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Studies on Microcapsules. XI. Electrophoretic Behavior of Polyphthalamide Microcapsules Containing Aqueous Solution of Bovine Serum Albumin

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Electrophoretic measurements were made on polyphthalamide microcapsules containing aqueous solution of bovine serum albumin and their membrane at various hydrogen ion concentrations. The microcapsules moved toward the anode at any pH, while the membrane migrated to the anode above pH 3.5 and to the cathode below this pH. These results were explained on the assumption that a fraction of albumin molecules is chemically incorporated into the microcapsule membrane through the microencapsulation and that the net electrical charge of the microcapsules is highly dependent on the charge of the outer surface of their membrane.

A previous paper of this series[1] has reported the electrophoretic behavior of polyphthalamide microcapsules containing aqueous solution of various polyelectrolytes. The microcapsules containing anionic or cationic polyelectrolyte, such as sodium heparinate or poly(1,2-dimethyl-5-vinyl pyridinium methyl sulfate), were found to move towards the anode or cathode according to the sign of charge on the polyelectrolyte encapsulated. When the microcapsules contained aqueous solution of amphionic polyelectrolyte, such as 2-methyl-5-vinylpyridine methyl acrylate-methacrylic acid copolymer, they migrated either to the anode or the cathode depending on the pH of the medium, showing the existence of an isoelectric point.

1) M. Shiba, Y. Kawano, S. Tomioka, M. Koishi, and T. Kondo, *Kolloid-Z. Z. Polym.*, submitted.

On the other hand, it was strongly suggested that bovine serum albumin, a typical amphoteric polyelectrolyte, participates in the interfacial polycondensation reaction between diamine and acid dichloride because albumin molecules, contrary to the amphionic polyelectrolyte molecules used in the previous paper,[1] have many amino groups which are able to react readily with acid chloride.[2] If it is really the case, a different electrophoretic behavior may be expected for microcapsules containing aqueous solution of bovine serum albumin from that for those containing aqueous solution of the polyampholyte.

This paper describes the experimental results on the electrophoresis of polyphthalamide microcapsules containing aqueous solution of bovine serum albumin and their possible implications.

Experimental

Preparation of Microcapsules. Polyphthalamide microcapsules containing aqueous bovine serum albumin solution used in this work were prepared by the same method as described in the previous paper[2] by making use of the interfacial polycondensation reaction between terephthaloyl dichloride and piperazine, and dispersed in water with the aid of dispersing agent. The microcapsule dispersion so obtained was dialyzed in a cellulose tubing (Visking) against distilled water at 40°C with shaking until a constant specific conductance of the dispersion was attained in order to remove any remaining ionogenic impurities. The dialyzed microcapsule dispersion could be conveniently stored in a refrigerator at 2—4°C before use. A fraction of the stored microcapsules was broken by subjecting to a high centrifugal field. This was done because it was expected that there may be a difference in surface charge between the outside and the inside of the membrane in view of the possibility that some of the albumin molecules encapsulated participate in the interfacial polycondensation reaction. The membranes thus obtained was washed and dispersed in buffer solution.

Measurement of Electrophoretic Mobility. Electrophoretic measurements of the microcapsules and their membrane were taken in a quartz flat microelectrophoretic cell. The media for the measurements were acetate buffer (pH 3—6) and HCl-sodium acetate buffer (pH 2—3), the ionic strength of which was maintained at 0.01 by the addition of NaCl throughout the measurement unless otherwise stated. For each measurement 40 specimens were timed in each direction to eliminate the polarization effect of the electrodes. Mobilities were taken at room temperature and corrected to water at 25°C.

Calculation of Zeta-Potential. At first sight, mobilities seemed to be convertible into zeta-potentials (ζ) by means of the simple Smoluchowski-Henry equation,[3]

$$\zeta = \frac{f \cdot \pi \cdot \eta}{D \cdot X} \cdot U$$

where U is the mobility, η the viscosity of the medium, D the dielectric constant, X the potential gradient, and f the numerical factor depending on the product of the reciprocal thickness of double layer and the particle diameter. However, some correction was necessary because the surface conductance was found to be of the order of $10^{-7}\Omega^{-1}$ which could not be neglected in the calculation. Zeta-potentials of the microcapsules were, therefore, calculated by the Henry equation,[4]

$$\zeta = \frac{6 \cdot \pi \cdot \eta}{D \cdot X} \cdot U[1 + \lambda'\{3f(\kappa a) - 2\}]$$

where κ represents the reciprocal thickness of double layer, a the microcapsule diameter, and $f(\kappa a)$ a function of κa. λ' is given by the following equation,

$$\lambda' = \frac{\mu_0 - \frac{2\mu_s}{a}}{2\left(\mu_0 + \frac{\mu_s}{a}\right)}$$

where μ_s is the surface conductance and μ_0 the specific conductance of the suspending medium. The surface conductance was evaluated by the method proposed by Fricke and Curtis.[5]

The Henry equation was also used for calculating the zeta-potential of microcapsule membrane. In this case, the value of a was assumed to be the same as the average diameter of the microcapsules though the shape of the membrane was not necessarily spherical. Therefore, the zeta-potential of the membrane should be regarded as being apparent.

Results and Discussion

Figure 1 shows the change in the specific conductance of the microcapsule dispersion during dialysis in a cellulose tubing against the volume of distilled water used. As the ionogenic impurities diffused out through the microcapsule membrane and the cellulose tubing, the specific conductance of the microcapsule dispersion decreased and then levelled off. The final value of specific conductance increased with increasing concentration of albumin encapsulated, being presumably due

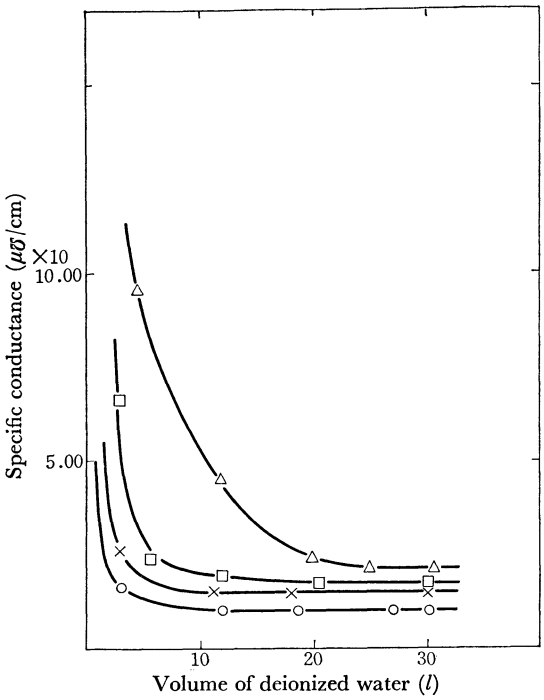

Fig. 1. Variation in specific conductance during dialysis against water of microcapsule dispersions.
Albumin Concentration:
(○) 0.5%, (×) 1%, (□) 2%, (△) 5%

2) M. Shiba, S. Tomioka, M. Koishi, and T. Kondo, *Chem. Pharm. Bull. (Tokyo)*, **18**, 803 (1970).
3) D. C. Henry, *Proc. Roy. Soc. London*, **133**, 106 (1931).
4) D. C. Henry, *Trans. Faraday Soc.*, **44**, 1021 (1948).
5) H. Fricke and H. J. Curtis, *J. Phys. Chem.*, **40**, 715 (1936).

to the dissociation of albumin molecules in water.

The microcapsule dispersions used in this work were those having the final specific conductance given in the figure.

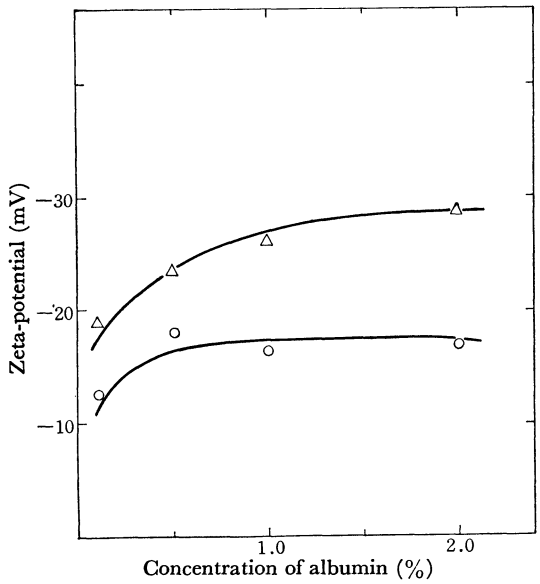

Fig. 2. Zeta-potential of microcapsules containing aqueous albumin solution as a function of albumin concentration in acetate buffers.
(○) pH 4.7, (△) pH 5.7

The zeta-potential of the microcapsules is given in Fig. 2 as a function of the concentration of albumin solution used in their preparation. The potential increased as the concentration of albumin increased and then became nearly constant above a concentration of 1% albumin irrespective of the pH of the medium. This tendency is quite similar to that observed for the microcapsules containing aqueous polyelectrolyte solution.[1)]

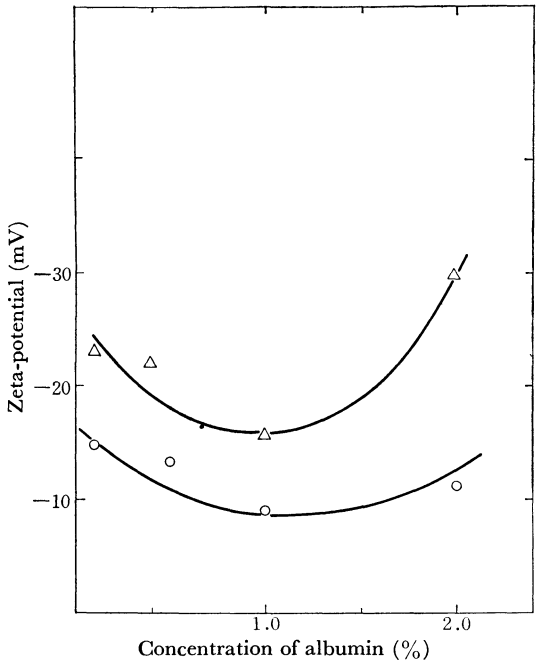

Fig. 3. Zeta-potential of microcapsule membrane as a function of albumin concentration used for preparing microcapsules in acetate buffers.
(○) pH 4.7, (△) pH 5.7

In Fig. 3 is plotted the zeta-potential of microcapsule membrane against the concentration of albumin solution. In contrast to the microcapsule themselves, the membrane showed a minimum zeta-potential when the albumin concentration varied. The appearance of the minimum may be interpreted as follows. An increase in the albumin concentration will increase the number of albumin molecules chemically incorporated in the membrane, causing an increase in the number of dissociable carboxyl groups. The increase in the number of dissociable carboxyl groups will facilitate the fixation of counter ions, thereby lowering the zeta-potential. However, it may outweigh the fixation of counter ions at high albumin concentration, resulting in an increase of negative charge of the microcapsule membrane.

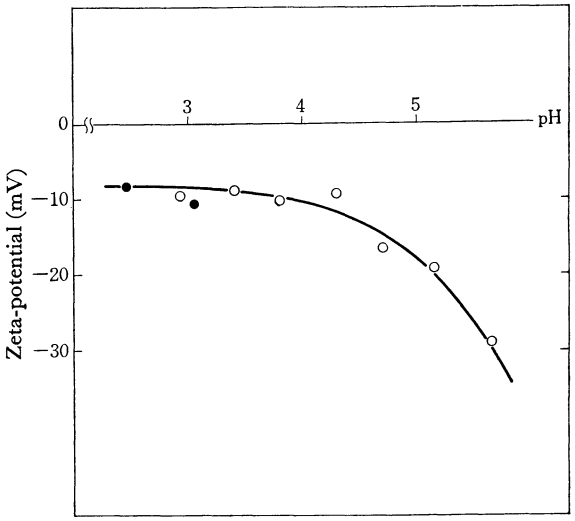

Fig. 4. Zeta-potential of microcapsules containing aqueous 1 wt% albumin solution as a function of pH in CH_3COONa–CH_3COOH (○) and CH_3COONa–HCl (●) buffers at a constant ionic strength of 0.01.

Figure 4 illustrates the zeta-potential *versus* pH curve of the microcapsules. They were negatively charged at all pH, even at lower pH than that of the isoelectric point of albumin (pH 4.8). This differs from what has been reported in the previous paper[1)] in that the microcapsules do not necessarily bear a charge of the same sign as that of albumin molecules encapsulated. The polyphthalamide microcapsules containing aqueous solution of cationic or anionic polyelectrolyte always moved towards the cathode or anode in an electric field in accordance with the sign of charge on the encapsulated polyelectrolyte.[1)]

The dependence of apparent zeta-potential of the microcapsule membrane on the pH of the medium is shown in Fig. 5. What is evident from this figure is a charge reversal of the membrane occurred in the vicinity of pH 3.5, indicating the existence of isoelectric point. This should be related to the chemical incorporation of albumin molecules into the membrane through the reaction with acid chloride.[2)]

Of the dissociable groups of albumin, the carboxyl groups of aspartic and glutamic acids give negative

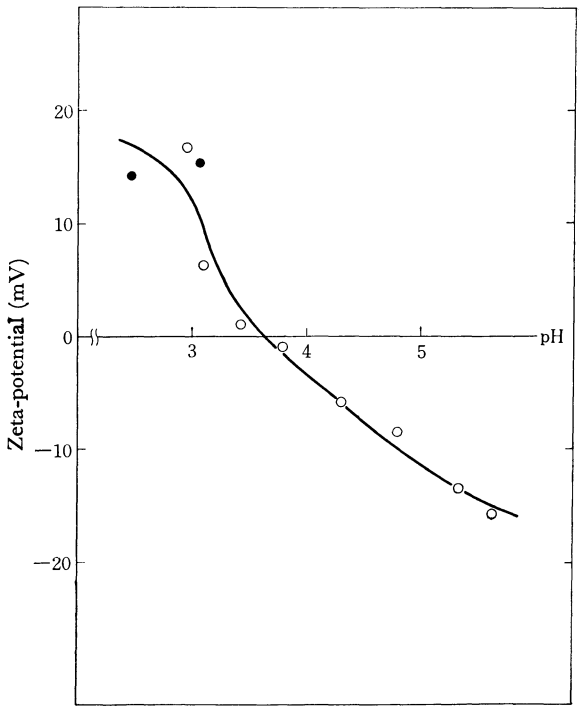

Fig. 5. Zeta-potential of microcapsule membrane as a function of pH in CH_3COONa–CH_3COOH (○) and CH_3COONa–HCl (●) buffers at a constant ionic strength of 0.01.

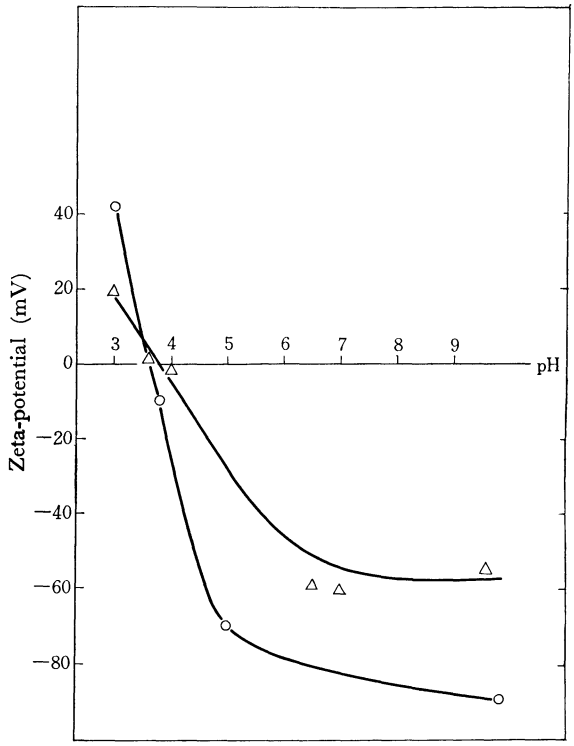

Fig. 6. Zeta-potential of microcapsules containing aqueous 1 wt% MPM solution as a function of pH in acetate buffers. Ionic strength: (△) 0.01 and (○) 0.001

charge (the dissociation of the hydroxyl groups of tyrosine and the sulfhydryl groups of cystein may also contribute to the negative charge at high pH) while the amino groups of lysine and arginine, and imino groups of histidine can produce positive charge. In view of this and the pK of each group,[6] it may be assumed that about 60% of the amino groups of lysine and arginine have reacted with acid chloride to shift the isoelectric point of the chemically incorporated albumin from pH 4.8 to 3.5.

As to the albumin molecules in the encapsulated solution, preliminary tests were made to check whether they were modified or not during the microencapsulation process. The Ouchterlony method,[7] which is frequently used in immunochemistry, and paper-electrophoresis proved that the albumin molecules are not subjected to any detectable changes immunochemically or electrophoretically during the microencapsulation process as compared with native albumin molecules.

Before making an attempt to elucidate the difference in the pH dependence of zeta-potential between the microcapsules and their membrane, an examination is made of the electrophoretic behavior of polyphthalamide microcapsules containing solution of a copolymer of 2-methyl-5-vinylpyridine-methyl acrylate and methacrylic acid (Fig. 6). This polymer (abbreviated as MPM) is practically insoluble in water in the range of pH 4—7.5.[8] However, the polymer is readily soluble in water outside of this pH region and has a net negative charge above pH 7.5 and a net positive charge below pH 4. Moreover, the polymer is unable to react with acid chloride. As is seen from the figure, the microcapsules containing aqueous MPM solution are negatively charged above pH 7.5 and positively charged below pH 4. It may be inferred, therefore, that the sign of charge of microcapsules is determined solely by that of amphoteric polyelectrolyte unless the membrane acquires electrical charge by chemically incorporating the polyelectrolyte.

TABLE 1. SIGN OF CHARGE OF ALBUMIN MOLECULES, MICROCAPSULES, AND THEIR MEMBRANE

Species	pH region		
	Below 3.5	3.5—4.8	Above 4.8
Encapsulated albumin	+	+	−
Microcapsules	−	−	−
Microcapsule membrane	+	−	−

In Table 1 are summarized the sign of charge of the encapsulated albumin, microcapsules, and their membrane in three pH regions. When the pH of the medium was above 4.8, all of the encapsulated albumin molecules, microcapsules, and their membrane were negatively charged. In the region of pH 4.8—3.5, the microcapsules and their membrane had a negative charge while the albumin molecules bore a positive charge. This may result from the screening of the positive charge of albumin molecules in the encapsulated solution by the negative charge of the membrane. Very curiously, however, the microcapsules were negatively charged below pH 3.5 in spite of

6) J. Steinhart and S. Beychok, "The Proteins," Vol. 2, Academic Press, New York (1964), p. 162.
7) O. Ouchterlony, *Pror. Allergy*, **5**, 1 (1958).
8) T. Ida, S. Kishi, S. Takahashi, and I. Utsumi, *J. Pharm. Sci.*, **51**, 1061 (1962).

positive charges on the encapsulated albumin molecules and the membrane. This makes it necessary to consider the distribution of charged sites in the membrane.

It is evident that polyphthalamide molecules constituting the microcapsule membrane form and grow on the organic solvent side of the organic solvent-water interface.[9] The presence of albumin molecules in the water phase to be encapsulated will not change the features of the polycondensation reaction except that albumin molecules may participate in the polymerization. The amino groups of albumin molecules are very likely to react at the interface with acid chloride molecules coming from the interior of the organic phase to form amide linkages since albumin molecules can spread in a more expanded state at the oil-water interface than at the air-water interface[10] with the amino groups directing towards the oil phase.[11] As the polycondensation reaction proceeds, the amount of acid chloride reaching the interface will decrease with time and thereby will increase the number of amino groups remained unreacted of the following albumin molecules. The number of dissociable amino groups in the microcapsule membrane will increase, therefore, in the direction from the outside to the inside of microcapsules whereas the dissociable carboxyl groups are uniformly distributed in the membrane. As the result, the negative charge on the outer surface of the membrane may screen and surpass the positive charge on the inner surface and albumin molecules in the encapsulated solution. Based on this assumption, the positive zeta-potential observed for the microcapsule membrane below pH 3.5 (Fig. 5) may be interpreted as indicating that the breakdown of the microcapsules by centrifugation turned their membrane inside out, probably because the inner surface is likely to be more hydrophilic than the outer surface, thus making prevail the positive charge due to the amino groups of albumin incorporated in the membrane over the negative charge arising from the dissociation of the carboxyl groups.

9) P. W. Morgan and S. L. Kwolek, *J. Polymer Sci.*, **40**, 299 (1959).
10) A. E. Alexander and T. Teorell, *Trans. Faraday Soc.*, **35**, 727 (1939).
11) J. T. Davis, *Biochem. J.*, **56**, 509 (1954).