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Effect of the Ionic Strength on the Behavior of Sodium Chondroitin Sulfate C in Aqueous Solutions*[1]

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Viscosity and light-scattering investigations were carried out in aqueous sodium chloride solutions at 25°C on a sample of sodium chondroitin sulfate C from shark cartilage. The weight-average molecular weight of 74000 was obtained. The molecular dimensions, obtained from the viscosity-partial specific volume, from the light scattering-partial specific volume, and from the light scattering-viscosity, were found to fit well for a long-rod model with a length of about 800 Å and a diameter of about 10 Å at the infinite ionic strength.

Sodium chondroitin sulfate is known to be an unbranched linear anionic polyelectrolyte. It has been considered that the molecule is fully stretched at a very low ionic strength by intramolecular electrostatic repulsion, but that it begins contracting with the increase in ionic strength because of the suppression of the repulsive force by ion-pair formation or by the electrostatic screening effect of the ion atmosphere, until at last it becomes randomly coiled.[1]

Chondroitin sulfate has three isomers, as is schematically illustrated in Fig. 1.[2] The B type of chondroitin sulfate has an axial carboxyl and sulfonic group, the A type, an equatorial carboxyl and an axial sulfonic group, and the C type, an equatorial carboxyl and sulfonic group. These differences in primary structure are considered to influence the behavior of the molecules in aqueous solutions. It has been known, actually, that the solubility in aqueous ethanol[3] is greater for the A and C types than for the B type; the value of pK_a on the potentiometric titration curve[3] is larger for the B type than for the A type, and the affinity of Ca^{2+} and Mg^{2+} under physiological conditions is greater for the B type than for the A type.[4] Furthermore, the affinity of a trivalent cation,

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1) M. B. Mathews, *Arch. Biochem. Biophys.*, **43**, 181 (1953).

2) M. B. Mathews, *ibid.*, **104**, 394 (1964).

3) M. B. Mathews, *Biochim. Biophys. Acta*, **35**, 9 (1959).

4) E. Buddecke and R. Drezenick, *Z. Physiol. Chem.*, **327**, 49 (1962).

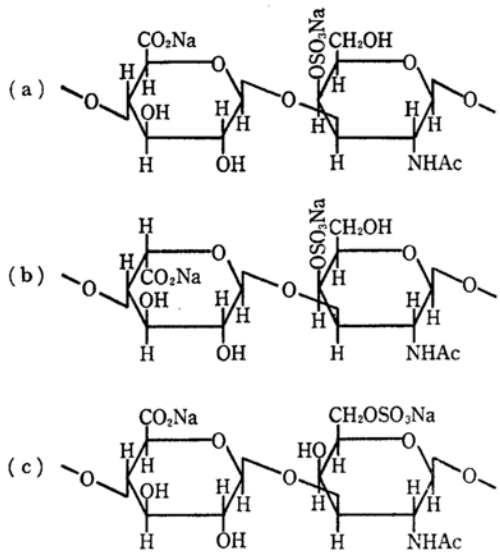

Fig. 1. Schematic illustration of disuccharide units of sodium chondroitin sulfates.
(a) A type; (b) B type; (c) C type

$Co(NH_3)_6^{3+}$, is greater for the B type than for the A type at a fixed ionic strength.[5] At a very low pH, where the ionization of carboxyl groups is mostly suppressed, there is not so large a difference in the affinity for $Co(NH_3)_6^{3+}$ among the three types of chondroitin sulfates at a low ionic strength; with an increase in the ionic strength, the affinity increases in the order of B>A>C types.[2] Further, only the B type of chondroitin sulfate is resistant to testicular hyaluronidase and possesses anticoagulant properties.[6]

It may be supposed from these facts that the C-type chondroitin sulfate has the least flexible structure of the three isomers, while the B type is the most flexible molecule. Recent studies of optical rotatory dispersion[7] have shown that a Cotton effect exists on the ultraviolet ORD curve with the C-type chondroitin sulfate, and an induced Cotton effect in the visible region is observed for the complex of this polymer with acridine orange. Comparing this phenomenon with other, similar phenomena shown by the helical polyglutamic acid,[8] tobacco mosaic virus,[9] and DNA,[10] chondroitin sulfate C may be supposed to have a helical structure at a suitable ionic strength.

In this paper, the effects of the ionic strength on the behavior of sodium chondroitin sulfate C prepared from shark cartilage is discussed quantitatively on the basis of viscosity measurements and light-scattering (Zimm-plot method) experiments.

Experimental

Materials and Solvent. The sodium chondroitin sulfate of the C type was kindly furnished by the Seikagaku-Kogyo Co., Ltd.,[11,12] the preparation being extracted from shark cartilage, fractionated, and carefully purified to remove any electrolyte except sodium chloride. The molecular weight of this preparation was 50000, as determined by the results of the terminal analysis, using D-galactosamine hydrochloride as a standard. The water content was 5.46%, and the specific viscosity of a one percent solution of this preparation was 2.03 at 30°C in a physiological solution of sodium chloride. Table 1 shows this preparation contained scarcely any diffusible ion species other than sodium ions.

For the purpose of serial experiments, a 1% stock solution was prepared by the dry-weight method and kept in a refrigerator. Water was distilled and refined by the ion-exchange method for the viscosity measurements. For light scattering, this water was distilled again with an all-glass unit.

Viscosity Measurements. The viscosity was measured at 25°C with a dilution viscometer of the Ubbelohde type. The filtering of the solution was omitted, because the solution was filtered automatically in the course of the experiment by the glass filter attached to the viscometer. The ionic strength and pH were adjusted with sodium chloride, sodium hydroxide, and hydrochloric acid.

Refractive Increment, dn/dc. The refractive increment, dn/dc, was measured with a Shimadzu electrophotometric differential refractometer at 25°C; the calibration was done by referring to a potassium chloride solution.[13] So as to remove errors due to the difference between the temperature of the solution and that of the solvent, and also because of the existence of bubbles, reading was carried out an hour after setting.

Light-scattering Measurements. A Shimadzu electrophotometric light-scattering photometer was employed. All the measurements were done with an unpolarized light of the 436 mμ wavelength, *in vacuo* and at the constant temperature of 25°C, controlled with circulated water. The apparatus was calibrated with the reduced scattering intensity at a 90° angle of benzene,[14] $R_{90}=48.5\times10^{-6}$. For this purpose, a reagent-grade commercial product of benzene was distilled twice with sodium metal, and filtered directly into the cell through a Cella Filter (Mittel) under pressing. A cylindrical glass was used as a solution cell, and its illuminated volume was corrected with a fluorescein solution. The solution and the solvent

5) M. B. Mathews, *Biochim. Biophys. Acta*, **37**, 288 (1960).
6) J. A. Cifonelli, J. Ludowieg and A. Dorfman, *J. Biol. Chem.*, **233**, 541 (1958).
7) A. L. Stone, *Biopolymers*, **3**, 617 (1965).
8) L. S. Streyer and E. R. Blout, *J. Am. Chem. Soc.*, **84**, 1411 (1961).
9) N. S. Simmons and E. R. Blout, *Biophys. J.*, **1**, 55 (1960).
10) D. F. Bradley, I. Tinoco, Jr., and R. W. Woody, *Biopolymers*, **1**, 239 (1963).
11) T. Furuhashi and K. Uchida, *Seikagaku*, **33**, 537 (1961).
12) M. B. Mathews, *Biochim. Biophys. Acta*, **58**, 92 (1962); the specimen used in our study was also employed in his study.
13) A. Kruis, *Z. Phys. Chem.*, **34B**, 13 (1936).
14) C. L. Carr, Jr., and B. H. Zimm, *J. Chem. Phys.*, **18**, 1616 (1950).

TABLE 1. ANALYSIS OF SODIUM CHONDROITIN SULFATE C (N104)
(by Mr. T. Furuhashi,[11] Seikagaku Co., Ltd.)

		Method
pH	6.0	1% in distilled water
Uronic acid (as a glucronic acid)	38.46%	Dische's treatment using carbazole
Hexosamine (as a galactosamine)	33.88%	Modified Elson-Mogan procedure (Boas procedure)
Ester sulfate (SO_4)	20.94%	Chelate method
Total nitrogen	2.78%	Micro Kjeldahl method
Sodium ion	9.28%	Chelate titration
Potassium ion	negative	Ammonium oxalate method
Free sulfuric ion	negative	Nephelometry using barium sulfate
Chloric ion	negative	Direct titration using mercurous nitrate
Peptide or protein	negative	Biuret reaction
$[\alpha]_D^{28}$ (1% in water)	−13.4	Hitachi model POB polarimeter
Paper electrophoresis	one spot	0.1 M acetate buffer (pH 6), 12 v/cm, 90 min dying with toluidine blue

were optically clarified with Membrane Filter No. 15 under pressure. The pressure was given by the air, which was washed by passing a dilute alkaline solution and distilled water through it. The solution was prepared at each concentration by direct dilution in the cell, and the decrease in the concentration was determined by weighing the whole cell. The density decrease of the solution with dilution was neglected in this case. By measuring the refractive index of the solution before and after the filtration, it was confirmed that there is no appreciable decrease in the polymer concentration in the course of the filtration. The ionic strength and the pH were adjusted by the same method as has been described for the viscosity measurements. The scattering intensity was read about an hour later after setting.

Results

The relation between the reduced viscosity and the polymer concentration are shown in Fig. 2 at various ionic strengths. As the ionic strength was decreased, an unusual phenomenon characteristic of polyelectrolytes was observed at low concentrations. Nevertheless, extrapolation to zero concentration was possible except in the case of an extremely low ionic strength. The value of $[\eta]$ thus obtained decreased with an increase in the ionic strength. At a fixed ionic strength, the value of $[\eta]$ was largest in a neutral solution, middle in an alkaline solution, and the smallest in an acidic solution.

The results of light scattering are shown in Figs. 3, 4, 5, and 6. The experimental data were analyzed according to the equation for a two-component system:[15]

$$\frac{Kc}{R_\theta} = \frac{1}{\bar{M}_w}\left(1+\frac{16\pi^2}{3\lambda^2}\langle\bar{S^2}\rangle \sin^2(\theta/2)\right) + 2A_2c \qquad (1)$$

and

$$K = 2\pi^2 n_0^2 (dn/dc)^2/(\lambda_0^4 N) \qquad (2)$$

R_θ is the reduced scattering intensity of the solution at a scattering angle of θ, from which the term of the solvent is subtracted. c is the polymer concentration, M_w is the weight-average molecular weight of the molecule, dn/dc is the refractive index of the medium, and λ_0 is the wavelength *in*

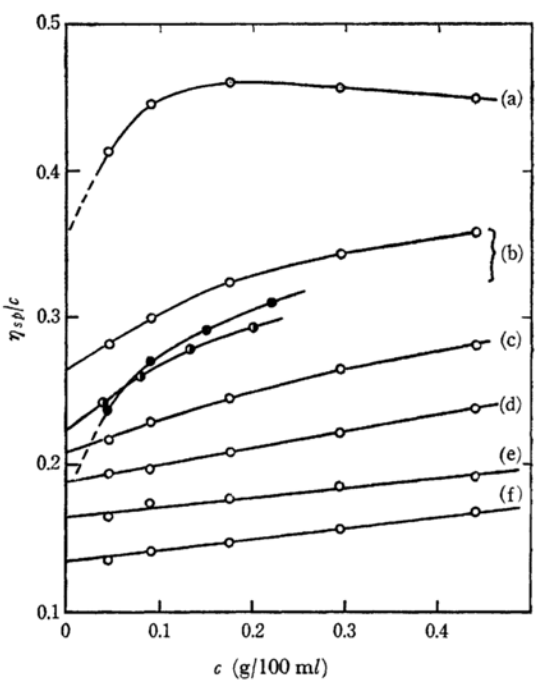

Fig. 2. Viscosity data for sodium chondroitin sulfate C obtained by dilution at a fixed ionic strength at 25°C.
(a), J=0.0035; (b), J=0.01 (○, pH 5.4; ●, pH 2.7; ◑, pH 11.5); (c), J=0.025; (d), J=0.05; (e), J=0.2; (f), J=1.0

15) B. H. Zimm, *ibid.*, **16**, 1099 (1948).

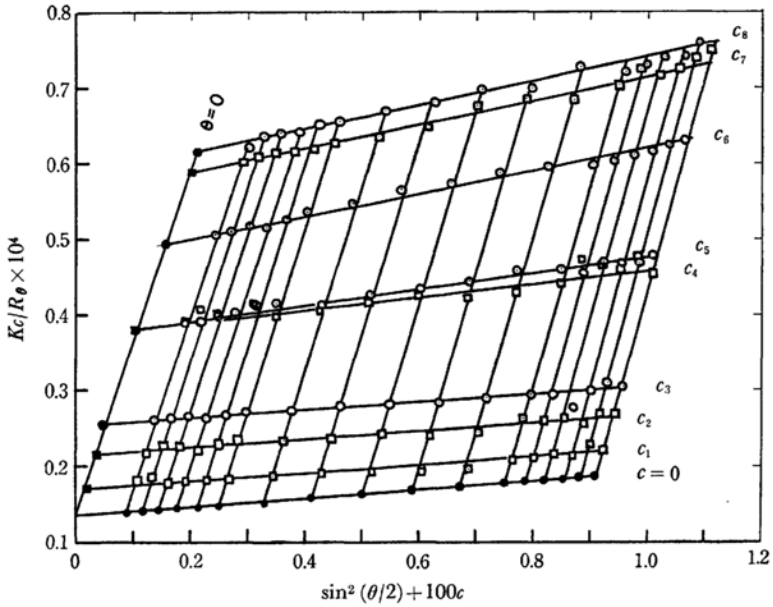

Fig. 3. Zimm plot for sodium chondroitin sulfate C in 0.01 M NaCl (pH 5.4) at 25°C. Polymer concentration: c_1, 0.01646%; c_2, 0.0341%; c_3, 0.04817%; b_4, 0.09824%; c_5, 0.1011%; c_6, 0.1545%; c_7, 0.2014%; c_8, 0.2112%

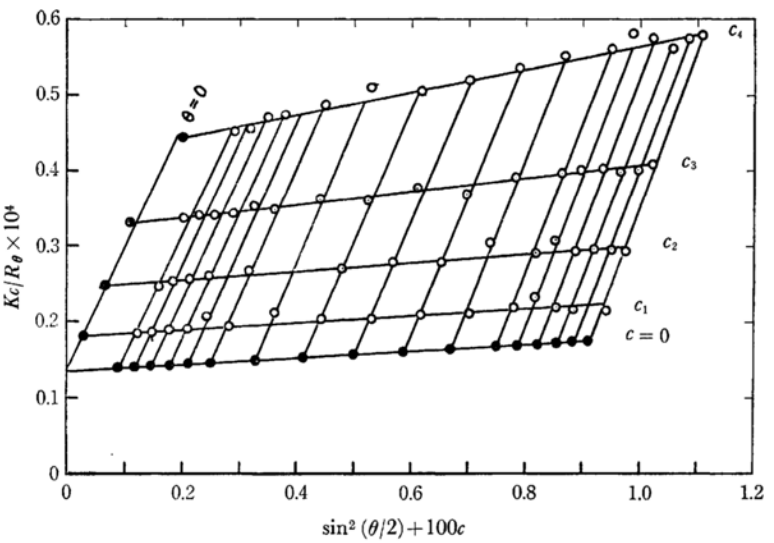

Fig. 4. Zimm plot for sodium chondroitin sulfate C in 0.01 M NaCl (pH 3.5) at 25°C. c_1, 0.03089%; c_2, 0.06355%; c_3, 0.1124%; c_4, 0.2014%

vacuo (4360 Å was used in this experiment). The wavelength, λ, in the medium is related to λ_0 as follows:

$$\lambda = \lambda_0/n_0 \tag{3}$$

$\langle\bar{S}^2\rangle$ is called the mean-square radius of gyration, which is the mean-square distance between the center of gravity and the respective mass element in the molecule; from it the molecular dimensions can be estimated. The following relation exists in the case of a long, rigid rod:[16]

$$\langle\bar{S}^2\rangle = L^2/12 \tag{4}$$

where L is the length of the rod. In the case of a randomly coiled molecule:

$$\langle\bar{S}^2\rangle = R^2/6 \tag{5}$$

where R is the root mean-square end-to-end distance of the flexible molecule.

In the Zimm plots of Figs. 3—6, the Kc/R_θ is

16) T. Neugebauer, *Ann. Phys.*, **42**, 509 (1943).

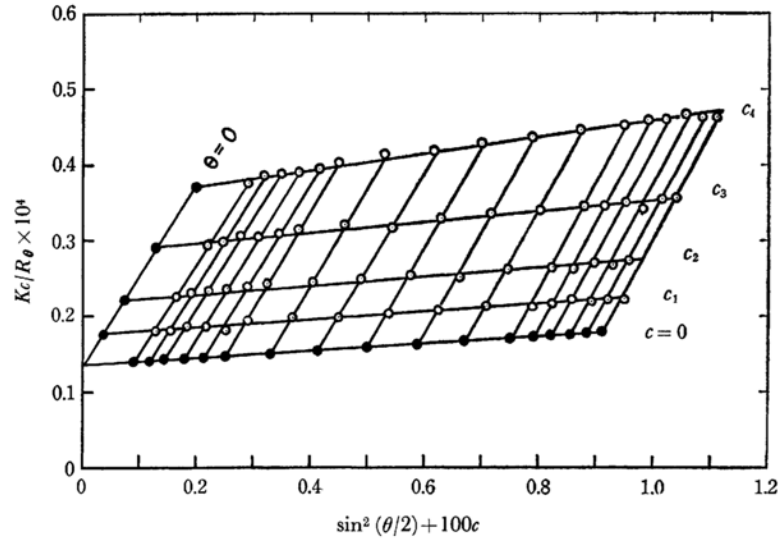

Fig. 5. Zimm plot for sodium chondroitin sulfate C in 0.025 M NaCl at 25°C. c_1, 0.03919%; c_2, 0.0764%; c_3, 0.1306%; c_4, 0.2014%

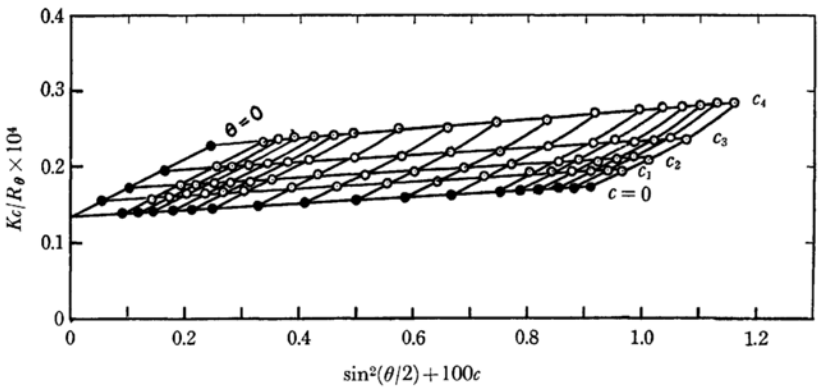

Fig. 6. Zimm plot for sodium chondroitin sulfate C in 0.2 M NaCl at 25°C. c_1, 0.05507%; c_2, 0.1028%; c_3, 0.1653%; c_4, 0.2464%

plotted as a function of $\sin^2(\theta/2)+100\,c$, where the value of 100 is an arbitrarily-selected constant. In Fig. 3, the open circles and squares are referred to independent experiments in order to show the reproducibility of the data. The refractive increment, dn/dc, was constant in every case within the limits of experimental error; its averaged value, 0.155, was used throughout.

On the basis of Eq. (1), the molecular weight was calculated from the intercept of the $c=0$ and $\theta=0$ lines on the ordinate; the mean-square radius of gyration, $\langle S^2 \rangle$ from the ratio of the initial slope of the $c=0$ line to the intercept on the ordinate, and the second virial coefficient, from the initial slope of the $\theta=0$ line. The results are collected in Table 2.

A constant molecular weight of 74000 was obtained in every case, and both the mean-square radius of gyration and the second virial coefficient decreased with an increase in the ionic strength or with a decrease in the pH of the solution at a fixed ionic strength. On the other hand, the molecular weight, estimated from the viscosity data according to this equation:[17]

$$[\eta] = 3.1 \times 10^{-4} \overline{M}_w{}^{0.74} \qquad (6)$$

was 80000 in a 0.15 M phosphate buffer (pH 7.0) containing 0.2 M NaCl at 25°C. The relation in Eq. (6) has been obtained for the A-type chondroitin sulfate; the molecular weight obtained from Eq. (6) is known to accord fairly well with that obtained from the results of terminal analysis for A-type molecule. However, the former is two or three times larger than the latter for the C type. In our case, the molecular weight determined by the

17) M. B. Mathews, *Arch. Biochem. Biophys.*, **61**, 367 (1965).

TABLE 2. EXPERIMENTAL RESULTS OF VISCOSITY AND LIGHT SCATTERING MEASUREMENTS FOR SODIUM CHONDROITIN SULFATE C IN VARIOUS IONIC STRENGTHS AT 25°C

J	0.0035	0.01*	0.025	0.05	0.2	1.0	∞
$[\eta]$	3.55	2.66	2.08	1.88	1.64	1.35	1.25
$[\eta]^{2/3}/[\eta]_\infty^{2/3}$	2.005	1.654	1.404	1.313	1.198	1.055	1.000
$\langle\bar{S}^2\rangle\times10^{14}$	—	848	744	—	641	—	580
$\langle\bar{S}^2\rangle/\langle\bar{S}^2\rangle_\infty$	—	1.462	1.283	—	1.105	—	1.000
$\langle\bar{S}^2\rangle^{1/2}$ (Å)	—	291	273	—	253	—	241
$A_2\times10^3$	24.5	11.5	5.88	—	1.9	—	1.3

$dn/dc = 0.155$, $\bar{M}_w = 74000$

* $\langle\bar{S}^2\rangle$ and $[\eta]$ at $J=0.01$ (pH 3.5 or 2.7) is in the same order as that at $J=0.2$ as seen from Figs. 13 and 2, respectively. A_2 at $J=0.01$ (pH 3.5) is 8.00×10^{-3}.

results of terminal analysis is 50000, while that obtained from the viscosity is 80000, according to Eq. (6). The value of 80000 is about 1.6 times as large as the value of 50000. The molecular weight, 74000, obtained by light scattering is rather closer to the value, 50000, by the results of terminal analysis.

Discussion

Treatment as a Flexible Coil. The intrinsic viscosity tabulated in Table 2 is plotted as a function of the reciprocal root of the ionic strength, $1/\sqrt{J}$, in Fig. 7; then the intrinsic viscosity, $[\eta]_\infty$, at the infinite ionic strength was estimated. In the same manner, the mean square of the radius of gyration, $\langle\bar{S}^2\rangle$, from light scattering is plotted as a function of $1/\sqrt{J}$ in Fig. 8, and $\langle\bar{S}^2\rangle_\infty$ was estimated at the infinite ionic strength.

On the other hand, the second virial coefficient, A_2, in Table 2 is plotted against the reciprocal ionic strength, $1/J$, and the reciprocal root of the ionic strength, $1/\sqrt{J}$, in Figs. 9 and 10 respectively. As the A_2 value is small at the infinite ionic strength, it can be considered that the medium is practically

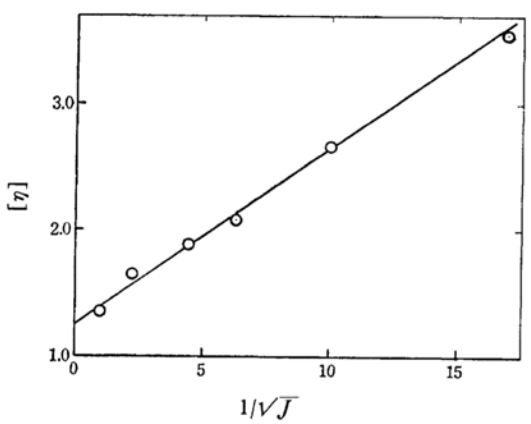

Fig. 7. Intrinsic viscosity of sodium chondroitin sulfate C plotted against reciprocal root of ionic strength $1/\sqrt{J}$.

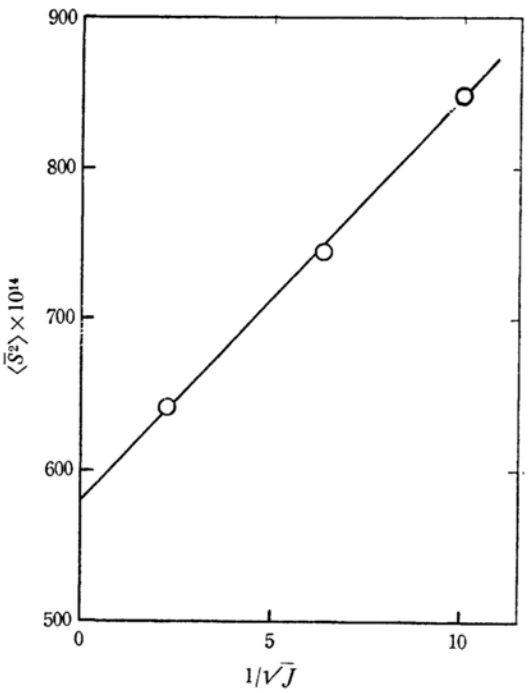

Fig. 8. Mean square radius of gyration $\langle\bar{S}^2\rangle$ plotted against reciprocal root of ionic strength $1/\sqrt{J}$.

a θ solvent at the ionic strength, and $[\eta]_\infty$ and $\langle\bar{S}^2\rangle_\infty$ may be regarded as $[\eta]_\theta$ and $\langle\bar{S}^2\rangle_\theta$ respectively.

The expansion factor of the macromolecule, α, is defined as the ratio of the radius of gyration to that in the θ solvent:

$$\alpha = \langle\bar{S}^2\rangle^{1/2}/\langle\bar{S}^2\rangle_\theta^{1/2} \quad (7)$$

Flory[18] has defined it as:

$$\alpha = [\eta]^{1/3}/[\eta]_\theta^{1/3} \quad (8)$$

Therefore, α^2 corresponds to $[\eta]^{2/3}/[\eta]_\infty^{2/3}$ or

18) P. J. Flory, "The Principles of Polymer Chemistry," Cornell Univ. Press (1953); P. J. Flory and T. G. Fox, *J. Am. Chem. Soc.*, **73**, 1904 (1951).

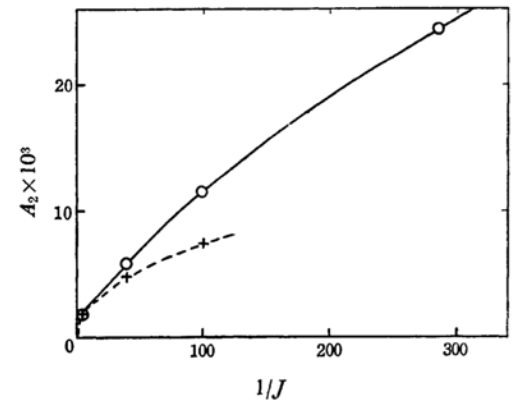

Fig. 9. Second virial coefficient A_2 plotted against reciprocal ionic strength $1/J$.
○, Experimental
+, Theoretical value by Eq. (10)

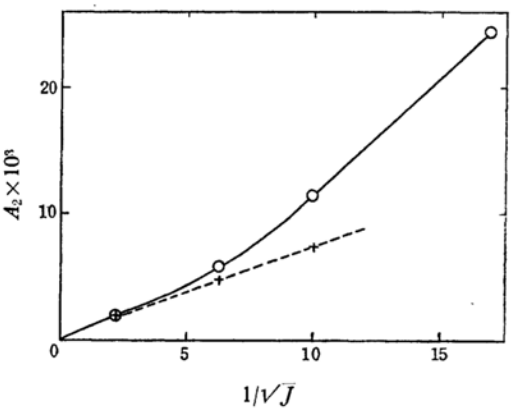

Fig. 10. Second virial coefficient A_2 plotted against reciprocal root of ionic strength $1/\sqrt{J}$.
○, Experimental
+, Theoretical value by Eq. (10)

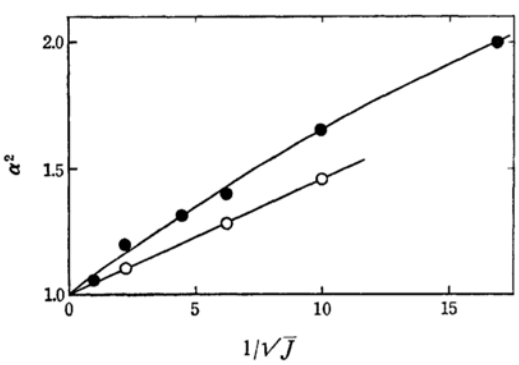

Fig. 11. Expansion factor α^2 obtained from viscosity (●) and light scattering (○), plotted against $1/\sqrt{J}$.

$\langle\bar{S}^2\rangle/\langle\bar{S}^2\rangle_\infty$. These quantities are plotted as a function of $1/\sqrt{J}$ in Fig. 11. With the decrease in ionic strength, the α^2 value obtained from the intrinsic viscosity increases more steeply than that obtained from light scattering. The discrepancy between the two curves can easily be expected to be even more severe when α is estimated from another relation obtained recently[19]:

$$\alpha \doteqdot [\eta]^{1/2.43}/[\eta]_\theta{}^{1/2.43} \tag{9}$$

The second virial coefficient calculated theoretically on the basis of the light-scattering data ($\langle\bar{S}^2\rangle$, $\bar{M}_w$, and α) and the following Flory relation,[19] is plotted as a function of $1/J$ and $1/\sqrt{J}$, together with the experimental second virial coefficient, in Figs. 9 and 10 respectively.

$$A_2 = (16\pi N/3^{3/2})[\langle\bar{S}^2\rangle^{3/2}/\bar{M}_w{}^2] \ln [1+(\pi^{1/2}/2)(\alpha^2-1)] \tag{10}$$

There is a large difference between the theoretical and experimental results here. It may, however, be meaningless to discuss the virial coefficient further, since no clear explanations have been obtained for many polyelectrolytes, such as sodium carboxymethyl cellulose,[20] sodium polyphosphate,[21,22] and potassium polyvinyl sulfonate.[22,23]

Considering a flexibly-coiled molecule as a sphere, the intrinsic viscosity (ml/g) of the spherical molecule can generally be expressed as follows:

$$[\eta] = 2.5\cdot\frac{4}{5}\cdot R_e\cdot\frac{N}{M} \tag{11}$$

where R_e is the effective radius of the sphere. By Kirkwood and Riseman's treatment,[24] the radius, R_e, is related to the radius of the gyration of the flexible coil of an adequately large degree of polymerization as follows:

$$R_e = 0.875\langle\bar{S}^2\rangle^{1/2} \tag{12}$$

Accordingly, the root mean-square radius of gyration $\langle\bar{S}^2\rangle^{1/2}$ is obtained through Eqs. (11) and (12) as 130 Å at the infinite ionic strength by consulting the value given in Table 2, $[\eta]=125$. On the other hand, the value of 241 Å was obtained as the value of $\langle\bar{S}^2\rangle^{1/2}$ from light scattering. In view of these facts, this molecule can not be considered as a flexible coil of a spherical shape.

Treatment as an Ellipsoid or a Rigid Rod. The intrinsic viscosity, $[\eta]$ (ml/g), is related to the hydrodynamic volume, V_h, molecular weight, M, and Avogadro's number, N, by:

19) T. A. Orofino and P. J. Flory, *J. Phys. Chem.*, **63**, 283 (1959).
20) H. J. Trap and J. J. Harmans, *ibid.*, **58**, 757 (1954); N. S. Schneider and P. Doty, *ibid.*, **58**, 762 (1954).
21) U. P. Straus and P. Ander, *J. Am. Chem. Soc.*, **80**, 6494 (1958); U. P. Straus and P. L. Wineman, *ibid.*, **80**, 2366 (1958).
22) N. Ise, *J. Phys. Chem.*, **67**, 382 (1963).
23) H. Eisenberg and E. Casassa, *J. Polymer Sci.*, **47**, 29 (1960); H. Eisenberg and D. Woodside, *J. Chem. Phys.*, **36**, 1844 (1962).

TABLE 3. MOLECULAR DIMENSIONS OF CHONDROITIN SULFATE C ESTIMATED FROM INTRINSIC VISCOSITY-PARTIAL SPECIFIC VOLUME, LIGHT SCATTERING-PARTIAL SPECIFIC VOLUME AND INTRINSIC VISCOSITY-LIGHT SCATTERING

J		0.01	0.025	0.2	∞
a (Å)	vis.	507	461	420	380
	L.S.	504	473	438	422
b (Å)	vis.	5.27	5.52	5.80	6.08
	L.S.	5.28	5.46	5.67	5.78
f	vis.	96.2	83.5	72.4	62.5
	L.S.	95.5	86.6	77.2	73.0
L (Å)	vis.	1014	922	840	760
	L.S.	1008	946	876	843
D (Å)	vis.	8.61	9.01	9.47	9.93
	L.S.	8.62	8.92	9.26	9.44
$\langle\bar{S}^2\rangle^{1/2}$	vis.	293	266	243	219
	L.S.	291	273	253	241
	f(vis.)	293	267	244	220
	f(L.S.)	293	268	245	222

$$[\eta] = \nu \frac{N}{M} V_h \tag{13}$$

Moreover, V_h is connected with the partial specific volume, $\bar{v}_2$, of the solute by:

$$V_h = \frac{M}{N} (\bar{v}_2 + \delta_1 v_1^\circ) \tag{14}$$

where v_1° is the specific volume of the pure solvent and δ_1 is the adsorption coefficient of the solvent to the solute. The ν in Eq. (13) is expressed by Simha[25] for prolate ellipsoids with the axial ratio, f, as follows:

$$\nu = \frac{f^2}{15(\ln 2f - 3/2)} + \frac{f^2}{5(\ln 2f - 1/2)} + \frac{14}{15} \tag{15}$$

The values of ν have been computed and tabulated by Scheraga[26] for various f values. According to Eqs. (13), (14) and (15), the hydrodynamic volume and the axial ratio can be estimated from the experimental values of $[\eta]$, M, and $\bar{v}_2$, supposing $\delta_1 = 0$. If the molecules are thus assumed to be prolate ellipsoids, the two semi-axes, a and b, of the ellipsoid can be obtained from the values of the hydrodynamic volume and the axial ratio according to these equations:

$$V_h = \frac{4}{3} \pi a b^2 \tag{16}$$

$$f = a/b \tag{17}$$

If the molecules are, on the other hand, assumed to be rigid rods with the same volume and length as the ellipsoid, then the length, L, and the diameter, D, of the rod will be:

$$L = 2a \tag{18}$$

$$D = (2/3)^{1/2} 2b \tag{19}$$

and the radius of gyration will be:

$$\langle\bar{S}^2\rangle^{1/2} = L/12 \tag{20}$$

The results obtained in this manner are shown in Table 3 on the lines designated as (vis.) and in Fig. 12 for various ionic strengths, J.

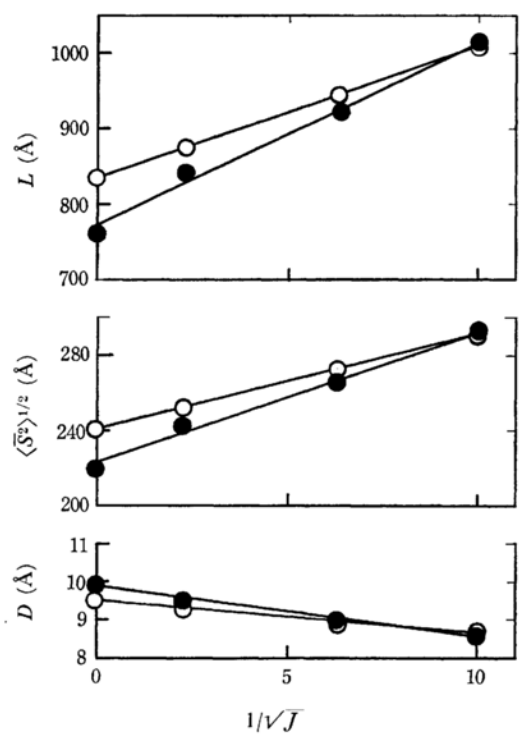

Fig. 12. Molecular dimensions of chondroitin sulfate C obtained, assuming a rigid rod.
○, light scattering-partial specific volume
●, intrinsic viscosity-partial specific volume

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On the other hand, the length, L, and the diameter, D, of the rod can also be calculated from the radius of gyration by light-scattering experiments and from the molecular volume obtained from the specific volume, according to Eq. (20) and this equation:

$$V_h = \pi(D/2)^2 L \tag{21}$$

These results, too, are shown in Table 3, on the lines designated as (L. S.) and in Fig. 12 for various values of the ionic strength, J.

As for the radius of gyration, it has been shown that the $\langle\bar{S}^2\rangle^{1/2}/([\eta]M)^{1/3}$ quantity can be expressed as a function of the axial ratio, f, only.[27] Therefore, the value of $\langle\bar{S}^2\rangle^{1/2}$ can be calculated by using the values of the axial ratio, f(vis.) or f(L. S.), together with the $[\eta]$ values and with $M=74000$. These results, shown in Table 3, show a fairly good agreement with each other.

As Fig. 12 shows, the results from the viscosity-partial specific volume and from the light scattering-partial specific volume show a certain disagreement with each other when the ionic strength is large. Even at the infinite ionic strength, however, the radius of gyration, 241 Å, obtained from light scattering, is much closer to the value as a rigid rod (219 Å) than to the value as a flexible coil (130 Å).

When we assume that the length of a repeating disuccharide unit is of the order of 10 Å, the residue weight, 490, and the molecular weight, 74000, will give a rough estimate of 1500 Å for the length of the polymer chain. Accordingly, the rod length of 843 Å obtained from light scattering or that of 760 Å obtained from the intrinsic viscosity must mean that the molecule is two-fold, if the polymer chain is assumed to be extended in full. If the polymer is, however, assumed to have a helical structure, as has been emphasized in an optical rotatory dispersion experiment,[7] sodium chondroitin sulfate C can probably be considered to be a helical rod with 5.0—5.6 Å of pitch per repeating disuccharide unit.

Consideration at Finite Polymer Concentrations. The slopes on the plot of Kc/R_θ against $\sin^2(\theta/2)$ at various concentrations:

$$g = \mathrm{d}(Kc/R_\theta)/\mathrm{d}\sin^2(\theta/2) \tag{22}$$

are considered to be related to the dimentions of the solute.

They are plotted against the polymer concentration in Fig. 13. It is apparent that the magnitude of the slope is unchanged up to a certain critical polymer concentration, and then steeply increases. It reaches a limiting value, when the ionic strength is low. This phenomenon can probably be considered to be related to its unusual viscosity behavior at low polymer concentrations, and also to the metachromatic color change,[28,29] observed at higher polymer concentrations. Furthermore, the chondroitin sulfate C is known to develop a non-Newtonian viscosity. It is, however, not sure at present that this behavior of chondroitin sulfate C is due to its rod shape, to the association of the molecules, or to the orientation of the highly-charged, rod-like macromolecules.

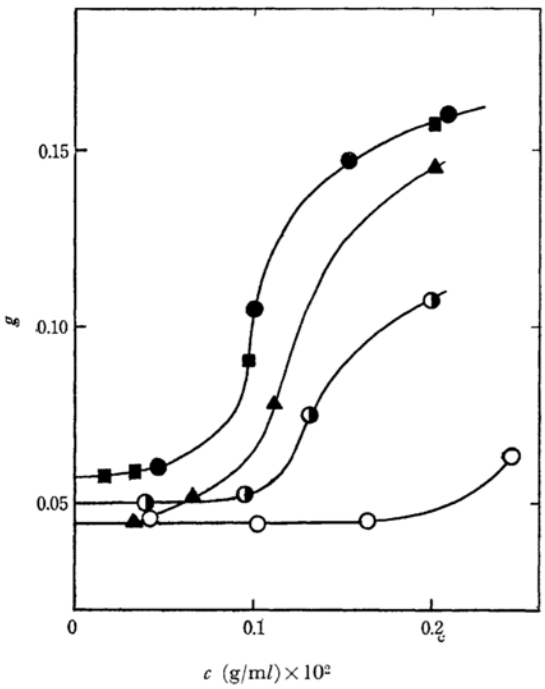

Fig. 13. Slopes on the plot of Kc/R_θ *vs.* $\sin^2(\theta/2)$ at various polymer concentrations.
●, ■, $J=0.01$; ▲, $J=0.01$ (pH 3.4); ◑, $J=0.025$; ○, $J=0.2$

The authors wish to express their thanks to the Seikagaku Kogyo Co., Ltd., for furnishing the material and to Mr. Tamotsu Furuhashi for his analysis of the preparation.

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