

# Preparation and Properties of Macromolecular Complexes Consisting of [2-(Diethylamino)ethyl]dextran Hydrochloride and Potassium Metaphosphate

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## SYNOPSIS

In aqueous solution [2-(diethylamino)ethyl]dextran hydrochloride (EA) was reacted with potassium metaphosphate (MPK) to form a series of water-insoluble macromolecular complexes (MC) at different hydrogen-ion concentrations (EA-MPK system). EA was also reacted with MPK in the presence of  $\text{CaCl}_2$  (EA-MPK- $\text{CaCl}_2$  system). The structure and properties of MC obtained were compared with each other; elemental analysis, IR spectroscopy, solubilities, thermogravimetric analysis, and scanning electron microscopy were used to characterize these complexes. The molecular structure and properties of each MC were dependent on the hydrogen-ion concentration and whether the  $\text{Ca}^{2+}$  ion coexisted. It was suggested that MC prepared at acidic pH were composed of a relatively loose network including a small quantity of MPK, whereas those prepared at neutral and alkaline pH were composed of a relatively tight network including a large quantity of MPK. This seemed to be due to changes in the degree of dissociation and the conformation of EA and MPK with the hydrogen-ion concentration. MC in the EA-MPK- $\text{CaCl}_2$  system were supposed to have a rather tightly bound network structure due to the  $\text{Ca}^{2+}$  ion as compared with those in the EA-MPK system. © 1993 John Wiley & Sons, Inc.

## INTRODUCTION

The mixing of oppositely charged polyelectrolytes in aqueous solution leads to the formation of macromolecular complexes (MC). There have been many studies on the preparation, properties, and applications of MC consisting of an organic polycation and an organic polyanion, and these complexes are expected for functional and biomedical materials.<sup>1,2</sup> We have previously reported on the preparation, structure, and properties of a number of MC consisting of organic macromolecules<sup>3-5</sup> and on the membrane application of MC containing [2-(diethylamino)ethyl]dextran hydrochloride (EA).<sup>6</sup> The permeability of solute through EA-containing MC membrane was controlled by the hydrogen-ion concentration. However, little has been reported so

far on the polyionic interaction between an organic macromolecule and an inorganic macromolecule.<sup>7,8</sup> We have also studied novel chemical reactions for the formation of MC consisting of an inorganic macromolecule and organic and/or inorganic macromolecules.<sup>9,10</sup> In addition, MC prepared in the presence of  $\text{CaCl}_2$  were found to have a more tightly bound network structure than those prepared in the absence of  $\text{CaCl}_2$ .<sup>11</sup>

Thus, as a part of a series of studies on MC consisting of an organic macromolecule and an inorganic macromolecule, we make a report on the MC consisting of EA and potassium metaphosphate (MPK). The molecular structure and properties of MC obtained are compared with each other in view of the dependence of the degree of dissociation and the conformation of EA and MPK on the hydrogen-ion concentration. Moreover, this paper deals with the preparation of MC in the presence of  $\text{CaCl}_2$  as well as in the absence of  $\text{CaCl}_2$ , focusing on the  $\text{Ca}^{2+}$  ion dependence of the structure and properties of them.

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## EXPERIMENTAL

### Materials

EA (intrinsic viscosity 1.49 dL/g in 1 mol/L NaCl at 25°C; molecular weight  $2.5 \times 10^6$ ; nitrogen content 5.33 wt %, degree of substitution 1.28 mol/anhydroglucose unit) was supplied by Meito Sangyo Co., Nagoya, Japan. MPK (compositional formula  $K_{n+2}P_nO_{3n+1}$  ( $n = 30$ ), phosphorus content 26.15 wt %) was of food-additive grade from Taihei Kagaku Sangyo Co., Osaka, Japan. Methanol was distilled just before use. The other chemicals were reagent grade and used without further purification.

### Preparation of Macromolecular Complexes

In both the EA-MPK and EA-MPK-CaCl<sub>2</sub> systems, MC were prepared at pH 1.0, 3.0, 6.5, and 9.0, considering that EA has three apparent pK values, 6.07, 9.00, and 14.<sup>6</sup> As solvent, 0.2 mol/L NaCl aqueous solution was adopted, and the concentration of each solution was 5 g/L. The mol ratio of calcium (CaCl<sub>2</sub>) to nitrogen (EA) in the polycation solution of the EA-MPK-CaCl<sub>2</sub> system was 1 : 1. A polycation (or polyanion) solution, which was adjusted to the desired pH with HCl or NaOH, was added dropwise to a 100 mL polyanion (or polycation) solution, which was adjusted to the same pH, at a rate of 50 mL/30 min with stirring at  $22 \pm 2^\circ\text{C}$ . By this procedure, we obtained the coagulation curves of MC for both systems. Then, MC to be characterized were newly prepared in the titration order of polyanion to polycation at pH 1.0, 3.0, 6.5, and 9.0 for the EA-MPK and EA-MPK-CaCl<sub>2</sub> systems. The mol ratios of the reactive group of MPK to that of EA and those of EA + CaCl<sub>2</sub> in the reaction mixture are

listed in Table I. After standing for 30 min, the precipitate was separated by centrifugation, washed with methanol, and dried *in vacuo* at room temperature until a constant weight was attained. The nitrogen content was determined by the Kjeldahl method, and the phosphorus content was determined as PO<sub>4</sub><sup>3-</sup> from the absorbance at 400 nm (Hitachi 100-10 spectrophotometer) by the phosphorus vanadomolybdate method. The calcium content was determined with a Shimadzu AA-640 atomic absorption spectrophotometer.

### Characterization of Macromolecular Complexes

IR spectra in KBr pellets were recorded on a Hitachi 270-50 IR spectrophotometer. The solubility of each MC was examined by immersing it (10 mg) in different solvents (5 mL) for 24 h. The miscibility limit of each MC in a ternary solvent mixture, NaBr-acetone-H<sub>2</sub>O, was determined according to the procedure described in the previous paper.<sup>12</sup>

Thermogravimetric analysis (TGA) was performed in air with a heating rate of 5°C/min and a flow rate of 100 mL/min with a Seiko Instruments SSC-5000 thermal analyzer and a thermogravimetric module. Scanning electron microscopy was carried out with a JEOL JSM-840 at 10 kV in the usual way.

## RESULTS AND DISCUSSION

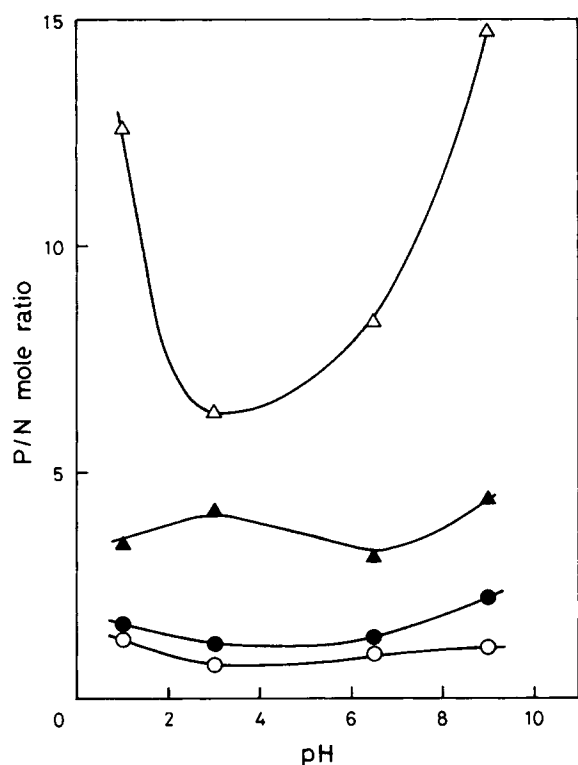
As a polycation (or polyanion) solution was added dropwise to a polyanion (or polycation) solution, the reaction mixture became turbid gradually and MC was coagulated and precipitated. The mol ratios of phosphorus to nitrogen (P/N) and phosphorus

**Table I** Preparation Conditions, Yield, and Results of Elemental Analyses of MC

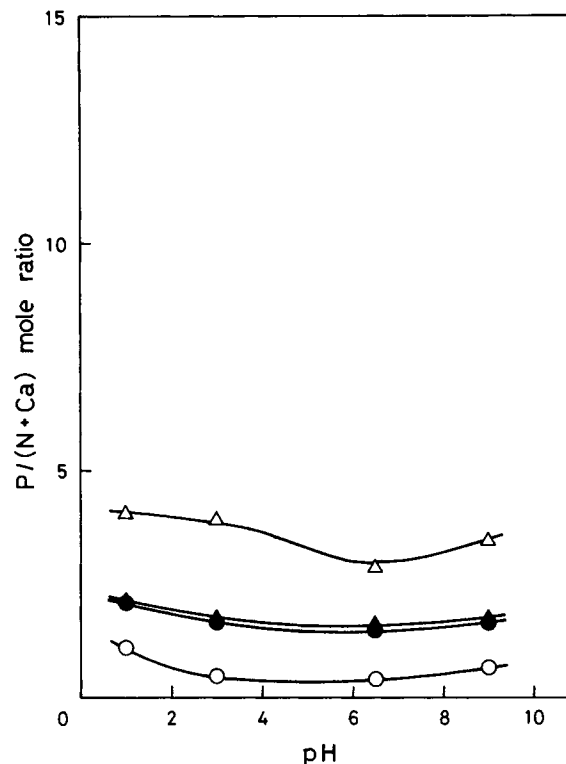
Sample code <sup>a</sup>	Conditions			Yield (g)	Phosphorus Content (wt %)	Nitrogen Content (wt %)	Calcium Content (wt %)	P/N in Structural Unit of MC	P/(N + Ca) in Structural Unit of MC
	[H <sup>+</sup> ]	P/N	P/(N + Ca)						
1-A	pH 1.0	2.50		0.76	11.7	3.5		1.5	
1-B	pH 3.0	2.50		0.76	13.6	3.4		1.8	
1-C	pH 6.5	2.50		0.78	15.7	3.2		2.2	
1-D	pH 9.0	2.50		0.63	18.8	2.5		3.4	
2-A	pH 1.0		2.10	0.73	18.2	2.9	11.8		1.2
2-B	pH 3.0		1.67	0.72	18.0	2.7	14.0		1.1
2-C	pH 6.5		1.57	0.66	15.8	1.9	14.1		1.1
2-D	pH 9.0		1.67	0.67	16.4	1.5	15.6		1.1

<sup>a</sup> Series 1: MPK solution was added dropwise to EA solution (100 mL). Series 2: MPK solution was added dropwise to EA + CaCl<sub>2</sub> solution (100 mL).

to the sum of nitrogen and calcium [ $P/(N + Ca)$ ] at the beginning and endpoints of coagulation for the EA-MPK and EA-MPK- $CaCl_2$  systems are shown in Figures 1 and 2, respectively. When the polycation solution was added to the polyanion solution,  $P/N$  mol ratios at pH 1.0 and 9.0 were remarkably higher than those at pH 3.0 and 6.5 in the EA-MPK system. This coagulation behavior is in disagreement with expectations based on changes in the degree of dissociation of EA and MPK with the hydrogen-ion concentration: The lower the pH, the lower is the degree of dissociation of MPK; the higher the pH, the lower is the degree of dissociation of EA. When the polyanion solution was added to the polycation solution, on the contrary,  $P/N$  or  $P/(N + Ca)$  mol ratios for each system were approximately constant.  $P/(N + Ca)$  mol ratios in the EA-MPK- $CaCl_2$  system are lower than  $P/N$  mol ratios in the EA-MPK system. This result can be attributed to the fact that the EA-MPK- $CaCl_2$  system contains the  $Ca^{2+}$  ion, but it is not clear in detail. Further investigation on this point should be made.



**Figure 1** Beginning and end points of coagulation in the EA-MPK system: (○, △) beginning point of coagulation; (●, ▲) endpoint of coagulation; (○, ●) polyanion solution was added dropwise to polycation solution; (△, ▲) polycation solution was added dropwise to polyanion solution.



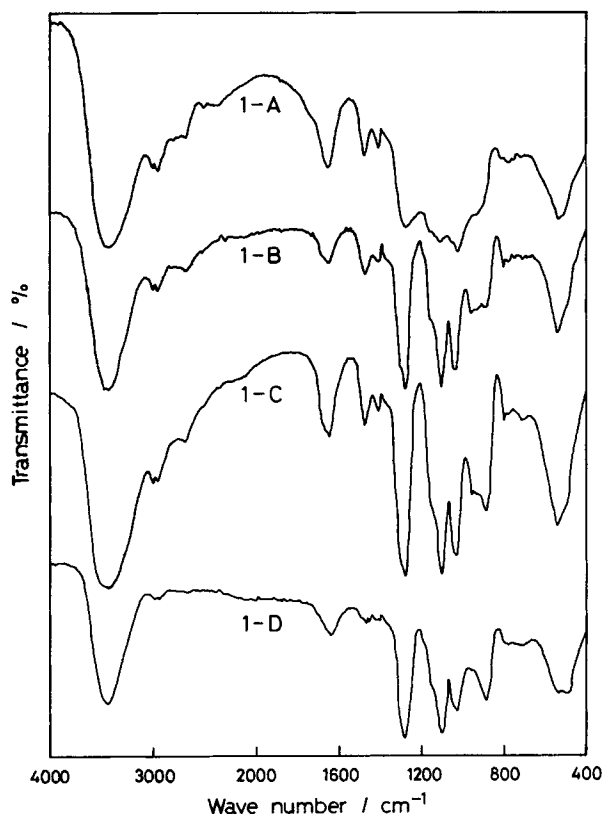
**Figure 2** Beginning and end points of coagulation in the EA-MPK- $CaCl_2$  system: (○, △) beginning point of coagulation; (●, ▲) endpoint of coagulation; (○, ●) polyanion solution was added dropwise to polycation solution; (△, ▲) polycation solution was added dropwise to polyanion solution.

In consideration of these coagulation properties, the preparation of MC was achieved by adding the polyanion solution to the polycation solution up to the completion of coagulation at pH 1.0, 3.0, 6.5, and 9.0 for the EA-MPK and EA-MPK- $CaCl_2$  systems in order to obtain invariable products for characterization.

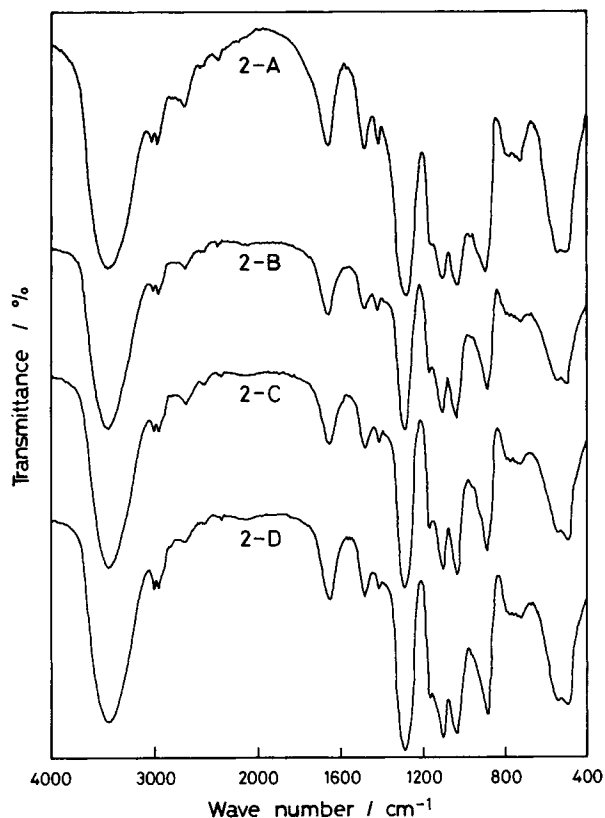
All MC thus obtained in the reaction mixture were white and gelled products, and the higher the preparation pH, the more brittle is the MC. Preparation conditions, yield, phosphorus, nitrogen, and calcium contents, and  $P/N$  or  $P/(N + Ca)$  mol ratio of each MC obtained after the completion of coagulation are given in Table I. The yield is high at lower pH and low at higher pH. The compositions of MC are different from one another according to the preparation conditions. In the EA-MPK system, the phosphorus content, reflecting the MPK content, increases with decreasing the hydrogen-ion concentration, whereas the nitrogen content, reflecting the EA content, decreases with decreasing the hydrogen-ion concentration. Therefore, the  $P/$

N mol ratio increases with decreasing the hydrogen-ion concentration. This is essentially consistent with changes in the degree of dissociation of EA and MPK with the hydrogen-ion concentration. In the EA-MPK-CaCl<sub>2</sub> system, the nitrogen content decreases with decreasing hydrogen-ion concentration; also, the phosphorus content decreases with decreasing hydrogen-ion concentration. Consequently, P/(N + Ca) mol ratios are approximately equivalent. The Ca<sup>2+</sup> ion that is a counterion of MPK seems to act as the compensation cation for EA in the EA-MPK-CaCl<sub>2</sub> system.

IR spectra of MC in the EA-MPK and EA-MPK-CaCl<sub>2</sub> systems are shown in Figures 3 and 4, respectively. They are roughly similar to the spectra of the mixture of EA and MPK, differing from each other only in detail. In the EA-MPK system, the absorption bands at 1280 and 890 cm<sup>-1</sup>, which are assigned to the P=O and P-O-P groups in MPK, respectively, are intense for the MC prepared at higher pH, whereas they are considerably weak for the MC prepared at pH 1.0. This is interpreted in terms of the fact that the degree of dissociation



**Figure 3** IR spectra of macromolecular complexes in the EA-MPK system. Sample codes correspond to those in Table I.



**Figure 4** IR spectra of macromolecular complexes in the EA-MPK-CaCl<sub>2</sub> system. Sample codes correspond to those in Table I.

of MPK extremely decreases and MPK can be hydrolyzed into orthophosphoric acid at pH 1.0. In the EA-MPK-CaCl<sub>2</sub> system, on the other hand, the absorption bands at 1280 and 890 cm<sup>-1</sup> are clearly detected even for the MC prepared at pH 1.0. This is consistent with the result of elemental analysis described above, and it is thought that the Ca<sup>2+</sup> ion serves as trap for MPK. The absorption bands at

2700 and 1420 cm<sup>-1</sup>, assigned to the  $\text{—NH}^+$

and  $\text{—CH}_2\text{—N}^+$  groups in EA, are observed for all of MC in both systems. However, the former absorption is weak for the MC prepared at pH 9.0,

since the major cation site is not the  $\text{—NH}^+$

group but the  $\text{—N}^+\text{—}$  group. MC is, generally, in-

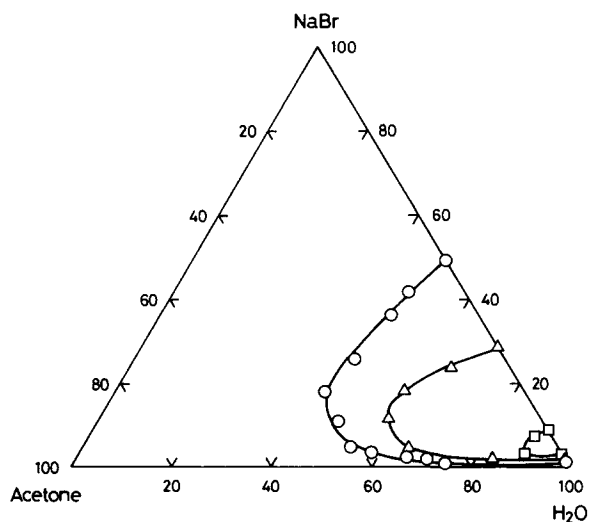
soluble in common organic solvents such as methanol, acetone, and chloroform. Table II lists the solubilities of our MC in single solvents. All MC are

**Table II Solubilities of MC in Various Solvents**

Solvent	1-A	1-B	1-C	1-D	2-A	2-B	2-C	2-D
<i>n</i> -Propanol	—	—	—	—	—	—	—	—
Ethylene glycol	—	—	—	△	—	—	—	—
Formalin	△	△	△	△	—	—	—	—
Formic acid	△	△	△	△	—	—	—	△
Acetic acid	—	—	—	—	—	—	—	—
Propionic acid	—	—	—	—	—	—	—	—
Hydrobromic acid	○	○	○	○	○	○	○	○
Aqueous ammonia	△	△	△	△	—	—	—	—
<i>n</i> -Butylamine	—	—	—	—	—	—	—	—
Diethylamine	—	—	—	—	—	—	—	—
<i>N,N</i> -Dimethylformamide	—	—	—	—	—	—	—	—
<i>N,N</i> -Dimethylacetamide	—	—	—	—	—	—	—	—
Dimethyl sulfoxide	—	—	—	—	—	—	—	—
Nitrobenzene	—	—	—	—	—	—	—	—

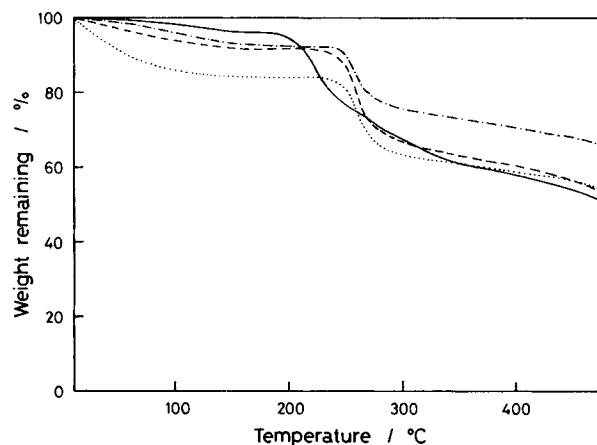
Sample codes correspond to those in Table I. (—) Stable; (△) unstable; (○) soluble.

soluble in hydrobromic acid. However, MC in the EA-MPK system are only swollen in such organic solvents as formalin, formic acid, and aqueous ammonia, and MC in the EA-MPK-CaCl<sub>2</sub> system are insoluble in every organic solvent. The addition of CaCl<sub>2</sub> to the EA-MPK system in forming MC reduces the solubility of MC produced. It is well known that MC are very soluble in specific ternary solvent mixtures that consist of water, water-compatible organic solvents, and microions. Figure 5 shows phase diagrams of MC in the EA-MPK system in the ternary solvent mixture, NaBr-acetone-H<sub>2</sub>O.

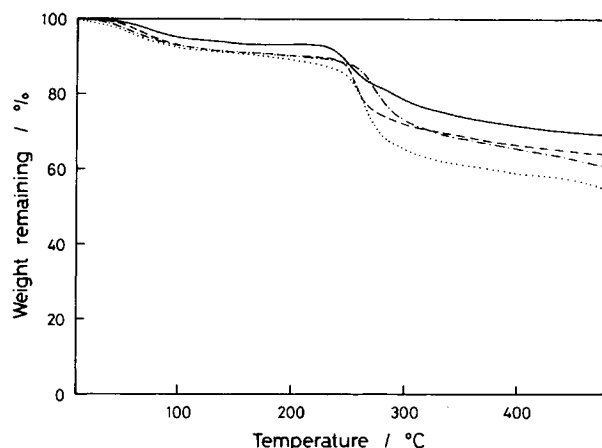


**Figure 5** Phase diagrams of macromolecular complexes in the ternary solvent mixture, NaBr-acetone-H<sub>2</sub>O, at 30°C: (○) 1-A; (△) 1-B; (□) 1-C.

There are small regions in the solvent composition field where the complexes remain in solution to yield a homogeneous liquid. The miscibility limit region of MC prepared at lower pH is large, whereas that of MC prepared at higher pH is small and the MC prepared at pH 9.0 is insoluble. The higher solubility of MC prepared at the lower pH depends probably on a small number of polyionic linkages because of the decrease in the degree of dissociation of MPK. Changes in the degree of dissociation of EA and MPK with the hydrogen-ion concentration appear to affect the solubility of MC extensively. On the other hand, no MC in the EA-MPK-CaCl<sub>2</sub> system are soluble at all. The bonding through the Ca<sup>2+</sup> ion seems to be so tight that NaBr cannot break it. After



**Figure 6** Thermogravimetric curves for macromolecular complexes in air in the EA-MPK system: (—) pH 1.0; (---) pH 3.0; (·····) pH 6.5; (- · - ·) pH 9.0.

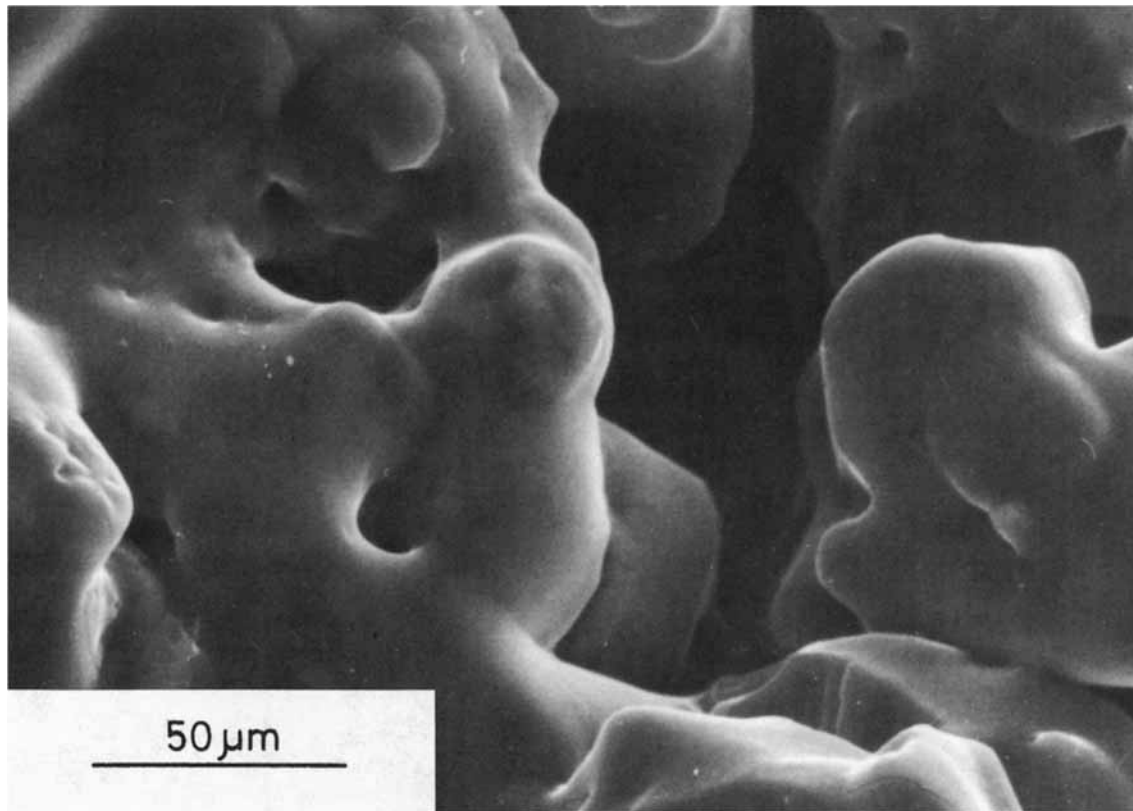


**Figure 7** Thermogravimetric curves for macromolecular complexes in air in the EA-MPK-CaCl<sub>2</sub> system: (—) pH 1.0; (---) pH 3.0; (·····) pH 6.5; (- - -) pH 9.0.

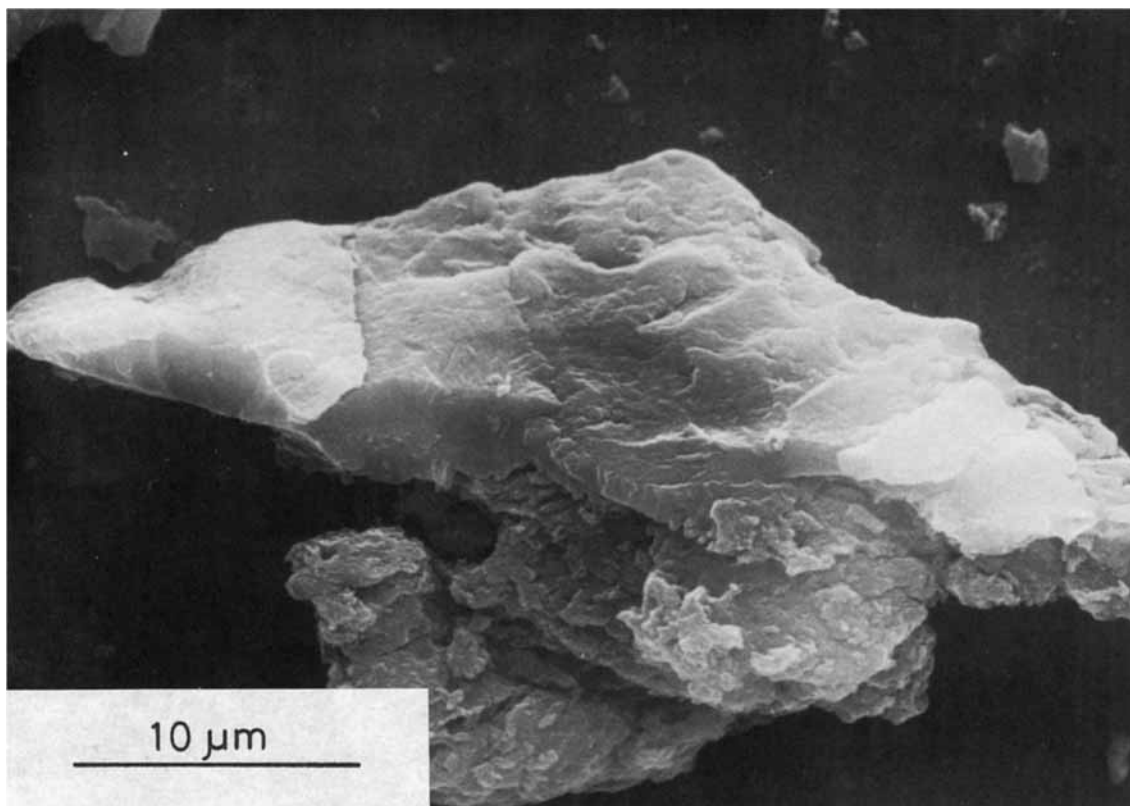
all, these results of solubility measurements support the concept that the network structure of each MC is dependent on the hydrogen-ion concentration and whether the Ca<sup>2+</sup> ion is present in the reaction mixture although all MC have the common components.

The strength and thermal stability of three-di-

mensional polymers result from the fact that the network preserves its structural integrity even after some bonds are broken. Then, TGA studies of MC in air were conducted to evaluate their thermal stabilities; 6–14 mg of MC was used for each analysis. The thermogravimetric curves for MC in the EA-MPK and EA-MPK-CaCl<sub>2</sub> systems are presented in Figures 6 and 7, respectively. The results show a loss of weight in the initial stage of heating, presumably due to loss of water absorbed on the hygroscopic complexes. They also show that MC begin to lose weight at about 200–240°C, which appears to correspond to the commencement of thermal decomposition at the main chain of polysaccharide.<sup>13</sup> Although it was anticipated that the binding force in the EA-MPK-CaCl<sub>2</sub> system is stronger than that in the EA-MPK system due to the Ca<sup>2+</sup> ion, like in the glycol chitosan-MPK-CaCl<sub>2</sub> system,<sup>11</sup> MC in the EA-MPK-CaCl<sub>2</sub> system decomposed at almost the same temperature as those in the EA-MPK system except at pH 1.0. Only MC prepared at pH 1.0 in the EA-MPK-CaCl<sub>2</sub> system have a significantly high decomposition temperature as compared with those in the EA-MPK system. Then, the morphology of MC prepared at pH 1.0 in the EA-MPK and



**Figure 8** Scanning electron micrograph of macromolecular complex prepared at pH 1.0 in the EA-MPK system.



**Figure 9** Scanning electron micrograph of macromolecular complex prepared at pH 1.0 in the EA-MPK-CaCl<sub>2</sub> system.

EA-MPK-CaCl<sub>2</sub> systems was estimated by scanning electron microscopic observation. As shown in Figures 8 and 9 of the SEM picture, the MC prepared at pH 1.0 in the EA-MPK system are covered with a smooth surface, whereas those in the EA-MPK-CaCl<sub>2</sub> system have a rough surface and sharp edges. It seems that the Ca<sup>2+</sup> ion plays an important role in the formation of MC that have a rather tightly bound network structure.

As aforementioned, it has become apparent that the molecular structure and properties of MC consisting of EA and MPK are different from one another according to the preparation conditions and that the Ca<sup>2+</sup> ion contributes to the solubility and stability of MC. This kind of investigation has been little studied in the field of macromolecules. It should be studied further on complexes containing the inorganic macromolecule or the Ca<sup>2+</sup> ion.

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