Polyelectrolyte Complexes of [2-(Diethylamino)ethyl]dextran Hydrochloride with Sodium Carboxymethylcellulose

Yasuo KIKUCHI* and Takashi KODA

Department of Materials Science and Engineering, Faculty of Engineering, Ōita University, 700-Dannoharu, Ōita 870

(Received July 24, 1978)

Sodium carboxymethylcellulose(*anti*-thrombogenic material)(CMC) reacted with [2-(diethylamino)ethyl]-dextran hydrochloride(*anti*-cancer material)(EA) having an opposite charge and three groups of different basicity at different hydrogen ion concentrations to form novel water-insoluble precipitates, the so-called polyelectrolyte complex(PEC) comprised of both biomedical materials, focusing on the coagulation of precipitate(PEC) produced. The mole ratio N(EA)/Na(CMC) of the reaction mixture in solution at the start coagulation increased with a lowering of hydrogen ion concentration. This depended on the change of the degree of dissociation of EA and CMC with changing hydrogen ion concentration. The nitrogen contents which describe the mole ratios of EA/CMC in each PEC thus prepared were determined to range from 1.75 to 4.51. It was established that the hydrogen ion concentration and mixing mole ratio of N/Na in solution play an important role in determining the composition ratio of EA/CMC in the PEC. The results of IR, elemental analyses, solubility measurements, degree of swelling in water, color reaction with Toluidine Blue, and blood clotting test for PEC, revealed that the molecular structure of the various PEC's differed according to the hydrogen ion concentration and the mole ratio of reaction mixture in solution, though all PEC's were prepared from the same starting materials. It appears that the degree of dissociation and conformation of EA and CMC change with hydrogen ion concentration. The blood clotting tests were performed on a slightly swelled tablet of PEC where it was found that the PEC suppressed coagulation of the blood.

The mixing of oppositely charged polyelectrolytes give rise to the formation of a polyelectrolyte complex whose properties, conformations, and biomedical characters, in general, are sensitive to such reaction conditions as the composition of the reaction mixture, the hydrogen ion concentration, the order of mixing, and the polyion concentration at which the reaction is conducted.[1,2]

The mechanism of polyion interaction, the probable structures of the PEC, and properties have been investigated in detail by Michels *et al.*[3,4]

To date, however, few papers[5] have dealt with the polyion interaction between polysaccharide, or polysaccharide and synthetic macromolecules of importance becasue of the similarities to biological systems, membranes, and industrial and biomedical applications.[6] In a previous paper[7] the chemical reaction of EA and sodium dextran sulfate (*anti*-thrombogenic material) which is a biomedical material was reported.

This paper deals with a novel chemical reaction of [2-(diethylamino)ethyl]dextran hydrochloride with sodium carboxymethylcellulose, focusing on the coagulation of the PEC produced, the general characteristics, and the process of clot formation.

Experimental

Sodium carboxymethylcellulose(*anti*-thrombogenic material, weak acid polyelectrolyte, intrinsic viscosity 0.401 dl/g in 1 M NaCl at 25 °C, sodium content 7.79%) and [2-(diethylamino)ethyl]dextran hydrochloride(*anti*-cancer material, weak basic polyelectrolyte, nitrogen content 4.97%, degree of substitution 1.11 mol/A.G.U.†) prepared by molecular weight 250000 of dextran(described later in detail), were obtained from Wako Junyaku Co., Ltd., and from Meito Sangyo Co., Ltd., Japan, respectively.

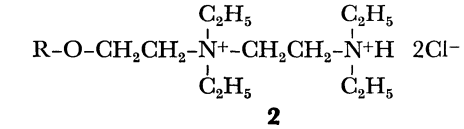
CMC and EA were highly-purified and used without further purification. CMC and EA (2 g/1000 ml) were dissolved in aqueous solution. The reaction were conducted at pH 3.0, 7.0, and 11.0, the pH of solution being adjusted to 7.0 and 11.0 with sodium hydroxide and to 3.0 with hydrochloric acid. The EA solutions were added dropwise to the CMC solutions, adjusted to the same pH as those of the EA solutions at a rate of 50 ml/30 min with stirring. The water-insoluble precipitate, the so-called PEC comprised of both biomedical materials, were thus obtained. After aging for a half hour, the precipitate was washed with water and methanol, centrifuged, and dried in vacuo at 50 °C to constant weight.

The blood clotting test was conducted according to the procedure of Imai and Nose;[8] PEC 200 mg was pressed (8 t/4.9 cm^2) in a vacuum for 10 min to make a sample tablet. The ACD blood of type A (Red Cross Hospital Blood Center) was kept in a thermostat at 4—6 °C for 3 days. The ACD blood was prepared by adding the blood to an anticoagulant citrate dextrose solution consisting of sodium citrate, citric acid and dextrose.

Elemental analyses of the PEC were performed at the Institute of Physical and Chemical Research.

The structures of **1** and **2** of EA have been proposed by Meito Sangyo Co., Ltd., Japan:

1



2

R: dextran residue.

Both groups are present in each molecule of EA *i.e.* three groups of different basicity, are reflected in the form of the titration curves in a previous paper,[7] quarternary (strong basic) and two tertiary ammonium groups in **1** and **2**, respectively.[9] Potentiometric titration studies of polybases for

† Determined at Meito Sangyo Co., Ltd., Japan.

EA have supplied information regarding the properties of the substances in solution.[10] The titration curves obtained in these experiments have been reported in a previous paper.[7] The ratio of groups **2** to group **1** was approximately 0.78 : 1. On the basis of the inflection points in the potentiometric titrations and the start, before and after of coagulation of PEC in the reaction system, subsequent experiments were conducted at pH 3.0, 7.0, 11.0, and a mole ratio N/Na of the reaction mixture in solution in Table 1.

The purple coloration is due to the reaction of CMC with a solution containing Toluidine Blue, utilized to demonstrate the CMC elution from PEC and the change of structure produced.

Results and Discussion

Experimental conditions, yields and elemental analyses for the PEC prepared at the beginning and end point, and before and after coagulation of PEC in the reaction system are given in Table 1, respectively.

The nitrogen contents which describe the molar ratios of EA/CMC in each PEC increased with an increased in the mole ratio of N/Na of the reaction mixture in solution and with a decrease in hydrogen ion concentration a direct consequence of the fact that the degree of dissociation of CMC and EA are dependent on the hydrogen ion concentration, *i.e.* the composition ratio of EA/CMC in the PEC was sensitive to the reaction conditions. Thus, the pH as well as the mole ratio of EA to CMC in the reaction mixture, played an important role in changing the ratio of the reactive group of CMC to that of EA in the PEC produced.

As seen in Fig. 1, the mole ratios N/Na of the reaction mixture in solution at the beginning point of coagulation increase with an increase in pH, *i.e.* a larger amount of EA is necessary to coagulate the CMC solution containing PEC with a lowering of hydrogen ion concentration. The reason for this is that the degree of dissociation of EA decreases and that of CMC increases with a lowering of hydrogen ion concentration, *i.e.* the decreasing of the positive net charge of EA and the increasing of the negative charge of CMC.

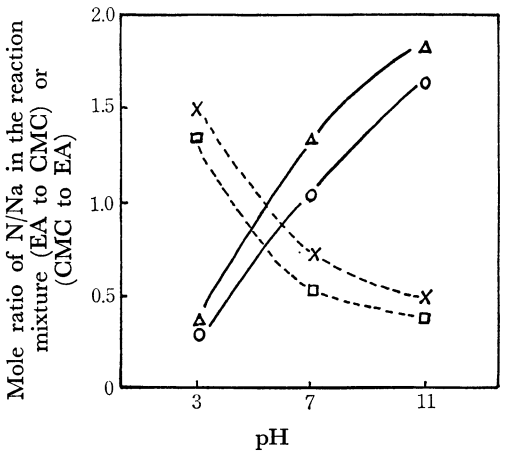


Fig. 1. Beginning and end points of coagulation. ○: Beginning point of coagulation, △: end point of coagulation (EA to CMC, N/Na).
×: Beginning point of coagulation, □: end point of coagulation (CMC to EA, Na/N).

The IR spectrum of PEC is similar to that of a mixture of CMC and EA, differing only in detail. How-

Table 1. Experimental conditions,[a] polymer yield and elemental analyses of polyelectrolyte complexes[b]

Sample code	Amount of EA or CMC solution ($\frac{\text{ml EA or ml CMC}}{\text{100 ml CMC or EA}}$)	Mole ratio of N/Na in solution	Yield of polymer (g)	Nitrogen content (%)	Sodium content (%)	Chlorine content (%)
A-1[c]	19.1	0.20	0.09	2.05	—	—
A-2[c,f]	29.5	0.31	0.18	1.75	—	—
A-3[c,g]	34.0	0.36	0.21	1.83	—	—
A-4[c]	200.0	2.10	0.24	2.27	0.02	0.08
B-1[d]	76.5	0.80	0.20	3.74	—	—
B-2[d,f]	99.5	1.04	0.26	3.84	—	—
B-3[d,g]	128.0	1.34	0.37	3.80	—	—
B-4[d,h]	200.0	2.10	0.37	4.10	0.05	0.77
C-1[e]	76.5	0.80	0.17	4.14	—	—
C-2[e,f]	155.0	1.62	0.38	4.34	—	—
C-3[e,g]	176.0	1.84	0.44	4.42	—	—
C-4[e]	200.0	2.10	0.45	4.51	0.19	0.13
D-1[c]	188.5	0.56	0.47	2.43	0.11	0.0
D-2[d,i]	188.5	0.56	0.30	3.82	0.06	0.07
D-3[e]	188.5	0.56	0.26	3.99	0.14	0.0

a) Concentration of EA and CMC, 2.0 g/l. A, B, C series: EA solution was added dropwise to CMC solution. Amount of CMC solution was 100 ml. D series: CMC solution was added dropwise to EA solution. Amount of EA solution was 100 ml. b) The analyses were performed at the Institute of Physical and Chemical Research. c) Both CMC and EA solutions were adjusted to pH 3.0. d) Both CMC and EA solutions were adjusted to pH 7.0. e) Both CMC and EA solutions were adjusted to pH 11.0. f) Coagulation just occured. g) End point of coagulation. h) Carbon content, 50.67%, hydrogen content, 7.77%. i) Carbon content, 50.59%, hydrogen content, 7.79%.

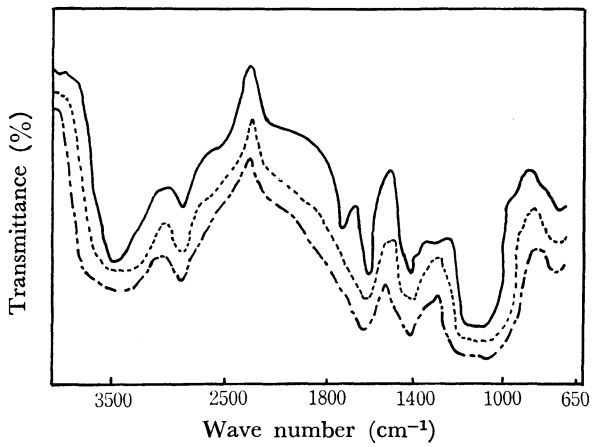


Fig. 2. IR spectra of polyelectrolyte complexes.
—— : Polyelectrolyte complex prepared at pH 3.
---- : Polyelectrolyte complex prepared at pH 7.
—·— : Polyelectrolyte complex prepared at pH 11.

ever, the PEC prepared at pH 3 had an absorption band at 1740 and 1240 cm^{-1}, which appeared neither in the PEC prepared at pH 7 and 11.0 nor in the mixture of EA and CMC. These absorption bands had been assigned to COOH group.[11] Furthermore, the absorption band at 3500 cm^{-1} (assigned to the OH group) of PEC prepared at pH 3 shifted to a higher wave number. In consideration hydrogen bond of formation between the OH group and other reaction groups, it is reasonable to expect shifts in frequency. In addition, the sodium and chlorine contents in the PEC are smaller than those in CMC or EA, and the nitrogen content distinctly different (Table 1). The results suggest that the amino groups in EA participate in the binding with CMC, probably through the $-COO^-$ groups, and that the molecular structures of PEC are different from one another. Actually, the PEC prepared at low hydrogen ion concentration differed appreciably from the PEC prepared at higher hydrogen ion concentration in such properties as the degree of swelling and the color reaction with Toluidine Blue as described later.

The resulting purple stain with Toluidine Blue provides a very sensitive test for CMC. Only the PEC of series A prepared at pH 3 gave a blue coloration, but not a blue purple coloration with Toluidine Blue as shown in Table 2.

The *anti*-thrombogenic CMC in the PEC produced did not elute into the solution except for PEC of series A as shown in Table 2.

PEC's are insoluble or only partially soluble in dimethyl sulfoxide or *N*,*N*-dimethylformamide on heating, partially soluble in a ternary solvent mixture (acetone/KBr/H_2O=20 : 20 : 60 wt %) on heating, and soluble in formic acid or a ternary solvent mixture (HCl/dioxane/H_2O=48 : 47 : 5 wt %) on heating. The difference in solubility however, is in discernible. The PEC's prepared at low hydrogen ion concentration are however, more soluble than those prepared at high hydrogen ion concentration.

TABLE 2. COLOR REACTION WITH TOLUIDINE BLUE[a]

Sample code	Color of solution	Color of PEC
EA	blue green	—
CMC	blue purple	—
A-1	blue purple	blue
A-2	blue purple	blue
A-3	blue purple	blue
A-4	blue purple	blue
B-1	blue green	white
B-2	blue green	white
B-3	blue green	white
B-4	blue green	white
C-1	blue green	white
C-2	blue green	white
C-3	blue green	white
C-4	blue green	white
D-1	blue purple	blue
D-2	blue green	white
D-3	blue green	white

a) Sample code corresponds to that in Table 1.

TABLE 3. SWELLING WITH WATER

Sample code	Degree of swelling
A-1	No swelling
A-2	No swelling
A-3	No swelling
A-4	No swelling
B-1	Slight swelling
B-2	Slight swelling
B-3	Slight swelling
B-4	Fairly large swelling
C-1	Slight swelling
C-2	Slight swelling
C-3	Slight swelling
C-4	Fairly large swelling
D-1	No swelling
D-2	Slight swelling
D-3	Fairly large swelling

a) Sample code corresponds to that in Table 1.

Swelling tests were performed with all PEC listed in Table 3. All the PEC prepared at pH 3 did not swell entirely. The degree of swelling of PEC became larger with decreasing hydrogen ion concentration and increasing mole ratio N/Na of the reaction mixture.

These experimental results support the differences in molecular structure according to the experimental conditions of hydrogen ion concentration and mole ratio of the reaction mixture, although the PEC have common constituents. It appears that the degree of dissociation and conformation of EA and CMC changes with hydrogen ion concentration.

The blood clotting tests were performed on a PEC tablet (A-4, B-3, C-2, D-1, D-2) prepared in solution (Table 1) by gravimetric measurement[8] of the amount of clot formed at the appropriate time intervals, after the addition of calcium chloride solution (0.1 M, 0.015 ml) to ACD blood (0.15 ml, A type, storage time 3 d) which had been in contact with the ma-

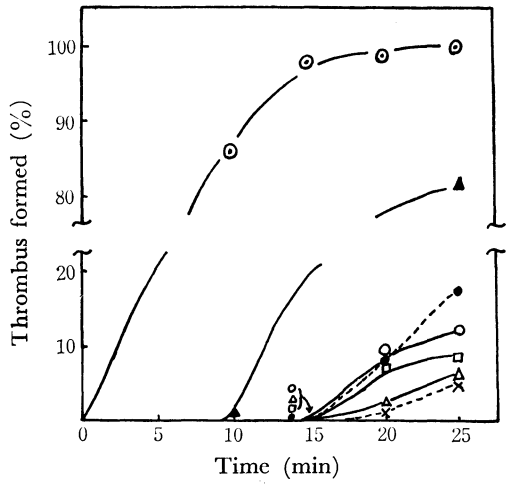


Fig. 3. Percentage of the thrombus formed on polyelectrolyte complexes compared with that on glass.
⊙: Glass, ▲: polyvinyl chloride, ●: C-2, ○: B-3, □: D-1, △: A-3, ×: D-2.
Sample codes C-2, B-3, D-1, A-3, and D-2 correspond to those in Table 1.

terials. The lightly swelled PEC could not perform the blood clotting test because of the soaking of blood into the tablet and therefore, the tests were performed on a PEC tablet with a small degree of swelling in A, B, C, and D series. The quantities of the clot formed on the PEC tablet are smaller than those formed on glass or PVC tablets. The PEC has an *anti*-thrombogenic character and supresses the coagulation of blood. Differences in *anti*-thrombogenic behavior among them is however, hardly discernible.

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