Polyelectrolyte Complexes of Heparin with Chitosan

We have reported on the chemical reaction of chitosan and heparin of 140 I.V./mg in a previous paper.[1] This paper deals with the results of our study on polyelectrolyte complexes of chitosan and heparin, with different sulfur content, of 100 I.V./mg. The dilute aqueous solution of highly sulfated dextrorotatory mucopolysaccharide (heparin) coreacted in dilute acetic acid solution with poly(N-deacetylated chitin) (chitosan) to form a water-insoluble hydrous precipitate which Miekka[2] called "polysalt."

The experimental conditions, yield, and elemental analyses for the polyelectrolyte complexes are given in Tables I and II. As seen in Table II, the sulfur content in the polyelectrolyte complex formed in the lower concentration range became greater with increase in the concentration of heparin, but the sulfur content in higher concentration range was at random. Further, the theoretical mole ratio of N/S in the structural units of the polyelectrolyte complexes 1 to 10 was estimated to be 0.34 to 1.18, on the basis of nitrogen content 8.2% of the chitosan structural unit, and with the heparin sample of sulfur content 7.42% (in the polyelectrolyte complex). That is to say, the concentration of the reactants, heparin and chitosan, played an important role in determining the composition ratio of chitosan to heparin in the polyelectrolyte complex produced.

TABLE I
Experimental Conditions and Yields of Polymers for Polyelectrolyte Complexes

Expt. no.	Concentration of chitosan solution, g/100 ml	Amount of chitosan solution, ml	Concentration of heparin solution, g/100 ml	Amount of heparin solution, ml	Mole ratio of N/S in solution	Yield of polymer, g
1	1	20	8	1.25	5.32	0.127
2	1	20	8	2.5	2.66	0.273
3	1	20	8	5	1.33	0.363
4	1	10	8	5	0.67	0.059
5	1	2.5	8	5	0.33	0.052
6	0.1	200	0.8	12.5	5.32	0.119
7	0.1	200	0.8	25	2.66	0.234
8	0.1	200	0.8	50	1.33	0.429
9	0.1	100	0.8	50	0.67	0.229
10	0.1	25	0.8	50	0.33	0.050

Our IR spectroscopic studies revealed that the polyelectrolyte complexes had an absorption band around 1520 cm^{-1}, which appeared neither in chitosan nor in heparin nor in a mixture of both. Nakanishi[3] has assigned a band around 1500 cm^{-1} to the N—H stretching frequency resulting from the $—NH_3^+$ group. Polyelectrolyte complexes failed to show this absorption, but instead a band appeared around 1520 cm^{-1}. We, therefore, propose that the band around 1520 cm^{-1} is due to an $—NH_3^+$ group participating in the polyelectrolyte complex formation, and hence that the $—NH_3^+$ group participates in the binding of heparin, probably through its SO_4^- group. Another absorption band was found around 1720 cm^{-1}, which became stronger with increase in the sulfur content of polyelectrolyte complexes. We could not give any assignment to this band, however.

We were unable to find a solvent for our polyelectrolyte complexes. Thus, all the polyelectrolyte complexes were found to be insoluble, even when heated in either of two ternary solvent mixtures of certain compositions water/hydrochloric acid/dioxane (5:45:50 vol-%) and water/potassium bromide/acetone (60:20:20 wt-%) and in a single solvent dimethyl sulfoxide, though they were only partially soluble in formic acid. All the polyelectrolyte complexes formed in the more

dilute solutions were more soluble in them than those formed in the more concentrated solutions. None of the polyelectrolyte complexes dissolved in water, most of them having swelled to a limited extent.

The colorimetric reaction with toluidine blue appeared to be throughout the interior of the polyelectrolyte complex, indicating an even dispersion of heparin in the polyelectrolyte complex.

Blood tests[4] were performed on samples 2 and 8 in Table 1 by measuring gravimetrically the amount of thrombus formed at an appropriate interval of time, after calcium chloride solution was added to ACD blood which had been in contact with test material in a glass tube for 10 min. The test material has previously been dipped in a 0.9% NaCl solution for a certain time of various lengths. A firm clot did not form until the tenth day (no. 2) or the 20th day (no. 8). The blood thrombus was formed on clean glass in a 37°C constant-temperature bath 8 min after calcium chloride solution was added to ACD blood (2 ml), and its amount was measured at 107 to 143 mg.

TABLE II
Sulfur Contents[a] and Mole Ratio of N/S in Polyelectrolyte Complexes

Expt. no.[b]	Sulfur content, %	Weight of heparin, %	Weight of chitosan, %	Mole ratio of N/S
1	5.99	76.8	23.2	0.73
2	5.42	69.5	30.5	1.06
3	5.84	74.9	25.1	0.81
4	5.41	69.4	30.6	1.06
5	6.12	78.5	21.5	0.66
6	5.23	67.1	32.9	1.18
7	5.36	68.7	31.3	1.09
8	6.30	80.8	19.2	0.57
9	6.84	87.7	12.3	0.34
10	6.82	87.4	12.6	0.35

[a] The analysis for sulfur content was performed in the Rikagaku Research Institute, Wako-shi, Japan.

[b] Experiment number corresponds to that in Table I.

The mechanism of polyion interaction, the probable structures of the polyelectrolyte complexes, and their properties have been investigated in considerable detail by Michels et al.[5,6]

A typical experimental procedure was as follows. The heparin was of 100 I.V./mg (sulfur content 7.42%) from Katayama Kagaku Co., Japan. When the concentrated solutions of chitosan and heparin were mixed under experimental conditions 1 to 5, a final colloidal precipitate was formed. The polyelectrolyte complexes so obtained had a wide variety of sulfur content, even under about the same experimental conditions, subtly affected by the rate of dropping of the solution and stirring of the reaction system.

The detailed structures and the antithrombogenic character of polyelectrolyte complexes are currently under investigation.

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Received August 8, 1975
Revised November 11, 1975