Creaming of Phosphatide Stabilized Fat Emulsions by Electrolyte Solutions

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Creaming effects of electrolytes on emulsions of cottonseed and soybean oils have been investigated. The emulsions were stabilized with egg yolk phosphatides or with soybean phosphatides of different degrees of purity. The differences in creaming of the emulsions by the electrolyte solutions apparently were due to the differences in composition or purity of the phosphatide emulsifiers, and to the differences in properties of the respective oils. Emulsions of cottonseed or soybean oils stabilized with chromatographically homogeneous lecithin creamed to a greater extent than did emulsions of the same oils stabilized with impure phosphatides. In the latter, emulsions of cottonseed oil were slightly less creamed than emulsions of soybean oil.

IN A PREVIOUS STUDY (1), the electronically counted number of particles of specific diameters in fat emulsions developed for intravenous nutrition was found to be critically dependent upon the elapsed time of dilution of the emulsion with saline electrolyte. Because of the extreme dilution required by the electronic counting method, it could not be visually established whether creaming or coalescence occurred and was the cause of the change in particle count. Recently, Le Veen *et al.* (2) have shown that when certain sera are mixed *in vitro* with a fat emulsion, creaming or complete breaking of the emulsion may occur, and ascribe this effect to a globulin. It would appear that the electrolytes of serum or plasma may be equally significant factors in emulsion stability, based on the effect noted with saline in determining particle count. It must be assumed that the protein and other components of circulating plasma to some extent "buffer" the effect of plasma electrolytes on fat emulsions; however, the creaming and breaking effects of electrolytes on certain types of emulsions is well known. Van den Temple (3) reporting on o/w emulsions of monochlorobenzene and paraffin oil, found that the chlorides of sodium, potassium, and magnesium caused coagulation and finally breaking, the rate increasing with salt concentration. The cause of the breaking, according to Van den Temple (4) and to Martin and Hermann (5) involved discharge of the double layer potential. Electrolytes may affect the rate of creaming, and van Gils and Kraay (6) found that latex was creamed in the increasing order Li > Na > K among monovalent ions, and Ba = Ca > Sr among bivalent ions. The available reports do not include the effects of electrolytes on emulsions of vegetable oils, nor on emulsions stabilized with phosphatides.

In the phenomenon of creaming, droplets of the dispersed phase of an emulsion accumulate as a "cream" layer, and the continuous phase from which the dispersed phase is withdrawn becomes clearer, with less concentration of dispersed phase than originally. The continuous phase usually remains as an emulsion. Because turbidity of an emulsion decreases with creaming, measurements of transmitted light in the clearer phase seem to be a useful method of determining cream formation, as discussed by Griffin and Behrens (7).

The present investigation reports the creaming effect of solutions of some of the electrolytes found in human plasma on emulsions of two types of vegetable oils stabilized with egg phosphatides. The mechanism of creaming as a rate function, or correlation of light transmission with any of the parameters involved in rate functions, is beyond the scope of this study.

EXPERIMENTAL MATERIALS

Emulsions—Six emulsions were used, four prepared by high pressure homogenization as described previously (8), and two commercial products. The components of the various emulsions are shown in Table I.

The egg lecithin stabilizer in SR-151 and SR-183

TABLE I—COMPONENTS OF VARIOUS EMULSIONS

Emulsion	Lipid Phase	Stabilizer
SR-151	Soybean, 20%	Egg lecithin, 1%
SR-182	Soybean, 20%	Egg phosphatide, 1.2%
SR-183	Cottonseed, 20%	Egg lecithin, 1%
SR-181	Cottonseed, 20%	Egg phosphatide, 1.2%
Emulsion *A*[a]	Soybean, 20%	Egg phosphatide, 1.2%
Emulsion *B*[b]	Cottonseed, 10%	Soybean phosphatide, 2%

[a] Intralipid, a commercial emulsion; the composition is listed on the label. [b] Lipofundin, a commercial emulsion; the composition is listed on the label.

Received April 4, 1967, from the Southern Regional Research Laboratory, Southern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, New Orleans, LA 70119

Accepted for publication July 18, 1967.

This investigation was supported by funds from the Office of the Surgeon General, U. S. Department of the Army, Washington, D. C.

was chromatographically homogeneous (9); the egg phosphatide in SR-182 and SR-181 was not fractionated by column chromatography, and hence was less pure than the egg lecithin. The purity of the stabilizers of the commercial emulsion was not known, but when isolated from the emulsions, were nonhomogeneous, as shown by thin-layer chromatography. The isotonic aqueous phases were solutions of glycerol in all emulsions except emulsion *B* which contained sorbitol.

Electrolytes—All reagents were A. R. grade. Distilled water solutions of NaCl, KCl, $CaCl_2$, and $CaCl_2$–NaCl were made at salt concentrations of 0.25, 0.5, 1, 2, and 5%. The $CaCl_2$–NaCl solutions contained chemically equivalent weights of the two salts. A solution that simulated human plasma electrolytes was prepared with the following composition per liter: $CaCl_2$, 0.277 Gm.; KCl, 0.373 Gm.; $MgCl_2 \cdot 6H_2O$, 0.305 Gm.; $NaHCO_3$, 2.268 Gm.; $NaHPO_4 \cdot 12H_2O$, 0.358 Gm.; and NaCl, 6.546 Gm.; at pH 8.18. A fine precipitate which formed on standing was removed by filtration.

Method—Four milliliters of the respective salt solutions was mixed with 0.2 ml. of emulsion in a cell. After standing for 30 min., light transmission measurements of the solution–emulsion systems were made with a Lumetron photoelectric colorimeter, model No. 402E, operating through a constant voltage transformer and with galvanometer at direct reading. A monochromatic filter, 550 μ, was used. Under the conditions of operation, the light transmission of the freshly mixed emulsion–salt solution was 0%, after separation of the cream layer, 100%. The ratio of the per cent light transmission of a particular emulsion–salt solution after 30 min. of standing and the maximum per cent transmission of the clearer phase of a creamed emulsion was calculated. A ratio of 1.0 represents maximum creaming. Duplicate determinations of transmission were made, and the calculated results represent the average ±2%.

The transmission of the various emulsions, when diluted with distilled water, increased approximately 10–15% in 30 min. Only those increases above 15% were considered to be due to the salt solutions.

RESULTS AND DISCUSSION

The amount of creaming of the various emulsions was calculated as explained, and the calculated ratios are given in Table II. A ratio of 1.00 represents maximum creaming, and values less than 1.00 indicate some degree of creaming.

The increases in transmission of the four SR emulsions as creaming progressed, due to clearing of the dispersed phase, are plotted in Figs. 1–4. Where coincidence of data occurred, only one curve was plotted. The increases in transmission were due to decrease of dispersed phase, as electronic count of the dispersed particles (1) decreased by 400/μl./min.

Emulsions Stabilized with Homogeneous Lecithin—Emulsions SR-151 and SR-183, which were stabilized with pure lecithin but which differed in oil phase, had similar patterns of creaming with the various concentrations of salt solutions.

Upon dilution with each solution of NaCl, both emulsions were completely creamed. Results obtained with the KCl solutions were essentially similar to those obtained with NaCl.

Creaming was negligible at the lower concentrations of $CaCl_2$, with the exception of a slight amount observed in the soybean oil emulsion with the 2%

TABLE II—RATIO OF LIGHT TRANSMISSIONS OF EMULSIONS CREAMED BY VARIOUS CONCENTRATIONS OF ELECTROLYTES[a]

	Ratio of Light Transmission					
Salt and Concn., %	SR-151	SR-181	SR-183	SR-182	Emulsion *A*	Emulsion *B*
NaCl						
0.25	1.0	0.12	1.0	0.10	0.13	0.13
0.5	1.0	0.21	1.0	0.12	0.16	0.13
1.0	1.0	0.33	1.0	0.75	0.56	0.17
2.0	1.0	0.15	1.0	1.0	0.80	0.15
5.0	1.0	0.12	1.0	1.0	0.46	0.14
KCl						
0.25	0.80	0.13	1.0	0.10	0.13	0.11
0.5	1.0	0.14	1.0	0.10	0.12	0.11
1.0	1.0	0.28	1.0	0.12	0.15	0.11
2.0	1.0	0.18	1.0	0.65	0.39	0.13
5.0	1.0	0.12	0.97	0.92	0.30	0.16
$CaCl_2$						
0.25	0.10	0.25	0.13	0.14	0.12	0.17
0.5	0.10	0.24	0.14	0.14	0.19	0.12
1.0	0.10	0.30	0.13	0.12	0.13	0.12
2.0	0.40	0.20	0.14	0.25	0.21	0.13
5.0	1.0	0.20	1.0	1.0	1.0	0.13
$CaCl_2$–NaCl						
0.25	0.10	0.40	0.14	1.0	0.17	0.78
0.5	0.10	0.20	0.12	0.40	0.10	0.36
1.0	0.20	0.16	0.12	0.40	0.14	0.26
2.0	1.0	0.14	0.86	1.0	0.46	0.28
5.0	1.0	0.20	1.0	1.0	1.0	0.30
Plasma electrolytes	1.0	0.10	...	...	0.10	...

[a] Calculated as ratio of per cent light transmission 30 min. after mixing and maximum possible transmission of the continuous phase; a value of 1.0 represents maximum creaming.

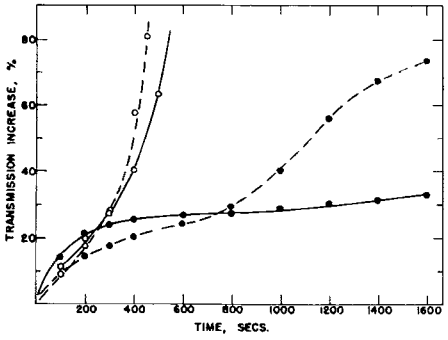

Fig. 1—Increase in transmission of SR emulsions with a 1% solution of NaCl. Key: ——, cottonseed oil emulsions; - - - -, soybean oil emulsions; ○, egg lecithin; ●, egg phosphatide.

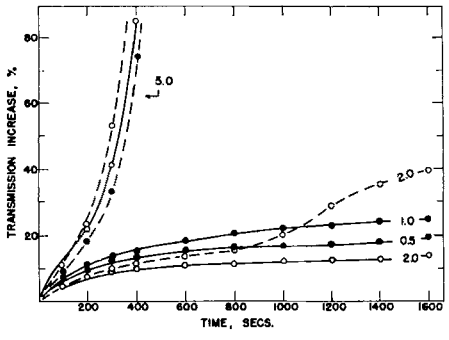

Fig. 3—Increase in transmission of SR emulsions with solutions of $CaCl_2$ of various concentrations. Key: ——, cottonseed oil emulsions; - - - -, soybean oil emulsions; ○, egg lecithin; ●, egg phosphatide.

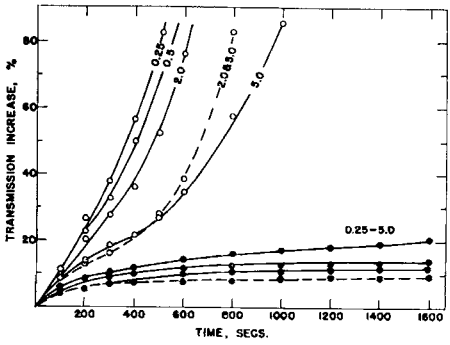

Fig. 2—Increase in transmission of SR emulsions with solutions of NaCl of various concentrations. Key: ——, cottonseed oil emulsions; - - - -, soybean oil emulsions; ○, egg lecithin; ●, egg phosphatide.

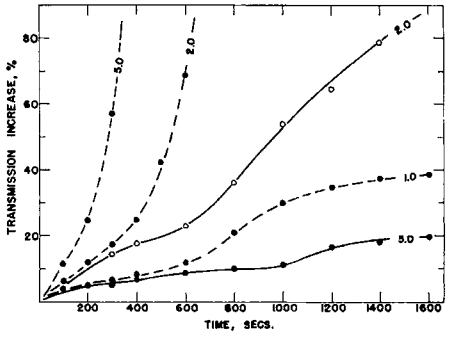

Fig. 4—Increase in transmission of SR emulsions with solutions of $CaCl_2$–NaCl of various concentrations. Key: ——, cottonseed oil emulsions; - - - -, soybean oil emulsions; ○, egg lecithin; ●, egg phosphatide.

solution; both emulsions were creamed by 5% solution of $CaCl_2$.

Creaming occurred in both emulsions with the 2% and 5% solutions of the mixed salts $CaCl_2$–NaCl.

Emulsions Stabilized with Crude Phosphatides—Emulsion SR-181, cottonseed oil, generally was less creamed than the soybean oil emulsion, SR-182, with all of the electrolytes.

Creaming in the two emulsions was negligible with the 0.25% and 0.5% concentrations of NaCl, but quite different with the higher concentrations. Creaming in the soybean oil emulsion increased to about the same extent as it did in the soybean oil emulsion stabilized with pure lecithin, whereas the cottonseed oil emulsion was relatively unaffected.

Similarly, the soybean oil emulsion was creamed by the higher concentrations of KCl, $CaCl_2$, and $CaCl_2$–NaCl, but the cottonseed oil emulsion was not.

Emulsions A and B—Emulsion *A* and emulsion SR-182, which are of similar composition, show similar patterns of creaming with the various concentrations of NaCl, KCl, and $CaCl_2$, except that emulsion SR-182 creamed to a greater extent with the higher concentrations of electrolytes. Emulsion SR-182 was more unstable than emulsion *A* with $CaCl_2$–NaCl.

There was little or no creaming of emulsion *B* at any of the concentrations of NaCl, KCl, and $CaCl_2$, and only a moderate amount with the concentrations of $CaCl_2$–NaCl. The oil content of emulsion *B* (10%) is less than that of the other emulsions and the phosphatide content larger, and the results may not be directly comparable with those of the other emulsions.

Plasma Electrolytes—Of the limited number of determinations with the mixture of plasma electrolytes, the soybean oil emulsion stabilized with pure lecithin was creamed, but the electrolyte had no effect on the other emulsions tested.

DISCUSSION

Creaming of the emulsions as a function of electrolyte concentration varied between emulsions of the same oil stabilized with pure lecithin and with crude phosphatide, and also between solutions of the various electrolytes. In some emulsions creaming was most complete with the 5% concentration of electrolyte; in others, with lower concentrations. In general, the amount of creaming was directly

proportional to electrolyte concentration. The inequality in creaming of the same emulsion with solutions of two different salts at identical concentrations apparently is explained by molarity of the cations (6).

The results obtained with the four SR emulsions indicated that the differences in stability of the emulsions to electrolytes apparently were due to the differences in properties of soybean and cottonseed oils, and to the differences in composition, or purity, of the stabilizers. Emulsions of cottonseed oil stabilized with chromatographically homogeneous egg lecithin or with egg phosphatides appeared to be more stable to electrolytes than were emulsions of soybean oil stabilized with the same emulsifiers. Also, emulsions of either oil stabilized with crude phosphatides were more stable to electrolytes than were emulsions stabilized with pure lecithin. The instability of the emulsions with NaCl, KCl, and $CaCl_2$ was marked with soybean oil–pure lecithin (SR-151), and was progressively less with cottonseed oil–pure lecithin (SR-183), soybean oil–phosphatide (SR-182), and cottonseed oil–phosphatide (SR-181).

Yeadon *et al.* (10) found that purified egg lecithin did not stabilize autoclaved fat emulsions in which a solution of dextrose was the aqueous phase. Zeringue *et al.* (8) produced a stable autoclaved fat emulsion stabilized with chromatographically homogeneous lecithin by substituting glycerol as the isotonic agent in the aqueous phase and adjusting the pH to 6.6–6.8. The marked instability of emulsions formulated with chromatographically homogeneous lecithin in the presence of electrolytes suggests that the electrolytes to which this type of emulsion is stable are quite limited and that salts known for their "salting out" effect bring about changes in the aqueous phase which are unfavorable to chromatographically homogeneous lecithin.

Although emulsion SR-182 and emulsion *A* were similar in composition, the egg phosphatide used in the emulsion *A* formula appeared to be nonhomogeneous, and could not be directly compared with the phosphatide in SR-182 which was quite highly purified. The greater amount of creaming of the SR-182 emulsion could be due to a difference in the composition of the two phosphatides, which lead to differences in the effects of the electrolyte solutions.

The creaming effects of the various salts with emulsion *B* were somewhat similar to those of emulsion SR-181, but differed considerably from those of the other emulsions. Emulsion *B*, although stabilized with phosphatide, differed in composition from the previously described emulsions. Soybean phosphatides were used as the stabilizer, sorbitol in place of glycerol as the isotonic phase, and a lower concentration in the oil phase, 10% cottonseed oil, as compared to 20%. The ratio of phosphatide to oil in the emulsion *B* formulation, 2.5% phosphatide to 10% oil, was considerably greater than the ratios of 1–1.2% phosphatide to 20% oil in the SR egg phosphatide-stabilized emulsions. These differences in composition may have contributed to the differences in creaming noted between emulsion *B* and SR emulsions.

It appeared that the electrolytes used caused agglomeration of the emulsions rather than coalescence. By visual inspection, cream formed in the cells and rose to the top surface. This cream could be redispersed, whereas coalescence would have resulted in free oil separation.

The relatively small amount of creaming that was observed in the phosphatide stabilized cottonseed oil emulsions essentially confirms the failure of dog blood sera to cream a similar emulsion (11). A conclusion of the latter study was that sera albumins did not complex with the fat component. It might be postulated from the present study that the sera electrolytes had but little effect on fat creaming because of the type of phosphatide used.

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