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The Influence of Cross-Linking Time on the Adsorption Characteristics of Microcapsules Containing Activated Charcoal Prepared by Gelatin–Acacia Coacervation

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In this study, the effect of cross-linking time on the adsorption characteristics of microcapsules containing activated charcoal (ACMC) was investigated. The microcapsules were prepared by the gelatin–acacia coacervation procedure. Activated charcoal powder was used as an adsorbent, and creatinine as a model adsorbate. The following results were obtained. The adsorption rate on ACMC could be controlled by changing the cross-linking time of the coacervate. A stable semipermeable membrane was formed by gelatin–acacia coacervation at cross-linking times greater than 60 min. Accordingly, the oral administration of ACMC should be available as a supporting technique in the treatment of patients with renal failure.

Keywords——activated charcoal; adsorption; artificial kidney; creatinine; coacervation; cross-linking time; hemodialysis; microcapsule; molecular sieving effect; semipermeable membrane

The artificial kidney, which is based on the principle of hemodialysis,[1] is now widely used for the treatment of patients with renal failure. Despite the success of this treatment, there are some physical and economical disadvantages[2] in these hemodialysis procedures, because two or three therapies are needed per week. Furthermore, a long time is required for each dialysis. Thus, simpler and less costly artificial kidneys[3] are desirable.

In order to support hemodialysis, the use of adsorbents such as activated charcoal[4] or ion-exchange resins given orally is typical. However, there are some disadvantages to this simple dosage form of therapy since the adsorbents come into direct contact the nutriment[5] or the mucous membrane in the gastrointestinal tract. However, it is considered that these problems might be solved by placing activated charcoal (AC) inside semipermeable microcapsules.[6]

In this study, the adsorption characteristics of microcapsules containing activated charcoal (ACMC) were tested *in vitro* to assess the suitability of the capsules for medical use through oral administration. Such microcapsules were prepared by the gelatin–acacia coacervation procedure,[7] and the influence of cross-linking time on the adsorption rate was theoretically evaluated. In addition, the molecular sieving effect[8] of the semipermeable membrane of ACMC was experimentally investigated.

Experimental

Materials——Activated Charcoal powder G.R. (No 2186, Merck Co., Ltd.) was used as an adsorbent, and creatinine (Tokyo Kasei Kogyo Co., Ltd.) as a model adsorbate.

Gelatin (Japan Leather Co., Ltd.) used had the following characteristics: molecular weight (M_r), 4.6×10^4; isoelectric point (pI), 6.3; the melting point of a 10% solution, 28 °C. Acacia (Kanto Chemical Co., Ltd.), which had an ash content of 3.2%, was used as a 10% solution.

To investigate the molecular sieving effect of these microcapsules, L-thyroxine sodium salt (M_r=798.5 Nakarai Chemicals Ltd.), β-cyclodextrin (M_r=1135 Wako Pure Chemical Industries, Ltd.), ovalbumin (M_r=45000 Tokyo

Kasei Kogyo Co., Ltd.), and bovine serum albumin (M_r=68000 Sigma Chemical Co.) were also used.

Specific Surface Area of Activated Charcoal——Activated charcoal powder was treated under nitrogen gas at 600 °C. After being washed with double-distilled water, the powder was dried at 110 °C for 24 h. This activated charcoal (100 mg) was placed in creatinine solution of various concentrations between 0.5 and 60 mol/m^3. The mixture was incubated at 37 °C, for 2 h, and the equilibrium concentration of creatinine was determined by the Folin–Wu method.[9] The procedure was as follows: 3 ml of deproteinizing agent (Creatinine-Test Wako Kit) was added to the supernatant of suspension at 37 °C. After 10 min, the suspension was centrifuged at 2500 rpm for 10 min, and 2 ml of solution was pipetted from the upper phase. To this solution, 1 ml of hygeric acid reagent and 1 ml of standarized 0.5 N sodium hydroxide were added, and the samples were kept at 37 °C for 20 min in a water bath. The creatinine content of the solution was measured with the spectrophotometer at 520 nm. Finally, the equilibrium concentration of creatinine was calculated from a standard curve.

Microencapsulation of Activated Charcol——The procedure for microcapsule preparation[10] is schematically shown in Fig. 1. The microcapsules were prepared by gelatin–acacia coacervation. In these experiments, gelatin and acacia solutions were prepared by dissolving 1.5 g of gelatin and 1.5 g of acacia each in 15 ml of purified distilled water. Activated charcoal (2 g) was suspended in gelatin solution at 40 °C and the suspension was agitated with a magnetic stirrer at 1000 rpm for 10 min. An equal amount of acacia solution at 40 °C was gradually added to the suspension, and stirring was continued for 10 min. Then 70 ml of distilled water was added, and the suspension was maintained at 40 °C for 15 min. The pH of the mixture was gradually adjusted to the desired pH (3.0—4.0) by dropwise addition of 3.5 ml of 10% acetic acid. At this point, coacervation occurred, and a gelatin coating was formed on the surface of the activated charcoal. This mixture was cooled to 5 °C by immersion in a water bath.

The coacervates were gradually hardened with 0.5 ml of form aldehyde solution. Next, the gelatin and acacia were strongly cross-linked on the surface of the activated charcoal, when the pH of the mixture was increased above 9 by addition of 10% sodium hydroxide. Stirring continued at 50 °C for 10 min, then the hardened coacervates were filtered off and washed with distilled water. The wet mass was added to acetone solution, and the sediment was filtered off and dried at room temperature for 24 h to yield activated charcoal microcapsules having a semipermeable membrane.

In these experiments, the cross-linking times used were 15, 60, 120 and 240 min.

Measurement of the Size and Shape of ACMC——The shape and surface morphology of the microcapsules after coaervation were examined under scanning electron microscope (model JSM T-200, JEOL Ltd.). Dry samples were coated with gold film approximately 30 nm thick under a high vacuum. Maximum magnification was 10000X.

The size distribution and mean diameter of ACMC were determined with a CAPA-500 particle analyzer (Horiba Co., Ltd.).

Measurement of Adsorption Characteristics of ACMC——The flow apparatus[11] used to measure the amount of adsorbate present on the ACMC is illustrated in Fig. 2.

Microcapsules containing 0.1 g of activated charcoal or the same amount of the powder were placed in the center of a cylindrical column (1.0 cm diameter and 10 cm length). Both sides of the column were filled with glass wool and connected to polyvinyl tubing. A 1.8×10^{-6}% (w/v) creatinine solution in double-distilled water was continuously circulated through the column by means of an RV-2 type roller pump (Furue Science Co.) at a flow rate of 1.0 ml/min at 37 °C. A half milliliter of sample solution was pipetted out at 5, 15 and 30 min, and 1, 2, 4, 8 and 24 h after the start

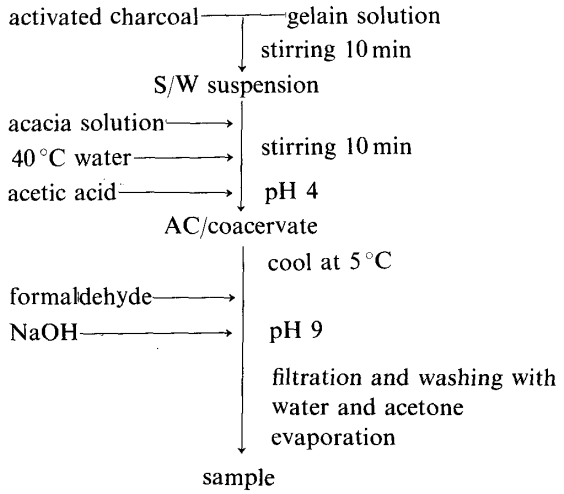

Fig. 1. Schematic Diagram of the Preparation of Microcapsules (ACMC) Containing Activated Charcoal by the Coacervation Method

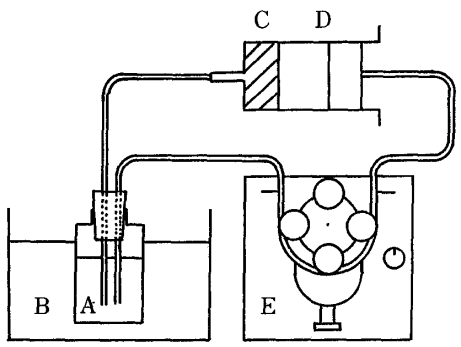

Fig. 2. Schematic Diagram of the Circulation Column Methods for Adsorption Experiments

A, creatinine solution; B, water bath; C, AC or ACMC; D, column; E, roller pump.

of recirculation, and the adsorbed amount of creatinine was calculated from the decrease of creatinine concentration in the solution.

The adsorbed amounts of L-thyroxine sodium salt, cyclodextrin, ovalbumin, or bovine serum albumin were similarly measured.

Measurement of the Stability of ACMC——The stability of ACMC was evaluated in terms of the disintegration ratio of ACMC.[12] ACMC were incubated in the standard first buffer solution (pH = 1.2) at 37 °C for 2 h, and the disintegration ratio of ACMC was determined by the spectrophotometric method.

Results and Discussion

Specific Surface Area of Activated Charcoal

The relationship between the amount of liquid physically adsorbed on a solid and the equilibrium concentration at constant temperature were established by Langmuir. The Langmuir adsorption isotherm is given by the following equation,[13]

$$Q=\frac{aQ_sC}{1+aC} \tag{1}$$

where Q is the amount of liquid adsorbed on a solid, (mol/g), Q_s is the equilibrium amount of liquid adsorbed on a solid (mol/g), C is the equilibrium concentration of liquid (mol/cm^3), and a is a constant. The amount of creatinine adsorbed per unit mass of activated charcoal (adsorbent) at 37 °C is plotted on the vertical axis against the equilibrium concentration of creatinine on the horizontal axis in Fig. 3-(a).

By inverting Eq. 1 and multiplying through by C, it may be written for plotting as

$$\frac{C}{Q}=\frac{1}{Q_s}C+\frac{1}{aQ_s} \tag{2}$$

Fig. 3-(b) shows the Langmuir adsorption isotherm plot of creatinine on the activated charcoal. A plot of C/Q against C should yield a straight line, and Q_s can be obtained from the reciprocal slope. The equilibrium amount of creatinine adsorbed on the activated charcoal, Q_s, was 1.73×10^{-3} (mol/g) in this experiment. If Q_s is known, the specific surface area, S_s (cm^2/g) of the activated charcoal can be calculated from the next Eq.,[14]

$$S_s=S_aNQ_s \tag{3}$$

where S_a is the adsorption area occupied by a creatinine molecular, and N is Avogadro's number. Substituting $S_a=34.7\times10^{-16}$ (cm^2) into the Eq. 3, it can be calculated that the specific surface area, S_s is approximately 360 (m^2/g) for the activated charcoal used in this experiment.

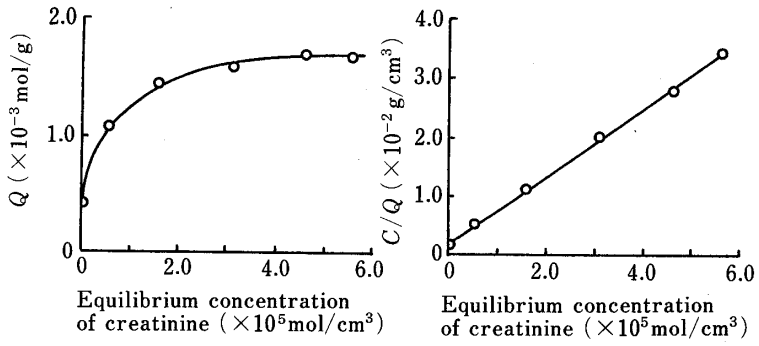

Fig. 3. Adsorption Isotherms of Creatinine on Activated Charcoal (AC) at 37 °C

(a) Amount of creatinine adsorbed per unit mass of activated charcoal plotted against the concentration of creatinine.

(b) Langmuir adsorption isotherm plot of creatinine on the activated charcoal.

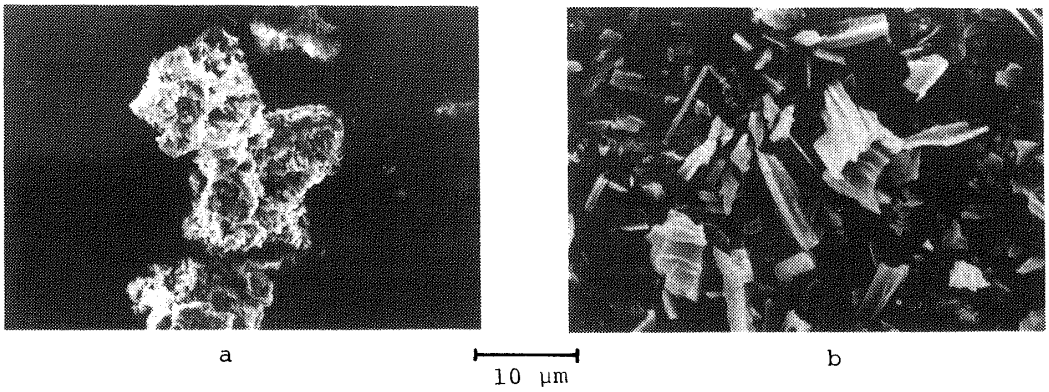

Fig. 4. Scanning Electron Micrographs of Microencapsulated and Uncoated Activated Charcoal

(a) Microencapsulated activated charcoal (ACMC).
(b) Uncoated activated charcoal (AC).

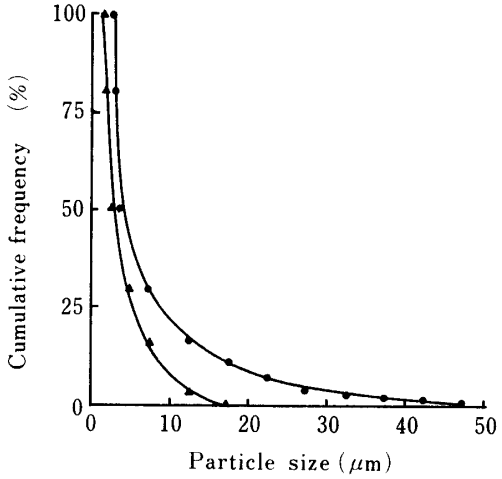

Fig. 5. Cumulative Curves of Particle Size Distribution

▲, activated charcoal (dp = 2.95 μm); ●, microcapsules containing activated charcoal (dp = 3.54 μm), cross-linking time = 120 min.

Shape and Size of ACMC Prepared by Gelatin–Acacia Coacervation

Scanning electron micrographs of microencapsulated and uncoated activated charcoal are presented in Fig. 4. The ACMC prepared in this procedure were mostly spherical and the surface was smooth. It is apparent from Fig. 4 that the activated charcoal was densely covered with gelatin–acacia coacervates, and a semipermeable membrane having many small holes was formed on the surface of the charcoal.

Cumulative curves of particle size distribution measured with a particle analyzer are shown in Fig. 5. Significant differences in the size distribution curves were found between the two samples. The mean diameter of microcapsules (dp = 3.54 μm) is larger than that of uncoated activated charcoal powder (dp = 2.95 μm). The thickness of the microcapsule wall can be calculated from the difference in mean diameter between ACMC and AC, and was about 245 nm.

Influence of Cross Linking Time on the Adsorption Profile of Creatinine on ACMC

Figure 6 shows the influence of cross-linking time on the adsorption profile of creatinine on ACMC. The adsorption rate of creatinine on AC is larger than that on ACMC. As the cross-linking time increases, the adsorption rate of creatinine decreases because the microcapsules prepared at coacervation longer times have smaller size pores at their surfaces for diffusion into the surrounding activated charcoal. In the preliminary experiment, we confirmed that there was no adsorption of creatinine on the membrane of gelatin–acacia

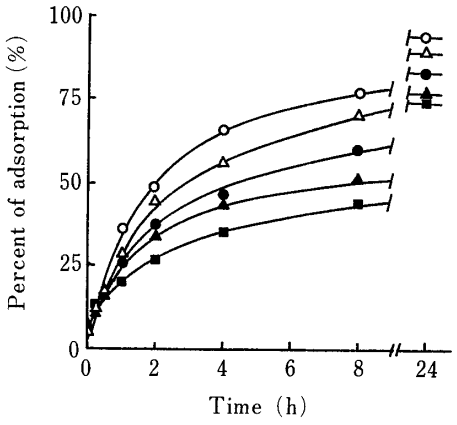

Fig. 6. Effect of Cross-Linking Time on the Adsorption Pattern of Creatinine on ACMC or AC

○, uncoated; △, 15 min; ●, 60 min; ▲, 120 min; ■, 240 min.

coacervates.

The mechanism of adsorption has been studied in detail by many authors,[15] but it is not easy to describe quantitatively the adsorption profiles of microcapsules. As the adsorption profile for semipermeable membranes gives apparent first-order kinetics, first-order adsorption plots were tried. The microcapsules tended to show apparent first-order adsorption at the initial stage for a short period, but the kinetics became more complex thereafter. Since polymeric matrixes sometimes show a dependence of adsorption on the square root of time, plots of this type were also examined. However, the plots were not linear, but sigmoidal. Accordingly, the mean adsorption rate of ACMC from zero till 2 h was adopted in this study. For a sample of ACMC, the mean adsorption rate is defined as,

$$\left(\frac{dQ}{dt}\right)_m = \frac{S_1 \Delta C}{R_T} \tag{4}$$

where $(dQ/dt)_m$ is the mean adsorption rate (mol/g·s) from zero till 2 h, ΔC, the difference in creatinine concentration (mol/cm^3) between the outside and inside of the microcapsules at the initial stage, S_1 the specific surface area (cm^2/g) of activated charcoal occupied by creatinine molecules, and R_T the total adsorption resistance (s/cm) of ACMC. Under the experimental conditions used, $S_1 = 1.66 \times 10^5$ (cm^2/g). When an adsorbate diffuses from the surrounding medium to the core of a microcapsule, the total adsorption resistance, R_T, is equal to the sum of the adsorption resistance encountered in the semipermeable membrane of the microcapsule, R_{mc}, and in the aqueous diffusion layer at the surface of activated charcoal, R_{ac}.

$$R_T = R_{ac} + R_{mc} \tag{5}$$

The characteristics of the microcapsules fraction used in the adsorption investigation are summarized in Table I. Furthermore, Fig. 7 shows the relationship between the adsorption resistance of ACMC and the cross-linking time. The total adsorption resistance of ACMC increases with increasing cross-linking time (Fig. 7). It is interesting to note that the ratio R_{mc}/R_T (Table I) also depends on the cross linking time; the resistance of the microcapsule is equal to 53.03% of the total resistance at a cross-linking time of 240 min. It would appear that the absolute molecular diffusion rate depends on the surface state of the semipermeable membrane of the microcapsule, which therefore influences the adsorption characteristics of ACMC.

Molecular Sieving Effect of the Semipermeable Membrane of ACMC

Figure 8 shows the molecular sieving effect of the semipermeable membrane of ACMC at the cross linking time of 1 h. Five substances, which vary in molecular weight from 10^2 to 10^5, were used. The adsorption efficiency, η_A, was calculated according to the following equation,

TABLE I. Calculated Values Relating to the Adsorption Resistance of ACMC and AC

Cross-linking time (min)	$t_{50\%}$ (h)	$(dQ/dt)_m$ (mol/g·s)	R_T (s/cm)	R_A (s/cm)	R_{mc} (s/cm)	R_{mc}/R_T (%)
Uncoated	2.2	7.10×10^{-9}	3.72×10^{8}	3.72×10^{8}	—	0
15	3.0	6.49×10^{-9}	4.06×10^{8}	3.72×10^{8}	0.35×10^{8}	9.27
60	4.5	5.20×10^{-9}	5.08×10^{8}	3.72×10^{8}	1.36×10^{8}	26.77
120	6.5	4.36×10^{-9}	6.07×10^{8}	3.72×10^{8}	2.35×10^{8}	38.71
240	12.0	3.33×10^{-9}	7.93×10^{8}	3.72×10^{8}	4.21×10^{8}	53.09

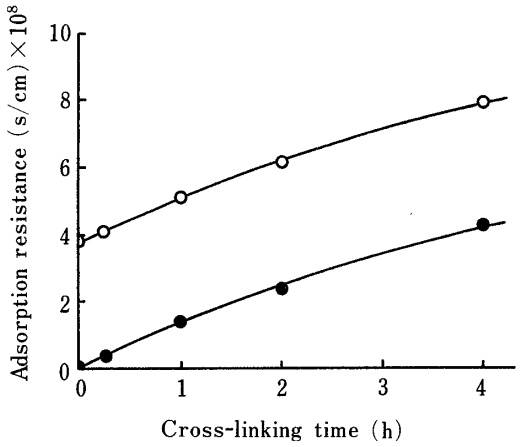

Fig. 7. Relationship between Adsorption Resistance of ACMC and the Cross-Linking Time

○, total adsorption resistance, R_T; ●, adsorption resistance of microcapsules, R_{mc}.

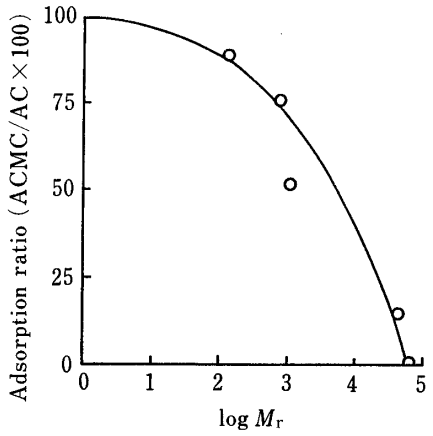

Fig. 8. Relationship between Adsorption Ratio of ACMC/AC and Molecular Weight of Adsorbate

Cross-linking time = 120 min.

$$\eta_A = \frac{Q_{ACMC}}{Q_{AC}} \times 100 \qquad (6)$$

where Q_{AC} is the amount adsorbed on the activated charcoal, and Q_{ACMC} is the amount adsorbed on the activated charcoal microcapsule. In the case of creatinine and L-thyroxine sodium, a little difference was found between AC and ACMC. However, the adsorption efficiency decreased markedly with increase of molecular weight of the adsorbate from 10^3 to 10^5. The values of adsorption efficiency were 51.7% and 14.7% for β-cyclodextrin and ovalbumin, respectively. Furthermore, no adsorption of bovine serum albumin on ACMC could be detected in this experiment.

Thus, higher molecular weight substances such as nutriment and enzymes in the gastro-intestinal tract should not be able to pass through the semipermeable membrane of ACMC, and ACMC should have an excellent molecular sieving effect *in vivo*.

The Effect of Cross-Linking Time on the Distintegration of ACMC

Figure 9 shows the relationship of disintegration of the semipermeable membrane of ACMC with cross linking time. The cross-linking time greatly influenced the disintegration of ACMC in the standard first buffer solution (pH = 1.2). The disintegration of ACMC prepared with a 5 min cross-linking time was approximately 14%. The distintegration decreased markedly with increasing cross-linking time, and then leveled off at about 1.5% after 1 h. This result indicates that the semipermeable membrane of microcapsules will be stable for a long time after oral administration when the cross-linking time of coacervation is increased above 1 h.

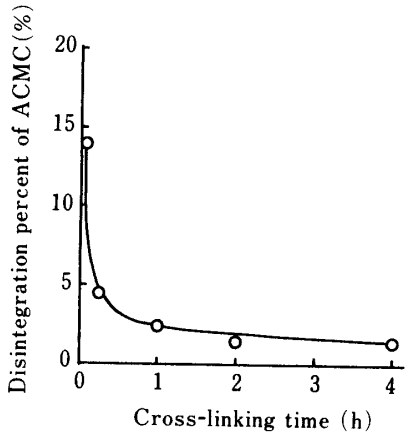

Fig. 9. Effect of Cross-Linking Time on the Disintegration Percent of ACMC

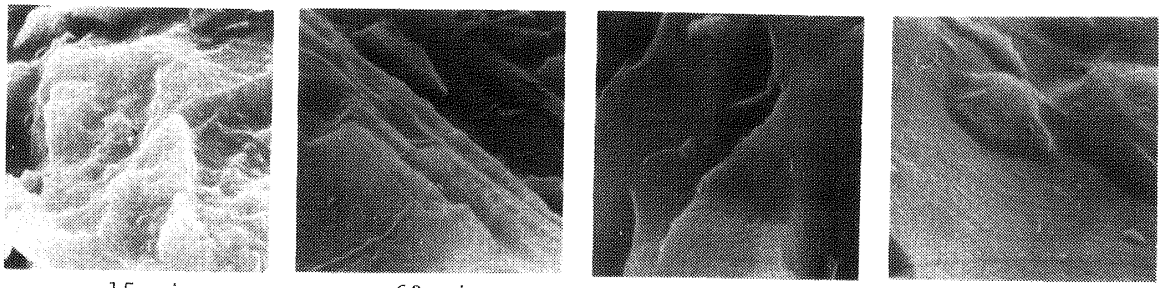

Fig. 10. Scanning Electron Micrographs of ACMC Surfaces after Various Times of Cross-Linking

Scanning electron micrographs of samples prepared at various cross-linking times are shown in Fig. 10. Rough and irregular surfaces were observed on the microcapsules prepared with a 15 min cross-linking time. The microcapsules prepared with a 60 min cross-linking time were covered with gelatin–acacia all over the activated charcoal, but the surface had some large holes. When the cross-linking time was increased beyond 120 min, a smooth-walled membrane was formed on the surface of the activated charcoal, and uniform and thick-walled microcapsules were obtained. From these results, it is clear that the disintegration of microcapsules is dependent on the nature of the surface produced upon coacervation.

Conclusions

The results on the adsorption characteristics of ACMC can be summarized as follows.

1. A stable semipermeable membrane could be formed on the surface of activated charcoal by gelatin–acacia coacervation.
2. The adsorption rate on the ACMC could be controlled by changing the cross-linking time of coacervates.
3. High-molecular-weight substances such as nutriments or enzymes should not pass through the semipermeable membrane of ACMC *in vivo*, because the adsorption efficiency of model substances was very low *in vitro*.
4. It can be presumed from the *in vitro* data that a long cross-linking time (more than 60 min) yields ACMC which are stable in the gastro–intestinal tract.

Finally, the above mentioned properties suggest that the oral administration of microencapsulated activated charcoals (ACMC) is available as a supporting technique in the treatment of patients with renal failure.

References

1) A. L. Babb, L. Grimsrud, R. L. Bell and S. B. Layno, "Chemical Engineering in Medicine and Biology," Plenum Press, New York, 1977, p. 289.
2) M. Odaka, H. Hirasawa, Y. Tabata, H. Kobayashi and H. Sato, *Nihon Rinsho*, **42**, 1774 (1984).
3) K. Oota, *Igaku No Ayumi*, **5**, 484 (1978).
4) G. Gioldano, R. Esposito and M. Pluvio, *PEDTA*, **10**, 136 (1973).
5) N. Naomi, Y. Shimamori, M. Umebayashi and M. Nakano, *Chem. Pharm. Bull.*, **32**, 699 (1984).
6) K. Ishibashi, K. Yamada, Y. Noda, Y. Nozawa, A. Miyagishima and F. Higashide, *Yakuzaigaku*, **44**, 57 (1984).
7) D. J. Burgess and J. E. Carless, *J. Colloid Interface Sci.*, **98**, 1 (1984).
8) M. Morishita, M. Fukushima and Y. Inaba, "Microencapsulation Processes and Applications," ed. by Jan E. Vandegaer, Plenum Press, New York and London, 1973, pp. 115—126.
9) R. W. Bonsnes and H. H. Taussky, *J. Biol. Chem.*, **158**, 581 (1945).
10) N. Nakabayashi, S. Yamanaka, E. Masuhara, S. Koshikawa, M. Suenaga and N. Matsui, *Kobunshi Ronbunshu*, **34**, 323 (1977).
11) F. Watamabe and M. Hasatani, *Kagaku Kogaku Ronbunshu*, **10**, 461 (1984).
12) O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
13) I. Langmuir, *J. Am. Chem. Soc.*, **40**, 1361 (1918).
14) F. Paneth, *Z. Phys. Chem.* (Frankfurt am Main), **201**, 408 (1922).
15) E. Berl and E. Weingaertner, *Z. Phys. Chem.* (Frankfurt am Main), **A173**, 35 (1935).
16) R. Senjkovic and I. Jalsenjak, *Pharm. Acta Helv.*, **57**, 16 (1982).