# *Interaction between Surface Active Agents and Proteins. III. Precipitation Curve of the System Sodium Dodecyl Sulfate-Egg Albumin at Various pH's and the Determination of the Concentration of Protein by the Titration Using Surfactant*

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## Introduction

As described in Part I[1], we observed in the reaction of egg albumin and sodium dodecyl sulfate (SDS) in the acetate buffer solution at pH 4.2 that the amount of precipitate formed changed with the mixing ratio of egg albumin to SDS (albumin/SDS). In this reaction a large amount of white precipitate was formed when albumin was in excess, and the amount of precipitate decreased with an increase in the relative amount of SDS. Furthermore, there was no precipitate when the relative amount of SDS exceeded a certain mixing ratio. We drew a curve, named precipitation curve, by plotting the precipitation percentage against the mixing ratio albumin/SDS.

The present paper is a report of the precipitation curve when the pH of the buffer solution was lower than 4.2. In addition, we made a study of this curve when the neutral salt solution instead of the buffer solution was used.

Further, we investigated the possibility of a rapid determination of the concentration of the protein solution by the titration using surfactant solution, taking advantage of the above fact; namely, that the protein and surfactant react quantitatively.

## Experimental

**Materials.**—SDS, egg albumin and horse serum albumin used in this study were obtained by the method described previously[1,2].

**Precipitation Curve.**—We determined the precipitation curve of the system egg albumin-SDS at pH's 4.2, 3.6, 3.1, and 2.3 and at constant ionic strength ($\mu$) 0.30, using the same method as in Part I. Further we determined the precipitation curve of this system in the sodium chloride solution at $\mu$ 0.30, and the curve in distilled water.

**Determination of Protein.**—As is seen in Fig. 1, there is a precipitate when the concentration of SDS is below a certain mixing ratio of albumin/SDS, but no precipitate when the concentration of SDS exceeds this mixing ratio. We tried to titrate the protein solution by surfactant solution taking the point where the precipitate disappeard as the end point. When the concentration of one of the two, i.e., albumin or SDS, is known, the concentration of the other can be found by the titration. In this study definite amounts of protein solutions varying in concentration were titrated by SDS solution of known concentration. Plotting the volume (X cc.) of SDS used up to the end point of titration against the concentration (Y%) of albumin, a relation between these two quantities was found. Using this relation, the concentration of albumin solution, the value of which is unknown, can be found through the titration by SDS solution. Of course, by conducting this titration in the reverse way, the amount of SDS can be determined by the titration using protein solution.

**Procedure of Titration.**—Albumin solutions of known concentration were prepared using acetate buffer* of known pH and ionic strength, or using sodium chloride solution. The concentration of protein was first determined by the Kjeldahl method, and then this protein solution was successively diluted with the same medium in order to have a series of solutions varying in concentration.

SDS solution of a known concentration was prepared using the same medium as used in the preparation of albumin solution.

Taking exactly 2.00 cc. of the albumin solution of known concentration, 1.00% SDS solution was gradually added. The egg albumin solution turned turbid by the first drop of SDS. Continuing the titration, the turbidity increased, and then the precipitate appeared. On the further addition of SDS, the precipitate dissolved and a transparent solution was obtained. This was taken as the end point of the titration.

When the SDS solution was added to the serum albumin solution, no precipitate appeared until a few drops of SDS solution were added[1]. With the addition of SDS the precipitate first increased in amount and then disappeared in the same way as in the titration of egg albumin.

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1) K. Aoki and J. Hori, This Bulletin, 29, 104 (1956).

2) K. Aoki, H. Shimosato and Y. Kamino, *Bull. Nagoya City Univ.*, 1, 67 (1955).

## Results

**Precipitation Curves.**—Fig. 1 shows precipitation curves of the system egg albumin-SDS at pH's 4.2, 3.6, 3.1, and 2.3 and at $\mu$ 0.30. In this figure we find that in the region of albumin excess plots are on the same line *ab* regardless of pH, while in the other region they lie on separate lines*. By use of plots on the curve *abc* in Fig. 1, we can calculate the number of SDS molecules which are bound to one molecule of egg albumin. The method of calculation is the same as described in Part I. The result is shown in Fig. 2.

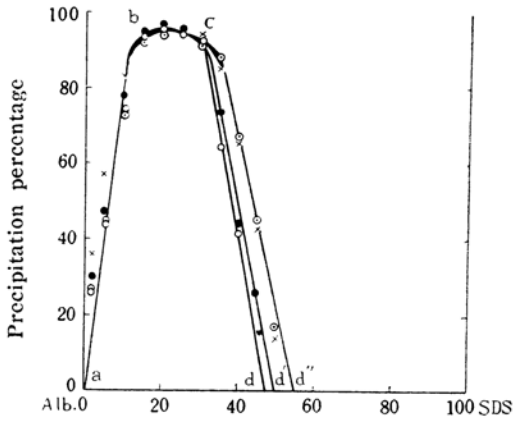



Fig. 1. Precipitation curves of the system egg albumin and sodium dodecyl sulfate in acetate buffer at 0.30 ionic strength. PH's are as follows; ○ 4.2, ● 3.6, ⊙ 3.1, and × 2.3.

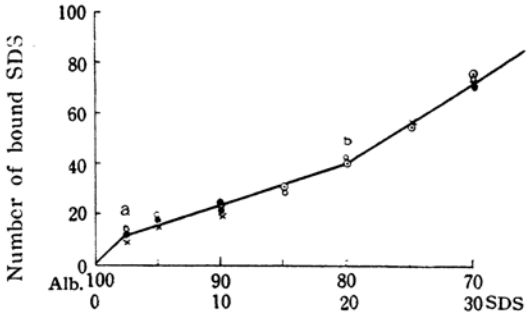



Fig. 2. Number of SDS molecules bound to one molecule of egg albumin. PH's are as follows; ○ 4.2, ● 3.6, ⊙ 3.1, and × 2.3.

Fig. 3 shows the precipitation curve of the system egg albumin-SDS in 0.30 N sodium chloride solution. Fig. 4 shows the precipitation curve of the same system in distilled water. As is shown there is a small precipitation curve even when distilled water is used. On the contrary, there is no precipitation curve in the system serum albumin-SDS when sodium chloride solution or distilled water is used.

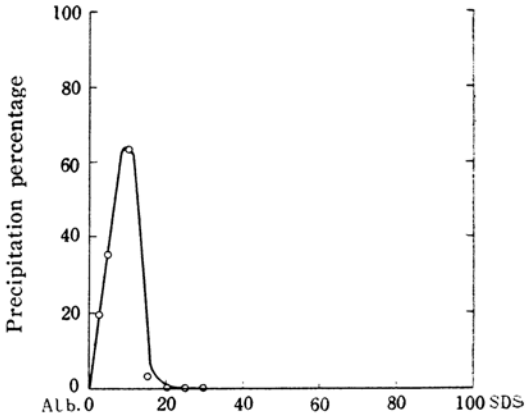



Fig. 3. Precipitation curve of the system egg albumin-SDS in 0.30 N sodium chloride solution.

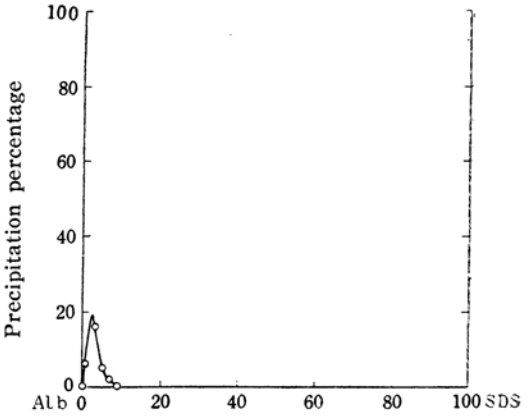



Fig. 4. Precipitation curve of the system egg albumin-SDS in distilled water.

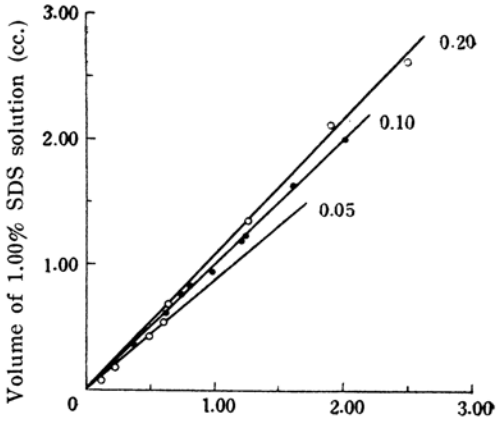
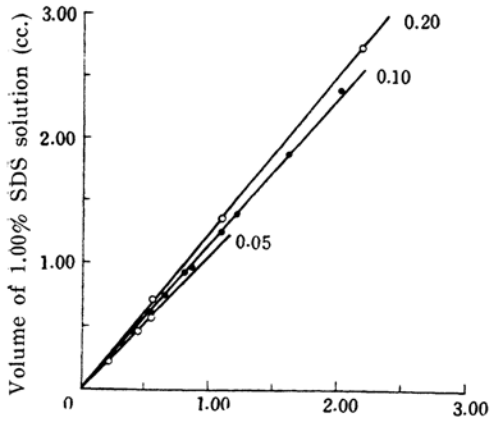



Fig. 5a. Titration of egg albumin by SDS in acetate buffer at pH 4.2 and ionic strengths 0.20, 0.10, and 0.05.

* This buffer was composed of hydrochloric acid and sodium acetate.

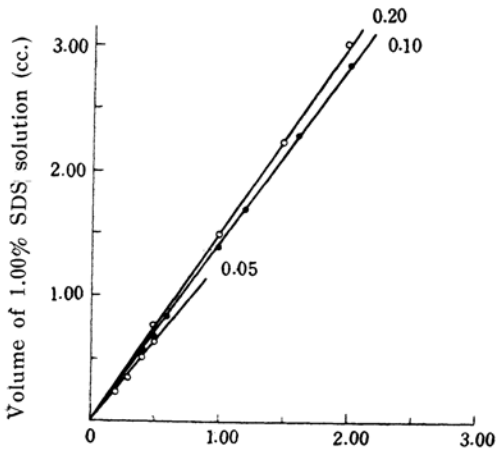
* Below pH 3.0 plots fall on a common line *cd''*.

**Titration of Egg Albumin.**—Some results obtained are shown in Figs. 5a, 5b, 5c and 6. Fig. 5a shows the result obtained at pH 4.2 and at three different ionic strengths. The abscissa represents the concentration of egg albumin used, and the ordinate represents the volume of the 1.00% SDS solution required to titrate 2.00 cc. of albumin solution. A linear relation was obtained between these two quantities. Slopes of these straight lines vary according to the ionic strength, and the lower the ionic strength the smaller the slope. At pH 3.6 and 3.1 also, straight lines having the same characteristics as those in Fig. 5a were obtained.




Concentration of egg albumin (%)

Fig. 5b. Titration of egg albumin by SDS in acetate buffer at pH 3.6 and ionic strengths 0.20, 0.10, and 0.05.



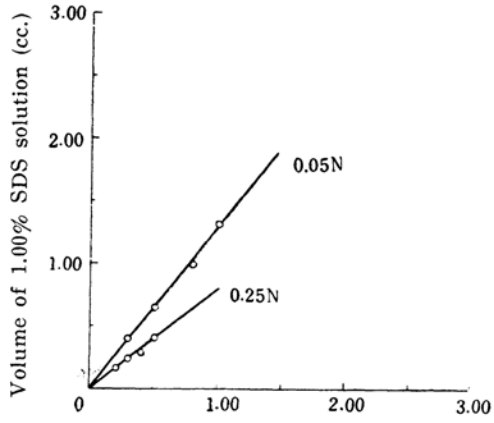

Concentration of egg albumin (%)

Fig. 5c. Titration of egg albumin by SDS in acetate buffer at pH 3.1 and ionic strength 0.20, 0.10, and 0.05.

Fig. 6 shows the results obtained when 0.50 N and 0.25 N sodium chloride solutions were used. In these cases also a linear relation was obtained, and the slope of the straight line varied with the concentration of sodium chloride. The pH of sodium chloride solution containing protein was 5.2.

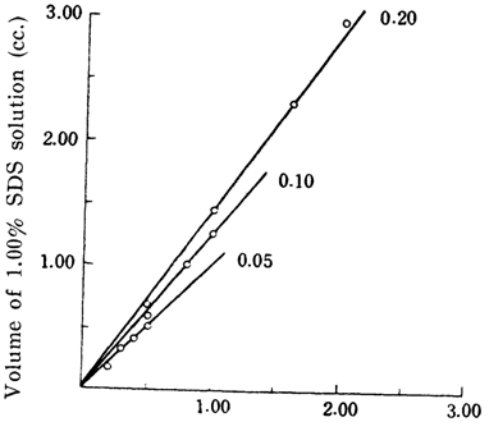
As is seen in Fig. 4 there is a precipitation curve when distilled water is used. Therefore the titration of egg albumin in distilled water was thought possible; the results, however, were not satisfactory because the greater error accompanies the titration.

**Titration of Serum Albumin.**—The results at pH 4.2 is shown in Fig. 7.




Concentration of egg albumin (%)

Fig. 6. Titration of egg albumin by SDS in sodium chloride solution.




Concentration of serium albumin (%)

Fig. 7. Titration of horse serum albumin by SDS in acetate buffer solution at pH 4.2 and ionic strengths 0.20, 0.10, and 0.05.

## Discussion

**Precipitation Curve of Egg Albumin in Acetate Buffer.**—Precipitation curves were found in the pH region between 4.2 and 2.3 and $\mu$ 0.30. Fig. 2, which is to be interpreted as described in Part I, shows that four series of plots fall near the same curve. This indicates that, regardless of pH, about forty molecules of SDS are bound to one molecule of egg albumin in the region of albumin

excess. As will be shown elsewhere[3], the number of positive charges on one egg albumin molecule should be constant and 42 for pH<6. The above result is considered to be an experimental verification of the fact that the number of positive charges on one egg albumin molecule is constant in this pH range.

**Titration of Protein.**—As was shown, the concentration of albumin is proportional to the volume of the 1.00% SDS solution required in the titration. The proportionality constant changes according to the conditions of the titration, i.e., pH and ionic strength of the buffer or the concentration of sodium chloride solution. As is seen in Figs. 5a, 5b, 5c, 6, and 7, the greater the ionic strength becomes at the same pH the more SDS is necessary. This fact is observed whether buffer or sodium chloride solution is used, for either egg albumin or serum albumin.

There is a limitation in the ionic strength of the medium to be used. When the ionic strength is too large, SDS does not dissolve completely, because of the limited solubility of surfactant due to the increase in the concentration of salt. When the ionic strength is too small, reproducible results cannot be obtained since the end point becomes somewhat obscure. The following are the media to be used; acetate buffer solution of an ionic strength between 0.50 and 0.05, or the sodium chloride solution of an ionic strength between 0.50 and 0.25. These values were found when the room temperature was about 20°C, and are affected by temperature.

When buffer solution is used, there is no limit of pH other than that the pH must be smaller than that of the isoelectric point of the protein*. It is interesting, however, that there was a precipitate in sodium chloride solution, although the pH of the egg albumin solution was 5.2 and was on the alkaline side of the isoelectric point of this protein. This is a difference in behavior observed between the buffer solution and the neutral salt solution.

Considering the results described above, it is concluded that a rapid determination of the concentration of the protein solution is possible. As is clear in Figs. 5a, 5b, 5c, 6 and 7, the accuracy of the titration using SDS is ±0.03 cc. In other words, the concentration of the protein is determined within an error of ±2%.

**Difference between Egg Albumin and Serum Albumin.**—Comparing the results of the titration by SDS, a difference is found between egg albumin and horse serum albumin. On horse serum albumin, no precipitate appeared until the addition of a few drops of SDS. But on egg albumin a precipitate occurred with the first drop. Therefore, egg albumin and serum albumin can be distinguished from each other by the titration using SDS.

When horse serum albumin and SDS were mixed in distilled water, there was no precipitate; this phenomenon agrees with the observation by Putnam and Neurath[4]. When egg albumin and SDS were mixed in distilled water, there was a little precipitate. This fact shows another difference between these two proteins.

## Summary

(1) The precipitation curve of the system egg albumin-SDS was studied in the pH region between 4.2 and 2.3, and at constant ionic strength 0.30. It was found that the number of SDS molecules bound to one molecule of egg albumin is constant and this is considered to be a verification of the fact that the number of positive charges on one egg albumin molecule is constant in this pH region.

(2) A rapid determination of the protein concentration by the titration using SDS is possible within an error of ±2%.

(3) Egg albumin and horse serum albumin react in different ways with SDS in distilled water and in sodium chloride solution.

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3) K. Aoki, unpublished.

* When the pH is lower than 2, egg albumin is gradually denatured.

4) F.W. Putnam and H. Neurath, *J. Am. Chem. Soc.*, 66, 692 (1944).