used to describe nutrients or nutrient-enriched foods that can prevent or treat disease. The term functional food, which originated in Japan in the 1980s, is defined as "any food or ingredient that has a positive impact on an individual's health, physical performance, or state of mind, in addition to its nutritive value" (*1*). The criteria that a food or ingredient must satisfy to be classified as functional are that it should be naturally occurring, be consumed as part of the daily diet, and exert a pharmacological action to prevent or control a specific disease (*1*, *2*). It should be noted, however, that functional foods are not medicines.

Examples of ingredients considered to be "functional" include β-carotene, lutein, fiber, fatty acids, flavonoids, probiotics, plant stanols, and phytoestrogens. Unfortunately, however, a number of the aforementioned ingredients are water-insoluble; it is frequently difficult to formulate them in a manner that is both convenient and palatable for oral administration. Fortunately, however, as most of these water-insoluble functional materials display some affinity for oil, it may be feasible for them to be formulated in an oil−water dispersion such as an emulsion or a microemulsion. Although there is much information in the literature regarding the formulation of food emulsions (*3*−*5*), in comparison there are far fewer data available on the preparation of microemulsions suitable for use in food products (*5*−*7*).

This is despite the fact that microemulsions offer several advantages over emulsions, such as the fact that they are transparent (due to their small domain size), form spontaneously, and are thermodynamically stable. Microemulsions are composed of an oil, water, surfactant(s), and usually a cosurfactant, typically a short-chain alcohol. The microstructure of these systems can be described as being oil-in-water (o/w), bicontinuous, or water-in-oil (w/o), depending upon the relative amounts of each component present. The ability of a microemulsion to solubilize large quantities of lipophilic and hydrophilic materials (simultaneously if necessary) make microemul-

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sions excellent vehicles for a wide variety of uses. For example, microemulsions have been utilized as solubilizers for flavors (*8*), phytosterols (*9*), vitamin and minerals (*6*), antioxidants (*7*), and drugs (*10*, *11*). In addition, microemulsions have been used as microreactors (*12*) and in food beverages (*13*).

Among the disadvantages of a microemulsion are the relatively high concentrations of surfactant needed (and the toxicity issues that this raises), the requirement for the presence of a cosurfactant (most of which are not food-acceptable), and the fact that most oils suitable for food use are both large (in terms of their molecular volume) and semipolar in nature, making them difficult to solubilize in a microemulsion.

The aim of this work was to prepare microemulsions using only food-acceptable surfactants, a (predominately) nonaqueous polar phase, and oil. In the present study soybean lecithin, a crude mixture of phospholipids, was chosen as the primary surfactant because it is a naturally occurring, widely used food emulsifier/surfactant (number E322). Unfortunately, however, phospholipids when used as sole surfactant do not generally form microemulsions when the two immiscible phases are water and oil, preferring instead to form liquid crystalline (LC) phases (*14*, *15*). For microemulsion formation in aqueous systems using a phospholipid as the primary surfactant, a cosurfactant (*16*, *17*) or a hydrotrope (*18*) the function of which it is to destabilize the LC phases, is normally required. However, it is possible, and indeed more beneficial, to use a second surfactant in the place of a cosurfactant. In the present study it was decided to use a micelle-forming nonionic ethoxylated sorbitan ester, Tween 80 [polyoxyethylene (20) sorbitan monooleate], as second surfactant.

In addition, it was decided to investigate whether it is possible to replace the aqueous phase with another polar solvent and obtain a microemulsion. Although there is some work investigating the replacement of water in self-assembling systems with other nonaqueous polar solvents such as ethanediol, glycerol, and propylene glycol (*20*), this approach is not widely investigated. In the present study it was decided to use propylene glycol as the nonaqueous polar solvent because it has been classified by the U.S. Food and Drug Administration (FDA) as an additive that is "generally recognized as safe" for food use and as a solvent for food colors and flavors. However, as propylene glycol is unfavorable in large quantities (the limit in human food is 1 g/kg) for food purposes (*21*), its partial substitution of the aqueous phase may be desirable.

One of the most commonly used classes of oil suitable for food use is the triglycerides, most of which are large and semipolar in nature. Because of their large molecular volume, triglycerides do not readily lend themselves to microemulsion formation (*22*). Triglycerides instead tend to promote LC phase formation in mixtures of surfactant and water (*23*). By way of contrast, smaller molecular volume oils, such as the ethyl ester ethyl oleate, do not so readily promote LC phases (*24*). In the present study a range of food-acceptable triglyceride and ethyl ester oils were examined for their ability to form microemulsions. Of the oils tested, only the triglyceride tributyrin possessed unknown food acceptability.

MATERIALS AND METHODS

The lecithin used in the present study, Epikuron 200, was from soybean and was obtained from Lucas Meyer. According to the manufacturer, Epikuron 200 contains 97 wt % phosphatidylcholine and has a fatty acyl chain composition of $C_{16:0}$ = 13.3 wt %, $C_{18:1}$ = 3.0 wt %, $C_{18:2}$ = 66.9 wt %, and $C_{18:3}$ = 6.6 wt %. A lower grade soybean lecithin, namely, Epikuron 170, 68−72 wt % phosphatidylcholine (manufacturer's information), which contained less phosphatidylcholine and more phosphatidylethanolamine, was also used. Tween 80, propylene glycol, soybean oil (a triglyceride reported by the manufacturer to contain 50−57 wt % linoelic acid, 5−10 wt % linolenic acid, 17−26 wt % oleic acid, 9−13 wt % palmitic acid, and 3−6 wt % stearic acid; all other acids present in trace quantities), isopropyl myristate (IPM), and tributyrin were purchased from Sigma Chemicals. Miglyol 812N (MCT, a triglyceride reported by the manufacturer to contain between 50 and 65.0 wt % caprylic acid and 30.0−45.0 wt % capric acid; caproic, lauric, and myristic acids were each present at <2 wt %) was supplied by Hüls and ethyl oleate by Croda Chemicals. Deionized water was used throughout the study sourced from an Elga Option 3 water purifier.

Phase Behavior. Existence of a clear, one-phase microemulsion region in a three- or four-component mixture was determined by the construction of ternary and pseudo-ternary phase diagrams, respectively. Initial mixtures of surfactant and polar solvent were prepared by the addition of a known amount of surfactant, and when applicable Tween 80 as second surfactant, and a polar solvent into screw-capped vials. Note that when a mixture of surfactants was used, the symbol K_m denotes the weight ratio of the (primary) surfactant and (secondary) surfactant used. The concentration of surfactant used (and when applicable, the second surfactant) ranged from 10 to 90 wt % in 10 wt % intervals, making a total of nine initial surfactant and polar oil mixtures for titration. Nine samples was the number previously determined to be necessary to determine phase behavior with reasonable precision using this methodology (*25*). Although when a phase boundary needed further clarification, additional samples with intermediate surfactant concentrations were made. The mixtures of surfactant (and second surfactant) and polar solvent were allowed to stand overnight before being checked for clarity and for signs of birefringence by visual inspection through cross-polaroids. The presence of birefringence was taken to indicate the presence of a LC phase (*25*). Oil was then added dropwise by weight and with stirring to the surfactant and polar phase mixture. The appearance of the resultant mixture was checked for clarity and for the absence of birefringence. Only one-phase, clear, non-birefringent phases were classified as microemulsions. In most cases the limit of the region of microemulsion existence was signaled by the appearance of a cloudy solution. In cases when the three-component mixture exhibited birefringence, the sample was classified as liquid crystalline, although the type and stability of these phases were not established. Phase diagrams were constructed from the experimental data obtained using weight percent (wt %) of the components. The extent of microemulsion formation was calculated as a percentage of the total area by drawing the phase diagrams on graph paper of an appropriate grid size, cutting out the various areas, and weighing them on an accurate three-decimal place balance. This process was repeated on three occasions, and the values thus obtained were estimated to be accurate to within a few percent.

It should be noted that, in a number of instances, more than one batch of oil was investigated for its phase behavior. In no case was any significant difference observed in the phase behavior upon changing the batch of the oil, suggesting that slight variations in oil composition do not have much influence on phase behavior.

RESULTS AND DISCUSSION

Three-Component Systems. *Medium-Chain Triglycerides.* **Figure 1** shows the results obtained for the phase behavior of the systems composed of Epikuron 200, propylene glycol, and, respectively, one of the four triglyceride oils examined. The most significant observation is that, regardless of the nature of the oil present, it is possible to form three-component lecithin-based microemulsions by replacing water with propylene glycol. By way of contrast it is not possible to form lecithin-based microemulsions using water and the same oils without the addition of a cosurfactant (*26*), typically a short-chain alcohol. Unfortunately, short-chain alcohols are not suitable in foods due to toxicity considerations, although the use of ethanol is permitted for food use in certain parts of the world.

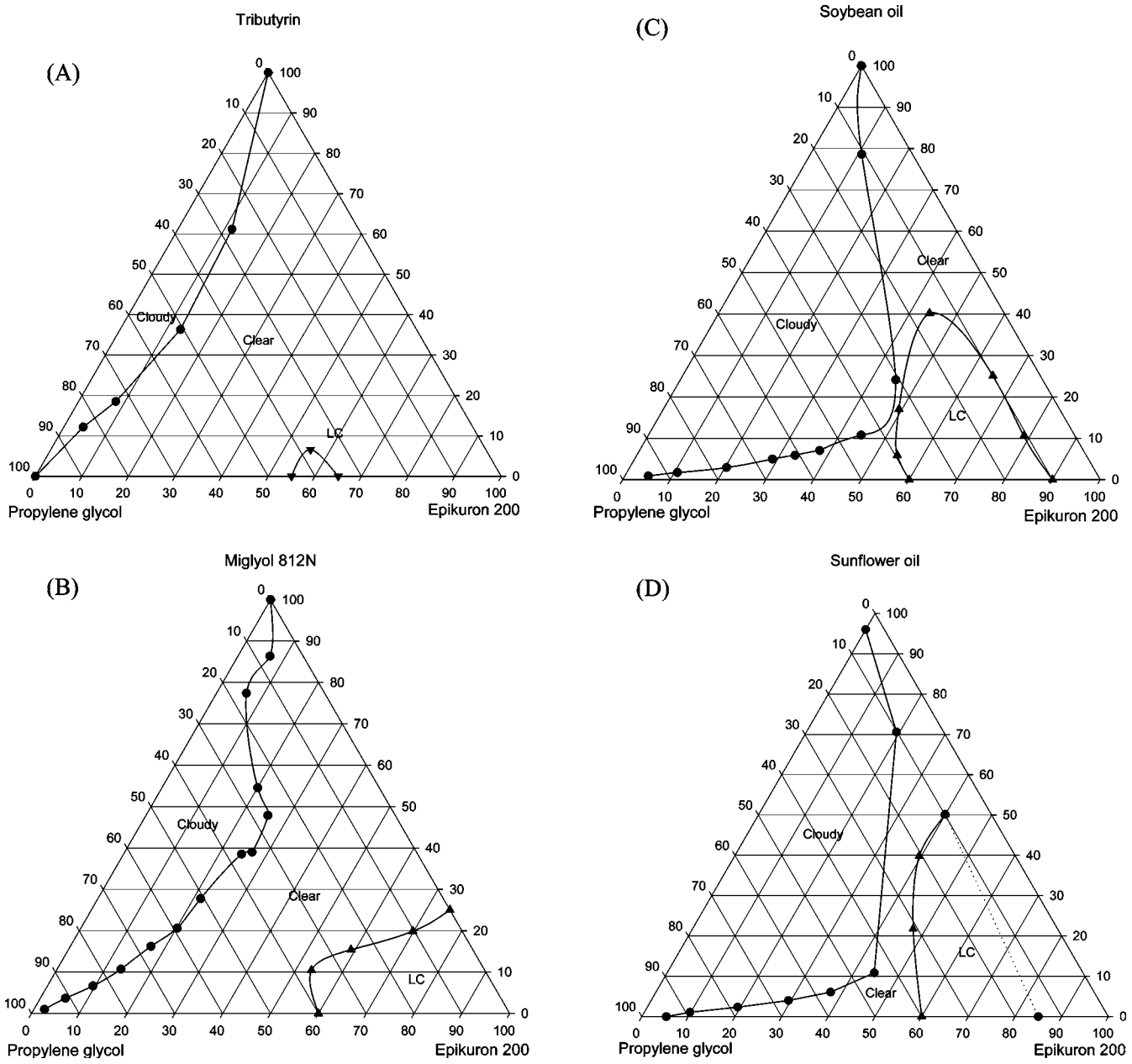

Figure 1. Ternary phase diagrams of lecithin (Epikuron 200), propylene glycol, and triglyceride oil (**A**) tributyrin, (**B**) Miglyol 812N, (**C**) soybean, or (**D**) sunflower (dotted line represents estimated phase boundary).

Table 1. Molecular Masses and Volumes of Triglyceride and Ethyl Ester Oils

oil	mol mass (g mol^{-1})	mol vol[a] at 298 ± 5 K (Å^3)
soybean oil	881	1592
Miglyol 812N	554	1019
tributyrin	302	486
ethyl oleate	310	592
isopropyl myristate	270	

[a] Molecular volumes were obtained from Malcolmson et al. (*37*). Molecular masses and molecular volumes for soybean oil and Miglyol 812N were determined by assuming an average composition based on manufacturer's available composition data.

It is clear from **Figure 1** and from **Tables 1** and **2**, which list, respectively, the volume and molecular masses of the oils studied and the percent area of the phase diagram that was a clear, one-phase "microemulsion" region, that there is a definite inverse relationship between the volume/molecular mass of the triglyceride oil and the extent of the formation of the one-phase, clear microemulsion region. The very large molecular volume of the triglycerides, soybean and sunflower oils, is due to their possession of three long (ranging in length from 16 to 22 carbons) acyl chains attached to a glycerol backbone. In terms of their phase behavior, there is little difference between the extents of the clear, one-phase regions, with both sunflower and soybean oils showing predominately large LC and two-phase cloudy regions. **Figure 1A** shows that the system containing tributyrin, the smallest triglyceride, contained a very large clear, one-phase region and exhibited only a very small LC region, occurring at between 55 and 65 wt % Epikuron 200. This result is in direct contrast with that obtained with the large chain triglycerides, soybean and sunflower oils, with which only very small clear regions were seen (**Table 2**; **Figure 1C**,**D**), presumably because these large-volume oils cannot readily penetrate the hydrophobic acyl chains of the Epikuron 200 monolayer.

Table 2. Percentages of Clear, One-Phase Microemulsion Region for the Multicomponent Systems Tested

system	% of clear, one-phase region[a]
Epikuron 200/propylene glycol	
tributyrin	83
Miglyol 812N	58
soybean	34
sunflower	36
Epikuron 200/propylene glycol	
isopropyl myristate	73
ethyl oleate	62
Epikuron 170/propylene glycol	
Miglyol 812N	60
ethyl oleate	66
Epikuron 200/Miglyol 812N	
propylene glycol/water (9:1 w/w)	32
propylene glycol/water (8:2 w/w)	10
propylene glycol/ethanol (9:1 w/w)	72
Epikuron 200:Tween 80/propylene glycol/ethyl oleate	
Km 1:2	68
Km 1:3	65
Epikuron 200:Tween 80/propylene glycol/Miglyol 812N	
Km 1:2	53
Epikuron 170:Tween 80/propylene glycol/ethyl oleate	
Km 1:2	74
Km 1:3	61

[a] Estimated to be accurate to a few percent.

In place of an extensive microemulsion region, large LC regions were formed at high surfactant (i.e., 60−90 wt %), low oil concentrations, together with very large, cloudy multiphase regions. Interestingly, it is well-known that triglyceride oils (such as soybean) are incorporated at only very low levels into phospholipid bilayers (*27*).

In the system containing the medium-chain triglyceride, Miglyol 812N (**Figure 1B**), the extent of microemulsion existence was intermediate to that of the other triglycerides as both the LC and multiphase regions were smaller than those observed when using soybean and sunflower oil, but the clear region was not as great as that seen with tributyrin. The observation that increasing the size of the oil from tributyrin to sunflower oil reduces the ability of the system to form a microemulsion is in accordance with that found by other workers when using water as the polar phase (*24*, *26*). Interestingly, however, it has not been established beyond doubt that the same sequence of phase behavior would be expected upon replacement of water with an alternative polar solvent.

To our knowledge this is the first time that microemulsions containing large molecular volume oils have been formed using phospholipids as sole surfactant. Usually, phospholipids do not form microemulsions in the presence of water without the addition of a cosurfactant such as a short-chain alcohol (e.g., butanol or propanol).

Ethyl Esters. The effect of replacing the triglyceride oil with an ethyl ester is shown in **Figure 2**. In agreement with the results obtained with the triglycerides, IPM (**Figure 2A**), the slightly smaller ethyl ester oil in terms of volume and molecular mass (**Table 1**) produced the largest clear, one-phase region (**Figure 2B**; **Table 2**). Interestingly, the system containing IPM exhibits no LC region. This observation is in contrast to that seen with the triglycerides, when even tributyrin produced an, albeit small, LC region and suggests that it may be of benefit to use IPM as oil when lecithin-based microemulsions are prepared. Unfortunately, however, no safety data are available on the suitability of IPM for food use, although it has been used as a flavoring agent (*28*). In contrast, ethyl oleate, which has been shown to have a good safety profile for food (*29*), produced a relatively large LC region and a microemulsion region comparable to that observed when using the medium-chain trigylceride as oil (**Figure 1B**).

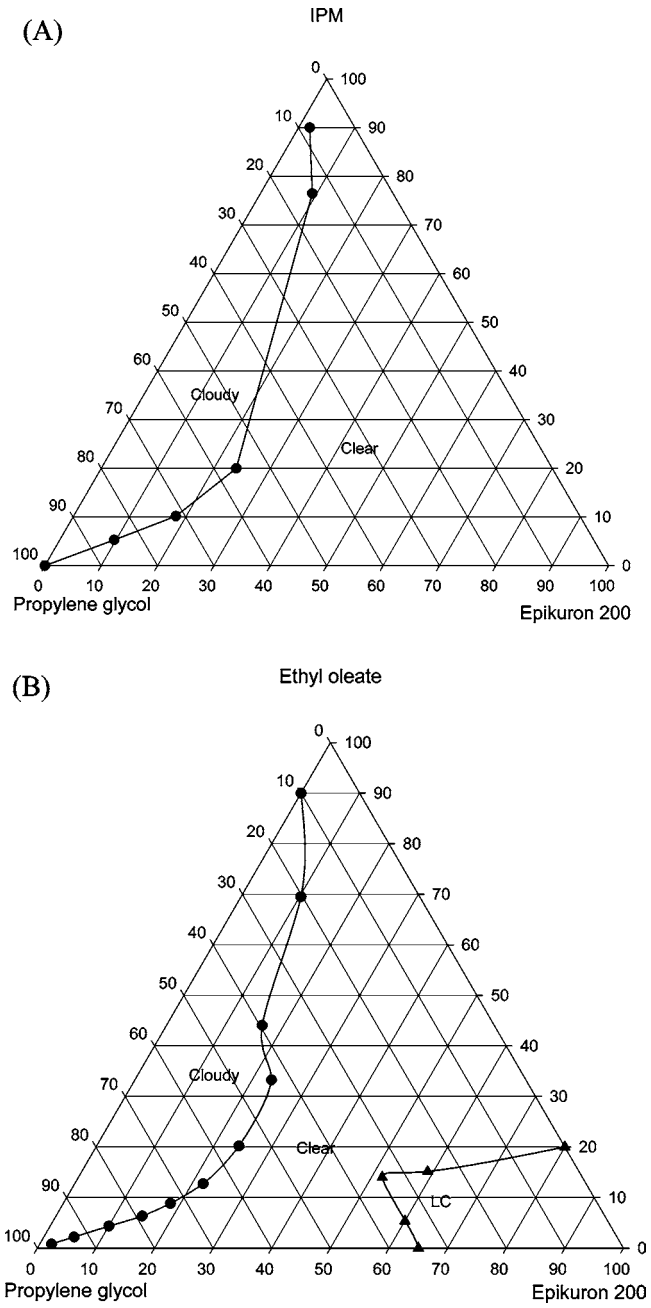

Figure 2. Ternary phase diagrams of lecithin (Epikuron 200), propylene glycol, and ethyl ester oil (**A**) isopropyl myristate (IPM) or (**B**) ethyl oleate.

Lecithin Purity. It has been previously reported using water-based systems that using a less pure lecithin may have an advantage in the preparation of microemulsions, primarily because of a reduced tendency to form LC phases (*30*). In the present study the effect of lecithin purity was examined by replacing Epikuron 200 in the systems prepared with MCT (Miglyol 812N) and ethyl oleate with the less pure lipid, Epikuron 170. The results of this study can be seen in **Figure 3**. First, it is clear that the results obtained using the two types of lecithin are comparable with the exception that the extent of the LC region was greatly reduced when Epikuron 170 was used. This beneficial effect possibly arises because of the higher

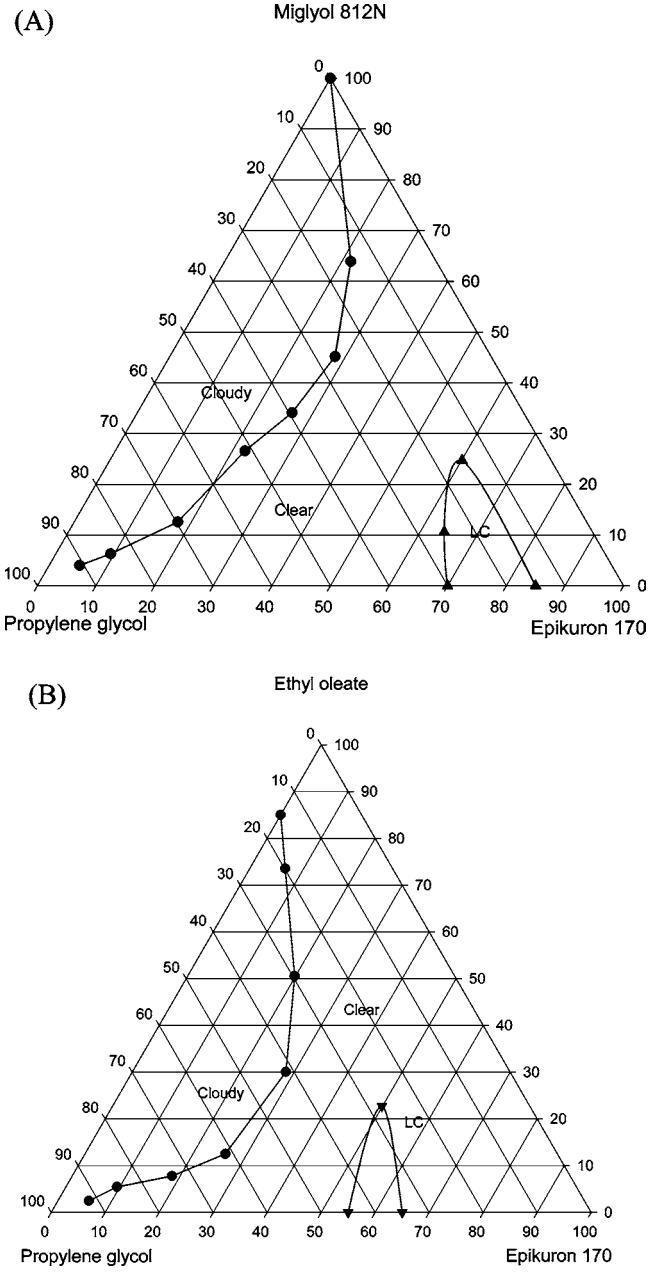

Figure 3. Ternary phase diagrams of low-grade lecithin (Epikuron 170), propylene glycol, and (**A**) Miglyol 812N or (**B**) ethyl oleate.

proportion of non-bilayer-forming lipids, such as phosphatidylethanolamine (10−13 wt %), present in Epikuron 170. These lipids may be thought of as acting as cosurfactants, destroying the extent of the LC phase and promoting the formation of a microemulsion region. It may be beneficial, therefore, in the formulation of microemulsions for food use to use the lower grade lecithin, in terms of both cost and reduction of the LC phase.

Four-Component Systems. *Partial Replacement of Propylene Glycol.* In this study it is clear that the use of propylene glycol as polar solvent, in most cases, negates the requirement for a cosurfactant in the production of lecithin-based microemulsions. However, in an attempt to reduce the amount of propylene glycol present in the formulation, a study was performed to determine how much water could be added to the propylene glycol and still produce a large clear, one-phase microemulsion region. As can be seen from **Figure 4**, which shows the effect of replacing 10 and 20 wt % of the propylene

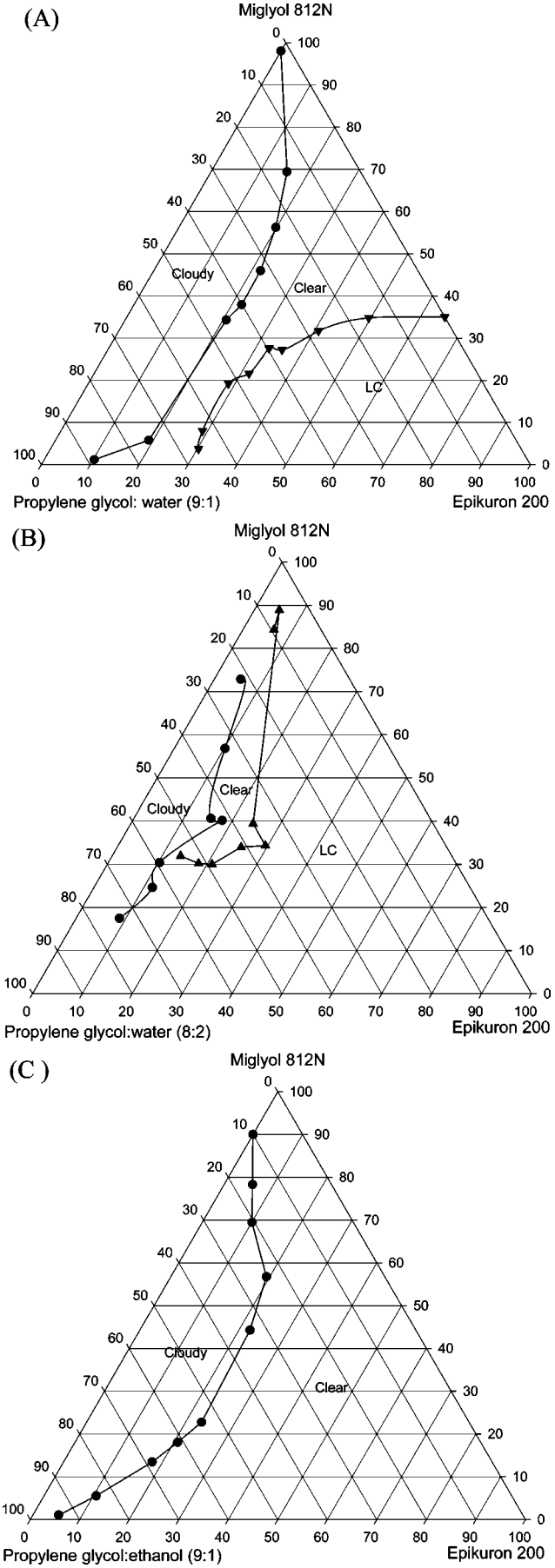

Figure 4. Pseudo-ternary phase diagrams of lecithin (Epikuron 200) and Miglyol 812N with (**A**) propylene glycol/water (9:1 w/w), (**B**) propylene glycol/water (8:2 w/w), and (**C**) propylene glycol/ethanol (9:1 w/w).

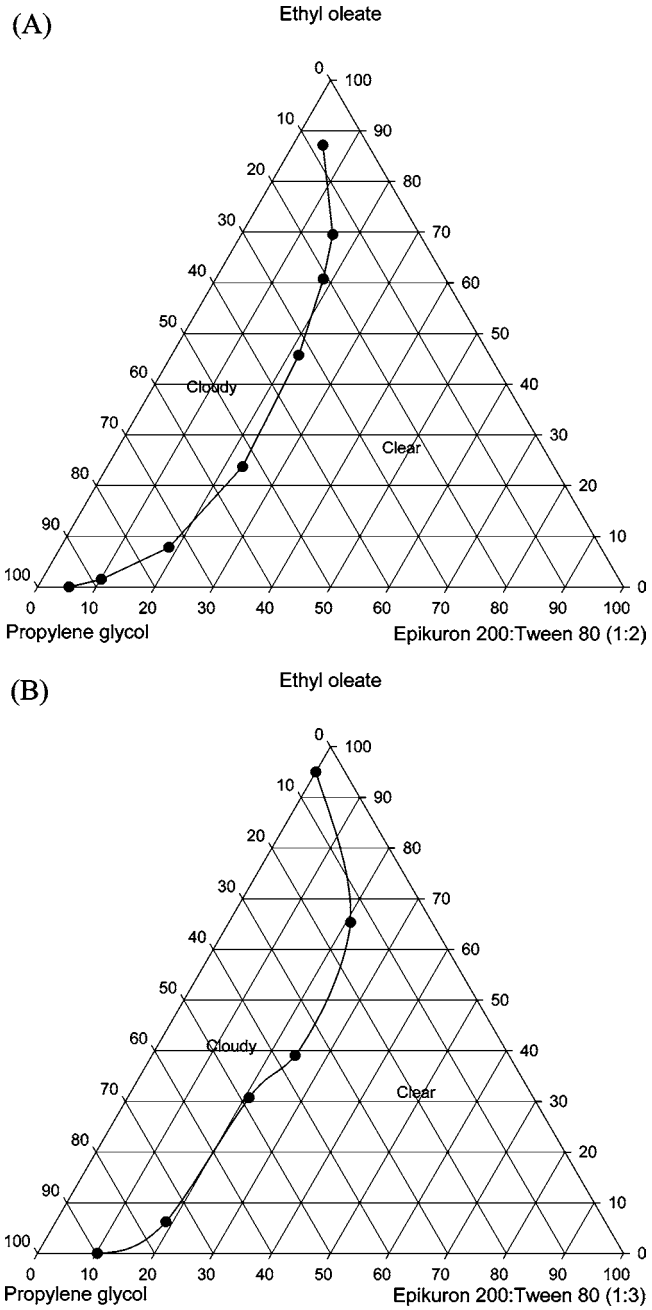

Figure 5. Pseudo-ternary phase diagrams of propylene glycol, ethyl oleate, and lecithin (Epikuron 200)/Tween 80 mixture (**A**) K_m of 1:2 or (**B**) K_m of 1:3.

glycol with water, that even as little as 10 wt % water significantly reduced the extent of the clear region and increased the LC region (**Figure 4A**; **Table 2**). Interestingly, the addition of water did not greatly alter the extent of the cloudy one-phase region. The increase in the extent of the LC phase was, however, expected due to the natural tendency of phosphatidylcholines, the main component of Epikuron 200, to rearrange themselves into the form of bilayers in the presence of water.

By way of contrast, the replacement of 10 wt % of the propylene glycol by ethanol (**Figure 4C**; **Table 2**) was shown to totally abolish the LC phase, although, again, the cloudy multiphase region remained largely unaltered. The reduction of the LC phase was undoubtedly due to a destruction of the packing of the lecithin molecules caused by the penetration of the ethanol molecules into the lecithin monolayer. However, in addition, ethanol may be acting as a cosolvent, reducing the likelihood of microemulsions being present over a wide range of compositions (*31*).

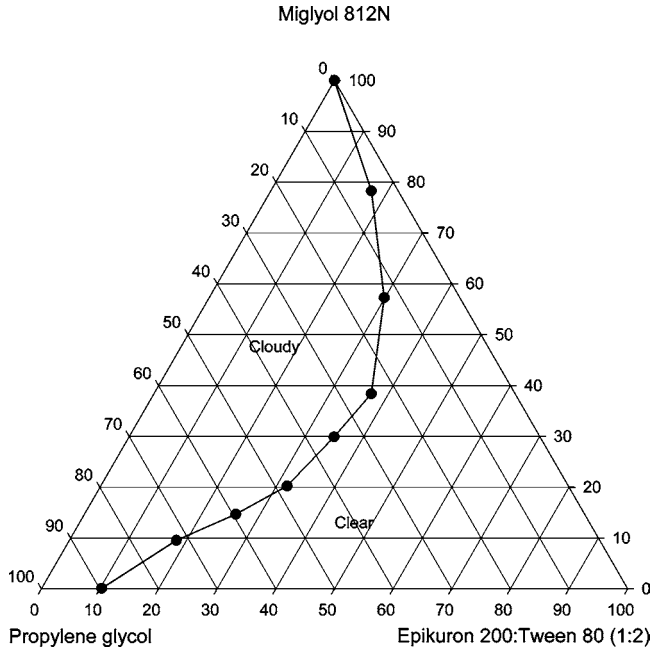

Figure 6. Pseudo-ternary phase diagrams of propylene glycol, Miglyol 812N, and lecithin (Epikuron 200)/Tween 80 at a K_m of 1:2.

Partial Replacement of Epikuron 200 with Tween 80. The addition of a second surfactant has been reported in some cases to promote microemulsion formation (*32*). The main benefit in using a second surfactant as cosurfactant derives from the fact that the resultant microemulsion can be diluted without altering or destroying the microemulsion (*8*). In the present study the food-acceptable, micelle-forming surfactant Tween 80 (HLB 15) was used to replace some of the primary surfactant, Epikuron 200, primarily in an attempt to reduce the extent of the LC phases formed in the three-component systems investigated. Epikuron 200/Tween 80 weight mixing ratios (K_m) of 1:2 and 1:3 were used. The results of this study can be seen in **Figures 5**−**7** and **Table 2**. Parts **A** and **B** of **Figure 5** show the phase diagrams obtained for a system prepared from propylene glycol, ethyl oleate, and Epikuron 200/Tween mixtures at K_m of 1:2 and 1:3, respectively. Although there was no LC region observed in either of these systems, the extents of the cloudy, multiphase region were slightly enhanced compared to the system prepared in the absence of Tween 80. Interestingly, there was little difference in the phase diagram produced with the two K_m studied, with the exception of a slight increase in the extent of the cloudy, multiphase region when the amount of Tween present was increased from 66 to 75 wt %. The phase diagram obtained upon substitution of MCT (Miglyol 812N) for ethyl oleate is shown in **Figure 6**. When ethyl oleate was used, no LC region was observed, although the extent of the cloudy, multiphase region had increased.

Parts **A** and **B** of **Figure 7** show the phase diagrams obtained for a system prepared from propylene glycol, ethyl oleate, and Epikuron 170/Tween mixtures at K_m of 1:2 and 1:3, respectively. The results obtained for these systems were very similar to those obtained for the equivalent system prepared using Epikuron 200 (**Figure 5**A,**B**). The only slight advantage of using the less pure Epikuron 170 was a slight reduction in the extent of the cloudy, multiphase region.

It appears from this limited study that the replacement of some of the lecithin with Tween 80 is beneficial in reducing the extent

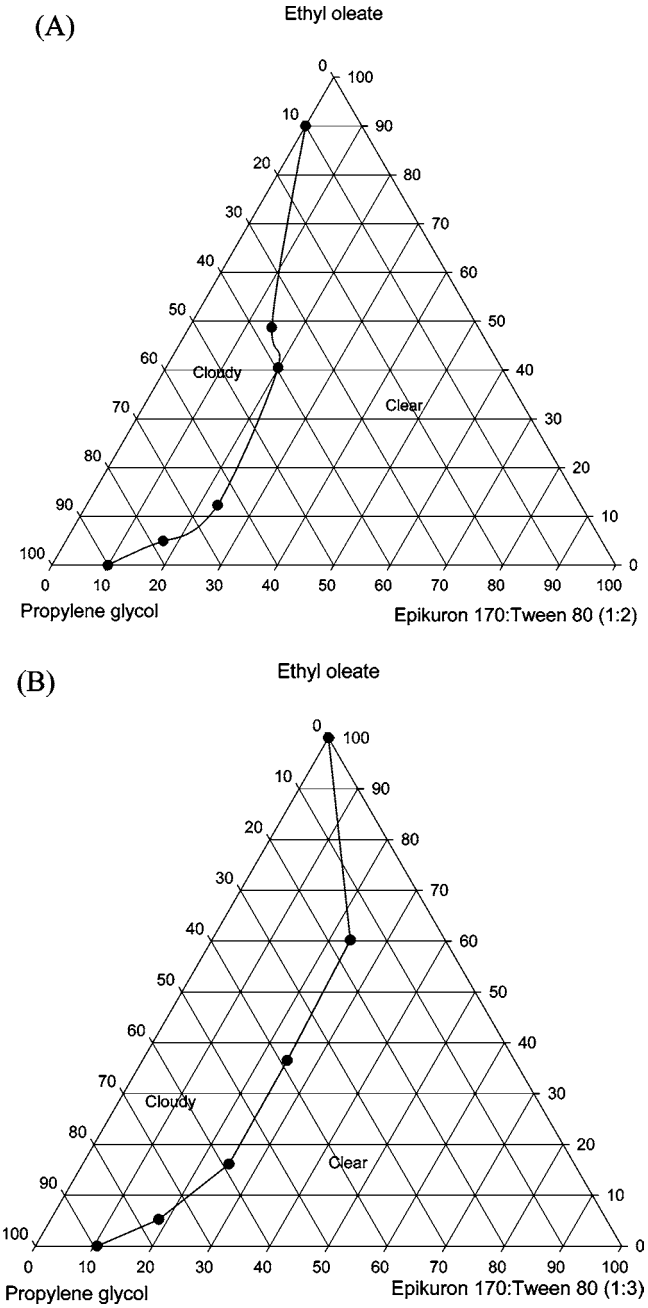

Figure 7. Pseudo-ternary phase diagrams of propylene glycol, ethyl oleate, and lecithin (Epikuron 170)/Tween 80 mixture (**A**) K_m of 1:2 or (**B**) K_m of 1:3.

of the LC phase, which is present when no nonionic surfactant is used. There is also an increase, albeit not great, in the extent of the microemulsion region. The perceived benefits of replacing some of the lecithin with Tween 80 may become more obvious in systems in which water is used as the polar phase. However, with the expense involved in using pure lipid, using a mixture may also serve as a more economical approach.

Microemulsion Formation. From the phase behavior studies reported above it appears to be a relatively trivial task to form lecithin-based microemulsions suitable for use in food products in the presence of the nonaqueous solvent propylene glycol. Although the concept of microemulsions as vehicles in the food industry is not a new one, many of the microemulsions reported as being suitable for food use are complex five-component systems (*9*, *33*). Perhaps not surprisingly, therefore, little success has been achieved in the commercial exploitation of microemulsions as vehicles in the food industry.

It is not possible, however, to unambiguously establish the existence of a microemulsion solely from visual studies such as those reported here. To definitively confirm the existence of a microemulsion, other studies such as light and neutron scattering and NMR self-diffusion measurements need to be made (*34*, *35*). Unfortunately, the similarity of the refractive indices of lecithin (1.456 at 488 nm), propylene glycol (1.431 at 488 nm), and oil (e.g., ethyl oleate, 1.455 at 488 nm) (values determined in-house with the method outlined in ref *36*) coupled with the fact that lecithin exhibits some solubility in propylene glycol makes light-scattering experiments extremely difficult to perform. Indeed, preliminary light-scattering experiments on a number of three-component Epikuron 200/propylene glycol/oil systems, at an arbitrary lecithin concentration of 25 wt %, did not show the presence of droplets or any significant change in the scattering of the sample upon altered composition. As it was not possible to draw any conclusion from this light-scattering study, preliminary neutron-scattering studies have been performed on the Epikuron 200/propylene glycol/ethyl oleate system using fully deuterated propylene glycol in place of (protonated) propylene glycol. These studies clearly demonstrate the existence of microemulsions over a wide range of propylene glycol/ethyl oleate concentrations when Epikuron 200 concentrations of $\leq$25 wt % were used (higher concentrations were not studied). This preliminary study supports the use of the term "microemulsion" for the description of the clear, one-phase systems observed in the present study, although it should be acknowledged that further research is needed to determine the range over which microemulsions rather than cosolvent systems are formed. Further work also needs to be performed to establish the ability of the microemulsions to incorporate ingredients known to be functional.

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