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Laws of Foam Formation and Foam Fractionation. II. The Influence of Different Association Conditions on Surfactants, Glycerides, Sugar, and Salts on the Foam Fractionation of Albumin*

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Abstract

The effect of various additives—nonionic surfactants, sodium caprylate, glycerides, sugar, and salts of sodium, calcium, and aluminum—on the separation of albumin from dilute solutions has been investigated. The results show the importance of the hydrophilic–lipophilic balance in the foam separation of albumin. This balance is influenced by the association conditions of surface-active substances with polymeric albumin. The liquid crystalline phase formed by surfactants and glycerides in association with protein is found to be most effective in the foam separation of albumin.

INTRODUCTION

In a previous communication (*1*), the effect of different operating parameters in a continuous foam fractionation of albumin was reported.

Proteins in solution produce various kinds of molecular arrangements as a consequence of their interaction with lipids and amphiphilic compounds (*2*, *3*). These different molecular arrangements in solutions have

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been found to effect the stability of foam (*10*). However, studies on foam separation of proteins in the presence of amphiphilic compounds and lipids would be of great value in separating proteins from dilute solutions.

This paper reports the effect of various additives on the molecular interaction with protein, together with their effects on the separation of albumin, by foaming. Various additives were used—nonionic surfactant (EMU 09), sodium caprylate, glycerides, sugar, and salts of sodium, calcium, and aluminum.

EXPERIMENTAL

Materials. The emulsifier, EMU 09 (Berol Co., Sweden), and sucrose (Sveska Sockerbolaget, Sweden) were commercial samples used without purification. As analytical reagents, calcium and sodium (Kebo Co., Sweden), and aluminum sulfate (Mallinckrodt, U.S.) were used. The monocapryline (98%) was synthesized from sodium ethylate and caproic acid. The purity of the sodium caprylate was checked by determination of its molecular weight. The apparatus and the experimental and analytical procedures were the same as those reported earlier (*1*).

Results

Effect of Emulsifier (EMU 09). The effect of the concentration of the emulsifier (EMU 09) is shown in Fig. 1. From Fig. 1 it is evident that the enrichment of the albumin in the foam depends on the concentration of the emulsifier. The maximum enrichment of the albumin was found at 50 ppm of EMU 09, and further addition of the emulsifier decreased the enrichment of albumin in the foam (Fig. 1, curve 1). The figure also shows the enrichment ratio of EMU 09 without albumin, which was found to decrease after 100 ppm EMU 09.

Effect of Sugar. The effect of sugar on the efficiency of albumin separation from a solution containing albumin, 150 ppm, was investigated at different sugar concentrations. The pH dependence of the separation of albumin remained unchanged in the presence of sugar, the most effective being pH 4.9. The enrichment ratio of the albumin in the foam was reduced from 1.33 to 1.29, and the efficiency parameter, $x_f F/x_b B$, was reduced from 7 to 5 by the addition of 200 ppm sugar. The enrichment ratio of the sugar remained unaffected in the foam. In the presence of both sugar and emulsifier (EMU 09, 100 ppm), the efficiency parameter, $x_f F/x_b B$, of the albumin separation decreased relative to the solution of albumin and

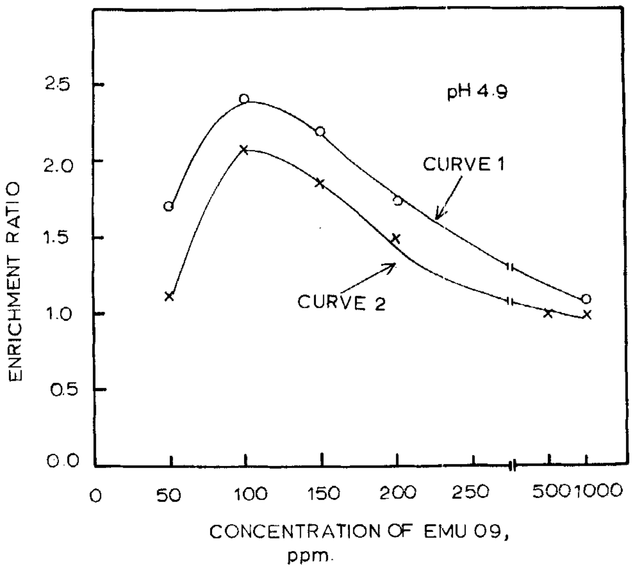

FIG. 1. Enrichment ratio (x_f/x_b) of albumin (○) and emulsifier (EMU 09) (×) at different concentrations of emulsifier (EMU 09). Albumin concentration is 150 ppm.

sugar. In the presence of the albumin and sugar, the separation efficiency obtained was only 2.5. The efficiency of the albumin separation decreased from 7 to 2.5 as the sugar concentration increased from 0 to 100 ppm in the presence of a mixture of an emulsifier (EMU 09, 200 ppm) and albumin (150 ppm) (Fig. 2). At higher sugar concentrations (200 ppm), no further decrease in efficiency took place.

The effect on the drain (B) and the overflow volume (F) was quite significant. The foam volume was found to decrease from 137 to 89 ml by the addition of 50 to 200 ppm of sugar to the solution of albumin (150 ppm) and EMU 09 (100 ppm). The changes in the enrichment ratio were not very significant (*9*).

From these results it appears that sugar plays a definite role in the separation of albumin from solutions containing a mixture of albumin and nonionic surface-active agents.

Effect of Monoglyceride. A solution of monocapryline (100 ppm) solubilized by the emulsifier showed an interesting behavior in albumin separation. The enrichment ratio of albumin in the foam reduced from 1.33 to 1.03 with the addition of 25 ppm monocapryline, whereas the

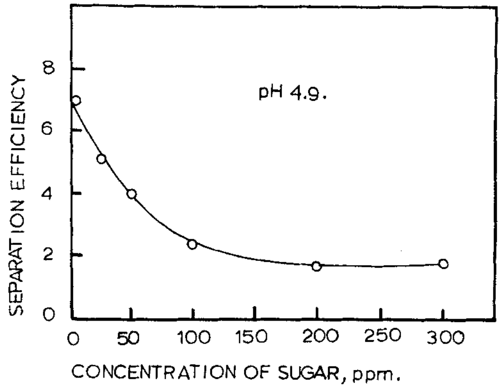

FIG. 2. Separation efficiency (x_fF/x_bB) of albumin (150 ppm) in the presence of emulsifier EMU 09 (100 ppm) at different concentrations of sugar.

further addition of monocapryline increased the enrichment ratio. The effect of monocapryline was found more significant on the volume of both the foam and the drain. With the addition of 25 ppm monocapryline, the foam volume decreased from 120 to 106 ml. Further addition of monocapryline increased the foam volume. The increment in the foam volume is large compared to the enrichment ratio. The efficiency of the foam separation was reduced from 7 to 2 by the addition of 25 ppm monocapryline, while further addition of monocapryline increased the efficiency (Fig. 3). Similar results regarding the albumin separation were observed with monocapryline additions using 50 ppm EMU 09.

The addition of 100 ppm sugar to the solution reduced the efficiency of the separation process by 50%. The monocapryline addition, together with the emulsifier (100 ppm) to the sugar and albumin solution, did affect the separation efficiency up to 25 ppm monocapryline. At higher concentrations of monocapryline (more than 25 ppm), an increase in the efficiency of the separation process was observed. Thus significant effects were observed on foam and drain volumes. The enrichment ratio, on the other hand, was not affected very much by the addition of sugar. No significant enrichment of sugar in the foam was observed. The enrichment ratio for the sugar in the foam was unity. The enrichment ratio for albumin and monocapryline was found to decrease with an increasing concentration of monocapryline. Experiments were carried out using EMU 09 and monocapryline to investigate the influence of different sugar concentrations. The efficiency parameter was found to decrease from 6 to 3 with an

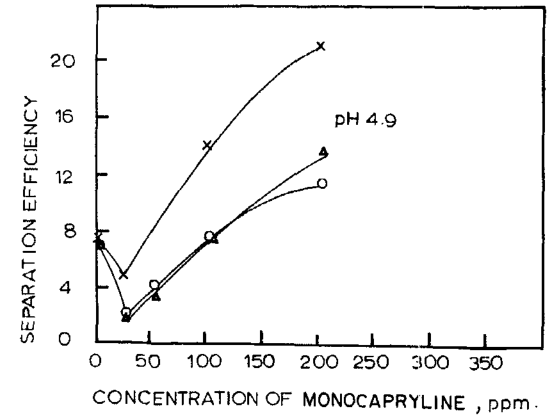

FIG. 3. *Separation efficiency* (x_fF/x_bB) *of albumin (160 ppm)* (○), *emulsifier* (EMU 09) (×), and monocapryline (▲) at different concentrations of monocapryline.

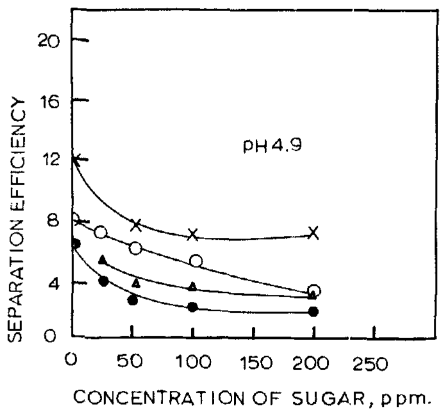

FIG. 4. Separation efficiency (x_fF/x_bB) of albumin (150 ppm) (○), emulsifier EMU 09 (100 ppm) (×), monocapryline (100 ppm) (●), and sugar (▲) in the presence of different concentrations of sugar.

increasing concentration of sugar; thereafter, it remained constant at sugar concentrations higher than 200 ppm (Fig. 4). The separation efficiencies of the other compounds present, viz., sugar, EMU 09, and glyceride, also decreased.

The effect of the concentration of EMU 09 on the separation of albumin

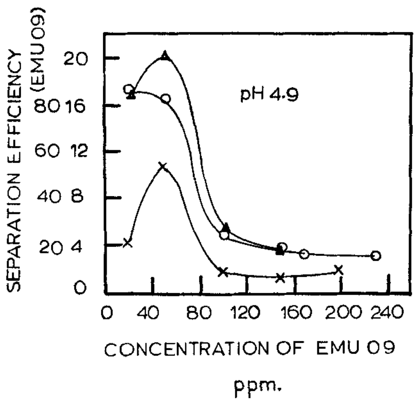

FIG. 5. Separation efficiency of albumin (150 ppm) (○), emulsifier (×), and monocapryline (▲) in the presence of different concentrations of emulsifier.

in the presence of monoglyceride (100 ppm) was also investigated (Fig. 5). The results were similar to those observed without monoglyceride. The initial efficiency of the protein separation increased up to 20 ppm EMU 09, after which the efficiency of the protein separation decreased. In the concentration range of EMU 09 of 60 to 100 ppm, the decrease in efficiency was very rapid; above 100 ppm the efficiency of the process became almost constant. The effect of a concentration of EMU 09 on the foam and drain volumes was also found to be significant. At 20 ppm EMU 09 the foam volume obtained was 150 ml, while at 150 ppm it was 120 ml. A decrease in the enrichment ratio of albumin occurred with an increase in the concentration of EMU 09, but the decrease in the enrichment ratio in the present study was not as great as that observed with a solution of protein and an emulsifier in the absence of monoglyceride.

Effect of Sodium Caprylate. The effect of sodium caprylate on the separation efficiency of albumin was characterized by the fact that the most effective separation was achieved at a concentration of 10 ppm sodium caprylate. The efficiency of the separation at pH 4.9 and 100 ppm albumin increased from 2.5 to 11.0.

The results show a decrease in separation efficiency (Fig. 6) at higher concentrations of sodium caprylate (above 10 ppm sodium caprylate). A sodium caprylate concentration between 30 and 50 ppm reduced the efficiency from 0.5 to 0.0.

From these results it is obvious that an anionic surface-active agent has

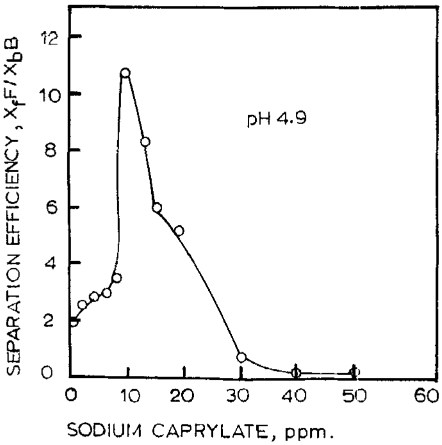

FIG. 6. Separation efficiency of albumin (100 ppm) at different concentrations of sodium caprylate.

a more pronounced effect on the separation of albumin than does a nonionic emulsifier.

Effect of Aluminum Sulfate. In the presence of aluminum sulfate (100 ppm), the effect of sodium caprylate on the separation of albumin diminished and there was no rise in the efficiency of the albumin separation. Also studied was the effect of aluminum sulfate at increasing concentrations in the presence of sodium caprylate (10 ppm) and albumin (100 ppm). The results indicated that the efficiency of the process decreased continuously with an increase in aluminum sulfate concentration. The initial efficiency of the process (*11*) was reduced to 3 by the addition of 25 ppm aluminum sulfate.

Effect of Calcium Chloride. The addition of calcium chloride to the albumin solution reduced the efficiency of the process. After an initial reduction of the albumin separation efficiency from 3.8 to 2.0, the efficiency of the process became almost constant. A wide concentration range of calcium chloride, 50 to 1000 ppm, was studied, but no appreciable effect on the efficiency was found.

Effect of Addition of Sodium Chloride. A wide concentration range of sodium chloride, 20 to 750 ppm, was studied. The results observed by the addition of sodium chloride to the albumin were similar to those observed by the addition of calcium chloride. Initially, the efficiency of the process was reduced, and thereafter it became constant with further additions of sodium chloride. The initial efficiency was 7.5 with 150 ppm albumin, and it was reduced to 4 by the addition of 100 ppm sodium chloride. Sodium chloride concentrations above 40 ppm produced no further decrease in the efficiency.

DISCUSSION

The results of the present investigation point to the importance of a hydrophilic-lipophilic balance for the efficiency of a foam separation of albumin, as well as to the influence of the associative conditions of surface-active substances on this balance of a polymeric substance.

The initial addition of a water-soluble nonionic emulsifier, poly-($\sim$9)-oxyethylene nonylphenol ether, gives rise to an increase in the foam separation due to an association in a more ordered structure than micelles. It is well known that surface-active substances of this kind associate to give liquid crystalline structures at concentrations below the critical micelle concentration (cmc) when the aqueous solution is in equilibrium with a hydrophobic substance of high polarizability (*4*). This is also the case with polymers (*5*). Apparently, the initial rise in the enrichment ratio, observed with the addition of the nonionic emulsifier (50 ppm), is due to the presence of a lamellar structure obtained as a result of the interaction between the polymeric albumin and the surface-active substance (*6*).

At higher concentrations of the surface-active substance, micellization takes place. The micelles do not exhibit a layered structure characteristic of the liquid crystalline phase which gives rise to the stable foams (*10*), nor do the micelles stabilize thin liquid films. This nonstabilization causes a pronounced decrease of the separation efficiency. In Fig. 1, curve 2, the enrichment ratio of EMU 09 is shown versus different concentrations of the emulsifier. Above 100 ppm EMU 09, the enrichment ratio decreases. It appears that the micellization of the nonionic surfactant becomes effective above 100 ppm EMU 09. From the results given in Fig. 1, curve 1, it seems evident that the maximum enrichment of albumin will be achieved below the cmc at 100 ppm EMU 09. These results clearly confirm the formation of an ordered structure by a nonionic emulsifier below the cmc in the presence of albumin (*11*).

The observed influence of sugar (causing a decrease in the efficiency of the process and the formation of less stable foam) can be explained by taking into consideration the studies by Erlander (*7*) and Nepper (*8*) on the steric stabilization of hydrosols. Nonionic polymers, such as dextrins, which have attached hydrophobic chains, can disperse and stabilize hydrophobic polymers in aqueous solutions (*7*). In the present experiments the albumin was studied at its isoelectric point (pH 4.9) where it becomes highly hydrophobic and tries to accumulate at the surface of the aqueous solution. Such an action promotes foam efficiency. The addition of sugar may disperse the albumin in the solution by attaching soluble moities. The dispersion of the albumin in the form of colloidal particles in the solution reduces the albumin at the surface; therefore, the stability of the foam is reduced and a decrease in the efficiency of the process is observed.

In the presence of both the nonionic surface-active agent and sugar, the efficiency of the process is found to decrease relatively more than it does in a solution of albumin and sugar alone. From these results it could be concluded that both nonionic compounds, sugar and EMU 09, compete for the dispersion of albumin in the solution.

The surface-active properties of EMU 09 depend on the interaction of the water molecules and the ether bridges of the hydrophilic part of the molecule. In a nonionic surfactant micelle, which has a hydrated layer around its hydrophilic part, the organized regions of the water molecules affected by the hydrated species are present beyond the periphery of the primary hydrated micelles and the bulk solution. The greater mobility of water molecules in the intermediate region, relative to that in pure water, may result from an electrostatic field effect on the hydrated layer. According to Erlander (*7*), the regions having a hydrated layer are impenetrable to the macromolecules, and only a region which is extended to a hydrated layer may provide pockets for dissolving the macromolecules. In the present study, sugar binds hydrophilically with albumin and disperses it into the solution by taking it from the liquid surface. The macromolecules formed with the sugar and albumin cross-link hydrophobically with the micelles of the nonionic surface-active agent, EMU 09. EMU 09, therefore, further disperses the albumin and helps to form a colloidal solution of bigger aggregates. This reduces the stability of the foam and decreases the efficiency of the process. This behavior of sugar with an emulsifier explains the influence of the sugar content on the efficiency of foam separation.

The influence of the monoglyceride, in combination with the nonionic emulsifier, results in an initial decrease in the efficiency of the process, followed by an increase. In order to explain this phenomenon, the difference in behavior of the emulsifier and the monoglyceride has to be considered. The emulsifier forms micelles at the cmc, and liquid crystalline phases only appear at lower concentrations in combination with a hydrophobic substance. The monoglyceride does not form micelles but does form a liquid crystalline phase in equilibrium with water containing molecularly dispersed glyceride in extremely low concentrations. Thus the reduction in the separation efficiency at low concentrations of the glyceride is explained by its solubilization into the micelles of the nonionic emulsifier which is present in the solution at its critical micellization concentration, 100 ppm. The cause of the decrease in the foam volume, from 120 to 104 ml, can be explained by taking this into consideration. The increase in foam separation efficiency at higher concentrations of monoglyceride is due to the tendency of the monoglyceride to form ordered structures. The increasing ratio of monoglyceride to emulsifier causes this tendency to be more pronounced. The increase in the foam volume clearly indicates the formation of a stable foam which is stabilized by the liquid crystalline phase (*10*).

The addition of the sugar (100 ppm) mixed with the glyceride and emulsifier has a similar effect as it does in the presence of the emulsifier with albumin. The dispersion of albumin into the solution and the formation of a colloidal solution could again be offered as an explanation of this phenomenon. But the decrease in efficiency when more than 25 ppm of the monoglyceride is present in the liquid crystalline phase does not appear to follow the same mechanism because sugar does not exhibit any surface-active property. It appears that the sugar interacts with the liquid crystalline phase formed by the monocapryline. In order to explain this, the study was extended to solutions containing albumin (150 ppm) with EMU 09 (100 ppm), monocapryline (100 ppm), and different concentrations of sugar. As a result, a decrease in the efficiency of the process with an increasing sugar concentration was observed (Fig. 4).

In a binary lamellar liquid crystalline phase, such as that formed from monoglyceride/emulsifier with water, the amphiphilic molecules like albumin at its isoelectric point can be dissolved mainly by incorporation in the hydrophobic region of the liquid crystalline phase. The water-soluble materials, such as sugar, can be dissolved primarily in the aqueous region or in the hydrophilic part of the liquid crystal.

The extent of solubilization of any of these components is limited in liquid crystals, as is the amphiphilic/water ratio, which is consistent with the stability of the lamellar phase. This leads to a breakdown of the lamellar liquid crystal with the formation of new phases. However, the lamellar phase formed in the solution with monoglyceride/emulsifier will be destroyed by dissolving the sugar in the hydrophilic part. This leads to an instability of the foam in the present study and a decrease in the separation efficiency.

The effect of the emulsifier concentration on the albumin separation in the presence of monocaprыline could also be explained by taking into consideration the presence of a liquid crystalline phase in the solution.

The presence of 100 ppm monocapryline with 20 to 40 ppm EMU 09 increases the efficiency of the process. However, a further increase in the in the concentration reduces the efficiency. This again can be explained by the formation of a liquid crystalline phase with a change in the monoglyceride/emulsifier ratio. A decrease in the ratio of monoglyceride and emulsifier promotes the micellar solution, which reduces the efficiency of the foam process.

The anionic soaps show a more specific interaction with protein (*5*). The initial fivefold increase in the efficiency when a weight ratio of only one-sixth of the soap compared to the albumin weight is added, most probably refers to the specific and more ionic character of the bonds between anionic soap and the acidic groups of the protein. Gravsholt (*5*) demonstrated strong interactions between the anionic surfactants and the macromolecules by using conductivity and viscosity techniques.

The decrease in the efficiency of the separation process in the presence of metal ions, aluminum, calcium, and sodium can be explained by considering a model suggested by Frank for aqueous electrolyte solutions (*8*). According to the model suggested by Frank (*8*), electrolytes help in forming colloidal solutions by dispersing the polymers into the aqueous solutions. The colloidal solution with small aggregates does not have the ability to form foam.

The present results shed light on the additive influence of different compounds on the foam separation of protein. Moreover, the liquid crystalline phase that is formed in the solution of nonionic emulsifier, monoglyceride, and polymeric albumin has a significant effect on the separation process.

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