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SYNERGETIC EFFECT OF SUCROSE AND ETHANOL ON FORMATION OF TRIGLYCERIDE MICROEMULSIONS

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ABSTRACT

The phase behavior of soybean oil, a nonionic surfactant (ethoxylated monodiglycerides) and an aqueous phase of water containing ethanol, and sucrose was investigated at 35 and 40°C. A minimum concentration of 20 wt% ethanol was required for the formation of isotropic solutions. Addition of sucrose to the aqueous phase decreased the amount of ethanol required to form these solutions. The solubilization mechanism of the oil was investigated by small angle x-ray diffraction and polarized light microscopy. A stable lamellar liquid crystalline phase was formed for a mixture of 75/25 surfactant/sucrose solution (2.5 wt% sucrose). This phase was destabilized with increased concentrations of sucrose and liquid crystalline phases having

hexagonal structures were favored at 8.75 wt% sucrose. At a ratio of 55/45 wt% of surfactant/sucrose solution (9 wt% sucrose) hexagonal structures were formed and could be destabilized or destroyed by addition of ethanol. The concept of stabilization and destabilization of liquid crystalline mesophases was applied to the solubilization of triglycerides in aqueous solutions. Two microemulsion regions were identified; oil-in-water (L_1) and water-in-oil (L_2) in systems containing soybean oil, ethoxylated monodiglycerides, and 20 wt% ethanol solution. At 55/45 wt% surfactant/20 wt% ethanol solution, 7.5 wt% of soybean oil was solubilized. Addition of 10, 20, and 30 wt% sucrose, at the same ratio of surfactant to ethanol solution, increased the solubility of the oil to 9, 13.5, and 18 wt% respectively. In addition, the size of the L_1 phase increased and moved to the aqueous corner of the phase diagram and the size of the L_2 phase decreased.

INTRODUCTION

Microemulsions have been extensively investigated during the last thirty years for potential applications in household products, lubricants, cosmetics, oil recovery (1-3), and food formulations (4). Lawrence et al. (5) have shown that isotropic solutions formed preferentially over liquid crystalline or two liquid phases in systems containing cetyl trimethylammoniumbromide/water/n-hexanol using cyclohexylammonmium bromide as a hydrotrope. Few systems based on triglycerides have been reported in the literature (6-10); however, Gulik-Krzywicki and Larsson (11) describe one containing triglyceride, monoglyceride, and aqueous phases in which they identified a limited area of water-in-oil microemulsion (L_2).

Vegetable oils such as soybean and peanut oils are difficult to solubilize in microemulsion systems because they contain long bulky alkyl chains (mainly C_{16}, C_{18}, and C_{20}) and preferentially form liquid crystalline mesophases. Alander and Warenheim (12,13) compared triglyceride behavior to that of ordinary hydrocarbons in microemulsion systems and found that triglycerides, in particular large molecules such as those found

in peanut oil, are more difficult to solubilize than hydrocarbons or alkyl esters of comparable size. Stable triglyceride systems have been reported by Friberg and Rydhag (7) which are formed with suitable hydrotropes such as urea and sodium xylene sulfonate. More recent studies (14,15) have shown that disaccharides such as trehalose and sucrose favor formation of hexagonal phases (H_{11}) at the expense of lamellar liquid-crystalline phases ($L\alpha$) in the presence of hydrated phosphatidylethanolamine. These sugars strongly decrease the transition temperature from $L\alpha$ to H_{11}. Ingrid and Helena (16) have concluded that a phase transition from cubic to hexagonal phase H_{11} occurs when sugar is added to a monoglyceride/water system. They reported that these phase transitions can result in an increased average wedge shape of the lipid molecule which is the ratio between the cross-section at the end of the hydrocarbon chain and the cross sectional area at the polar head group. Crowe et al. (17,18) however found that trehalose stabilizes the lamellar phase over the hexagonal phase.

This study reports the formation and phase behavior of microemulsion systems through the stabilization and destabilization of the liquid crystalline mesophases with sucrose and ethanol.

EXPERIMENTAL AND METHODS

Materials

Nonionic Surfactant, Ethoxylated monodiglycerides (Mazol 80 MGK, Lot # 95721) was from PPG Industries Inc. This surfactant is a mixture of stearates and palmitate partial esters of glycerine condensed with approximately 20 moles of ethylene oxide per mole of alpha monoglyceride reaction mixture. (Acid Value=2, Saponification Value=65-75, Hydroxyl Value=65-80, and Iodine Value=2). Soybean oil was obtained from Central Soya Inc.; ethyl alcohol (200 proof) from Pharmco Ltd.; and

sucrose from Sigma. The water was double distilled and filtered with a laboratory reagent grade water system (Continental Water System Corporation).

Phase Diagram

Microemulsion regions were determined in phase diagrams which were constructed by titrating either the oil phase-surfactant or aqueous phase-surfactant mixtures with the aqueous phase or the oil phase respectively. The microemulsion region boundaries were determined by visual observation for transparency, and all samples were vigorously stirred, centrifuged, and incubated at 35, and 40 ± 1 °C overnight, and checked for anisotropy using crossed polarized light. Liquid crystalline mesophase structures were photographed by a polarized light microscopy technique at a magnification of 150X.

Small Angle X-Ray Diffraction

Samples containing ethoxylated monodiglycerides and the aqueous phase were prepared in 13-ml test tubes with screw caps. The samples were heated in an oven (60°C) for 5 min., mixed vigorously, centrifuged, then stored at 40°C for one week. Samples for small angle x-ray diffraction were drawn into a fine glass capillary (1.0 mm diameter) and sealed using hard wax.

Small angle x-ray diffraction was carried out with a XDS 2000 diffractometer (Scintag Inc.)* with a Cu K_α line, a double set of slits on

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*Identification of certain equipment does not imply recommendation by the United States Department of Agriculture and the National Institute of Standards and Technology nor does it imply that the equipment is necessarily the best available for the purpose.

either side of the sample, and a pyrolytic graphite analyzer crystal resulting in a resolution of 0.25° full width at half maximum of the diffraction angle. The coherent structure under study corresponds to peaks occurring at small angles (0.5° - 3°). Under these circumstances, the observed peaks exist on a substantial background which slopes from the small angle due to the undeviated beam. In order to extract precise information (scattering angle, intensity and width) concerning the diffraction peaks, the scattering intensity as a function of angle was modeled as consisting of a very intense peak at 0° and the several observed diffraction peaks. The shape of each peak was a sum of a Gaussian and a Lorentzian function whose relative fractions were adjusted to best describe its shape. The position, intensity and width of each peak were adjusted using non-linear least square methods program developed by R. W. Erwin (Reactor Radiation Division, NIST, Gaithersburg, MD) so as to reproduce the observed scattering. Figure 1 presents a representative diffraction pattern and the fit obtained in this manner. This procedure resulted in reliable values of the parameters describing each peak (and for their integrated intensities) even in those cases in which the peak intensity was weak.

RESULTS AND DISCUSSION

Solubilization of triglycerides was studied for systems containing mesophases previously stabilized with sucrose and destabilized by ethanol. Phase behavior for systems containing ethoxylated monodiglycerides, ethanol, and various sucrose concentrations are shown in Figure 2. Mixtures with compositions in the region above the miscibility curve were isotropic, while those below the curve were anisotropic. The effect of both ethanol and sucrose on the formation of isotropic solutions at 20, 55, and

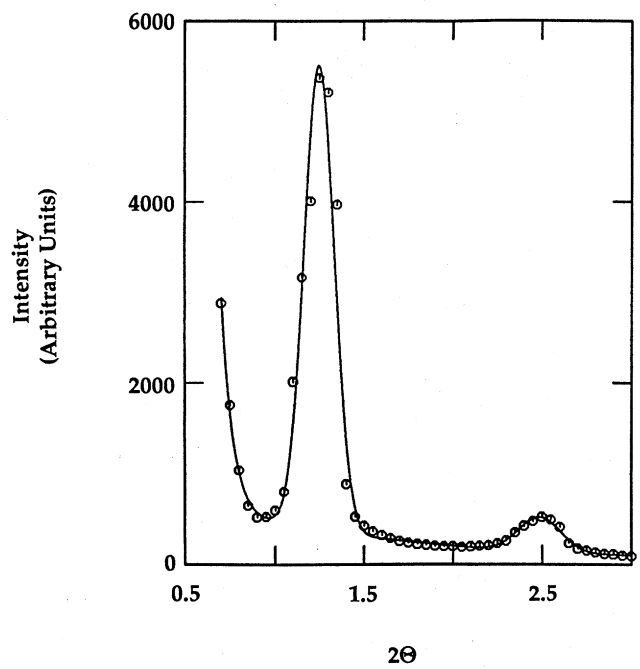

FIGURE 1: A typical x-ray diffraction pattern. The sample contains 75 wt% of ethoxylated monodiglycerides, and 25 wt% of 10 wt% of sucrose solution at 40°C. The symbols represent the data and the solid line the fit as described in text.

75wt% surfactant concentrations is demonstrated in Figure 3. It is clear that small amounts of sucrose can decrease the amount of ethanol required to form isotropic solutions, especially at higher surfactant concentrations. For example, at 75/0.5 wt% of surfactant/sucrose mixture, there was a 46% reduction of ethanol required to form an isotropic solution.

The effect of temperature and aqueous phase on the solubilization of soybean oil is shown in Figure 4. Regardless of temperature, the systems containing only sucrose or water have not improved the mutual solubility

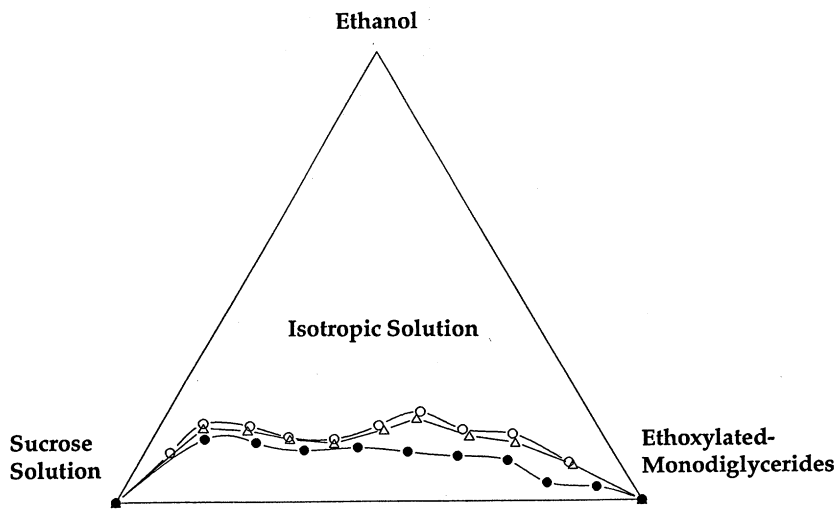

FIGURE 2: Phase equilibria of a system containing ethoxylated monodiglycerides, ethanol, and 0 (○), 1 (△) and 10 (●) wt% of sucrose at 35°C.

of the surfactant or the soybean oil (compare Figures 4a and 4b). Addition of ethanol at 35°C resulted in the formation of a microemulsion region L_1 which contained a significant amount of soybean oil and a corresponding increase in the L_2 region (compare Figures 4a and 4c). While at 40°C, an isotropic solution was detected continuously along the surfactant/aqueous phase axis. Addition of 10 wt% of sucrose to the ethanol solution (Figure 4d) further increased both L_1 and L_2 regions and improved the soybean oil solubility from 6 to 9 wt% in L_1.

To better understand the effect of sucrose on the structural behavior of microemulsion systems, two series containing 75 wt% and 55 wt% surfactant and various sucrose concentrations in the aqueous phase were investigated by small angle x-ray diffraction. The integrated intensities of

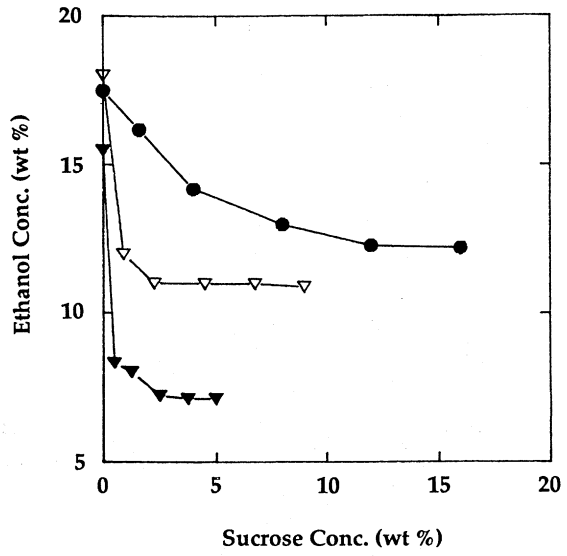

FIGURE 3: Effect of sucrose concentration on the formation of isotropic solutions containing 20 (●), 55 (▽), and 75 (▼) wt% ethoxylated monodiglycerides at 35°C.

the first diffraction peak versus the sucrose content for 75 wt% surfactant are summarized in Figure 5. Assuming that the intensities are taken as a measure of the relative amount of the ordered structures, the results showed that increasing the sucrose content stabilized the bilayer structure at 2.5 wt% (point B) compared to the system without sucrose (point A). Further addition of sucrose destabilized the bilayer structures resulting in a phase transition to hexagonal structures at 8.75 wt% (point C). This behavior was confirmed by polarized light micrographs (Figure 6) which correspond to the three points in Figure 5. The micrographs clearly show bilayer structures for points A and B which are converted to hexagonal structures with further addition of sucrose.

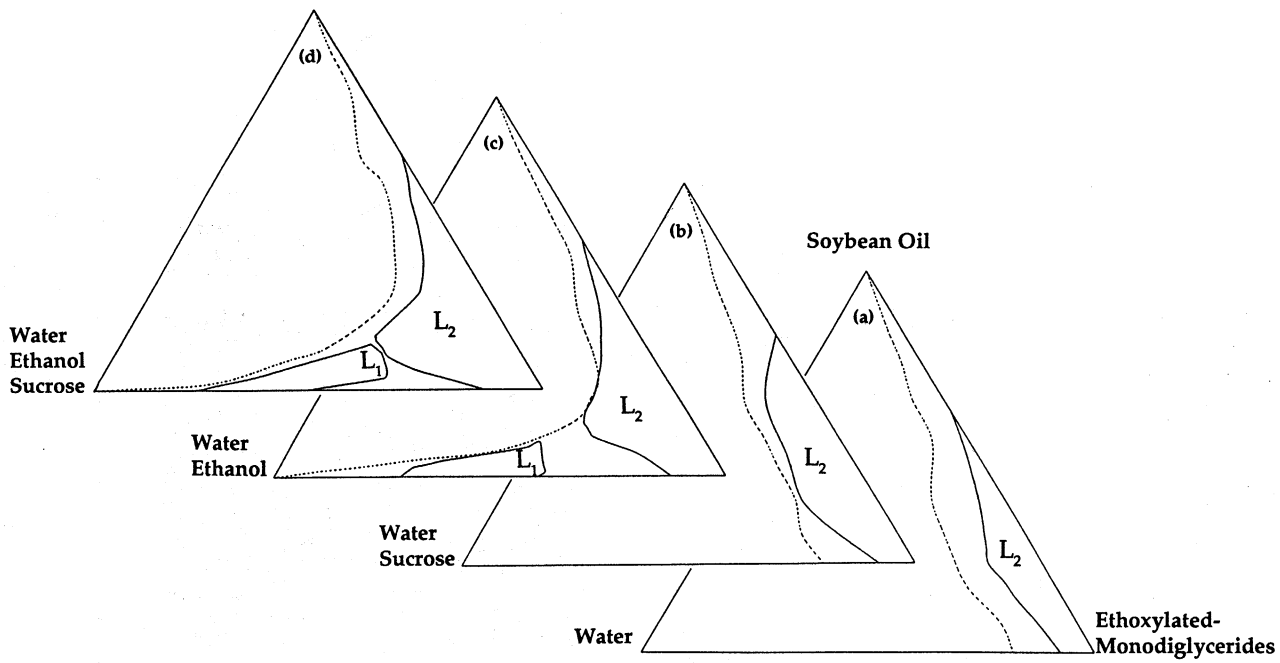

FIGURE 4: Effect of temperature and aqueous phase on regions. 35°C (——), and 40°C (.....) (a) water, (b) 90/10 wt% of water/sucrose, (c) 80/20 wt% water/ethanol, and (d) 70/20/10 wt% water/ethanol/sucrose.

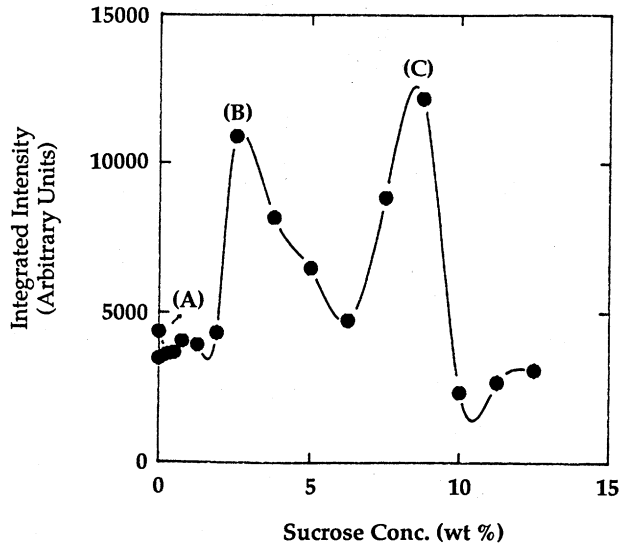

FIGURE 5: Integrated intensities of the first diffraction of small angle x-ray measurements of systems containing 75/25 wt% ethoxylated monodiglycerides/aqueous phase versus sucrose at 40°C.

FIGURE 6: Polarized light micrographs of systems in Figure 5. (A) lamellar liquid crystalline pattern oily streaks), 0 wt% sucrose (B) lamellar liquid crystalline pattern (maltesian cross), 2.5 wt% sucrose, and (C) hexagonal structure, 8.75 wt% sucrose. (150X, Bar=50 μm at 40°C).

The transition from cubic to reversed hexagonal phase is consistent with an increased average wedge-shape of the surfactant molecule from the polar head group towards the methyl end group (16). Similarly, in our studies, the hydrophilic part of the interface appears to become more rigid laterally, with addition of sucrose due to formation of additional hydrogen bonds. This effect and subsequent addition of ethanol to the system would likely result in increased disorder of the hydrocarbon chains.

Conversely, for systems containing a lower concentration of surfactant (55 wt%) and various amounts of sucrose (Figure 7), hexagonal structures (point A) were destabilized at concentrations as low as 0.25 wt% sucrose and the integrated intensity area decreased from 9500 to 2000. The hexagonal structures were stabilized again with increasing amounts of sucrose and the integrated intensity area increased to 6500 with 9 wt% of sucrose (point B). This structure was preserved by further addition of sucrose up to 15.75 wt%. while the integrated intensity decreased to 4000 (point C). This structural behavior was confirmed by polarized light microscopy (Figure 8).

It is known that liquid crystalline mesophases could be destroyed by adding short chain alcohols. In our study, ethanol was used as a cosolvent which has the properties of a chaotropic reagent (22). Strongly chaotropic reagents (e.g., trichloro- and tribromo-acetates) promote micellization of the lamellar or the hexagonal phases by favoring larger head groups (19). Such behavior of the lamellar phase composed of 75/25 wt% of surfactant/sucrose solution (Figure 5, point B) is demonstrated for various concentrations of ethanol (Figure 9). It is clear that ethanol destroys these structures and isotropic properties were detected in systems above 6.5 wt% ethanol.

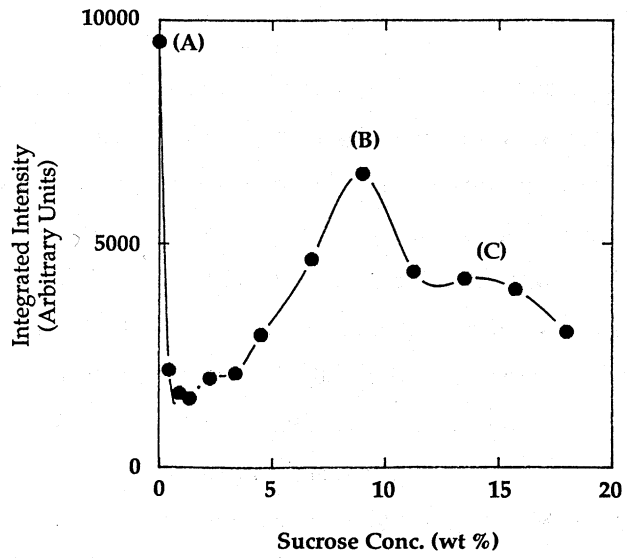

FIGURE 7: Integrated intensities of the first diffraction of small angle x-ray measurements of systems containing 55/45 wt% ethoxylated monodiglycerides/aqueous phase versus sucrose at 40°C.

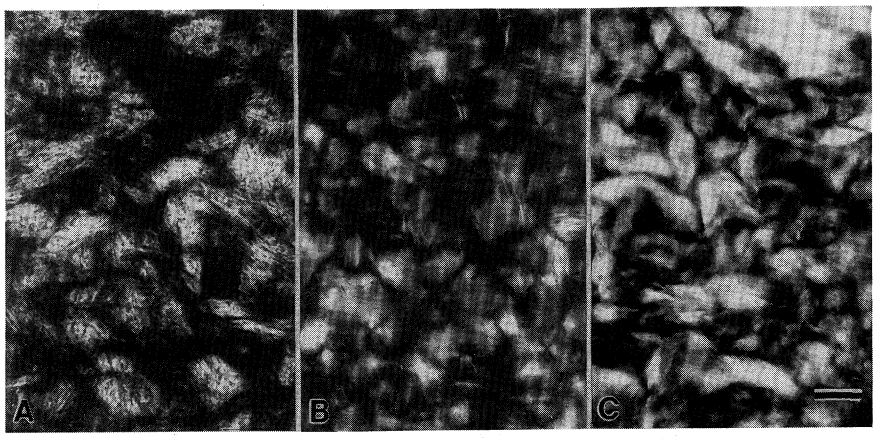

FIGURE 8: Polarized light micrographs of systems in Figure 7. (A) hexagonal liquid crystalline pattern, 0 wt% sucrose, (B) hexagonal pattern, 9 wt% sucrose, (C) hexagonal pattern, 15.75 wt% sucrose (150X, Bar=50 μm at 40°C).

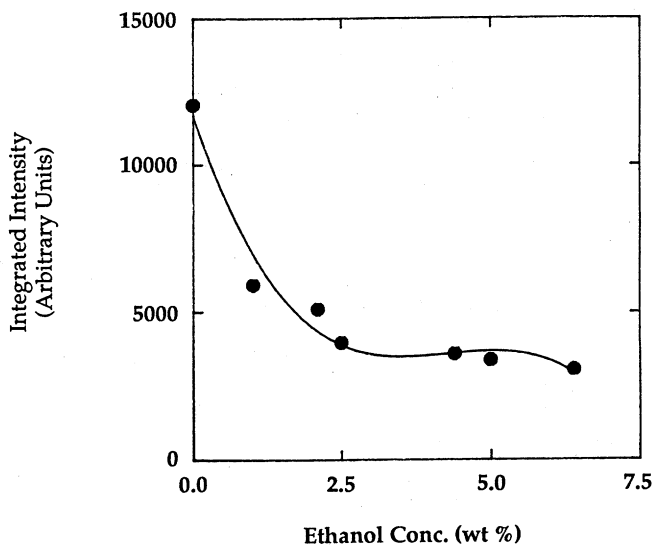

FIGURE 9: Integrated intensities of the first diffraction of small angle x-ray measurements versus ethanol. Systems contained 75 wt% of ethoxylated monodiglycerides, and 25 wt% of 10 wt% of sucrose solution at 40°C.

Fontell et al. (20) have reported that transition from lamellar or hexagonal phase to isotropic solution may be obtained by either a change in temperature or in composition. Such destruction of the hexagonal phase results in the formation of shorter rod aggregates. However, if the liquid crystalline phase has a lamellar structure, there will be a reduction in the size of the bilayer domains and an increase in their curvature. In our system, adding ethanol contributed to that transition in both the lamellar and hexagonal phases.

The stabilizing effect of sucrose combined with the destabilizing effect of ethanol on surfactant solutions provides an elegant technique to

solubilize poorly soluble triglycerides. Addition of various amounts of sucrose to systems containing soybean oil, ethoxylated monodiglycerides, and aqueous phases composed of a constant concentration of ethanol (20 wt%) does not significantly affect their phase behavior (Figure 10). Phases L_2 and L_1 were observed at 10 wt% sucrose and 35°C (Figure 10a). Increasing the sucrose fraction to 20 wt% (Figure 10b) resulted in a larger L_1 phase (more soybean oil was solubilized) and the L_2 phase became smaller. Further addition of sucrose to 30 or 40 wt% (Figures 10c and 10d) altered the phase diagram. The L_1 phase was shifted towards the aqueous corner and contained low surfactant concentrations and higher concentrations of solubilized soybean oil and the L_2 phase became even smaller.

This behavior revealed two major conclusions: (1) sucrose favored the formation of the L_1 phase and made the L_2 region smaller; and (2) high sucrose concentrations are needed at low surfactant content to solubilize more soybean oil. This effect is demonstrated in Figure 11 which shows the amount of solubilized soybean oil versus the sucrose concentration in systems with 20 wt% ethanol and various concentrations of surfactant. At 10 wt% surfactant, 27 wt% of sucrose was needed to solubilize 2 wt% of oil. When the surfactant concentration was increased up to 55 wt%, smaller amounts of sucrose, as low as 13.5 wt%, solubilized 18 wt% of oil.

Friberg and Kayali (21) have pointed out that triglyceride microemulsions could be obtained by forming a liquid crystalline phase containing triglycerides which were destabilized by addition of suitable compounds. Apparently, destabilization by itself is not sufficient to obtain optimum solubilization (compare Figure 4c with 4d). The hydrotrope used to destabilize the system would decrease both the hydrophilic and hydrophobic interactions of the surfactant molecules resulting in a relatively

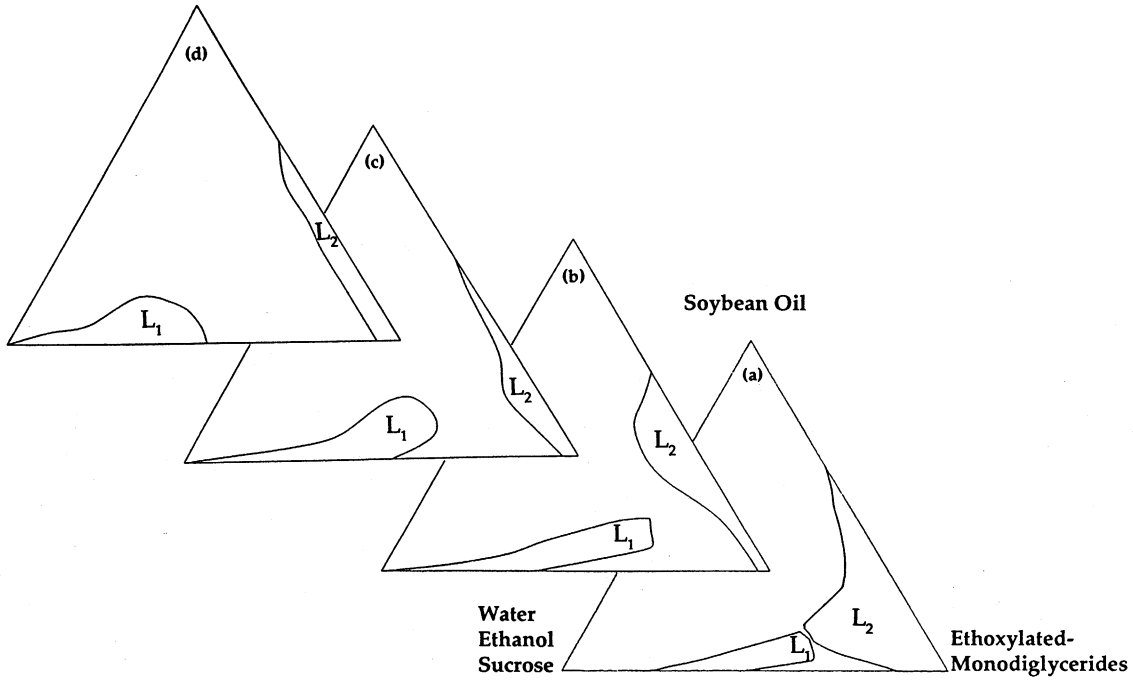

FIGURE 10: Microemulsion regions of systems containing ethoxylated monodiglycrides, soybean oil, and different aqueous phases, (a) 70/20/10, (b) 60/20/20, and (c) 50/20/30, and (d) 40/20/40 wt% of water/ethanol/sucrose at 35°C.

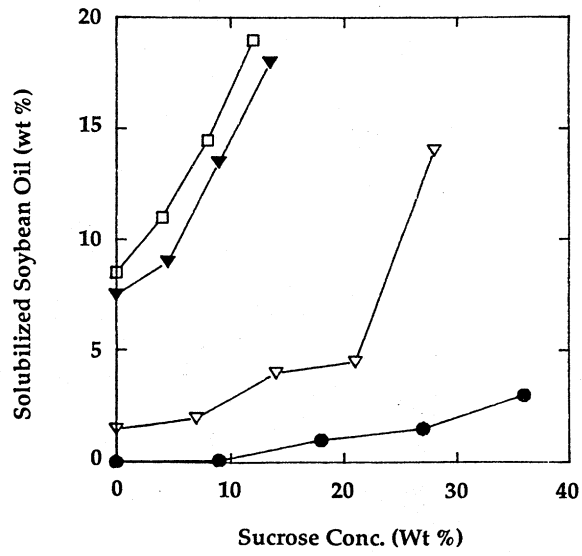

FIGURE 11: Effect of sucrose concentrations on the amount of soybean solubilized in systems containing 10 (●), 30 (▽), 55 (▼), and 60 (□) wt% surfactant.

unstable system. However, addition of sucrose appears to preferentially form a stronger hydrophilic matrix at the interface, resulting in increased solubilization of triglycerides.

Since sucrose has a high water solubility (68 wt% at 30°C), it promotes the formation of a strong network of hydrogen bonds with the polar head group of the surfactant and the hydrophobic chain of the surfactant remains disordered.

Such information on the synergetic effect of various hydrotropic agents on triglyceride microemulsion structure will assist in the solubilization of poorly soluble compounds for both food and non food applications.

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