Interaction between Surface Active Agents and Proteins. I. Precipitations formed by Mixing Sodium Alkyl Sulfates and Egg Albumin

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Introduction

Putnam and Neurath[1] studied the precipitation reaction between sodium dodecyl sulfate (SDS) and horse serum albumin by mixing solutions of SDS and serum albumin at lower pH than 4.75, the pH of the isoelectric point of this protein. They observed that white precipitate instantly formed when both solutions were mixed if there was an excess albumin, and that the precipitate was redissolved in the region of SDS excess and, further, that these two phenomena did not depend upon the protein concentration.

Pankhurst et. al.[2] studied the interaction between SDS and gelatin using that same method and Elkes et. al[3]. studied the interaction between sodium cetyl sulfate and hemoglobin. Both also found that there was a region wherein precipitation occurred when the surface active agent and the protein were mixed.

No investigation has been made of interactions between egg albumin and SDS, or its homologues. A study was made of the precipitation reaction between each one of these compounds and egg albumin at lower pH than that of the isoelectric point of egg albumin. The results obtained are reported in this paper.

1) F.W. Putnam and H. Neurath, *J. Am. Chem. Soc.*, **66**, 692 (1944).
2) K.G.A. Pankhurst and R.C.M. Smith, *Trans. Faraday Soc.*, **40**, 565 (1944).
3) J. Elkes and J.B. Finean, "Surface Chemistry", Butterworth, London (1949), p. 281.

Experimental

Materials.—SDS was prepared as follows. Purified dodecyl alcohol was sulfonated by chlorosulfonic acid; this solution was then neutralized by sodium hydroxide[4]. The crystal obtained after two recrystallizations was glossy white and scale-like. The melting point was 191–193°C and the purity was 99.8%*. The critical micelle concentration of the aqueous solution of this SDS was found to be 0.007–8 mol./l. at 25°C, by measuring the surface tension, the viscosity and tne specific conductivity[6].

SOS and SBS were synthesized in the same way. The purity of these compounds was of the same degree as that of SDS.

* We took the known quantity of this crystal. We added dilute hydrochloric acid and warmed it to hydrolyze this compound. Then barium sulfafe was precipitated by adding barium chloride solution. The purity of SDS was calculated from the amount of this precipitate[5].

4) W. Kimura and H. Taniguchi, *J. Soc. Chem. Ind. Japan*, **42**, 157 (1939).
5) K. Nishizawa and T. Tomizawa, ibid., **35**, 1368 (1932).
6) K. Aoki, *Bulletin of Nagoya City University*, in press.

Egg albumin was isolated by ammonium sulfate. After several repetitions of salting out, the obtained solution was dialyzed by running water and then electro-dialyzed. This solution was evaporated to dryness to obtain solid egg albumin. This egg albumin was proved to be electrophoretically pure[7].

Procedure.—We obtained the SDS solution having the known concentration by dissolving the known amount of SDS crystal into buffer solution, the pH and the ionic strength of which were known. We dissolved the known amount of egg albumin powder into the buffer solution. Using the micro-Kjeldahl method, the concentration of egg albumin in this solution was determined by finding the amount of nitrogen in it, taking the percentage of nitrogen in egg albumin as N=15.76%. We obtained the albumin solution having the desired concentration by diluting this solution.

Known volumes of SDS solution were placed in a series of test tubes or conical flasks, and then to each test tube the calculated volume of egg albumin solution was added. They were allowed to stand over night at room temperature, and supernatants were separated from precipitates by centrifugation. Concentrations of protein in these supernatants were found by the micro-Kjeldahl analysis. Knowing both the amount of protein which originally existed and the amount of protein found in the supernatant after removing precipitate, we knew the percentage of the protein turned into precipitate. (Hereafter we call this percentage "precipitation percentage".)

The buffer solution used consisted of sodium acetate and hydrochloric acid, its pH being equal to 4.2 and ionic strength (μ) 0.34.

Precipitation reactions between SOS and egg albumin, and between SBS and egg albumin were studied in the same way as between SDS and egg albumin.

Results

Precipitation Curve.—We obtained the relation between the precipitation percentage and the mixing ratio of SDS to egg albumin at pH=4.2, μ=0.34, whereby the concentration of egg albumin was kept constant. This relation is shown in Fig. 1. Observing this curve, which is hereafter termed "precipitation curve", the precipitation percentage of protein was found to increase first with the mixing ratio, and upon reaching its maximum, the precipitation percentage then decreased. When solutions of SDS and egg albumin were mixed and the amount of SDS was sufficiently rich, precipitation did not occur even when the pH of the solution was lower than that of the isoelectric point of the protein. We observed the same phenomenon as above when the concentration of protein used was between 0.5 and 2.0%.

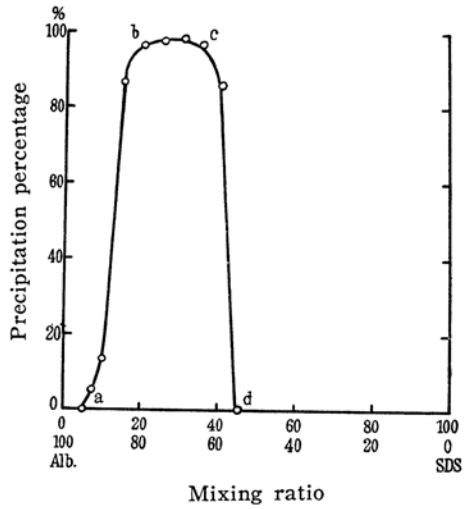

Fig. 1. Precipitation curve of the system of egg albumin and sodium dodecyl sulfate at pH 4.2 and ionic strength 0.34. Concentrations of egg albumin are follows: ⊙ 2.0%, ○ 1.0%, ● 0.5%.

In the region of albumin excess, the supernatants were not transparent, appearing slightly turbid. After these supernatants were left standing for a few days, we observed that a small quantity of precipitate had newly occurred.

Precipitate did not appear instantly when solutions of SBS and egg albumin were mixed. There was a slight daily increase in the amount of precipitate. Fig. 3 was calculated using the data obtained on mixtures left standing for ten days after mixing.

There was an instant precipitation when solutions of SOS and egg albumin were mixed. When supernatants were left standing for a few days after removing precipitate, a little precipitate appeared in the same way as in the SDS-egg albumin system. Figs. 2a and 2b show the precipitation curves of the system SOS-egg albumin. We can note in the region of SOS excess that the precipitation curves are different when the concentrations of SOS differ. In other words, when 1.0% of SOS solution is used, the precipitation percentage of protein is always near 100%; but when the concentration of SOS is over 1.5%, the precipitation curve descends, as in the SDS-egg albumin system.

Electrophoresis of Turbid Supernatant and Transparent Solution.—We electrophoretically analyzed supernatants after centrifugation and also solutions with variously different mixing ratios of SDS to egg albumin. We used Hitachi's electrophoretic apparatus and the experiments were carried

7) K. Aoki, ibid., in press.

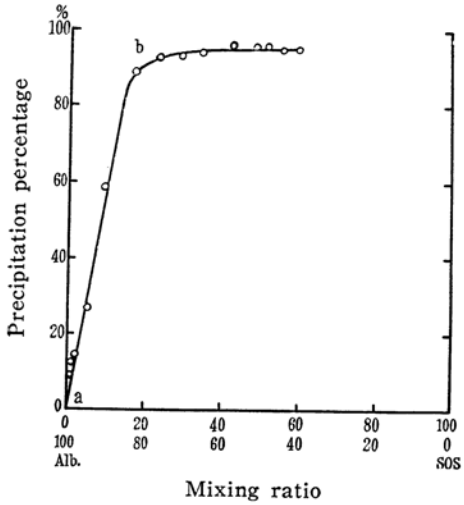

Fig. 2a. Precipitation curve of the system of egg albumin and sodium octyl sulfate at pH 4.2 and ionic strength 0.34. Concentration of egg albumin is 1.0%.

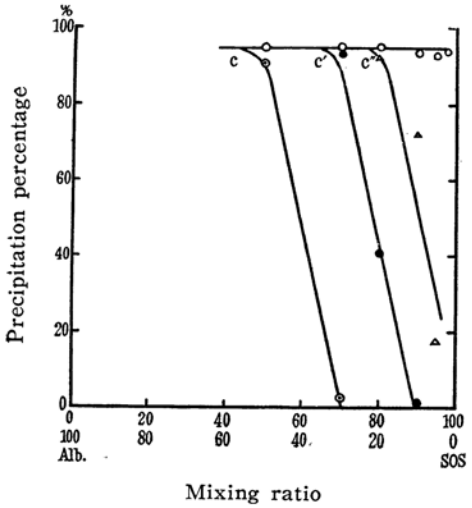

Fig. 2b. Precipitation curve of the system of egg albumin and sodium octyl sulfate at pH 4.2 and ionic strength 0.34. Concentrations of SOS are as follows: ⊙ 3.0%, ● 2.0%, △ 1.5%, ○ 1.0%.

out at 25°C, pH=3.6, and μ=0.1*. Some of the results are shown in Figs. 4a and 4b. At pH=3.6, a solution which contained only egg albumin moved toward the anode and a solution which contained only SDS moved toward the cathode. In cases where the concentration of egg albumin was richer than albumin/SDS =80/20 (mixing ratio) the supernatants after centrifugation were always turbid white. In

* The experimental method of electrophoresis is described in Part II.

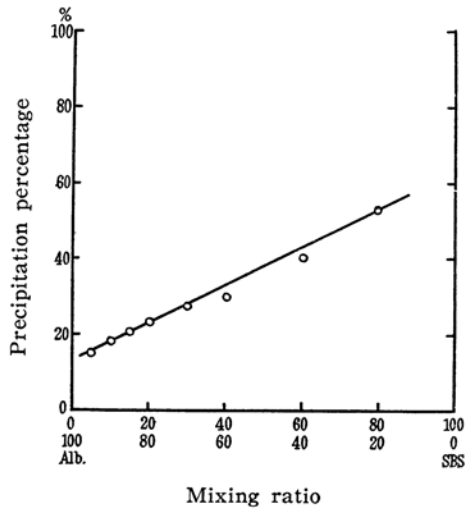

Fig. 3. Precipitation curve of the system of egg albumin and sodium butyl sulfate at pH 4.2 and ionic strength 0.34. Total concentration is 2.0%.

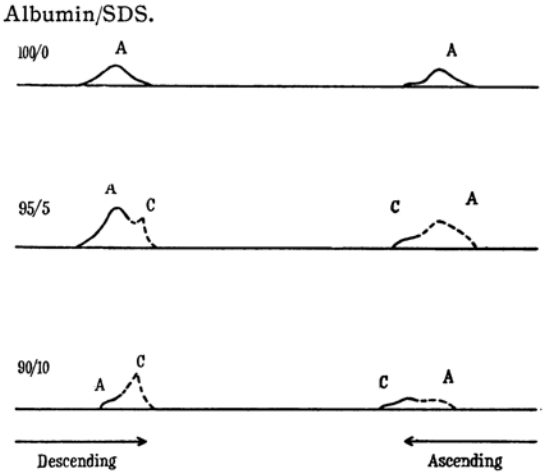

Fig. 4a. Electrophoretic pattern of the system egg albumin-sodium dodecyl sulfate at pH 3.6 and ionic strength 0.1. Both egg albumin and complex moved toward the anode. Photographs of albumin/SDS=95/5 and 90/10 were taken with larger inclination of diagonal slit. Broken line means that the light passed through the turbid phase.

electrophoretic experiments of these supernatants, we observed that all moved toward the anode and separated into two components, i. e. turbid complex and transparent egg albumin. When the ratio of albumin/SDS was less than 80/20, we observed only one complex which migrated toward the cathode. Further, when the ratio of albumin/SDS became less than 30/70 there appeared two components, i. e. SDS and complex.

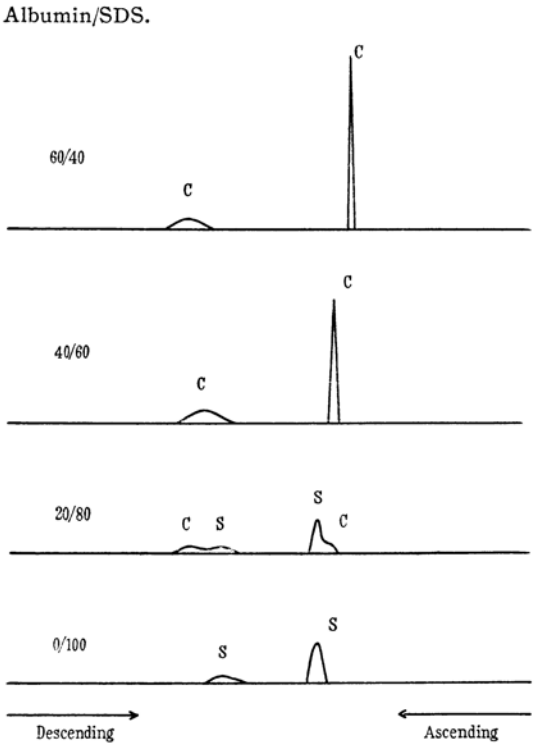

Fig. 4b. Electrophoretic pattern (continued). Both complex (C) and SDS (S) move toward the cathode.

method[8]. The dye used was Eastman's pinacyanol; the three buffer solutions used were acetate buffer solution at pH 4.2, phosphate buffer solution at pH 6.8 and carbonate buffer solution at pH 10.0*. We also studied the effect of ionic strength of sodium chloride solution on the CMC of SDS. As seen in Fig. 5a, the lowering effect of ionic strength of the three buffer solutions and sodium chloride solution upon the CMC of SDS is the same if the ionic strength of each solution is the same. Further, the curve shown in Fig. 5a coincides with the curve calculated by Corrin and Harkins[8]. This means that the lowering of the CMC does not depend upon the kinds and pH's of the buffer solutions nor upon the kinds of neutral salt solutions and that it depends only upon the ionic strength of the solution which dissolves the detergent. We can find in Figs. 5a and 5b that the CMC of SDS is 0.03% (0.0010 mol./l.) and the CMC of SOS is 1.39% (0.060 mol./l.) in the buffer solution at $\mu=0.34$.

Discussion

Number of SDS and of SOS Bound to One Molecule of Egg Albumin.—We can calculate the number of SDS bound to one molecule of egg albumin from precipitation

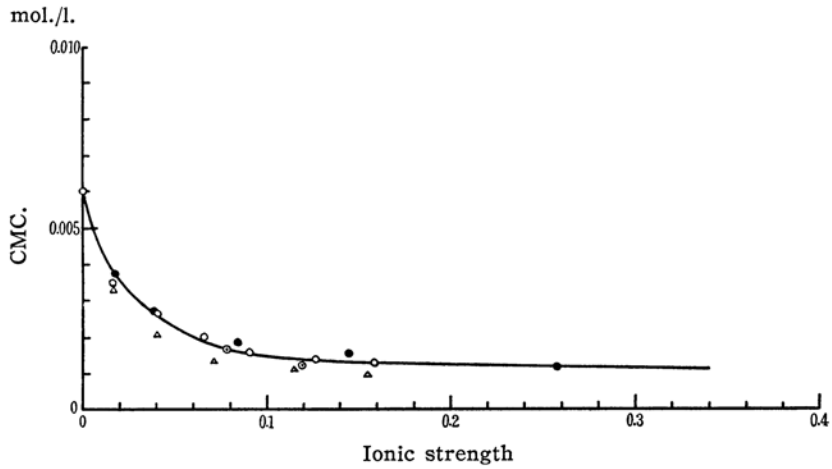

Fig. 5a. The effect of ionic strength upon the critical micelle concentration of sodium dodecyl sulfate.
● Acetate buffer solution at pH 4.2, ○ phosphate buffer solution at pH 6.8, ⊙ carbonate buffer solution at pH 10.0, △ sodium chloride solution.

Critical Micelle Concentration of SDS and of SOS in Buffer Solution.—We investigated the lowering of the critical micelle concentration (CMC) of SDS and of SOS caused by the increase in the ionic strength of buffer solution using the Corrin and Harkins, curve in Fig. 1. No precipitation occurred when barium chloride solution was added to supernatants obtained from mixtures the

8) M.L. Corrin and W.D. Harkins, *J. Am. Chem. Soc.*, **69**, 683 (1947).

* This will be desdribed later.

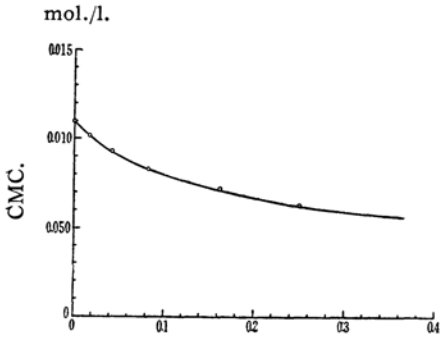

Fig. 5b. The effect of ionic strength of phosphate buffer solution at pH 6.8 upon the critical micelle concentration of sodium octyl sulfate.

compositions of which were between albumin/SDS=100/0 and 65/35, so we can assume that all of the added SDS are bound to egg albumin and form a complex, and that almost all of the complex thus formed exists in precipitate within this region of mixing ratio. White precipitate occurred when barium chloride solution was added to supernatants obtained from mixtures having the concentration of SDS over albumin/SDS=65/35, so we know that in this mixing ratio the added SDS exists both in the supernatant and in the precipitate.

We calculated the composition of precipitate, or the number of SDS bound to one molecule of egg albumin, in the region of albumin/SDS=100/0–65/35, taking the molecular weight of egg albumin as 46,000 and that of SDS as 288. The compositions of precipitate thus calculated at each mixing ratio are shown in Fig. 6a. This curve has two inflection points *a* and *b*, and the number of bound SDS at point *b* is about 40 which is considered the true number of SDS bound to one egg albumin molecule. This composition is expressed by AD_{40} (A: albumin, D: detergent). The numeral 40 is almost equal to 42 which is the number of positive charges on one egg albumin at this pH*.

In Fig. 6a, line *ab* is not horizontal. One reason is that supernatant contains not only unreacted excess albumin but also turbid complex, as seen in electrophoretic patterns of supernatants. We should not consider that the composition of the complex changes with mixing ratio in the region between points *a* and *b*, but should consider that all

* Line *a'b'* is more horizontal than line *ab*. The reason as follows: We added the amount of precipitate which was produced after standing a few days to that which was produced instantly when both solutions were mixed, and this value was used to calculate the precipitation curve.

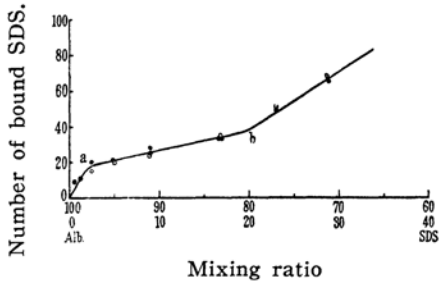

Fig. 6a. Number of SDS molecules bound to one molecule of egg albumin. Concentrations of egg albumin are as follows: ⊙ 2.0%, ○ 1.0%, ● 0.5%.

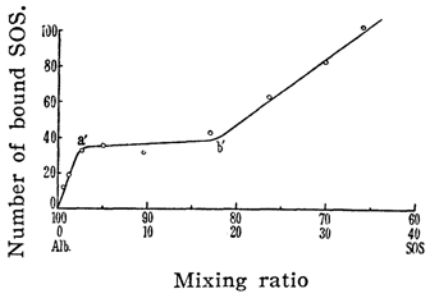

Fig. 6b. Number of SOS molecules bound to one molecule of egg albumin. Concentration of egg albumin is 1.0%.

the complex formed in this region have the same composition of AD_{40}. If we assume that all the complex formed exists in precipitate, line *ab* should be horizontal.

The curve showing the relation between the number of SOS bound to one molecule of egg albumin and the mixing ratio was calculated by using the precipitation curve of SOS shown in Fig. 2a. This curve is shown in Fig. 6b and is similar to that of the SDS-egg albumin system. The composition of precipitate calculated at point *b'* is also AD_{40}*.

We can deduce that there is no great difference in the region of albumin excess between the reactivity of SDS and that of SOS toward egg albumin.

Redissolution of Precipitate.—The precipitation curve of the SDS-egg albumin system and of the SOS-egg albumin system descends after reaching the maximum, with each position of the descending lines different. This means that the abilities of SDS and SOS to dissolve precipitate are different. As described above, at the point where only complex AD_{40} exists (point *b* in Fig. 1, albumin/SDS=80/20), 40 molecules of SDS bind to one molecule of egg albumin. Eighty molecules of SDS bind to one molecule of egg albumin at a point where the redissolution of precipitate begins (point *c* in Fig. 1,

albumin/SDS=67/33). A comparison of SDS molecules at these two points reveals that the latter is double that of the former. In the same way we can calculate the number of SOS molecules bound to one molecule of egg albumin at the point where only complex AD_{40} exists (point *b* in Fig. 2a) and at the points where redissolution of precipitate begins (points *c*, *c'*, *c''* in Fig. 2b). But here the ratio of bound SOS at two points is not double.

As previously noted, it was proved by the electrophoretic experiment that the complex was formed in supernatants and that the complex and SDS coexisted in the region where SDS was in excess. This means that the composition of the complex is limited.

Difference in Reactivity of SDS, SOS and SBS toward Egg Albumin.—As pointed out, these three compounds, viz. SDS, SOS, and SBS showed different kinds of behavior in their reactions toward egg albumin. The CMC of SDS is 0.03% and that of SOS is 1.39% in buffer solution at $\mu=0.34$. Therefore, within the concentration range of this study, SDS existed as a micellar colloidal solution, SBS behaved as a true solution, and SOS existed as a micellar colloidal solution or as a true solution according to its concentration.

There was no instant precipitation when SBS solution and egg albumin solution were mixed; but there was an instant precipitation when SDS or SOS solution and egg albumin solution were mixed. The main cause of this phenomenon is considered to be that SBS solution is a true solution and SDS and SOS solutions are colloidal.

In the SOS-egg albumin system, there are two kinds of precipitation curves depending upon the concentration of SOS; one descends and the other does not. Considering the fact that the CMC of SOS is 1.39% in a buffer solution at $\mu=0.34$, we can deduce that when the concentration of SOS is over the CMC, the curve descends, and that when the concentration of SOS is below the CMC, the curve is horizontal.

Difference in Egg Albumin and Horse Serum Albumin.—The precipitation curve of the egg albumin-SDS system begins at the original point in Fig. 1. But the precipitation curve of the horse serum albumin-SDS system begins to the right of the original point in Fig. 7. In addition Yang and Foster[9] recently found by dialysis equilibrium method that the reactivities of egg albumin and bovine serum albumin toward sodium dodecyl-benzenesulfonate were different. Thus, egg albumin and serum albumin behave in different ways toward the same detergent. That there are some differences in the structure of both proteins, may be the cause of this phenomena.

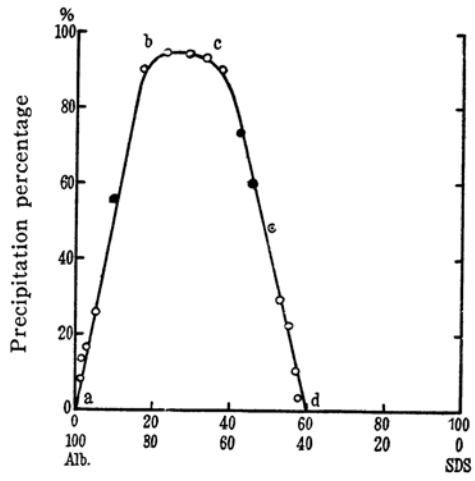

Fig. 7. Precipitation curve of the system of horse serum albumin and sodium dodecyl sulfate at pH 4.2 and ionic strength 0.34. Total concentration is 2.0%.

Summary

(1) Precipitation reactions between SDS and egg albumin, SOS and egg albumin, and SBS and egg albumin were studied. There was an instant precipitation when SDS and egg albumin solutions were mixed, and when SOS and egg albumin solutions were mixed, but not when SBS and egg albumin solutions were mixed. Comparing the precipitation curve of the SDS-egg albumin system and that of the SOS-egg albumin system, we can find that both detergents form a complex having the same formula AD_{40} (A: albumin, D: detergent) in the region of protein excess, and therefore we can conclude that there is no difference between the reactivities of SDS and SOS toward egg albumin. On the contrary, in the region where redissolution of precipitate occurs, the precipitation curves do not coincide. This means that the two detergents have different powers to dissolve precipitate. In addition, when the concentration of SOS differs, the power to dissolve precipitate differs.

(2) It was found electrophoretically that the complex is formed in turbid supernatants after centrifugation and also in transparent

9) J.T. Yang and J.F. Foster, *J. Am. Chem. Soc.*, **75**, 5560 (1953).

solutions the composition of which is in the region where no precipitation occurs.

(3) Observing the precipitation curves of the system horse serum albumin-SDS and the system egg albumin-SDS, we can see that there is a region where no precipitation occurs when a great excess of serum albumin is used; but there is not such a region when a great excess of egg albumin is used.

(4) We measured the lowering of the CMC of SDS and of SOS caused by the increase in the concentration of buffer solution. The lowering does not depend upon the kinds and pH's of buffer solutions but upon the ionic strength of buffer solution which dissolves detergent.

We are sincerely grateful to Professor Rempei Goto of the Institute for Chemical Research, Kyoto University for his kind guidance during this study.

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