The Influence of Food Emulsifiers on Fat and Sugar Dispersions in Oils. III. Water Content, Purity of Oils

Dorota Johansson* and **Björn Bergenståhl**
Institute for Surface Chemistry, S-114 86 Stockholm, Sweden

The influence of water on the interactions between fat and sugar crystals dispersed in triglyceride (vegetable) oils was qualitatively estimated from sedimentation and rheological experiments. The experiments were performed both with and without food emulsifiers (monoglycerides and lecithins) present in the oil. The effects of minor natural oil components (nontriglycerides) on the interactions and on emulsifier adsorption to the crystals were examined by comparing a commercial refined oil and a chromatographically purified oil. The results show that water generally increases the adhesion between fat and sugar crystals in oils and also increases the surface activity of the oil-soluble food emulsifiers. Minor oil components give a small increase in the adhesion between fat and sugar crystals in oils, but do not influence the adsorption of food emulsifiers in any systematic way.

KEY WORDS: Emulsifiers, fat, food, oils, rheology, sedimentation, sugar, triglycerides, water.

Fat-continuous food products, such as chocolate, margarine or butter, consist of a network of particles (fat crystals, sugar crystals, etc.) dispersed in a continuous oil phase. All of these products also contain emulsifiers, either naturally present or added as emulsion stabilizers, rheology regulators and/or fat crystallization regulators. The characteristic property of emulsifiers is their ability to absorb to interfaces. The adsorption of emulsifiers to aqueous/oil interfaces is well characterized. On the other hand, the adsorption of emulsifiers to crystals dispersed in oils is not as thoroughly described. This adsorption, and its influence on both the sedimentation stability and the rheology of oil-continuous dispersions, have been topics of our previous studies. The results are described in the first two articles of this series (1,2). These studies were mainly carried out in refined soybean oil (SBO) with water content of around 0.1–0.2%.

However, it is expected that traces of water can adsorb to fat and sugar crystals in oils and interact with the polar head groups of the present emulsifiers. Hence, water influences the adsorption of emulsifiers to the crystals as well as the interaction between the crystals. As water is always present, it is important to estimate the magnitude of its influence.

It has been shown in the literature that water can influence the adsorption of surfactants to particles dispersed in a nonpolar medium and, consequently, interactions between those particles (observed as changes in the colloidal stability of the systems) (3). Other studies show that increased water content increases the viscosity of chocolate (4–7). However, to our knowledge, no basic systematic studies have previously been carried out in oil-continuous food systems.

All refined vegetable oils contain minor components, such as mono- and diglycerides, fatty acids, phospholipids and sterols. Because the source, the treatment and the freshness of food oils can vary, their content of minor components also can vary. Therefore, it is important to estimate the influence of these components on the properties of oil-continuous foods. In our investigation, the influence of minor components on the interparticle interactions and on the adsorption properties of emulsifiers is examined in both a commercially refined soybean oil (SBO) and a chromatographically purified soybean oil (CPLSBO).

Since adsorption measurements are rather labor-intensive, we have chosen simple and quick sedimentation experiments for a qualitative estimation of the influence of water and minor oil components on oil-continuous dispersions. We have shown (1) a strong correlation between the magnitude of the changes in sedimentation properties and in the strength of adsorption, represented by the slope of the adsorption isotherms. There is also an obvious correlation between the sedimentation properties of diluted dispersions and the rheological properties of the corresponding concentrated systems (2).

EXPERIMENTAL PROCEDURES

All materials were dried in vacuum (pressure 10^{-2} torr) until their weights were constant over time to minimize the water content.

Emulsifiers. Two different emulsifiers have been used in this study—a pure phosphatidylcholine extracted from soybean oil and a pure, laboratory-prepared monoolein. Soybean phosphatidylcholine (PC) (Epikuron 200 with a purity of 98%, supplied by Lucas Meyer Company, Hamburg, Germany) has a molar mass of about 773 g/mole, an iodine value of about 100 and a HLB (hydrophilic-lipophilic balance) value estimated at about 8. It forms reversed micelles in nonpolar oils (8–11). It can only be slowly dispersed in oil due to the formation of stiff cubic liquid crystals at low water activities (12). Thus, the dispersion procedure is easier if some water is present in the oil. Monoolein (purity $\geqslant$ 99%, supplied by Grinsted Products AS, Århus, Denmark) has a molar mass of 366 g/mole, an iodine value of 50 and the HLB value of about 3.4. It is oil-soluble.

Crystals. A pure tristearin (supplied by Fluka, Buchs, Switzerland) was chosen as a model fat crystal. It was recrystallized in acetone to decrease the average particle size and to be certain that the β-polymorphic form of crystals was formed. The specific surface area of the crystals was about 7 m^2/g by BET adsorption with nitrogen gas. The crystals are nonpolar, with mostly hydrocarbon tails on the surface.

A ground and sieved saccharose (supplied by the Swedish Sugar Company, Arlöv, Sweden) was chosen as a model sugar crystal. The largest dimensions of the crystals were about 30 μm and the specific surface area (BET measurements with argon) was 1.15 m^2/g. The crystals are expected to be polar and also to be dominated by hydroxyl groups on the surface.

Oils. Experiments were performed in a refined soybean oil (SBO) from Karlshamns AB (Karlshamn, Sweden). The oil is highly unsaturated (about 2.5 double bonds in each

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*To whom correspondence should be addressed at Institute for Surface Chemistry, Box 5607, S-114 86 Stockholm, Sweden.

fatty acid chain on average) and has an iodine value of 132. It contains minor components, such as mono- and diglycerides, traces of phospholipids, fatty acids, tocopherols and cholesterol (13). Another set of experiments was performed in a specially purified soybean oil (CPLSBO) from Karlshamns LipidTeknik AB (Stockholm, Sweden). This oil was purified by a preparative high-performance liquid chromatography (HPLC) technique, and levels of the minor components in this oil were below the limits of detection for HPLC.

Sedimentation studies. Dried saccharose crystals (0.5 g or about 0.3 mL) were mixed with 4.5 g (about 4.9 mL) of refined or purified soybean oil. Dried tristearin crystals (0.05 g or about 0.05 mL) were mixed together with 4.95 g (about 5.4 mL) of the oils. The emulsifiers, when present, were dissolved in the oil phase before mixing with the crystals.

The following mixtures of emulsifiers were prepared: 0.1, 0.2, 0.5 and 1.0% of PC (corresponding to approximately 1.2, 2.4, 5.9 and 11.8 mmole/L), and 0.05, 0.1, 0.25 and 0.5% monoolein (approximately 1.25, 2.5, 6.2 and 12.4 mmole/L). In a few experiments (PC in CPLSBO) it was difficult to obtain uniform solutions, even after prolonged stirring.

Sedimentation experiments were performed in series, each with a different level of water. For the fat crystals, the water content was approximately 0, 0.2 and 2%. The latter level corresponds to an excess of water. For the sugar dispersions, the water levels were approximately 0, 0.05, 0.75, 1.5 and 2.3%. In both cases (fat and sugar crystals) water was added as the last component to the dry dispersions. After equilibration for about 24 h at room temperature ($\sim$20°C), the dispersions were allowed to settle (also at $\sim$20°C) until the sediment volumes were constant over time (about one week). The results, sediment volumes of dispersions, are presented as a function of water content in the samples. A separate curve is obtained for each emulsifier and its concentration. Each diagram corresponds to one type of crystal (tristearin or saccharose) and one type of oil (refined SBO or purified CPLSBO).

Rheology. A few rheological measurements were performed on the Bohlin VOR and Bohlin CS (controlled stress) rheometers (Bohlin Rheology, Lund, Sweden). Elastic modulus (G'), its limit of linearity (γ_{lin}) and yield value ($\tau_y = \gamma_{\text{lin}} \times G'$) were determined from the strain/stress sweep measurements. The measuring system was cone and plate, with a plate diameter of 30 mm and a cone angle of 5° (CP 5/30) for the VOR rheometer and the corresponding system (CP 4/20, 4°, 20 mm) for the CS rheometer. All experiments were performed at 20°C.

The following samples were prepared separately for the rheological measurements—dried samples of 25% tristearin in refined SBO and in purified (CPLSBO), and 25% tristearin in SBO with water content of about 0.2%.

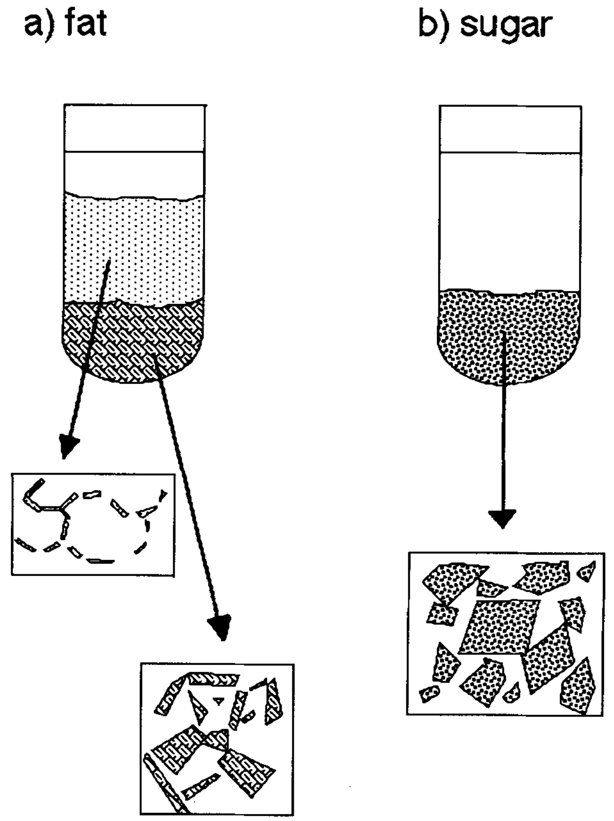

FIG. 1. The appearance of sediment samples. a, Fat crystals form three different sediment zones: A thin oil film without crystals on the top of samples; an upper sediment zone with a loose network of small crystals and relatively long interparticle distances; and a lower sediment zone with a tight network of big crystals and relatively short interparticle distances. b, Sugar crystals are bigger and heavier than the fat crystals. They form only two sediment zones: An upper oil zone without crystals and a sediment zone with a crystal network.

RESULTS

The appearance of sediment samples. The sugar and fat crystals appear different after sedimentation. The sugar samples form one sediment phase and an upper pure oil phase, as shown in Figure 1. Fat dispersion samples instead form two different sediment zones and a pure oil zone. The boundary between the upper sediment zone and the oil phase is rather sharp. The upper sediment phase, observed in a microscope, consists of small fat crystals in a kind of loosely packed network. The lower sediment zone is much denser and consists mainly of bigger particles or flocs, which compress the network to a certain degree due to gravitational forces.

Because the particles in the lower sediment zone are densely packed, the average distances between them are small and, consequently, they interact strongly with each other. Therefore, the lower sediment zone is more easily influenced by adsorption of emulsifiers to the particles than is the upper, loosely packed, sediment zone. The sugar crystals are bigger and heavier than the fat crystals. They form only one dense sediment zone because gravitation forces are important, even for the smallest particles in the samples.

Fat crystals in refined SBO. Results for the fat crystals dispersed in refined soybean oil are presented in Figure 2. The sediment volumes of dispersions are plotted as a function of the water content in the samples. Both upper and lower sediment volumes are presented in the Figure. The upper sediment volumes are not affected by addition of water, while the lower sediment volumes double when water content of the samples approaches about 2%.

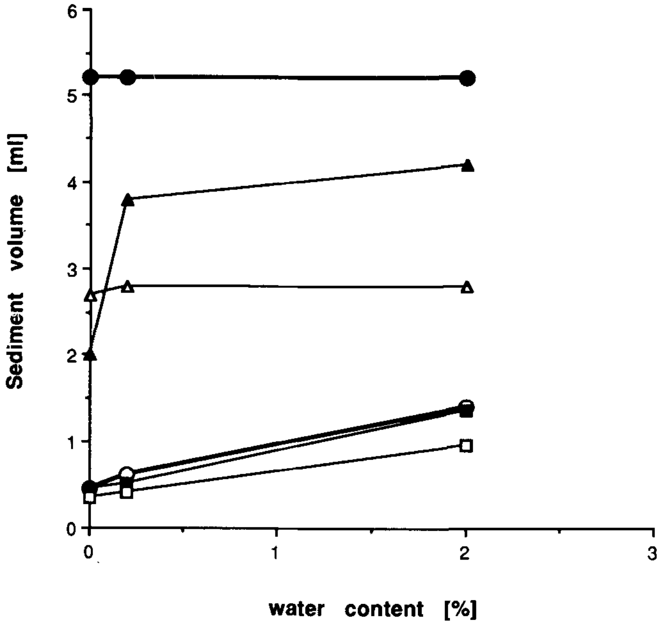

FIG. 2. Sediment volumes of fat crystals in refined SBO containing different emulsifiers and concentrations, as a function of water content in the samples. No emulsifier, lower sediment (○); no emulsifier, upper sediment (●); 1 mM phosphatidylcholine, upper sediment (▲); 10 mM phosphatidylcholine, upper sediment (△); 1 mM monoolein, lower sediment (■); and 10 mM monoolein, lower sediment (□).

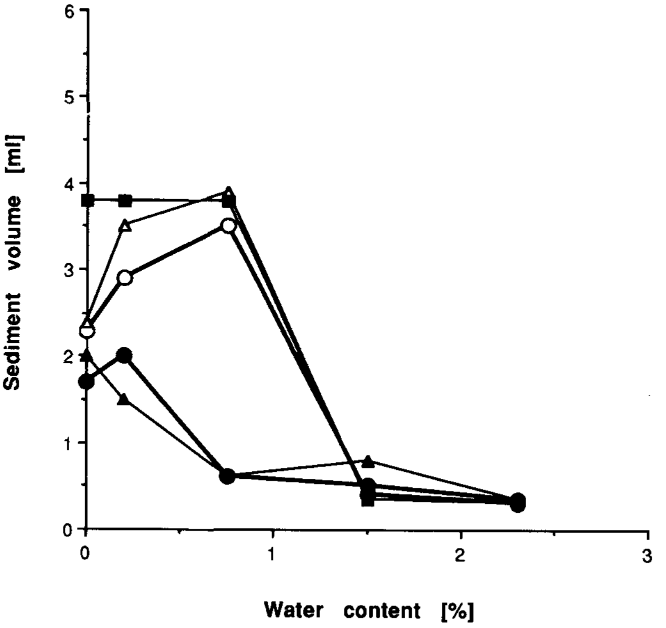

FIG. 3. Sediment volumes of sugar crystals in refined SBO containing different emulsifiers and concentrations, as a function of water content in the samples. No emulsifier (○); 1 mM phosphatidylcholine (●); 10 mM phosphatidylcholine (▲); 1 mM monoolein (△); and 10 mM monoolein (■).

Generally, the upper sediment volumes are about 5-10 times larger than the lower.

When monoolein is added to SBO, the upper sediment volumes are not changed, but the lower sediment volumes decrease. The decrease is small for the monoolein concentration of 1 mM, and it is slightly larger at higher concentrations (about 10 mM). The decrease is also a little more pronounced when water is present in the samples.

Soybean PC in the oil changes the appearance of the fat dispersions, only one sediment zone with a smaller total volume occurs. The decrease in sediment volume caused by PC becomes larger when its concentration increases and smaller when the water content in the samples increases.

Sugar crystals in refined SBO. The results are presented in Figure 3. Sediment volumes of sugar dispersions increase with 50% as the water content of the samples increases to about 1%. Further increases in the amount of water results in a dramatic decrease of sediment volumes to about 0.3-0.5 mL. This volume is roughly the same as the volume of the dry added sugar crystals.

Monoolein increases the sediment volumes of sugar dispersions. For a low content of monoolein (1 mM), this increase is more pronounced when water is present in the samples. Soybean PC reduces the sediment volumes of sugar dispersions. This decrease is also more pronounced when water is present. As the water content exceeds about 1%, the sediment volumes of the samples with and without emulsifiers fall to about 0.3-0.5 mL.

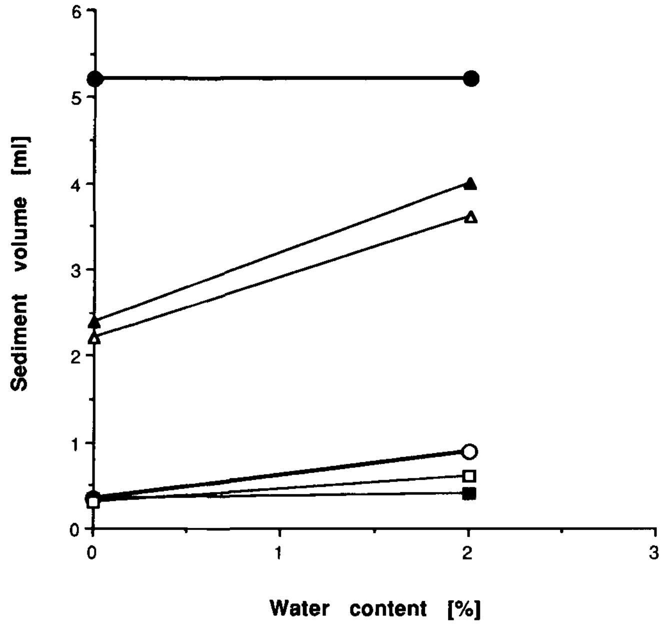

FIG. 4. Sediment volumes of fat crystals in CPLSBO containing different emulsifiers and concentrations, as a function of water content in the samples. No emulsifier, lower sediment (○); no emulsifier, upper sediment (●); 1 mM phosphatidylcholine, upper sediment (▲); 10 mM phosphatidylcholine, upper sediment (△); 1 mM monoolein, lower sediment (■); and 10 mM monoolein, lower sediment (□).

Fat crystals in CPLSBO. The results are presented in Figure 4. The appearance of fat crystal dispersions in CPLSBO is similar to that in SBO. The sediment volumes are somewhat more compact in CPLSBO than in SBO. This is even more pronounced when water is present in the samples. Some quantitative differences do occur when

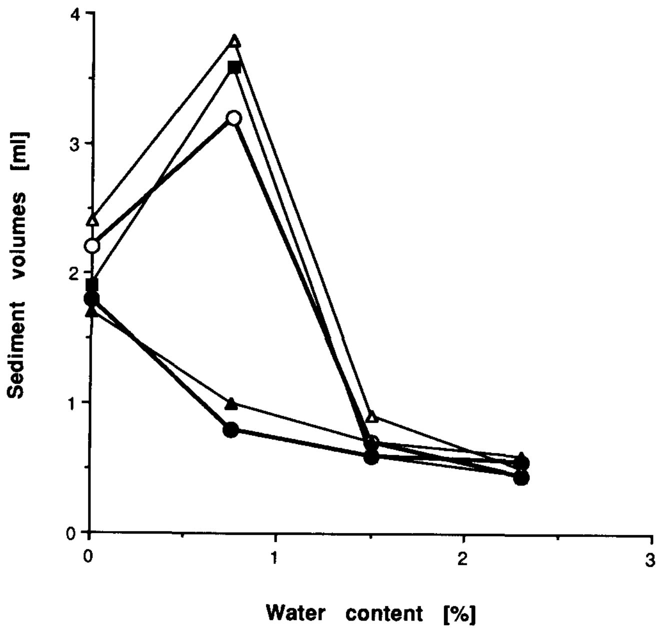

FIG. 5. Sediment volumes of sugar crystals in CPLSBO containing different emulsifiers and concentrations, as a function of water content in the samples. No emulsifier (○); 1 mM phosphatidylcholine (●); 10 mM phosphatidylcholine (▲); 1 mM monoolein (△); and 10 mM monoolein (■).

emulsifiers are added to the samples, but general trends remain the same.

Sugar crystals in CPLSBO. The results are presented in Figure 5. The appearance of sugar crystal dispersions in CPLSBO is also almost the same as in SBO for all samples examined. Again, sediment volumes are a little more compact in CPLSBO than in SBO, and this trend is more pronounced when water is added. The samples with emulsifiers also show qualitatively the same behavior in CPLSBO as in SBO.

Rheology. The rheological results are summarized in Table 1. The elasticity of a 25% dispersion of tristearin in SBO is 12 kPa (±4 kPa), and the yield value is 13.8 Pa (±2.4 Pa). When water (about 0.2%) is added to the above dispersion, the elasticity (about 10 kPa) is not significantly changed, and the yield value increases about 3–4 times (to about 50 Pa).

When SBO is exchanged for CPLSBO and the samples are free of water, both the elasticity and the yield value decrease to 6.6 ± 1.5 kPa and 7.6 ± 3.9 Pa, respectively. The decreases are relatively small but significant.

DISCUSSION

In discussing the results, it is necessary to use a relationship between changes in sediment volumes of dispersions and changes in adhesion between the crystals (14–22). When a dispersion settles, its volume depends on particle interactions in the system. If the interaction changes towards a more adhesive one, then a more voluminous sediment is obtained. On the contrary, if the interaction changes towards a more repulsive one, then the resulting sediment is more dense. We have shown previously (1) that the magnitude of the changes in sediment volumes corresponds to the slope of the adsorption isotherms.

The influence of water on fat crystal dispersions in oils. The presence of water increases sediment volumes of the examined fat dispersions. It is interpreted as water adsorption to fat crystals, which increases the polarity of their surfaces and the adhesion between them. About 0.2–0.3% water adsorbs from the air to the fat crystals, which is supported by increased crystal weight after equilibration with humid air.

Water is expected to adsorb preferentially to the polar areas on the crystals, and/or to the nonpolar areas by linking with the surface-active molecules. The adsorbed water increases the Hamaker constant and, therefore, the van der Waals adhesion between the crystals. Water bridges also can be formed between the crystals and contribute to increased adhesion. This interpretation is supported by the rheological measurements. The strength of interaction between the fat particles (*i.e.*, the yield value) increases about 3–4 times, and the elasticity remains unchanged.

A network model (23), as well as a correlation of yield value to interaction energy (24–26) is applied in the interpretation of the rheological data. According to these models, the particles in the actual dispersion are flocculated in a three-dimensional network of chains by means of attractive forces. The elasticity of such a system is proportional to the slope of the force/distance curve at the equilibrium distance, *i.e.*, to the elasticity of the interparticle bonds. Further, the yield stress is proportional to the depth of the energy minimum between particles.

As previously suggested (2), the elasticity of fat dispersions and the distances between crystals are mainly determined by van der Waals forces, while the yield value and crystal interactions are determined by other interactions (for example, water bridges). Because the elasticity in the system remains almost unchanged, van der Waals interactions and Hamaker constants in the system are not changed significantly by the presence of water, and the increase in the interaction energy is probably caused by formation of water bridges.

The influence of water on sugar crystal dispersions in

TABLE 1

Rheological Results (elasticity G′, its strain limit of linearity $\gamma_{\rm lin}$ and calculated yield value $\tau_y = G' \times \gamma_{\rm lin}$) for Dispersions of Tristearin (25% w/w) in SBO and CPLSBO

Oil	Water content (%)	G′ (kPa)	$\gamma_{\rm lin}$	τ_y (Pa)
SBO[a]	0.2	10.0 ± 0.5	5×10^{-3}	50.0 ± 2.5
SBO	0	12.0 ± 4.0	1.15×10^{-3}	13.8 ± 2.4
CPLSBO	0	6.6 ± 1.5	1.15×10^{-3}	7.6 ± 3.9

[a] Previous rheological data (2).

oils. The interactions between crystals change when the water content is raised (observed as an increase in sediment volumes). This is interpreted as water adsorption to sugar crystals. Adhesion between the crystals increases, probably mainly due to formation of water bridges. As the amount of water increases, the sugar surface becomes covered by a layer of sugar solution. When the water content approaches a certain value (in the actual case, about 1%), all the sugar (0.5 g ≈ 0.3 mL) melts and separates from the oil at the bottom of the samples.

The influence of water on the adsorption of emulsifiers to fat crystals in oils. Monoolein, which is oil-soluble, adsorbs weakly to fat crystals in oils, forming loosely packed layers on the surface (1). These layers give weak steric stabilization of the fat crystals, with somewhat denser sediments as a result. The changes are relatively small, and only the lower sediments are affected.

Water enhances the adsorption of monoolein to the crystals because the lower sediment volumes decrease more when water is present. The character of the surface activity of monoolein is sensitive to the presence of water. The observed increase in adsorption could be due to formation of reversed micelles by the monoglyceride.

Soybean PC, which is poorly soluble in the oil, adsorbs strongly to fat crystals in the oil, resulting in multilayers. The adsorption causes a strong decrease in sediment volumes of fat dispersions due to steric stabilization of the crystals (1). The influence of PC is so strong that the two different sediment zones in the fat samples merge.

As water is added to the samples, the influence of PC on the sediment volumes of the fat dispersions diminishes, probably due to weaker adsorption of PC to the crystals. This occurs especially at low concentrations of the emulsifier. A possible formation of aggregates, reversed micelles or liquid crystals by the lecithin in the soybean oil reduces its surface activity. This phenomenon is schematically presented in Figure 6. The emulsifier molecules cooperate in aggregates with their polar head groups direct towards each other and with hydrocarbon chains directed towards oil (reversed micelles, reversed hexagonal phase or cubic phase). Formation of reversed micelles by lecithin in different oils has been reported recently (8–11). Micelle formation leads to less adsorption to fat crystals because both processes are competitive. The driving force for adsorption is the same as the driving force for formation of reversed aggregates, *i.e.*, hydration of polar head groups and minimization of unfavorable contacts between water and nonpolar oil. Because micelle formation, adsorption and solubility are thermodynamically controlled processes, an intimate equilibrium exists between these three forms, as shown in Figure 6. As the concentration of PC increases, an adsorption plateau is reached. The emulsifier is present in excess. The adsorbed molecules are not removed from the surface in this case.

Influence of water on the adsorption of emulsifiers to sugar crystals in oil. Monoolein adsorbs strongly to sugar crystals in oils and increases both the adhesion between the crystals and the sediment volumes of the samples (1). This is probably due to a bridging mechanism.

The presence of water enhances the adsorption of monoolein and the possibility for different bridging mechanisms to occur. Thus, adhesion between the crystals also is enhanced when water is present in the samples. As the monoolein concentration increases, the adsorption plateau is reached and water no longer influences adsorption significantly.

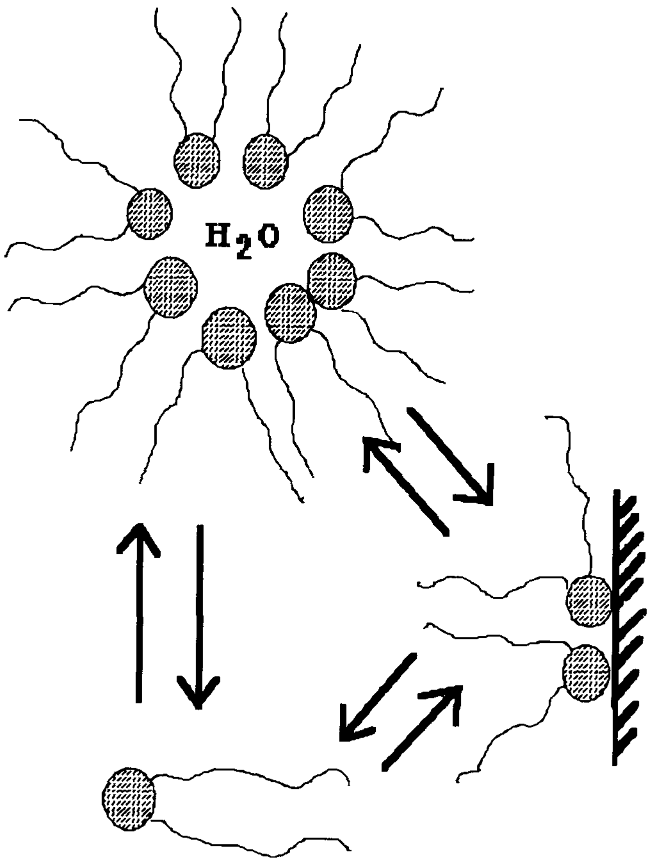

FIG. 6. A hypothetical equilibrium between three different states of an emulsifier in an oil phase—reversed micelles, adsorbed molecules and molecules in solution.

Soybean PC adsorbs strongly to sugar crystals in oils and decreases adhesion between the crystals and the sediment volumes of dispersions (1). Since PC has a strong affinity to water, its adsorption to the water-covered sugar surface increases. This is observed as decreasing sediment volumes of samples that contain water. As the water concentration exceeds about 1%, the sugar melts and forms a separate phase at the bottom of the samples.

Influence of minor oil components (nontriglycerides) on the fat and sugar crystal dispersions in oils. The influence of minor oil components on fat and sugar dispersions in oils is much less pronounced than the influence of water, but it increases when water is added. The adhesion between fat and sugar crystals is slightly stronger when minor components are present in the oil. The minor oil components show some surface activity and can therefore adsorb to the crystals, causing these effects. This behavior is somewhat enhanced by the presence of water, which also adsorbs to the crystals. For fat crystals, the sedimentation results are consistent with the rheological results. The latter also indicate a decrease in fat crystal adhesion (decreased yield value and elasticity) as the SBO is purified.

The sedimentation experiments show some quantitative differences between the refined (SBO) and the purified

(CPLSBO) oils when emulsifiers are present in the samples. However, it is difficult to make general statements about the influence of minor components on emulsifier adsorption to the crystals. If any differences arise, they seem to be of minor importance and qualitative behavior is about the same.

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