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# Studies on Microcapsules. XIX.[1] Effect of Phospholipid Treatment on Cation Uptake by Carboxylated Polyphthalamide Microcapsules

Kazuo Kamei[2a] and Tamotsu Kondo[2b,c]

*Research Laboratory, Banyu Pharmaceutical Company, Ltd.[2a] and Faculty of Pharmaceutical Sciences, Science University of Tokyo[2b,c]*

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Poly (phthaloyl L-lysine) microcapsules were prepared by interfacial polymerization techniques and treated by lecithin. Various mono-, di-, and trivalent cations were found electrophoretically to be bound by both intact and lecithin-treated poly (phthaloyl L-lysine) microcapsules dispersed in aqueous media. In both cases, cation binding was stronger with ions of higher valency and charge reversal of the microcapsules was observed at high concentrations of polyvalent cations. However, the treatment of the microcapsules with lecithin caused appreciable changes in the zeta-potential and cation binding of the microcapsules. That is, the zeta-potential was less negative and the cation concentration needed to produce the charge reversal was lower for the lecithin treated microcapsules than for the intact microcapsules. The zetapotential was converted into the surface charge density by means of a modified Gony-Chapman equation. Finally, the free energy of cation binding was evaluated from the surface charge density as a function of the cation concentration using Stern's adsorption theory.

The exact molecular organization of natural capsular membranes of biological cells is still unknown. However, it is already well established that proteins and lipids are major components of biological cell membranes. It is also known that the surface of most biological cells has a net negative charge in aqueous media. The most important functions of the membranes are those of protecting the interior material from the environment and controlling the flow of materials across the membrane.

Meanwhile, many attempts have been made to prepare artificial capsular membranes possessing the two functions by utilizing various techniques of microencapsulation.[3] Of the microencapsulation methods so far tested, the interfacial polymerization method seems to be the most promising one because it can provide us with various microcapsules consisting of a large variety of synthetic polymers, which have a diameter of the order of micrometers and a wall thickness in the nanometer range.

Poly (phthaloyl L-lysine) microcapsules prepared by making use of the interfacial polycondensation reaction between L-lysine, a basic amino acid, dissolved in water and *p*-phthaloyl dichloride dissolved in an organic solvent have been found to be negatively charged in aqueous media due to ionization of the carboxyl groups of lysine residues of the polyamide.[4] Although the microcapsules have, of course, the two functions which are prerequisite to any model membrane, it is still desirable to make their physicochemical properties more similar to those of natural biological cell membranes. In this paper, therefore, an attempt is made to coat the microcapsules with lecithin, a component of many biological cell membranes, and to see the effect of the coating on cation binding to the microcapsules.

## Experimental

**Materials**——Reagent grade L-lysine monohydrochloride and *p*-phthaloyl dichloride were used for preparation of poly (phthaloyl L-lysine) microcapsules.

1) Part XVIII: K. Takahashi and T. Kondo, *Colloid & Polymer Sci.*, in press.
2) Location: *a*) *Meguro-ku, Tokyo*; *b*) *Shinjuku-ku, Tokyo*; *c*) To whom all inquiries should be directed.
3) T.M.S. Chang, "Artificial Cells," Thomas, Springfield, 1972.
4) Y. Shigeri, M. Tomizawa, K. Takahashi, M. Koishi, and T. Kondo, *Can. J. Chem.*, **49**, 3623 (1971).

The phospholipid used for treatment of microcapsules was egg yolk lecithin of guaranteed reagent grade.

Chlorides of sodium, magnesium, calcium, barium, strontium, aluminum, and lanthanum were employed for electrophoresis measurements. All of these salts were of reagent grade.

**Preparation of Microcapsules**——Poly (phthaloyl L-lysine) microcapsules (PPLM) were prepared in a way similar to that described in the earlier paper.[4]

Ten milliliters of aqueous solution of 0.4 M L-lysine monohydrochloride in 0.65 M sodium carbonate solution were mechanically dispersed into 100 ml of mixed solvent (cyclohexane–chloroform 3: 1, v/v) maintained at −10° and containing 20% (v/v) sorbitan trioleate as an emulsifier to yield a W/O emulsion.

Without stopping agitation, 100 ml of 0.04 M *p*-phthaloyl dichloride in the mixed solvent was added to the emulsion and the agitation was further continued for 3 min. In this process, the surface of water droplets was covered with poly (phthaloyl L-lysine) membrane formed by the polycondensation reaction between L-lysine transferred from the aqueous phase to tha organic phase and *p*-phthaloyl dichloride in the organic phase. Hydrogen chloride formed during the polycondensation reaction was neutralized by sodium carbonate in the aqueous phase. About 200 ml of cyclohexane were then added to the microcapsule dispersion thus obtained in order to slow down the reaction.

The microcapsules were settled down by gentle centrifugation (150 g) and the supernatant was discarded. The settled microcapsules were then dispersed in cyclohexane. The dispersion was centrifuged, followed by removal of the supernatant. By repeating this procedure several times, the emulsifier and the unreacted dichloride were removed completely.

The washed PPLM were suspended in deionized water and the resultant dispersion was dialyzed in a dialysis bag (Visking) against deionized water at room temperature with frequent shaking to remove remaining unreacted L-lysine and inorganic salts. The dialysis was continued until a constant electrical conductance was attained for the outer liquid of the dialysis bag. The dialyzed PPLM were found to have a mean diameter of about 3 μm and to be negatively charged in aqueous media due to dissociation of the carboxyl groups of lysine residues.

**Preparation of Lecithin Dispersion for Treating PPLM**——Lecithin dispersion for treating PPLM was prepared by Huang's method.[5] A small quantity of egg yolk lecithin was suspended in the HCl–$CH_3COO$-Na–NaCl buffer, and the resultant suspension was sonicated for 5 hr. at 3° under a nitrogen atmosphere. The sonicated suspension was then ultracentrifuged at 100000 *g* for 1 hr at 3°. After the centrifugation, the supernatant was filtered through a glass filter and used for treating PPLM.

**Treatment of PPLM by Lecithin**——Twenty milliliters of the dialyzed microcapsule dispersion were mixed with 80 ml of a HCl–$CH_3COONa$–NaCl buffer at 25°. To this dispersion were added 100 ml of the lecithin dispersion and the mixture was stirred for 20 min to allow the microcapsules to react with lecithin.

Determination of the amount of lecithin taken up by PPLM was made in the following way. The mixture of the microcapsule dispersion and the lecithin dispersion was filtered through a glass filter after the reaction time of 20 min which was sufficient to bring the uptake into an equilibrium. The concentration of lecithin in the filtrate was determined colorimetrically.[6] The difference between the concentrations of lecithin before and after the reaction with PPLM gave the amount of lecithin captured by the microcapsules.

**Electrophoresis of Intact and Lecithin-treated PPLM**——Electrophoretic mobility measurements on intact and lecithin-treated PPLM were carried out at 25° using a microelectrophoresis apparatus (Karl Zeiss). The movement of the particles was observed at the stationary level in a quartz flat microelectrophoresis cell. For each measurement, 20 microcapsules were timed in each direction to eliminate the polarization of the electrodes. Samples for mobility measurements were prepared by mixing an appropriate volume of the microcapsule dispersion with buffered electrolyte solutions. The electrolyte concentration was varied from $10^{-8}$ to 1 M. When the concentration was below $10^{-2}$ M, the ionic strength of the solutions was adjusted to be 0.01 by the addition of NaCl.

In view of the size of PPLM (*ca.* 3 μm in diameter) and the ionic strength of the dispersion medium (0.01 or higher), the mobility was converted into the zeta-potential, ζ, by means of the Helmholtz-Smoluchowski equation:

$$\zeta = \frac{4\pi\eta u}{D} \qquad (1)$$

where $u$ is the mobility of PPLM, and $\eta$ and $D$ are the viscosity and dielectric constant of the dispersion medium.

## Results and Discussion

### Lecithin Uptake by PPLM

The amount of lecithin taken up by PPLM was dependent on the pH of the dispersion

5) C. Huang, *Biochemistry*, **8**, 344 (1969).
6) E.J. King, *Biochem. J.*, **26**, 292 (1932).

Table I. Amount of Lecithin Captured by PPLM

| pH | Number of lecithin molecules captured by one microcapsule $\times 10^{-9}$ |
|---|---|
| 3.30 | 1.02 |
| 3.80 | 2.31 |
| 4.30 | 2.20 |
| 5.20 | 0.89 |

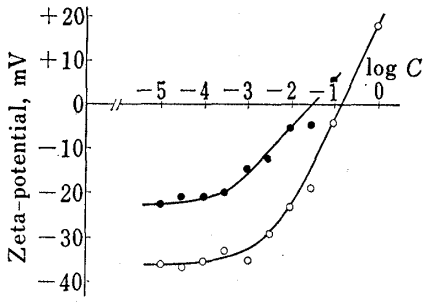


Fig. 1. Effect of $Mg^{2+}$ Concentration on Zeta-potential of PPLM at pH 4.0
○ : intact PPLM, ● : lecithin-treated PPLM

medium. Table I gives the number of lecithin molecules taken up per poly (phthaloyl L-lysine) microcapsule at several pH.

In view of the fact that the isoelectric point of egg yolk lecithin lies between pH 3.0—4.0,[7,8] it is interesting to note that the lecithin uptake by negatively charged PPLM shows a maximum around pH 3.8. This would indicate an important role in the uptake of hydrophobic bonding between the hydrocarbon chains of lecithin molecules and the hydrophobic parts of the polymers constituting PPLM though the electrostatic interaction of the positively charged choline groups of lecithin with the negatively charged carboxyl groups of the microcapsules should play a part.

Potentiometric titration data show that there are about $10^{11}$ lysine residues per poly (phthaloyl L-lysine) microcapsule and the degree of ionization of the carboxyl groups is about 0.2 at pH 4.0.[9] Accordingly, the maximum number of lecithin molecules bound to one microcapsule around this pH corresponds roughly to one fifth of the number of the ionized carboxyl groups.

**Zeta-potential**

The absolute value of the zeta-potential for both intact and lecithin-treated PPLM was found to decrease first and then increase after passing through the zero point of potential with increasing concentration of cations of added electrolytes, indicating the reversal of charge of the microcapsules at high cation concentrations. A remarkable valency effects was noted on the concentration of cation necessary to reverse the charge of PPLM; higher the valency, lower the charge reversal concentration. The lecithin treatment caused a reduction in the absolute value of the zeta-potential of PPLM when the cation concentration was lower than the value at the zero point of potential. It also caused a lowering in the cation concentration required to produce the charge reversal. Figure 1 shows a typical example of the zeta-potential *vs.* cation concentration curve at pH 4.0. In this figure, the abscissa refers to the logarithm of molar concentration of added cation.

In Fig. 2 is shown the effect of the pH of the medium on the zeta-potential *vs.* cation concentration relation for PPLM. Increase in the pH of the medium makes the zeta-potential of PPLM more negative since it enhances the dissociation of the carboxyl groups of the microcapsules. Nevertheless, the absolute value of the zeta-potential is lowered by the lecithin treatment.

These findings suggest strongly that the treatment of PPLM with lecithin causes a decrease in the surface charge density of the microcapsules.

7) M.C. Panghorn, *J. Biol. Chem.*, **188**, 471 (1951).
8) A. Watanabe, A. Fujii, Y. Sakamori, and A. Tamai, *Nippon Kagaku Zasshi*, **90**, 880 (1969).
9) K. Takahashi and T. Kondo, unpublished data.

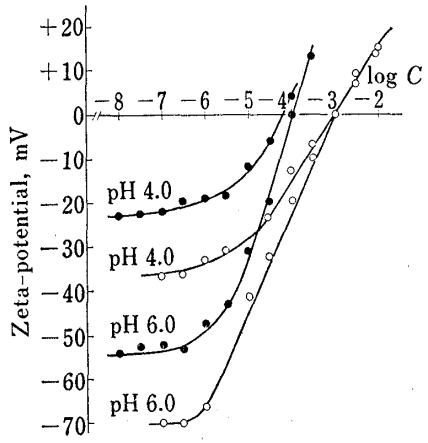


Fig. 2. Effect of pH on the Zeta-potential-$La^{+3}$ Concentration Relationship for PPLM

○ : intact PPLM, ● : lecithin-ttteaed PPLM

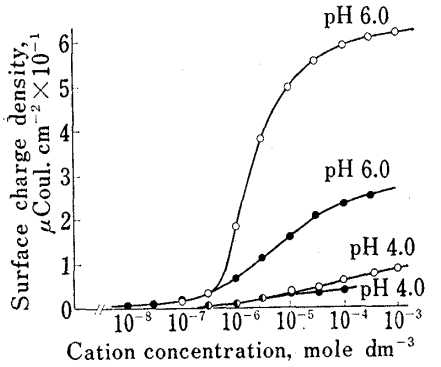


Fig. 3. Cation Binding Isotherms for $La^{+3}$ at pH 4.0 and 6.0, ○: intact PPLM

○ : imtact PPLM, ● : lecithin-treated PPLM

## Surface Charge Density

The zeta-potential was converted into the surface charge density in the electrokinetic plane of shear, $\sigma$, by a modified Gouy-Chapman equation for a flat double layer in the form[10,11]:

$$\sigma = (1+\sqrt{1-\alpha})\sqrt{\frac{2DkTNC}{1000\pi}}\sinh\frac{Ze\zeta}{2kT} \qquad (2)$$

where $C$ is the concentration of electrolyte in moles per dm$^3$, $D$ the dielectric constant of the medium, $N$ the Avogadro number, $k$ the Boltzmann constant, $T$ the absolute temperature, $Z$ the valency of the counter ion, and $e$ the electronic charge. The factor of $\alpha$ was introduced to take account for permeation of counter ions through the membrane. It is the fraction of the total space which is not available to counter ions in the surface membrane. In fact, observations of polyamide microcapsules by means of scanning electron microscopy indicate that they have a porous structure.[12,13] The value of $\alpha$ was assumed somewhat arbitrarily to be 0.5 in this work.

At virtually constant ionic strength, the reduction in the zeta-potential is a reflection of the fact that ion binding to the microcapsule surface is taking place. Now, we may regard the charge density of the microcapsule surface as the sum of the surface charge density of ions in the Stern layer and that of the diffuse double layer. On addition of a cation, in quantities insufficient to alter the ionic strength significantly, the surface charge density will remain constant but increased adsorption (cation binding) will occur in the Stern layer. Strictly speaking, the potential in the adsorption plane is the Srern potential, but this may probably be taken as equivalent to the zeta-potential at not too high values of potential. Then, taking $\zeta_0$ the potential corresponding to the initial surface charge density and $\zeta$ as corresponding to the surface charge density after addition of cation, we find from Eq (2).

$$\Delta\sigma = (1+\sqrt{1-\alpha})\sqrt{\frac{2DkTNC}{1000\pi}}\left(\sinh\frac{Ze\zeta_0}{2kT}-\sinh\frac{Ze\zeta}{2kT}\right) \qquad (3)$$

where $\Delta\sigma$ is the increase in the surface charge density in the Stern layer produced by cation binding. If we plot $\Delta\sigma$ against the concentration of an added cation, we have an adsorption isotherm. Typical adsorption isotherms are given in Fig. 3 where increased cation binding

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11) K. Takahashi, M. Koishi, and T. Kondo, *Kolloid-Z, u. Z. Polymere*, **251**, 232 (1973).
12) A.W. Jenkins and A.T. Florence, *J. Pharm. Pharmacol.*, **25**, *Suppl.*, **57**, (1973).
13) C.B. De Savigny and E.E. Ivy, "Microencapsulation," bd, Vandegaer, by Plenum, New York, 1974.

with increasing cation concentration is indicated. This figure also shows that the lecithin treatment causes a reduction in the amount of cation uptake by PPLM.

**Free Energy of Cation Binding**

Using Stern's adsorption theory,[14] in which the adsorption of cation is assumed to be essentially monomolecular onto widely spaced immonile noninteracting sites, we can deduce

$$\Delta\sigma = Zen = \frac{ZeN_a}{1+\frac{\exp(-\Delta G/kT)}{x}} \tag{4}$$

where $Z$ is the valency of cation, $e$ the electronic charge, $n$ the number of cations adsorbed per cm$^2$ on the surface of PPLM, $x$ the mole fraction of cation, $N_a$ the number of binding sites per cm$^2$ on the surface of the microcapsules, $\Delta G$ the electrochemical free energy of cation binding, $k$ the Boltzmann constant, and $T$ the absolute temperature. As $x=C/55.6$, if we put $\exp(\Delta G/kT)/55.6=K$, then we have

$$\Delta\sigma = \frac{ZeN_aCK}{1+CK} \tag{5}$$

This equation can be transformed to give

$$\frac{1}{\Delta\sigma} = \frac{1}{ZeN_a} + \frac{1}{CZeN_aK} \tag{6}$$

According to Eq. (6), a straight line will be obtained when $1/\Delta\sigma$ is plotted against $1/C$. Actually, the plot gave a linear relation in all cases studied in this work though not shown here. $N_a$ and $\Delta G$ can be evaluated from the intercept with the ordinate and the slope of the straight line. The free energy of cation binding was dependent on the valency of cation as shown in Table II, indicating ionic nature of the interactions between the anionic binding sites of intact and lecithin-treated PPLM and the cations. However, it was not affected by the lecithin treatment. The average number of binding sites per cm$^2$ at pH 4.0 was found to be $7.1\times10^{12}$ and $6.0\times10^{12}$ for intact and lecithin-treated PPLM, respectively.

Table II. Free Energy of Cation Binding to PPLM

| Cation | Intact PPLM $-\Delta G$ (kcal mole$^{-1}$) | Lecithin-treated PPLM $-\Delta G$ (kcal mole$^{-1}$) |
|---|---|---|
| $Mg^{2+}$ | 6.4[a] | 8.1[a] |
| $Ca^{2+}$ | 6.6[a] | 6.3[a] |
| $Ba^{2+}$ | 7.5[a] | 7.5[a] |
| $Sr^{2+}$ | 6.5[a] | 5.9[a] |
| $Al^{3+}$ | 8.9[a] | 9.2[a] |
| $La^{3+}$ | 9.0[a] | 8.9[a] |
| $La^{3+}$ | 10.1[b] | 9.5[b] |

a) evaluated at pH 4.0
b) evaluated at pH 6.0

In conclusion, it would be stated that lecithin molecules adsorb onto PPLM mainly through hydrophobic bonding to screen the negative charge of the microcapsules arising from ionization of the carboxyl groups of lysine residues. This is most evidenced by the facts that the amount of lecithin captured by the microcapsules shows a maximum at a pH around the isoelectric point of the phospholipid and that the number of binding sites for cation on the surface of the microcapsules is reduced by the lecithin treatment.

14) O. Stern, *Z. Elektrochem.*, **20**, 508 (1924).