Formation of Polyelectrolyte Complexes between Cellulose Derivatives and Their Blood Compatibility

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Synopsis

Formation of polyelectrolyte complexes (PECs) between cellulose derivatives in aqueous solution and their blood compatibility were examined. To this end, two types of quaternary ammonium cellulose derivatives, Q-Cell and Q-HEC, were prepared by treating cellulose and hydroxyethyl cellulose (HEC), respectively, with glycidyl trimethylammonium chloride. Anionic derivatives were carboxymethyl cellulose (CMC) with different degrees of substitution (DS) and cellulose sulfate (CS). *In vivo* blood tests were made by a method of peripheral vein indwelling suture. The results showed that the complex formation of Q-Cell did not follow a stoichiometric reaction, but Q-HEC reacted stoichiometrically with CMC and CS. It was also found that water-insoluble cellulosic PECs are soluble in formic acid, showing that the cellulosic PEC films can be prepared from formic acid solutions. The blood tests revealed that by the criteria of the test method employed in this work, cellulosic PECs had a good blood compatibility.

INTRODUCTION

Many of the water-insoluble polyelectrolyte complexes (PECs), which are obtained by mixing oppositely charged polyelectrolytes, have an antithrombogenic character and suppress the coagulation of blood. Since its first demonstration by Michaels,[1] a fairly large number of studies on the formation and properties of PECs have been reported from the viewpoint of biomedical use.[2-16] Cellulose can be easily converted into various types of anionic and cationic derivatives, which differ not only in the chemical structure of substituents but also in the degree of substitution (DS), and a very attractive materials from practical point of view. However, little study has been reported on the characteristics of PECs formed between cellulose derivatives as a biomaterial.

Recently, we found that water-insoluble cellulosic PECs are soluble in formic acid. Formic acid can also dissolve water-insoluble cellulose derivatives such as aminoethyl cellulose (AEC) and diethylaminoethyl cellulose

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(DAC) with low DS values, suggesting that the PECs containing these derivatives as one component can be prepared from formic acid solutions.

In the present work, the formation behavior of PECs from various types of cellulose derivatives and their blood compatibility were investigated. For this purpose, we prepared two types of quaternary ammonium cellulose derivatives, between which the ionizable groups are the same, but the flexibility of the substituents is different. Water-insoluble AEC and DAC, which are commercially available, were also used as cationic derivatives. Anionic derivatives employed were carboxymethyl cellulose (CMC) and cellulose sulfate (CS). Tests, *in vivo*, for blood compatibility were made by a method of peripheral vein indwelling suture.[17-19] By the criteria of this test, some cellulosic PECs were found to have an excellent blood compatibility.

EXPERIMENTAL

Materials

Cellulose used as starting material was Avicel (commercial cellulose powder manufactured by Asahi Chem. Ind. Co., Japan). Deionized water was used throughout the experiments. All other solvents and reagents were of highest purity available and used without further purification.

Preparation and Purification of Cellulose Derivatives

Two types of quaternary ammonium cellulose derivatives, Q-Cell and Q-HEC, were used as polycation components. A quaternary ammonium derivative of hydroxyethyl celulose (HEC), Q-HEC-4, was obtained from Union Carbide Co. (U.S.). According to the statement of the manufacturer, the approximate DS and molar substitution (MS)[20] value were said to be 0.4 and 1.8, respectively. Another Q-HEC sample with a higher DS value, Q-HEC-8, was prepared by further etherification of Q-HEC-4 with glycidyl trimethylammonium chloride (GMAC) in a 5*N* NaOH solution.[21] The other type of quaternary ammonium derivative, Q-Cell, was prepared by homogeneous etherification of cellulose with GMAC in a 10% LiCl/dimethylacetamide (DMAc) solvent.[22] A typical example was as follows: 12 g of cellulose was dissolved in 200 mL of LiCl/DMAc solution according to the method of Turback et al.[22] To the solution, 30 g of GMAC was added and then 1.1 g potassium tertiary butoxide in 30 mL of DMAc added dropwise at 105°C. After 30 min, the solution was cooled to room temperature and recovered by precipitation with ethanol.

CMC–Na samples with different DS values and a CS–Na sample with a DS value of 0.54 were kindly supplied from Daicel Chem. Ind. Ltd. (Japan). The samples AEC and DAC were purchased from Serva Chem. Co. (U.S.). All the sample derivatives were designated by attaching the number of DS $\times$ 10 to the code of each sample.

The water-soluble derivatives were deionized using a column packed with a mixture of anion- and cation-exchange resins and then freeze-dried. AEC and DAC were treated with aqueous ammonia solution and thoroughly washed with deionized water.

Characterization of Sample Derivatives

Contents of ionizable groups of the water-soluble derivatives, i.e., DS values were measured by conductometric titrations with a Mitsubishi Kasei Conductivity Apparatus Model CT-10 according to the procedure of Nakajima et al.[11-13] The results were consistent with those estimated by elemental analysis within an experimental error. In the case of water-insoluble derivatives like AEC and DAC, the DS values were determined by elemental analysis. The chemical structure of substitutents and DS values of the sample derivatives employed are summarized in Table I.

The degree of dissociation, α, of ionizable groups was determined by potentiometric titrations according to the procedure of Nakajima et al.[11-13] The apparatus used for potentiometric titrations was a Hitachi-Horiba pH-Meter Model F-7 II. The initial concentrations of polyelectrolyte were about 0.003 mol ionizable groups/L. Titrations were performed under N_2 atmosphere on both the polyelectrolyte solution and a reference solution containing no polyelectrolyte. The degree of dissociation was obtained from the difference between these two curves at a given pH value.[11] In Figure 1, the degrees of dissociation obtained in the presence and absence of 0.005*N* NaCl are plotted against pH for COOH of CMC. The effect of ionic strength on the dissociation was not so large, and the difference in charge density of the sample derivatives did not affect the degree of dissociation. The former result was not consistent with that by Sato and Nakajima.[11] The reason for this discrepancy is not clear at present. Subsequent experiments were carried out in aqueous solutions free from NaCl. With respect to $-SO_3H$ and $-N(CH_3)_3Cl$, which are strong electrolyte, α values were unity independent of pH.

Formation of Polyelectrolyte Complexes

The cellulose derivatives were dissolved at ca. 0.03 g/dL concentration in water. The pH values of the solution were adjusted with 0.1*N* HCl or 0.1*N* NaOH. The polycation solution at a fixed pH was then added at different ratios to the polyanion solution having the same pH. However, the pH value of the resulting mixture slightly changed, because the buffer solution was not employed in the present work. The pH value of the resulting mixture was used as that of the solution. The complex formation was followed by the measurement of turbidity of the mixtures.[11-13] Turbidity measurements were carried out at a wavelength of 430 nm with a Shimadzu Spectrophotometer Type UV-200S.

TABLE I
Characteristics of Cellulose Derivatives Employed

Sample code	Substituent	Degree of substitution
Q-Cell	$-CH_2CH(OH)CH_2N(CH_3)_3Cl$	0.41, 1.03
Q-HEC	$-(CH_2CH_2O)_n-CH_2CH(OH)CH_2N(CH_3)_3Cl$	0.40, 0.89
AEC	$-CH_2CH_2NH_2$	0.05
DAC	$-CH_2CH_2N(C_2H_5)_2$	0.18
CMC-Na	$-CH_2COONa$	0.32, 0.69, 0.85
CS-Na	$-SO_3Na$	0.54

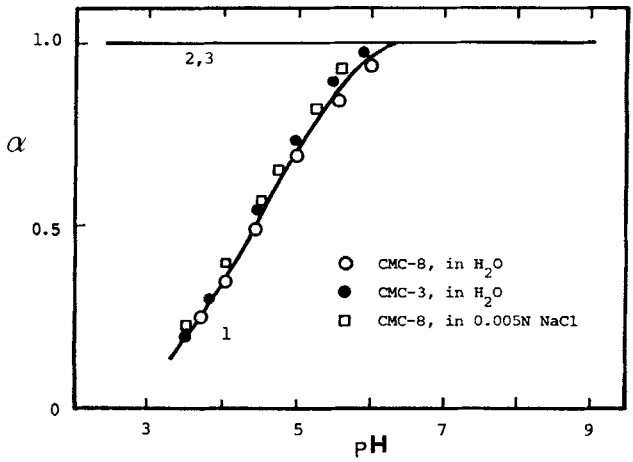

Fig. 1. Plots of the degree of dissociation α vs. pH: (1) for COOH groups of CMC; (2) for SO_3H groups of CS; (3) for $N(CH_3)_3Cl$ groups of Q-Cell and Q-HEC; (○) CMC-8 in H_2O; (●) CMC-3 in H_2O; (□) CMC-8 in 0.005*N* NaCl.

In vivo blood tests were made by a method of peripheral vein indwelling suture which was developed by one of the authors.[17] The specimen was prepared as follows. A polyester suture ranging from No. 2-0 to 1 (USP) with length of 10 cm is coated with a desired PEC. In the animal experiment (in this work, a dog), a 18-gauge needle is inserted into the peripheral vein (jugular and femoral vein) under general anesthesia. The suture is introduced through the needle into the lumen of the vessel. After the needle is withdrawn, the edge of the suture is ligated to the connective tissue near the puncture site of vessel. After an adequate time (in this work, 1 day), the dog is sacrificed by acute exsanguination from the aorta under general anesthesia with the adminsitration of heparin (2–3 mg/kg). The vein in which the suture is inserted is opened gently and the suture is examined visually. This test has been proved to be excellent in reproducibility and exact, because the blood stream is slow in peripheral veins and the thrombus formed on the test suture is seldom carried away from the surface.[17] Another advantage of this test method is that four materials can be examined at once in each animal.

The coating of the sample PEC on the polyester sutures was carried out by casting from formic acid solutions. The PEC-coated sutures were washed thoroughly with deionized water and stored in deionized water containing 20% ethanol.

RESULTS AND DISCUSSION

Formation Behavior of Cellulosic PECs

When a polycation is added to a polyanion solution, the mixing ratio of polycation R_c is given by

$$R_c = \frac{\mathrm{PC}}{\mathrm{PC} + \mathrm{PA}} \tag{1}$$

where PC and PA are the total numbers of ionizable groups of polycation and polyanion in the mixture, respectively. In Figure 2(a), the turbidity τ of the mixtures is plotted against the mixing ratio R_c of Q-HEC-4 solution to CMC-8 solution at pH 7.2. The effect of standing time after mixing on the turbidity is also shown in this figure. As is well known,[11-13] the maximum in turbidity curve, which appeared at an early stage of standing, became a minimum with increasing time of standing, but the mixing ratio at the maximum turbidity was the same as that at the minimum. It is obvious that such a decrease in turbidity is probably due to the sedimentation of parts of PECs formed.[11-13] The maximum yield of PEC can be obtained at the mixing ratio of maximum or minimum turbidity. These facts were also true for other systems.

The effect of mixing order on the turbidity curve is shown in Figure 2(b). As can be seen from the comparison with Figure 2(a), the order of mixing affected the pattern of the turbidity curve, but not the mixing ratio of maximum or minimum turbidity, being consistent with with those reported

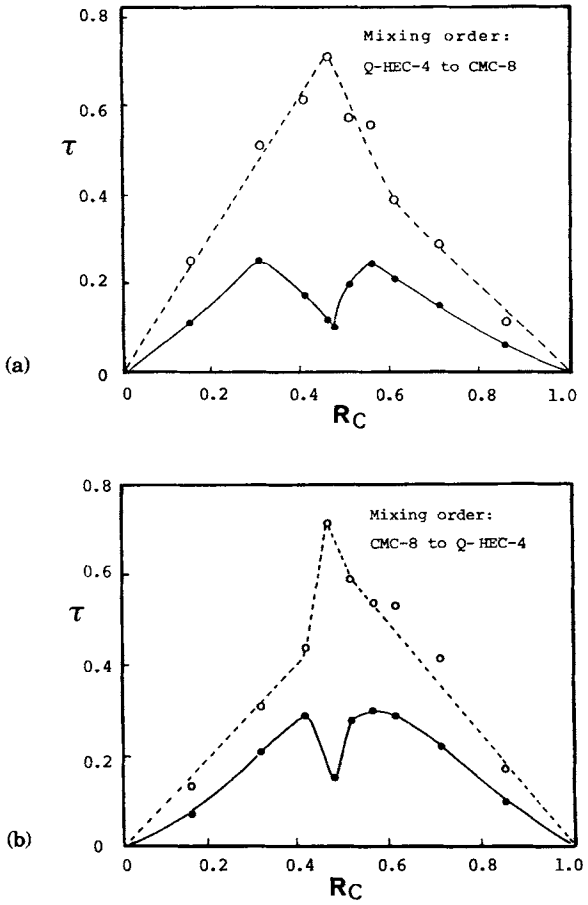

Fig. 2. Relation between turbidity τ and the mixing ratio R_c for the system of Q-HEC-4/CMC-8 at pH 7.2. (a) The order of mixing, Q-HEC-4 to CMC-8; (○) at 15 min after mixing; (●) at 24 h after mixing. (b) The order of mixing; CMC-8 to Q-HEC-4; (○) at 15 min after mixing; (●) at 24 h after mixing.

by Sato and Nakjima.[11] Therefore, in the subsequent experiments, the mixing order was fixed as follows: The polycation solution was added to the polyanion solution. Figure 3 shows an example of the change in turbidity curve against pH. In the system of Q-HEC-4 and CMC-8, water-insoluble PECs were not formed at below pH 2.0.

Stoichiometry of Complex Formation

If the complex formation follows a stoichiometric reaction, a perfectly neutral complex is formed at the mixing ratio of maximum turbidity and the following equation should hold[11-13]:

$$\mathrm{PC} \cdot \alpha_c = \mathrm{PA} \cdot \alpha_a \tag{2}$$

where α_c and α_a are the degrees of dissociation of polycation and polyanion, respectively, at a definite pH. In the systems studied here, however, it is enough if the value of α for COOH groups of CMC, α_{COOH}, can be known, since other ionizable groups are strong electrolytes, that is, the values of α are unity independent of pH. In the case of the systems containing CMC as polyanion, the composition of the complex by stoichiometric reaction, $(R_c)_{\mathrm{theor}}$, can be given by the following equation:

$$(R_c)_{\mathrm{theor}} = \frac{\mathrm{PC}}{\mathrm{PC} + \mathrm{PA}} = \frac{\alpha_{\mathrm{COOH}}}{1 + \alpha_{\mathrm{COOH}}} \tag{3}$$

Figures 4–7 show the pH dependences of R_c at the maximum turbidity for the systems of Q-Cell/CMC, Q-Cell/CS, Q-HEC/CMC, and Q-HEC/CS. In each figure, the pH dependence of the corresponding $(R_c)_{\mathrm{theor}}$ is also shown. From Figures 4 and 5, it can be seen that the complex formation of Q-Cell does not follow a stoichiometric reaction, independent of the charge density of the component derivatives. On the other hand, Figures 6 and 7 show that Q-HEC reacts stoichiometrically with CMC and CS, as observed in the flexible vinyl polymers.[11-13] In the case of Q-HEC, quaternary ammonium

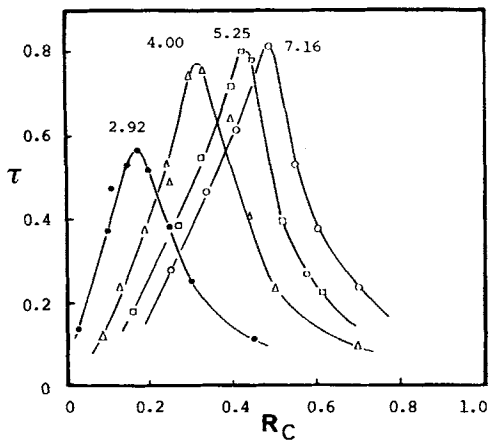

Fig. 3. Turbidity curves for the system of Q-HEC-4/CMC-8 at different pH values.

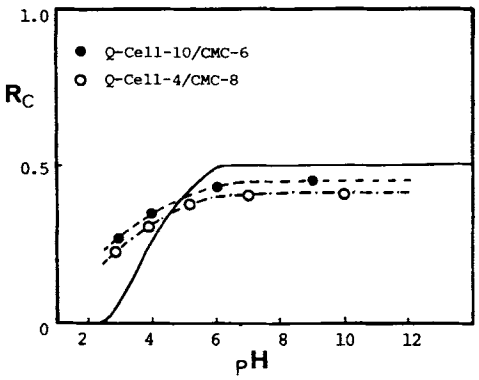

Fig. 4. Comparison between R_c and $(R_c)_{theor}$ plotted against pH for the system of Q-Cell/CMC: (●) Q-Cell-10/CMC-6; (○) Q-Cell-4/CMC-8.

groups are attached not directly to semirigid glucopyranose rings but to the flexible polyoxyethylene segments. The substituent of Q-Cell is not flexible as compared with that of Q-HEC (see Table I). Such a difference between the chemical structures of substituents may be considered to cause the difference between the reaction schemes of Q-Cell and Q-HEC.

In the systems containing CMC as polyanion, the complex formation is pH-dependent. However, our interest is the PECs formed at pH 7.4, i.e., the pH value of physiological salt solution, as discussed later. At this pH value, it is not necessary to take into account the degree of dissociation of CMC.

Solubility of Cellulosic PECs

In general, water-insoluble PECs are soluble in ternary solvent systems such as acetone/KBr/water, but insoluble in usual organic solvents.[1,2] As already mentioned, the cellulosic PECs are soluble not only in such ternary solvent systems but also in formic acid and certain concentrated aqueous salt solutions.[23] Formic acid can also dissolve water-insoluble cellulose derivatives such as AEC and DAC with low DS values. It is possible to prepare the PECs containing these derivatives as polycation components. This is also one of the advantages of formic acid as casting solvent of cellulosic PECs.

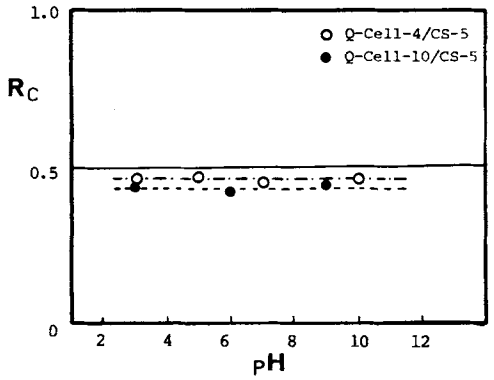

Fig. 5. Comparison between R_c and $(R_c)_{theor}$ plotted against pH for the system of Q-Cell/CS: (○) Q-Cell-4/CS-5; (●) Q-Cell-10/CS-5.

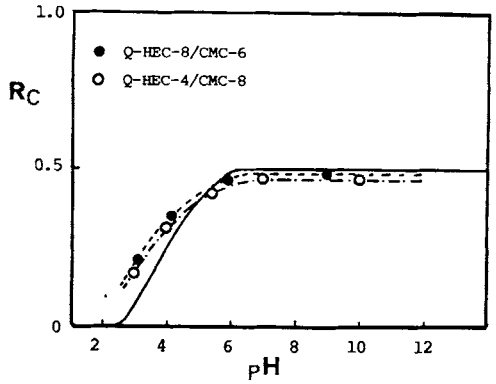

Fig. 6. Comparison between R_c and $(R_c)_{theor}$ plotted against pH for the system of Q-HEC/CMC: (●) Q-HEC-8/CMC-6; (○) Q-HEC-4/CMC-8.

Blood Compatability

The coating of the sample polymers on the polyester sutures used for the blood compatibility tests was carried out by casting from formic acid solutions. The formic acid solution of the sample polymers was prepared by dissolving the water-insoluble PECs formed at pH 7.4. In the case of PECs containing AEC and DAC, the weight ratio of polycation to polyanion component in the sample mixture to be dissolved was calculated from the DS values, assuming the stoichiometric reaction. The polymer-coated sutures were immersed in water, but the amount of water-soluble unbound polyanion component was negligible.

The results on the blood tests of cellulosic PECs thus prepared are summarized in Table II. To obtain reliable results, the tests were carried out at least twice on each sample. The symbols used to evaluate in vivo results have the following significance: The + symbols indicate the relative degree of the thrombus formation of the samples in three steps where +++ denotes the thrombus formation along the entire length of the coated suture surface. – indicates the case where no thrombus was observed. Figure 8(a)

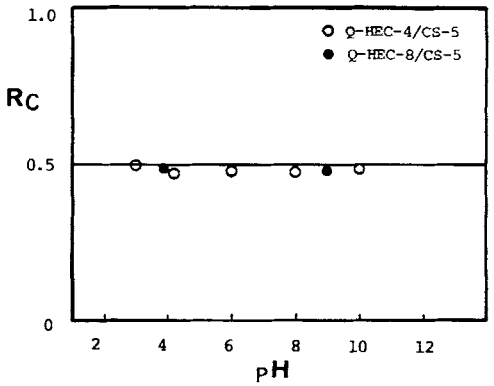

Fig. 7. Comparison between R_c and $(R_c)_{theor}$ plotted against pH for the system of Q-HEC/CS: (○) Q-HEC-4/CS-5; (●) Q-HEC-8/CS-5.

TABLE II
In Vivo Results on Blood Compatibility of Various Types of PECs from Cellulose Derivatives

Sample code	Thrombus formation[a]
Q-HEC-4/CMC-8	−
Q-HEC-4/CS-5	−
Q-Cell-4/CMC-8	−
AEC-0.5/CMC-3	+
DAC-2/CS-5	+
Polyester	+++

[a] See text for symbols.

shows a macroscopic view of a suture coated with Q-HEC/CMC, showing that no thrombus is observed around the suture (S). For the sake of comparison, a macroscopic view of a suture coated with wool keratin derivative, which was evaluated as ++,[18] is shown in Figure 8(b). It can be seen that, by the criteria of the present *in vivo* test, the cellulosic PECs have a relatively good blood compatibility.

The problem is whether or not a nonthrombogenic material can be distinguished from a thrombogenic, nonthromboadherent material by this test method. As already mentioned in the Experimental section, the tests were carried out by using peripheral veins in which the blood stream is slow, and the thrombus which is formed on the test suture may not easily shed from the surface. In fact, the validity of this test method has been proved by the experiments on denatured keratins from wool.[18,19] Recently, it has been also found that the results obtained here are in good agreement with those by Lee–White test using fresh dog's whole blood. The results will be reported with those by other *in vitro* test methods in a subsequent paper.[24]

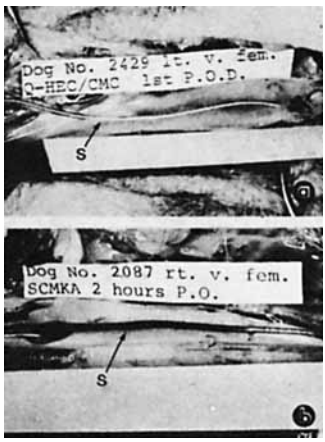

Fig. 8. Macroscopic views of polyester sutures coated with cellulosic PEC (a) and wool keratin derivative (b) after implantation in the fomoral vein of a dog for 24 and 2 h, respectively. (a) No thrombus was observed around suture (S); (b) in this case, the blood compatibility was evaluated as ++.

Among the PECs examined, the PECs containing quaternary ammonium cellulose derivatives as polycation component showed an excellent blood compatibility. However, the DS values were considerably different between the cationic derivatives employed. In order to discuss the effect of the chemical structure of cellulosic PECs on the blood compatibility, a further investigation must be carried out to clarify the effect of DS values of component derivatives on the blood compatibility. Cellulosic PEC is a kind of hydrogel,[3] and it is also important to examine the relationship between the water content of PECs and their blood compatibility. Furthermore, the system of Q-HEC and CMC, in which the complex formation is stoichiometric, is suitable for investigating the effect of the excess charge in PEC on the blood compatibility.[9,10] Such studies are in progress in our laboratory using not only *in vivo* but also *in vitro* tests for blood compatibility.

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