♉The Function of Phospholipids of Soybean Lecithin in Emulsions[1]

L. RYDHAG and I. WILTON, The Swedish Institute for Surface Chemistry, Box 5607, S-114 86 Stockholm, Sweden

ABSTRACT

A number of commercially available soybean lecithins were analyzed with respect to their phospholipid composition and emulsifying properties. A phosphatidylcholine (PC) from soybean swells to a lamellar liquid crystalline phase which incorporates slightly less than 50% of water. The swelling behavior of the commercially available soybean lecithins may be different depending on the concentration of other phospholipids such as phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidic acid (PA). In the presence of the negatively charged phospholipids PI and PA, the swelling of the lamellar phase of PC was dramatically enhanced while a lecithin with equal amounts of PC and PE and small quantities of PI and PA formed two liquid crystalline phases, i.e., a lamellar and a hexagonal phase. Stable o/w-emulsions can be prepared when the phospholipid composition is such that a lamellar liquid crystalline phase in equilibrium with the oil and water phases incorporates large amounts of water. The minimal amount of emulsifier required to stabilize the emulsions has been estimated to give an interfacial film of ca. 80 Å thickness which corresponds to a thickness of two double lipid layers in the interfacial film. The incorporation of large amounts of water is obtained if the lamellar layers contain dissociated ionic groups.

INTRODUCTION

Phospholipids from soybeans are permitted as food emulsifiers and they are also used as emulsifiers in pharmaceutical, cosmetic and technical preparations. Different compositions and total concentrations of phospholipids are declared by different suppliers of lecithins of commercial origin used in food. However, we have shown that large variations between batches occur (1).

Soybean lecithin is obtained as a by-product in the production of oil from soybeans. The phospholipids are removed from the raw soybean oil in a degumming procedure, i.e., those components which swell with water precipitate as a liquid crystalline phase which is removed from the oil. After evaporation of the water, the precipitated mixture contains ca. 65% phospholipids and ca. 35% raw soybean oil. This is what is known as the commercially available soybean lecithin. Depending on both the history of the soybeans themselves and the method used for their extraction, the soybean lecithin will vary both as to the composition and the total concentration of the phospholipids whereas the fatty acid pattern corresponds to that of the soybean oil.

It would obviously be highly desirable to establish whether the variability in composition of commercially available lecithins can be correlated with their properties as emulsifiers. In this paper, we report continued studies of the swelling behavior of different lecithins and show that this may be correlated to their composition as well as to their function as emulsifiers.

We have studied three commercial lecithins. Their phospholipid composition and fatty acid pattern were determined and are reported in Materials and Methods. The swelling properties were investigated by means of low angle X-ray diffraction patterns. Phase equilibria were determined

for a water-soybean emulsifier system. Knowledge of these made it possible to prepare emulsions under controlled conditions in a multicomponent system for which the phase equilibria were known. Emulsion stabilities were determined as a function of the o/w ratio and the amount of emulsifier in the model systems. We will show that, in addition to the correlation between phase equilibria and emulsion properties, the studies give some important insights into the stabilization mechanism of the o/w emulsions.

MATERIAL AND METHODS

Methods of Analysis

The emulsifiers were all of commercial origin, i.e., phosphatidylcholine (PC), well defined with respect to the polar group, and mixtures of phospholipids with a fatty acid pattern corresponding to that of soybean oil. In the mixed phospholipid preparations, the amounts of constituent phospholipids were determined according to the method described (1). After two-dimensional thin layer chromatographic (TLC) separations on silica gel plates, first with a basic eluent and then an acid one, spots on the plate corresponding to PC, phosphatidic acid (PA) and lysophosphatides were identified but only PC, PE, PI and PA were analyzed quantitatively for phosphorous according to the method described by Lowry and Tinsley (2). The amount of lysophosphatides was less than 2 mol %. The phospholipid compositions are shown in Figure 1.
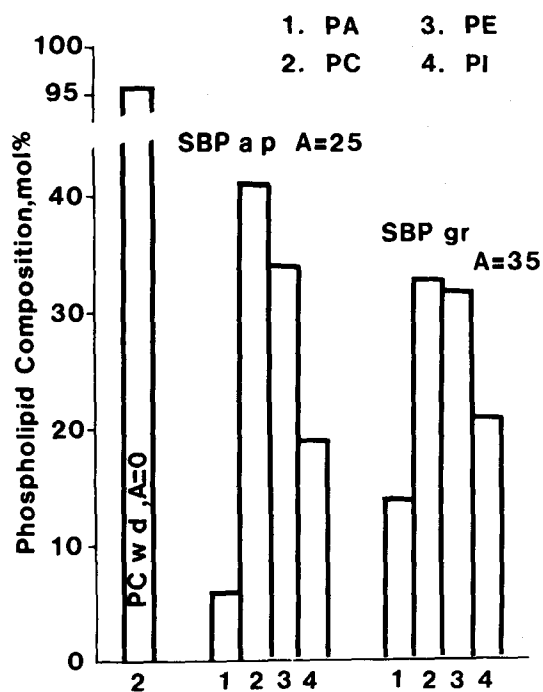

FIG. 1. The principal phospholipid composition of the soybean lecithins which were used as emulsifiers.

TABLE I[a]

Product	Fatty acid composition (wt. %)				
	C_{16}	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Phosphatidylcholine, well-defined (PCwd)	14.9	3.2	13.7	62.7	5.2
Soybean phosphatides, acetone-precipitated (SBPap)	21.5	4.3	7.2	60.9	6.1
Soybean phosphatides, granulated (SBPgr)	18.9	4.1	6.8	60.8	9.2
Soybean oil, refined, deod. (SBO)	11	4	25	50	8

[a]The fatty acid pattern of the soybean phosphatides and the soybean oil varies. The table shows the composition for the batches used in this investigation.

The fatty acid patterns of the soybean PC, the mixed phospholipid preparations and the soybean oil were determined by means of gas chromatographic analysis of the methyl esters according to Glass et al. (3). The results are summarized in Table I.

The influence of the batch variation of the phospholipid composition of a commercial lecithin has earlier been reported (1). The amount of negatively charged phospholipids varied, e.g., between 29 and 42 mol % in 6 different batches of an acetone-precipitated commercial lecithin.

Definition of A-Value

The pK_a values of PA are $pK_1 = 3.8$ and $pK_2 = 8.6$ in water at 25 C (4). Hence, while the PC and PE are neutral at pH 6-7 the PA and PI are partly dissociated and, thus, negatively charged, each molecule carrying one charge. The fraction of negatively charged phospholipids thus may be expressed as the sum of mol % (A) of PA and PI.

$$A = \frac{n_{PA} + n_{PI}}{n_{PA} + n_{PC} + n_{PE} + n_{PI}} \times 100,$$

where n_i is the amount of component i.

Emulsifiers

(a) PC wd is a well defined soybean lecithin containing 98 mol % PC (i.e., $A \approx 0$) delivered by Lucas Meyer, Hamburg, Germany. (b) SBPap is an acetone precipitation of a Yelkin® lecithin from Ross & Rowe, NY, and contains 90% phospholipids with A = 25. (c) SBP gr is a defatted and granulated lecithin of unknown origin in The Netherlands. It contains 78% phospholipids with A = 35.

Soybean lecithins contain mostly unsaturated fatty acids and hence all investigations have been made well above the chain melting temperature (cmt) which was -17 C for the well defined soybean PC in water.

The salts used were of p.a. quality, all water was twice distilled and the oil used was refined, deodorized soybean oil (SBO).

Phase Equilibria

The phase equilibria in the pseudo-ternary system water/ oil/phospholipid were determined thus: The phospholipids were dissolved together with the oil in petroleum ether and ethanol, after which the solvent was evaporated under reduced pressure. Following the addition of water, the mixture was repeatedly forced through a centrifuge tube with a narrow constriction or a similar arrangement of two syringes. The phases were then separated by ultracentrifugation at 150,000 G and identified by means of their low angle X-ray diffraction patterns at 20 C. The samples were handled in a nitrogen atmosphere because of the high degree of unsaturation in the fatty acid chains of both the phospholipids and the oil. Otherwise, the determinations were carried out according to previously described methods that have been used for ternary systems (5,6).

Emulsification Procedure

Emulsions of varying oil:water ratios and with emulsifier concentrations from 0.1 to 3% were prepared in a continuously working valve homogenizer under controlled conditions, i.e., time, pressure and temperature were kept constant. The apparatus is a modified hand-powered valve homogenizer which was improved by Tornberg and Lundh at the Technical University of Lund, Sweden (7). Before emulsification, the phospholipids were dispersed in the oil phase, after which the oil was mixed with the water phase in the homogenizer. Afterwards, the emulsion was transferred to a graduated glass cylinder and kept at constant temperature (20 C).

Characterization of Emulsions

The following terms describing different steps in the aggregation processes involving emulsion droplets will be used. Creaming: the dispersed oil phase flotate; flocculation: two or more droplets form an aggregate but keep their identity; coalescence: two or more droplets form a bigger one.

Emulsion stability was judged (a) by visual determination of the separation of the oil and water phases, and (b) by the determination of droplet size in the upper and lower parts of the sample volume immediately after the preparation of the emulsion as well as after 24 hr. Coalescence and creaming were determined by means of visual observation combined with drop size determinations. The droplet size determination was carried out by means of a Coulter Counter, Model TA, Coulter Electronics, Ltd., England. Flocculation was examined with the help of a light microscope equipped with polarization filters and interference contrast. Emulsion type was determined under the microscope after staining the water and oil phases with methylene blue and Sudan red, respectively. The emulsions were judged to be stable when neither phase separation, creaming, flocculation or coalescence had taken place after a period of 24 hr.

RESULTS

Phase Equilibria

X-ray diffraction measurements. The swelling behavior of the acetone-precipitated phospholipid mixture, SBPap in water, was investigated by means of low angle X-ray diffraction at 25 and 40 C. The higher temperature was chosen to check that all the saturated fatty acid chains of the SBPap were in a fluid state. The swelling was identical at 25 and 40 C.

A liquid crystalline phase with two-dimensional hexagonal symmetry characterized by Bragg spacings in the ratio of $1:1/\sqrt{3}:1/\sqrt{4}$ was observed at low water concentrations. This phase is a reversed hexagonal structure (RM), type F according to Fontell et al. (6) or H_{II} according to Luzzati (8). At water concentrations larger than 20%, a phase was formed which was characterized by Bragg spacings in the ratio of $1:1/2:1/3$. This corresponds to a lamellar phase (N), D according to Fontell et al. or L_α according to Luzzati. Figure 2 shows the repeat distance d of the liquid crystalline phases as a function of $1-C_a/C_a$, where C_a is the phospholipid concentration which is proportional to the inverse volume fraction of the phospholipids. As expected for a lamellar liquid crystalline phase, the d value increases linearly with this quantity between 20 to 64 wt. % water. The swelling corresponds to the case of

a lamellar phase incorporating all the water of the system. When the water concentration exceeds 64 wt. %, two phases exist in equilibrium, i.e., the lamellar phase (N) and a very dilute aqueous solution of the phospholipid mixture.

The swelling behavior and the phase equilibria have been established earlier for the well defined soybean PC, PCwd, and the granulated phospholipid mixture (9). The swelling of the three phospholipids are compared in Figure 3.

The PCwd swells to a lamellar phase which incorporates slightly less than 50 wt. % water. At larger water concentrations, two phases exist in equilibrium, the lamellar phase and a solution of the PCwd monomers in water. At maximal swelling with water the repeat distance is d = 64 Å (Fig. 3). The lamellar phase formed by the SBPap containing 25 mol % of charged lipids incorporates a maximum of 65 wt. % water. The repeat distance at this maximum

was d=124 Å. The SBPgr with 35 mol % of charged lipids also forms a lamellar phase containing 70 wt. % water at maximal swelling. The repeat distance, in this case, was also d = 124 Å.

Thus, the presence of charged phospholipids in the phospholipid mixtures renders the lamellar phases capable of incorporating larger amounts of water than the well defined phospholipid which contains undissociating molecules only.

The thickness of the lipid bilayer (d_1) may be obtained by extrapolation of the expression $1-C_a/C_a$ to zero concentration of water. d_1 is ca. 40 Å for all the emulsifiers used in this investigation. The slopes of the repeat distance to inverse volume concentration plots is slightly different for the mixed phospholipid preparations.

Phase Diagrams

The one-, two-, and three-phase areas in the soybean oil/water/phospholipid systems are shown in the phase diagram in Figure 4 which is given as a pseudo-ternary phase diagram. The solubility of the soybean oil in the lamellar phase of the acetone-precipitated phospholipid mixture is less than 10 wt. %. Upon addition of the phospholipid mixture to oil and water the emulsifier precipitates in the form of a lamellar liquid crystalline phase (N), in equilibrium with solutions of SBPap in water (L_1) and in soybean oil (L_2). It is well known that the solubility of PC in water is very low, ca. 1×10^{-10} M (10). For SBPap, the solubility in soybean oil is $\approx 1 \times 10^{-3}$ M (9). This means that practically all of the emulsifier was precipitated in the form of a lamellar phase in the water-oil system. In the presence of charged lipids, the lamellar phase is easily dispersible in the water phase (L_1).

Emulsions

Emulsion stability. Emulsions with varying oil:water ratios were prepared with emulsifier contents from 0.1 to 3 wt. %. In the systems investigated, o/w-emulsions were formed almost exclusively. W/o-emulsions were obtained only with the SBPgr emulsifier when the oil-to-water ratio exceeded 0.6. The dispersing and stabilizing properties of well defined PC and the different phospholipid mixtures were compared in several series of emulsions containing oil-to-water phase in the ratio 0.1 to 0.8. The results are summarized in Figures 5-11, which show the concentration regions in which oil separation, unstable and stable emul-
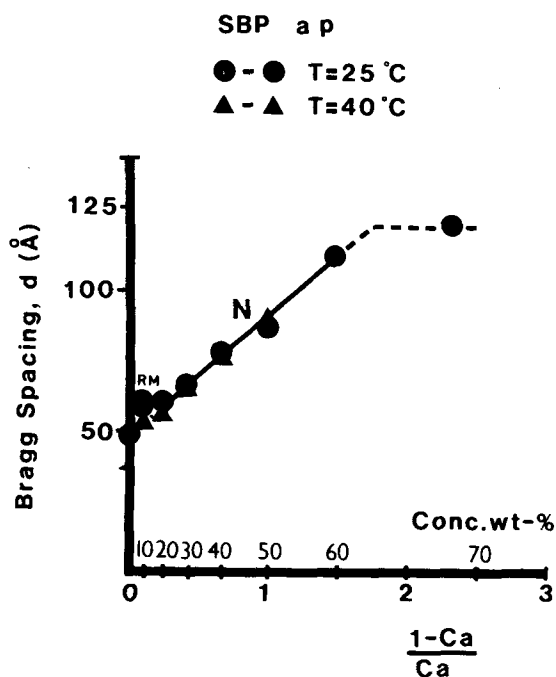

FIG. 2. The swelling behavior of the SBPap in water at (●) 25 and (▲) 40 C. N = lamellar phase. RM = reversed hexagonal phase.

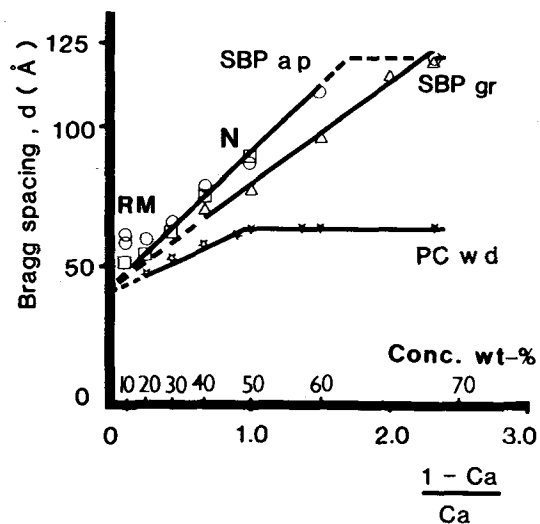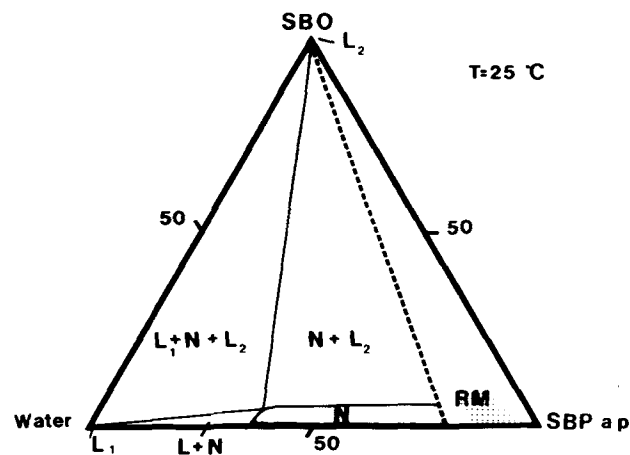

FIG. 3. The swelling behavior of the different soybean phospholipids in water (☆) PCwd, (△) SBPgr and (○,□) SBPap at 25 and 40 C, respectively. N = lamellar phase, RM = reversed hexagonal phase.

FIG. 4. The phase equilibrium for the pseudo ternary phase-diagram; SBPap-SBO-water at 25 C. L_1 = water solution of SBPap; L_2 = oil solution of SBPap; N = lamellar phase; RM = reversed hexagonal phase; L_1 + N and N + L_2 = two phase regions; L_1 + N + L_2 = three phase region.

EMULSIFIER : PC wd
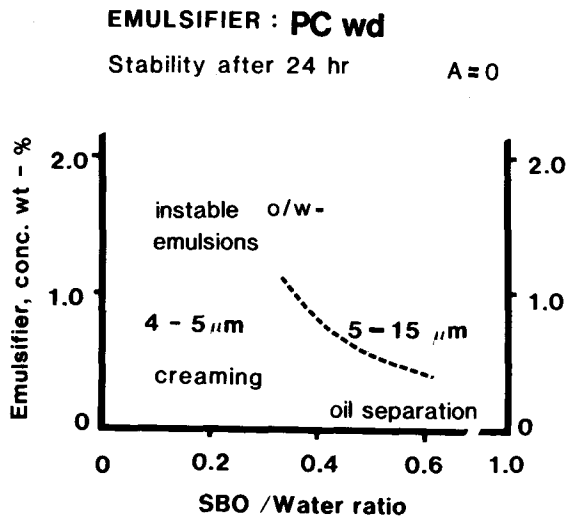
Stability after 24 hr   A = 0

FIG. 5. Emulsions with varying oil-to-water ratios and different concentrations of the emulsifier, PCwd were investigated within the concentration regions shown in the diagram. The mean globule size and the emulsion stability were determined after 24 hr storage at room temperature.

sions were observed. The mean globule sizes of the emulsions are also given in the diagrams.

The well defined soybean lecithin, PCwd, did not stabilize the o/w-emulsions with respect to creaming and coalescence (Fig. 5). At low emulsifier concentrations, oil separation occurred immediately after the emulsification. The mean globule size was 4-5 μm. Emulsions with a high oil/water phase ratio (0.4 - 0.6) and more than 0.5 wt. % emulsifier were very viscous and did not separate spontaneously within 24 hr. Their large mean globule sizes indicate that these emulsions were mechanically stabilized with respect to creaming while coalescence was not prevented. When the emulsions were stirred or centrifuged at low G-values, oil phase separated immediately.

The acetone-precipitated phospholipid mixture, SBPap, contained 25 mol % of the negatively charged phospholipids PA and PI, i.e., A = 25. In this system, a concentra-

tion region with stable o/w-emulsions was observed with a mean globule size varying between 3-5 μm (Fig. 6). At low concentrations of emulsifier and high oil-to-water phase ratios, oil separation occurred immediately after the emulsification. Between these regions there is an intermediate region with creaming and coalescence of oil droplets, the average drop size varying between 5-10 μm. The emulsifier concentration needed to stabilize the emulsions corresponds to ca. 1.25 wt. % of the oil phase.

The granulated phospholipid mixture contained 35 mol % charged lipids. Stable o/w-emulsions were obtained with oil-to-water phase ratios 0.1 to 0.6 and emulsifier concentrations of 0.5 to 2 wt. % (ca. 1.25 wt. % when calculated on the oil phase), the droplet size varying from 3-5 μm (Fig. 7). The mean globule size in the region where creaming occurred was 5-10 μm, which indicates coalescence of the emulsion droplets. The oil phase separated at low emulsifier concentrations and high oil-to-water ratios. When the emulsifier was dispersed in the water phase before emulsification, much more emulsifier was required to stabilize the emulsions (Fig. 8).

Influence of sodium chloride on the emulsion stability. The well-defined soybean lecithin, PCwd: in the presence of sodium chloride, very unstable emulsions were formed. An average droplet size of more than 25 μm indicates rapid coalescence of oil droplets followed by oil phase separation (Fig. 9).

The acetone-precipitated phospholipid mixture, SBPap: more than twice as much emulsifier was required to produce stable emulsions as in salt-free systems (Figs. 10 and 6). In a large concentration region of the system, the electrolyte gave rise to creaming followed by coalescence as shown by the large drop sizes.

The granulated phospholipid mixture, SBPgr: stable emulsions were formed when about twice as much emulsifier was used as in the salt-free system (Figs. 11 and 7). Creaming and coalescence occurred at low emulsifier concentration and a high oil-to-water phase ratio.

Interfacial area and interfacial film thickness. The total o/w interfacial area in the emulsion was calculated from the drop size distribution curves. If it is assumed that all surface-active lipid (the emulsifier) is located at the oil-

EMULSIFIER : SBP ap
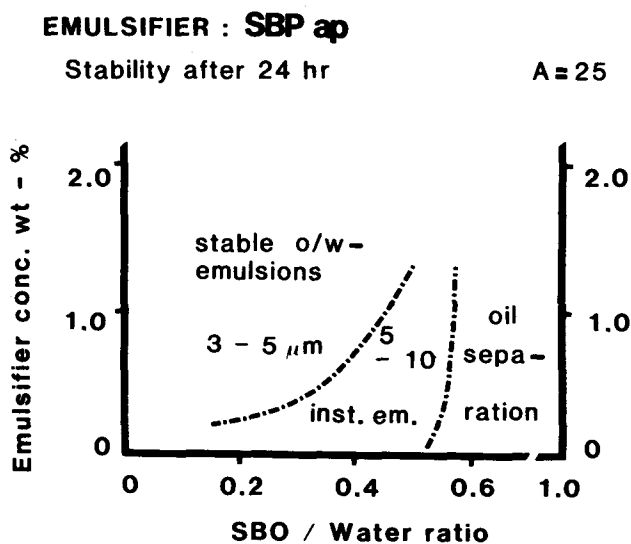
Stability after 24 hr   A = 25

FIG. 6. Emulsions with varying oil-to-water ratios and different concentrations of the emulsifier SBPap were investigated within the concentration regions shown in the diagram. The mean globule size and the emulsion stability were determined after 24 hr storage at room temperature.
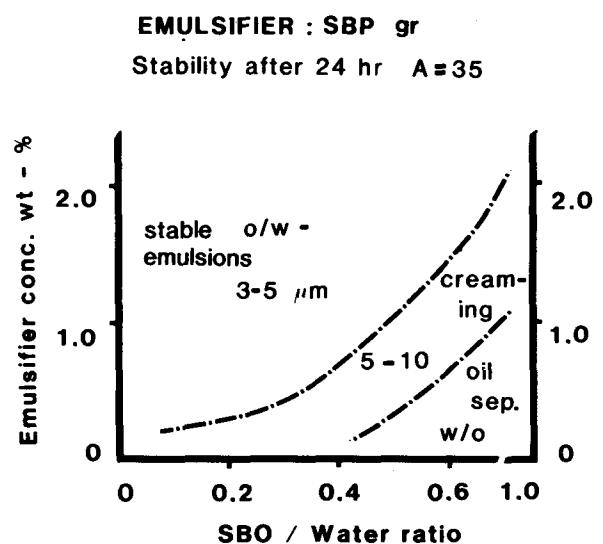
EMULSIFIER : SBP gr

Stability after 24 hr   A = 35

FIG. 7. Emulsions with varying oil-to-water ratios and different concentrations of the emulsifier SBPgr were investigated within the concentration regions shown in the diagram. The mean globule size and the emulsion stability were determined after 24 hr storage at room temperature.

water interfacial area of the emulsion, the thickness of the interfacial phospholipid film may be calculated. Film thicknesses for the different emulsifiers are plotted as a function of the oil-to-water phase ratio in Figures 12-14.

Figure 12 shows that, in spite of the large amounts of emulsifier and thick films, stable emulsions were not obtained with PCwd. SBPap (Fig. 13) stabilizes o/w-emulsions if the interfacial film thickness exceeds ca. 80 Å. At high oil-to-water phase ratios, the amount of emulsifier required to stabilize the o/w-emulsions increases. SBPgr (Fig. 14) also stabilizes o/w-emulsions if the interfacial film thickness exceeds ca. 80 Å. Thicker films are required to stabilize o/w-emulsions with high oil-to-water phase ratios.

The conditions of emulsification in the valve homogenizer, i.e., the shear stress, the interfacial tension and the viscosities of the oil and water phases, respectively, are such that the droplets produced vary between 3-5 μm. Thus, if the emulsifier is able to adsorb at the o/w interface at the moment of emulsification in such a way that the coalescence of droplets is prevented and stable emulsions are formed, the mean globule size will vary between 3-5 μm. The extensive swelling that takes place when the emulsifier contains charged lipids evidently makes such adsorption possible. The minimal amount of lipid that has to be adsorbed can be found by plotting the mean globule size as a function of the interfacial film thickness. Such plots are shown in Figures 15-17. For PCwd (Fig. 15) there is no correlation between amount adsorbed, emulsion stability and mean globule size. However, the diagrams for the mixed emulsifiers, SBPap and SBPgr (Figs. 16 and 17) show that when the concentration of the emulsifiers SBPap and SBPgr is high enough to form interfacial films with a thickness of ca. 80 Å or higher, the mean globule size approaches a minimal mean drop size of 3-5 μm and stable emulsions are formed. It is obvious from Figures 13 and 14 that this correlation is not obtained for emulsions with a high oil-to-water phase ratio.

EMULSIFIER : SBP gr

SBP dispersed in water
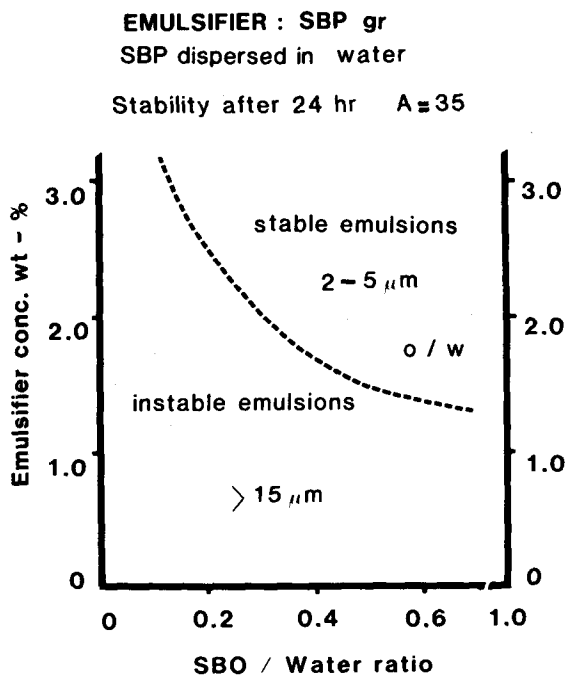
Stability after 24 hr   A ≡ 35

FIG. 8. Emulsions with varying oil-to-water ratios and different concentrations of the emulsifier SBPgr were investigated within the concentration regions shown in the diagram. The mean globule size and the emulsion stability were determined after 24 hr storage at room temperature. The emulsifier was dispersed in water before emulsification.

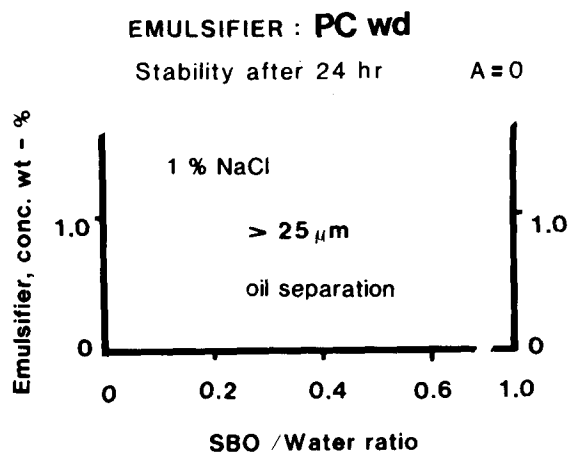**EMULSIFIER : PC wd**

Stability after 24 hr   A = 0

FIG. 9. Emulsions containing 1 wt. % NaCl with varying oil-to-water ratios and different concentrations of the emulsifier PCwd were investigated within the concentration regions shown in the diagram. The mean globule size and the emulsion stability were determined after 24 hr storage at room temperature.
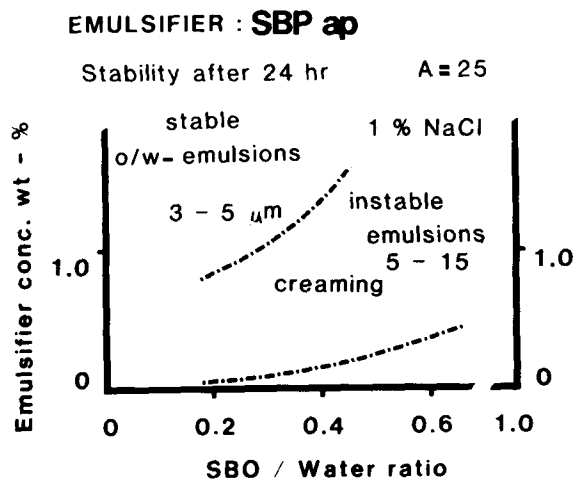

EMULSIFIER : SBP ap

Stability after 24 hr   A ≡ 25

FIG. 10. Emulsions containing 1 wt. % NaCl were investigated at different oil-to-water ratios and emulsifier concentrations of SBPap within the concentration regions shown in the diagram. The mean globule size and the emulsion stability were determined after 24 hr storage at room temperature.

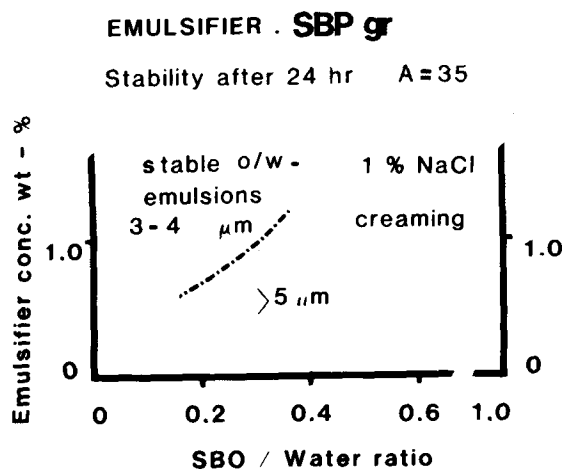**EMULSIFIER . SBP gr**

Stability after 24 hr   A = 35

FIG. 11. Emulsions containing 1 wt. % NaCl were investigated at different oil-to-water ratios and emulsifier concentrations of SBPgr within the concentration regions shown in the diagram. The mean globule size and the emulsion stability were determined after 24 hr storage at room temperature.
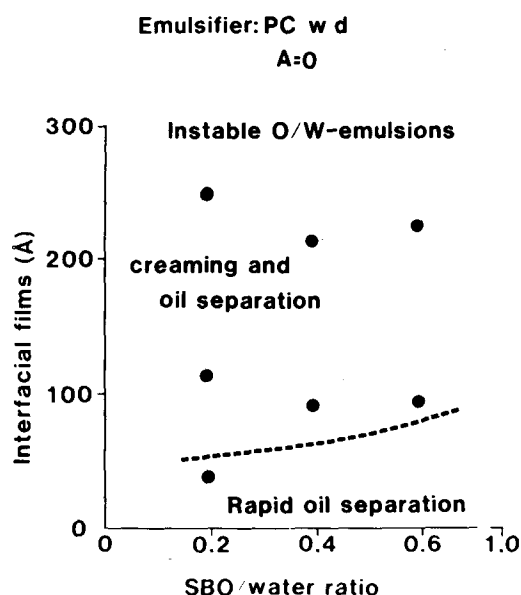
Emulsifier: PC w d

A=0

300┐   **Instable O/W-emulsions**

Interfacial films (Å)

200┤   **creaming and**

   oil separation

100┤

0┤

       Rapid oil separation

0   0.2   0.4   0.6   1.0

SBO/water ratio

FIG. 12. The thickness of the interfacial films as a function of the oil-to-water ratio for emulsions stabilized with the emulsifier PCwd. (o) Unstable emulsions.

DISCUSSION

It was first demonstrated by Friberg in 1969 that the presence of a lamellar liquid crystalline phase enhanced the stability of o/w-emulsions in the system of p-xylene-water-nonionic emulsifier (11). Later, Friberg and coworkers demonstrated that stabilization of o/w-emulsions by means of lyotropic liquid crystals formed by surface active substances could be obtained in different emulsifier systems where these surfactants were used as emulsifiers (12-14). This investigation gives some additional insight into the relationship between liquid crystal formation and emulsion stability.

It should be observed that surface active lipids below their chain melting temperatures (cmt) may form several gel phases that are of great importance for the emulsion

stability at such temperatures (15,16). We have only investigated the emulsion stability above the cmt of the phospholipids and hence no conclusions concerning the relative stability of emulsions stabilized by gel phases as compared to liquid crystalline phases can be drawn from this study.

It is well known that PC swells to a lamellar liquid crystal above its cmt (8). It has been reported that egg PE forms both a lamellar and a hexagonal structure of which the hexagonal packing dominates when the temperature is raised (8). PA swells with water to form a lamellar liquid crystal (17) which probably also is the case for the PI. At pH 6-7, PA and PI are partly dissociated. This introduction of charged groups causes the lamellar phase to incorporate more water (9,18). In the mixed emulsifier consisting of PA, PC, PE and PI only the lamellar packing was found to be simultaneously in equilibrium with the water (L_1) and oil (L_2) solutions. Thus, our results on the swelling behavior of the well defined soybean PC and the mixed phospholipid emulsifiers seem to agree well with the literature data reported for the individual phospholipids. The solubility of the nonpolar triglyceride, i.e., soybean oil, is less than 10 wt. % in the lamellar phase of well defined soybean PC and in the mixed phospholipid emulsifiers (Fig. 4 and Ref. 9) while the solubility of the emulsifier in the water and oil phases is extremely low.

This investigation shows clearly that more than a monolayer of the emulsifier is required to stabilize the o/w-emulsions (Figs. 10 and 11). Since the emulsifiers were dispersed in the oil phase before emulsification it may be assumed that they adsorb at the oil-water interface as soon as the new interface is created. When the amount of emulsifier exceeds a monolayer corresponding to an interfacial film thickness of ca. 20 Å, the excess will precipitate as a lamellar liquid crystalline phase due to the very low monomer solubility in both the water and oil phases. Figures 13 and 14 show that, in the stable emulsion, the film thickness is ca. 80 Å which corresponds to four monolayers. Interfacial films slightly less than 100 Å were earlier found in emulsions stabilized by PC mixed with small amounts of a soap, sodium stearate (9). There are a few points in Figure 17 indicating that even thinner films give stable emulsions. This raises the question of how reliable the judgment of
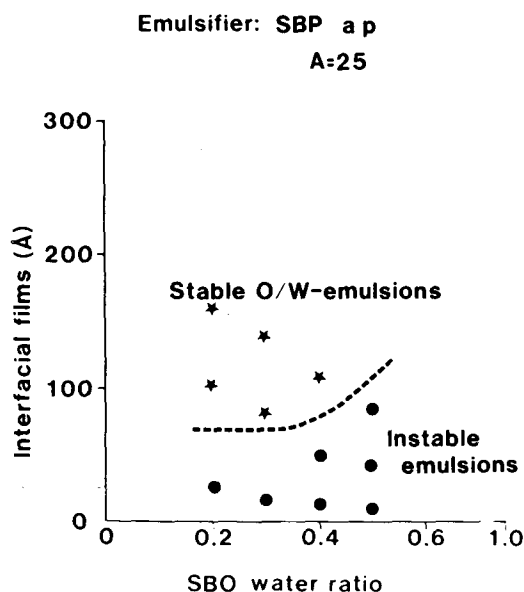
Emulsifier: SBP a p

A=25

300┐

Interfacial films (Å)

200┤

    Stable O/W-emulsions

100┤

                  Instable

                  emulsions

0┤

0   0.2   0.4   0.6   1.0

SBO water ratio

FIG. 13. The thickness of the interfacial films as a function of the oil-to-water ratio for emulsions stabilized with the emulsifier SBPap. (●) Unstable emulsions; (☆) stable emulsions.
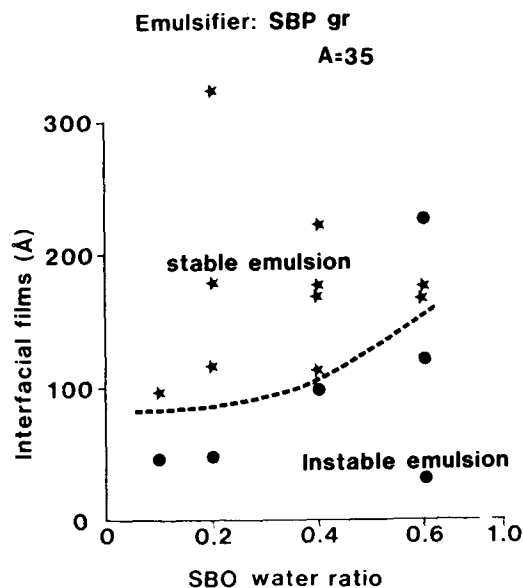
Emulsifier: SBP g r

A=35

300┐

Interfacial films (Å)

200┤   **stable emulsion**

100┤

                  Instable emulsion

0┤

0   0.2   0.4   0.6   1.0

SBO water ratio

FIG. 14. The thickness of the interfacial films as a function of the oil-to-water ratio for emulsions stabilized with the emulsifier SBPgr. (●) Unstable emulsions; (☆) stable emulsions.
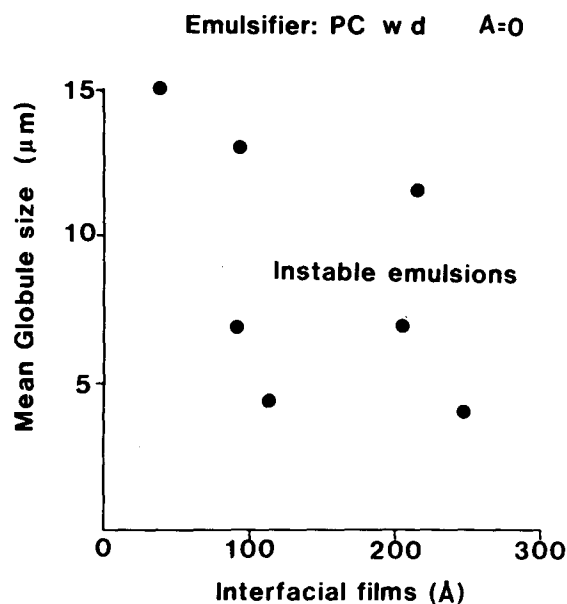
Emulsifier: PC w d   A=0

FIG. 15. The mean globule size of emulsions stabilized with PCwd as a function of the interfacial film thickness. (●) Unstable emulsions.

Emulsfier: SBP a p   A=25

FIG. 16. The mean globule size of emulsions stabilized with SBPap as a function of the interfacial film thickness. (●) Unstable emulsions; (☆) stable emulsions.

emulsion stability and the calculation of the total interfacial area are.

First of all, it should be noted that in the SBPap and SBPgr, emulsifiers contain 10 and 22 wt. %, respectively, of lipid-soluble material in addition to PA, PC, PE and PI. These compounds are probably surface active and may influence both the swelling behavior and the emulsion stability.

The total interfacial areas have probably been underestimated because the Coulter Counter does not detect particles less than 1 μm. However, only if it is assumed that at least 30-40% of the oil forms smaller droplets varying between 0.1 - 1 μm in size will the interfacial area be large enough to qualitatively change the conclusions. This appears highly unlikely.

Another possibility is that the emulsifier is partly dispersed in either the oil or water phase. Observations between crossed polarizers directly reveal that no birefringent material is present in the oil phase. When the mixed emulsifier SBPgr was dispersed in the water phase much more emulsifier was required to stabilize the emulsions. The emulsifier in this case forms stable dispersions, i.e., liposomes in the water phase which do not adsorb quantitatively at the o/w-interface (Fig. 8). The possibility that such dispersions are also formed in the emulsification process following the dispersion of the lipids in oil cannot be ruled out, although it appears unlikely since the minimal amount of emulsifier required to form stable emulsions is quite sharply defined. Further investigations are required to clarify whether phospholipids dispersed in an oil phase form dispersions of liposomes and emulsion droplet simultaneously.

Our results clearly show that the amount of charged lipids in the emulsifier mixture has a strong influence on the ability of the lamellar liquid crystalline phase to swell with water, i.e., as the fraction of charged lipids increases, a phase with larger and larger interplanar distances will be in equilibrium with the oil and water phases. The formation of these swollen lamellar structures also correlates well with an increased stability of the emulsions formed when the water, oil and liquid crystalline phase is subjected to an emulsifying procedure.

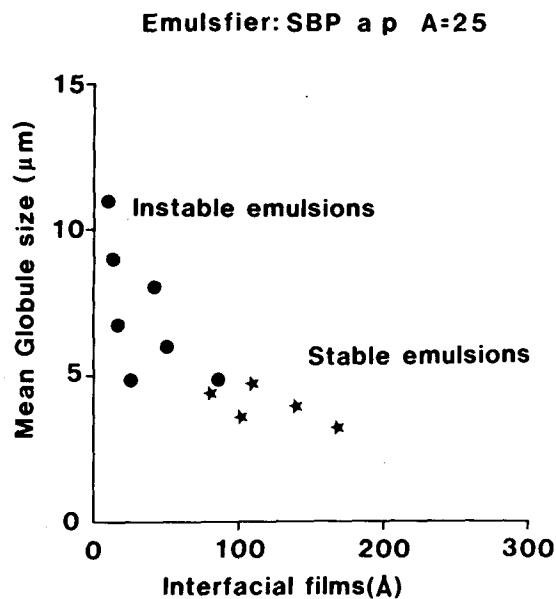These facts are clearly manifested in the difference

in behavior between the well defined PC and the mixtures. PCwd is not able to swell extensively with water but addition of a few mol % of sodium stearate causes extensive swelling (9,18). At the same time, the stability of the emulsions formed by this emulsifier, water and soybean oil increases (9). The SBPap and SBPgr both behave similarly to the PCwd containing sodium stearate.

The difference in swelling indicated in Figure 3 probably depends both on the ratios of the different phospholipids PA, PC, PE and PI, and on the presence of minor nonphospholipid compounds. The stabilizing properties of the emulsifiers SBPap and SBPgr are equally good when the emulsions are prepared with distilled water. In the presence of electrolyte, slightly more of the SBPap with $A = 25$ is required to stabilize emulsions as compared to the SBPgr
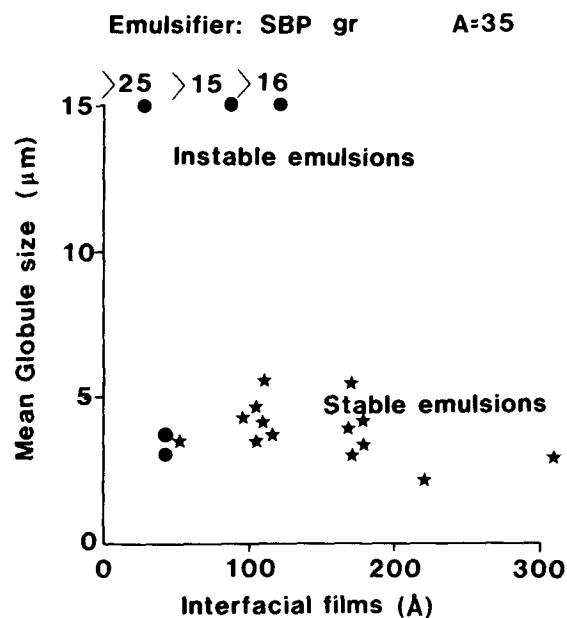
Emulsifier: SBP gr   A=35

FIG. 17. The mean globule size of emulsions stabilized with SBPgr as a function of the interfacial film thickness. (●) Unstable emulsions; (☆) stable emulsions.

with A = 35 (Figs. 10 and 11). According to the theory of colloidal stability, there are two types of forces which influence the coalescence (coagulation) of dispersed emulsion droplets, i.e., (a) the London-van der Waals attraction forces and (b) the electrostatic repulsion forces between electrical double layers. As these forces operate independently, the total interaction energy is obtained by summation. If the surface potential is reduced or the ionic strength is increased, the theory predicts that the potential energy barrier against coalescence is lowered (19). The decrease in stability against coalescence when salt is added indicates that the electrostatic repulsion forces originating from the amount of charged lipids in the emulsifier play an important role for the emulsion stability.

In the emulsions stabilized with the well defined PC, creaming and oil separation were observed (Fig. 5) whereas limited concentration regions with stable emulsions could be obtained with the mixed phospholipid emulsifiers (Figs. 6-11). These emulsifiers seem to adsorb more easily at the oil-water interface than the well-defined phospholipid (Figs. 12-14). One explanation may be that the charged PA and PI in the phospholipid mixtures are more soluble in water; hence, the diffusion to the oil-water interface is faster as compared to the electrically neutral substances.

Friberg has suggested that the presence of a multilayer structure on the surface of the oil droplets reduces the Van der Waals attraction forces between the dispersed oil globules (14). As a consequence of this the force, the tendency to coalescence will be reduced. In addition to the mechanism suggested by Friberg et al., our investigation demonstrates that the repulsion forces occurring from the charged molecules in the emulsifier also contribute to the prevention of coalescence; the droplet stability is greatly enhanced if lamellar liquid crystals are able to incorporate large amounts of water due to the presence of charged lipids which can be adsorbed at the o/w-interface.

REFERENCES

1. Biverstedt, A., and L. Rydhag, 9th Scand. Lipid Symposium, Visby, Sweden, 1977.
2. Lowry, R.R., and F.J. Tinsley, Lipids 9:7 (1974).
3. Glass, R.L., R. Jenness and H.A. Troolin, J. Dairy Sci. No. 8 (1965).
4. Hvolby, A., JAOCS 48:503 (1971).
5. Mandell, L., and P. Ekwall, Acta Polytech. Scand. Chem. 74:1 (1968).
6. Fontell, K., L. Mandell, H. Lethinen and P. Ekwall, Ibid. 74:1 (1968).
7. Tornberg, E., and G. Lundh, J. Food Sci. 43:5 (1978).
8. Luzzati, V., in "Biological Membranes," edited by D. Chapman, Academic Press, New York, 1968, chapt. 3.
9. Rydhag, L., Fette Seifen Anstrichm. 81:168 (1979).
10. Tanford, C., in "The Hydrophobic Effect," J. Wiley & Sons, New York, 1973.
11. Friberg, S., L. Mandell and M. Larsson, J. Coll. Interface Sci. 29:155 (1969).
12. Friberg, S., and L. Mandell, JAOCS 47:149 (1970).
13. Friberg, S., and L. Rydhag, Kolloid Z. Z. Polym. 244:233 (1971).
14. Friberg, S., P.-O. Jansson and E. Cederberg, J. Coll. Interface Sci. 55:3 (1976).
15. Larsson, K., and N. Krog, Chem. Phys. Lipids 10:177 (1973).
16. Larsson, K., Prog. Chem. Fats Other Lipids 16:163 (1978).
17. Luzzati, V., and A. Tardieu, Ann. Rev. Phys. Chem. 25:79 (1974).
18. Gulik-Krzywicki, T., A. Tardieu and V. Luzzati, Mol. Cryst. Liq. Cryst. 8:285 (1969).
19. Kitchener, J.A., and P.R. Musselwhite, in "Emulsion Science," edited by P. Sherman, Academic Press, London, 1968, chapt. 2.

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