The Interaction of Gelatin Molecule with Surface Active Ions

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Introduction

The interaction between proteins and synthetic detergents has been the subject of discussion of many investigators[1]. It has been reported that the interaction depends upon some factors, among which the ratio of detergent to protein seems to be of importance in elucidating its mechanism[2]. It has also been observed that cationic detergents precipitate only anionic proteins, namely, proteins on the alkaline side of the isoionic point, while anionic detergents precipitate only cationic proteins, namely, proteins on the acidic side of the isoionic point, and that the precipitate initially formed is dissolved when excess detergent is added. Pankhurst and Smith[3] assumed, basing their assumption on their investigation of the complex formation between gelatin and sodium dodecyl sulphate, that the precipitation took place when the monolayer of dodecyl sulphate ions was formed at basic groups of gelatin molecules and the precipitate was led to dissolve when the second adsorption layer was built up. Lundgren[4] also suggested such a mechanism of complex formation between proteins and detergents. Furthermore it has been evidenced that certain stoichiometric combinations can also take place between cationic detergents and proteins on the acidic side of an isoionic point as well as between anionic detergents and proteins on the alkaline side of the isoionic point[5,6,7,8,9].

In this paper we describe our experiment on the interaction of dodecyl amine hydrochloride and sodium dodecyl sulphate on gelatin at various values of hydrogen ion concentration, whereby we studied the phenomena first by determining the combined detergent to the precipitated gelatin and then by measuring flow properties of the mixed solutions.

Experimental

Materials.—The sample of gelatin used in this experiment was a commercial one, its ash content

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[1] F.W. Putnam, "Advances in Protein Chemistry", **4**, 80 (1948).

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

[3] K.G.A. Pankhurst and R, C. M. Smith, *Trans. Faraday Soc.*, **40**, 565 (1944); **41**, 630 (1945); "Surface Chemistry" London (1949) p. 109.

[4] H. P. Lundgren, *Textile Research J.*, **13**, 335 (1945).

[5] H.P. Lundgren, D.W. Elam and R.A. O'Connell, *J. Biol. Chem.*, **149**, 183 (1943).

[6] F. W. Putnam and H. Neurath, ibid., **159**, 195 (1945).

[7] H. Neurath and F.W. Putnam, ibid., **160**, 397 (1945).

[8] S.N. Timasheff and F.F. Nord, *Arch. Biochem. Biophys.*, **31**, 309 (1951).

[9] B.S. Harrap and J.H. Schulman, *Discussions Faraday Soc.*, No. 13, 197 (1953).

having been found to be 2.6% and the pH of its solution 5.3. This sample was purified by using ion exchange resins by which means the ash content of the gelatin was reduced to less than 0.09% and the pH at the isoionic point of the purified gelatin was found to be 4.8.

Dodecyl amine hydrochloride (DAH) was prepared from dodecyl amine supplied by Armour and Co. U.S.A., and recrystallized twice from acetone.

Sodium dodecyl sulphate (SDS) was a sample of the highest purity supplied by Nezu Chemical Laboratory, Tokyo.

Method of Analysis of Detergents.—SDS which remained uncombined in the mixed solutions was determined by volumetric precipitation process using DAH as a precipitant and eosin as an indicator. DAH was determined in the same manner by using SDS as a precipitant and rhodamin 6G as an indicator. In both cases, the concentration of the standard solutions was 0.001 mole per liter, and the end point of titration was determined by means of the disappearance of fluorescence of these indicators. Such method of analysis had been proposed by the investigators of Nezu Chemical Laboratory[10] and was proved to be applied to the solution containing gelatin with the error of about ±2% in our experiment.

Measurement of Viscosity.—The viscosity of the mixed solutions was measured by means of a horizontal capillary tube viscosimeter, the solution having been made to flow through the tube under suitable pressure differences at the two ends of the tube. The pressure difference was maintained constant during a set of measurements by means of a manostat. The radius of the capillary tube was equal to 0.031 cm. and its length 30.3 cm. The pressure range studied was from 0.5 cm. to 11 cm. in mercury manometer.

Results and Discussion

a) Complex formation.—Various amounts of detergents were added to a 0.5% gelatin solution at various values of pH of the solution, which had been controlled by adding normal HCl or NaOH to the solution. It was found that gelatin was precipitated in a certain range of the concentration of any one of the detergents at a certain value of pH of the solution. DAH precipitated gelatin at pH values greater than that of the isoionic point, while SDS precipitated gelatin at pH values less than that of the isoionic point. When precipitates appeared, the resulting mixture, after being allowed to stand for twenty four hours, was centrifuged or filtered, and the concentration of detergent of the supernatant or filtered solution was determined as described above; the amount of detergent combined with the precipitant was thus obtained by taking the difference of concentration which existed before and after the process. Figs. 1a. and 1b. show the amount of the combined detergent in relation to the ratio of detergent to protein.

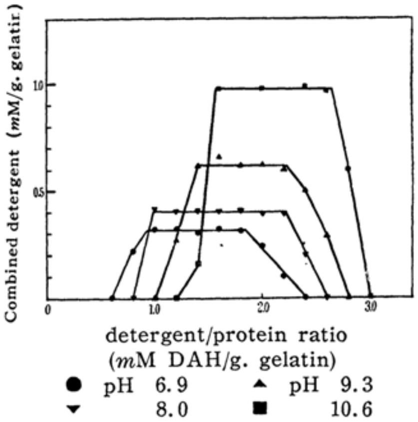

Fig. 1a. Amount of combined detergent (DAH) in relation to detergent/protein ratio.

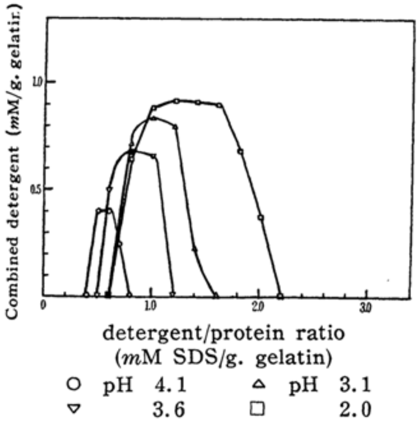

Fig. 1b. Amount of combined detergent (SDS) in relation to detergent/protein ratio.

As these figures indicate, the precipitation zone is greatly influenced by the pH of the mixed solution and, in the case of gelatin-dodecyl amine ion combination, the precipitation zone is displaced to the higher ratio of detergent to protein as pH becomes greater, while in the case of gelatin-dodecyl sulphate ion combination, the precipitation zone is displaced to the higher ratio of detergent to protein as pH becomes smaller. In both cases, it is to be remarked that the maximum precipitation and the complete dissolution of the precipitate take place at the definite ratio of the detergent concentrations, namely 1:2. It is also noticeable that SDS precipitates gelatin at much lower concentrations than DAH does.

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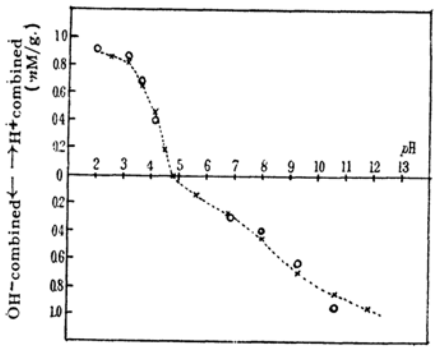
[10] T. Ino, T. Kondo, K. Meguro and O. Yoda, *J. Chem. Soc. Japan*, **76**, 220 (1955).



×···×··· Titration curve

○ Maximum value of combined detergent.

Fig. 2. Titration curve of gelatin and maximum value of combined detergent at each pH.

Fig. 2 shows that the maximum values of detergent combined at each pH are approximately corresponding to the acid or base combining power of gelatin, and therefore it is concluded that the first polar combination between gelatin molecules and detergent ions is accomplished at the point of the maximum precipitation.

b) Flow Properties.—The flow of gelatin solutions in presence and absence of detergent was found generally non-Newtonian, the rate of flow being expressed by the well known Bingham's equation[11], namely:

$$\frac{V}{t}=\frac{\pi\mu R^4}{8l}\left(P-\frac{4}{3}p+\frac{p^4}{3P^3}\right),$$

where, V means the volume of the solution passed through the capillary tube in time t, R the radius of the capillary tube, l the length of the tube, P the difference of pressures applied, μ the coefficient of mobility, p a constant equal to $\frac{2l}{R}f$ in which f means the yield value. When the applied pressure difference is large, the curve V/t *vs.* P becomes nearly linear, and it obeys the following equation:

$$\frac{V}{t}=\frac{\pi\mu R^4}{8l}\left(P-\frac{4}{3}p\right)$$

Putting herein $V/t=0$, we obtain the intercept at the axis P and from this we get the value of the yield value. From the slope of the asymptote we obtain the value of $\frac{\pi\mu R^4}{8l}$ and consequently the value of the coefficient of mobility μ : $\mu=1/\eta$, where η means the coefficient of viscosity. In this paper, we conventionally use the relative viscosity to water, η_r, instead of μ or η.

The relative viscosity and the yield value obtained of the gelatin solution first in absence of detergent are shown in Fig. 3, both as a function of pH. As this figure demonstrates, η_r and f run completely parallel to each other. Both the values have the minimum at the isoionic point and the maximum on both sides of the isoionic point. Such a characteristic feature was formerly found by Freundlich and Neu kircher[12] and recently studied in some detail by Stainsby[13].

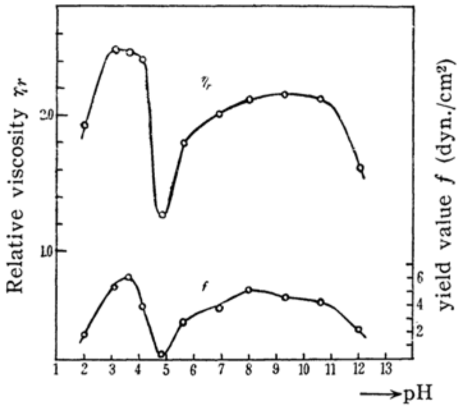
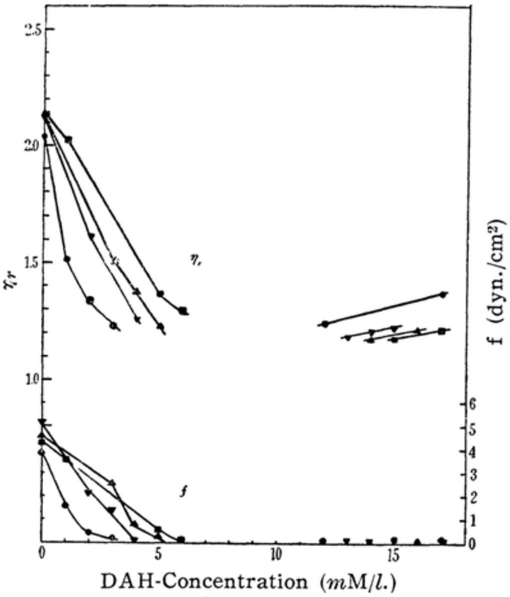


Fig. 3. Viscosity and yield value of gelatin solution as a function of pH. Solution: 0.5%, Temperature: 25°C.



● pH 6.9 ▼ pH 8.0 ▲ pH 9.3 ■ pH 10.6

Fig. 4a. Variation of viscocity and yield value of gelatin solution by addition of DAH. Solution: 0.5%, Temperature: 25°C.

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[11] E.C. Bingham, "Plasticity and Fluidity" New York, (1922) p. 222.

[12] H. Freundlich and H. Neukircher, *Kolloid-Z.*, **38**, 180 (1926).

[13] G. Stainsby, *Nature*, **169**, 662 (1952).

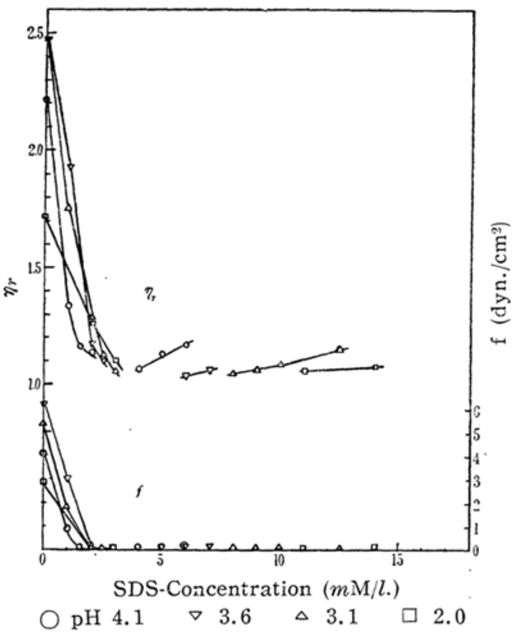

Fig. 4b. Variation of viscosity and yield value of gelatin solution by addition of SDS. Solution: 0.5%, Temperature: 25°C.

At the isoionic point, the macromolecule of gelatin is probably in the contracted form owing to the attractive forces between balanced charges, whereas, on both sides of the isoionic point, the molecule has a net charge, which may cause it to extend itself by repulsion. However, in more acidic or more basic solutions, the repulsive forces will be again reduced, owing to the increase of free ions in those solutions. The maximum of η_r and f on both sides of the isoionic point may be the result of the interplay of these two counter effects.

Fig. 4a shows how η_r and f of the solution change by the addition of DAH within certain range of detergent concentrations either before the precipitation or after the redissolution. Fig. 4b shows the similar change of η_r and f in the case of gelatin-SDS mixtures. In both cases, we find such a tendency that the values of η_r and f are greatly reduced as detergent is added until precipitation takes place and that, after redissolution of the precipitate initially formed, the mixture exhibits nearly Newtonian flow. The mechanism of the phenomena may be considered as follows. The more or less expanded gelatin molecule either at the acidic or alkaline side of the isoionic point may be changed into the folded, hydrophobic state by the continuous combination of detergent ions and consequently η_r and f decrease until precipitation takes place. On addition of excess detergent, however, the second adsorption layer of detergent ions will be formed by van der Waals attraction force between carbon chains, which makes the molecule hydrophilic and accordingly the precipitate once formed dissolves again into solution. The solution thus formed behaves itself similarly to the solution at the isoionic point without detergent.

It is remarkable that the relative viscosity of the mixed solutions, directly before the precipitate formation or directly after the dissolution of the precipitate, is, somewhat less than that of the solution at the isoionic point. This suggests that the gelatin molecule in such mixtures may be in more folded state than the molecule at the isoionic point. It is further noticed from our experiment that SDS reduces the viscosity of the gelatin solution more effectively than DAH does and consequently that SDS is more effective than DAH in precipitating gelatin from the solution. This agrees with the general character of proteins that they interact more strongly with anions than with cations.

Furthermore, the combination of detergent ions with cationic or anionic groups of the macromolecules of gelatin would have taken place mainly at the side chains and probably not at the backbone in the case of our experiment, because the interaction was studied in absence of any other inorganic salts except that very small amount of sodium chloride formed by the neutralization of detergent ion with HCl or NaOH.

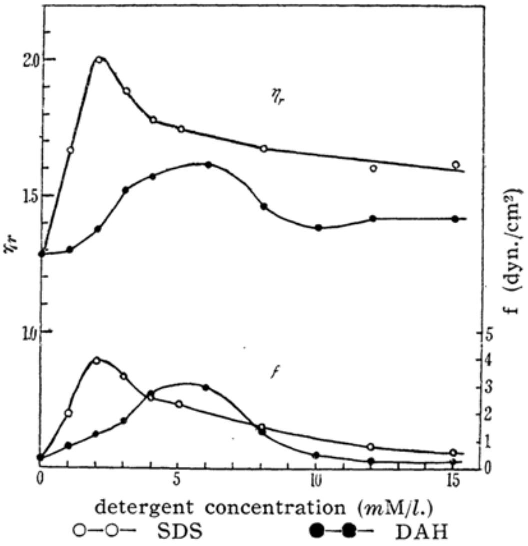

Fig. 5. Variation of viscocity and yield value of gelatin solution by addition of detergent at isoionic point. Solution: 0.5%, Temperature: 25°C.

Lastly, Fig. 5 indicates the change of η_r and f of the mixture in relation to the added DAH or SDS at the isoionic point of gelatin. As this figure shows, both η_r and f increase first with the addition of detergent, attain maxima and then decrease. Comparing these curves of η_r or f in this figure with those in Fig. 3, which show the change of η_r or f due to the addition of hydrogen ion or hydroxy ion to isoionic gelatin, we find a remarkable resemblance in the features of the corresponding curves. This suggests that the effect of detergent cations or anions upon gelatin bears a resemblance to that of hydrogen ion or hydroxy ion upon the same gelatin. It is thus quite presumable that either detergent cations or anions combine with a neutral gelatin molecule to some extent to give it net charges and consequently cause the molecule to extend itself. The appearance of maximum and decrease of η_r or f with increasing detergent concentration is probably due to the screening effect of counter ions.

Summary

The action of dodecyl amine hydrochloride and sodium dodecyl sulphate on gelatin was studied at various hydrogen ion concentrations of the mixed solution, once by determining the combined detergent ions to the precipitated gelatin and then measuring flow properties of mixed solutions.

The determination of the combined detergent ions showed that the complex formation between detergent and gelatin should have taken place according to the mechanism proposed by Pankhurst and his co-workers.

The non-Newtonian flow character of gelatin solution at pHs on both sides of the isoionic point, was greatly influenced by the addition of detergent cations or anions and the flow of the mixed solutions directly before the precipitate formation or after the precipitate dissolution became approximately Newtonian, which suggested that the gelatin molecule would have been brought into a more folded state by the successive combinations of detergent ions.

The viscosity coefficient as well as yield value of the gelatin solution at the isoionic point were found to increase by the addition of detergent cations or anions. This suggested that the folded isoionic gelatin molecule would have been expanded by the combination with either one of the detergent ions.

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