Ionic Polysaccharides. I. Adsorption and Fractionation of Polyelectrolytes on (Diethylamino)ethyl Cellulose[1]

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Abstract: Adsorption equilibrium on and elution from DEAE-cellulose have been studied for the ionic polysaccharide series: hyaluronate, chondroitin sulfate, and heparin. *Exhaustive* elution of a given polymer at a molar concentration M of NaCl is characterized by the parameter θ^*, the ratio of the weight adsorbed per gram of absorbent to the maximum adsorption capacity for that polymer. Elution data are fitted approximately by the linear relation: $1 - \theta^* = (M - M_1)/(M_0 - M_1)$, where M_1 and M_0 are constants, corresponding approximately to M values for initial desorption from a saturated surface ($\theta^* = 1$) and for complete elution, respectively. The θ^*–M relation appears not to depend on molecular weight for hyaluronate. The values of M_0 and M_1 increase with polymer charge density. The polymer desorption is exothermal, since hyaluronate solubility increases with temperature decrease. Stepwise elution of hyaluronate adsorbed on DEAE-cellulose leads to efficient molecular-weight fractionation. Breadth of distribution in selected fractions is characterized by the average value of 1.2 for the weight- to number-average molecular weight.

A search for a rapid, efficient molecular-weight fractionation method for ionic polysaccharides, particularly sodium hyaluronate, led us to an investigation of (diethylamino)ethyl cellulose (DEAE-cellulose) as a polymer adsorbent. Chromatographic investigations of sodium pectate adsorbed on DEAE-cellulose,[2] heparin on ECTEOLA-cellulose,[3-5] sodium hyaluronate on DEAE-Sephadex [cross-linked (diethylamino)ethyl dextran][6] or ECTEOLA-cellulose,[7] and keratan sulfate on ECTEOLA-cellulose[8] have given indications that some degree of separation by molecular weight was effected upon elution of the polymer. The eluent, either NaCl or HCl–NaCl mixtures, removed increasing molecular weights as the Cl^- concentration was increased, with use either of gradient or stepwise elution. Upon dilution of a solution containing hyaluronate, cetylpyridinium chloride, and sodium sulfate, hyaluronate fractions which decreased in molecular weight in successive dilutions were obtained as precipitates of the cetylpyridinium salt.[9] Fractionation by molecular weight was also indicated upon chromatographic elution of hyaluronate adsorbed on Celite (diatomaceous earth).[10] Of the chromatographic work mentioned, only the investigations of keratan sulfate[8] and heparin[5] contain sufficient published information to demonstrate the utility of cross-linked polysaccharide anion-exchange adsorbents for molecular-weight fractionation of polyelectrolytes. The present work presents more complete evidence than heretofore that elution from such an adsorbent provides efficient fractionation. In addition, a study of the equilibrium properties of the desorption reaction is presented.

(1) This investigation was supported by U. S. Public Health Service Research Grant GM08113 from the National Institute of General Medical Sciences.

(2) W. Heri, H. Neukom, and H. Deuel, *Helv. Chim. Acta*, **44**, 1939 (1961).

(3) ECTEOLA-cellulose is the reaction product of cellulose with epichlorohydrin and triethanolamine.

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(5) T. C. Laurent, *Arch. Biochem. Biophys.*, **92**, 224 (1961).

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(8) T. C. Laurent and A. Anseth, *Exptl. Eye Res.*, **1**, 99 (1961).

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Experimental Section

Materials. Glucuronic acid, its lactone, and cetylpyridinium chloride (CPC) were commercially available. DEAE-cellulose (Schleicher and Schuell) had a specified capacity of 0.87 mequiv/g; potentiometric titration with base gave 0.74 mequiv/g. Bovine vitreous humor hyaluronate (BVH), potassium salt, crude (Nutritional Biochemicals), contained about 50% hyaluronate and 7% protein. The intrinsic viscosity $[\eta]$ varied from 640 to 740 ml/g in different lots. Tumor hyaluronate from a human mesothelioma source was supplied by J. E. Scott. This material had been purified by precipitation with CPC and contained 73% by wt of dry hyaluronate and *ca.* 2% protein: $[\eta]$ 1310 ml/g in 0.2 M NaCl, 980 ml/g in 1 M NaCl. Chondroitin sulfate, crude (General Biochemicals), was about 54% pure and contained 10% protein: $[\eta]$ 36 ml/g. Heparin, crude (General Biochemicals), was 40% pure, 2% protein: $[\eta]$ 21 ml/g.

Analytical Methods. Hexuronic acid content was determined by the carbazole method[11] with omission of the ice-cooling step. Color development was carried out for 2 hr in a 25° bath. Calibration with glucuronic acid, whose purity was determined by potentiometric titration, gave for w, the hexuronic acid concentration in g/l., $w = 0.108(\alpha - \alpha_0)$, where α and α_0 are the optical densities of test solution and blank at 525 mμ in cells of 11.66-mm length. This result was confirmed by calibration with glucuronic acid lactone. The value of w for polymer samples was reproducible to about 2 mg/l.

The protein content was determined by use of the Lowry technique,[12] which assays aromatic amino acid content. Calibration of this method with bovine serum albumin gave for W, the protein concentration in g/l., $W = 0.36(A - A_0)$, where A and A_0 are the optical densities for sample and blank at 600 mμ in cells of 11.66-mm length.

Physical Measurements. Viscosities were measured in Cannon–Ubbelohde dilution viscometers in 0.2 M NaCl (unless otherwise stated). Values of $[\eta]$ were determined by extrapolation to zero polymer concentration or by use of the Huggins equation

$$\ln \eta_{rel}/c = [\eta] - k''[\eta]^2 c$$

where η_{rel} is viscosity of solution relative to solvent, c is polymer concentration, and k'' is a constant taken to be 0.15 for sodium hyaluronate in 0.2 M NaCl.

Weight-average molecular weights $\bar{M}_w$ of hyaluronate fractions in 0.5 M NaCl were determined by light scattering using an instrument described by Shultz,[13] with the modification that relative light

(11) Z. Dische, *J. Biol. Chem.*, **167**, 189 (1947).

(12) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *ibid.*, **193**, 265 (1951).

(13) A. R. Shultz, *J. Am. Chem. Soc.*, **76**, 3422 (1954).

intensities were obtained by direct dc measurement of photocurrent with a sensitive galvanometer. Intensities were measured at 11 angles from 30 to 135° with the incident vertically polarized beam of 4358-Å wavelength. Solutions were clarified by centrifugation at about 28,000*g* for 3 hr. The differential refractive index increment was found to be 0.140 ml/g for sodium hyaluronate dialyzed against 0.5 *M* NaCl. Molecular weights were obtained by the Zimm method[14] as modified for multicomponent systems by Casassa and Eisenberg.[15] Number-average molecular weights $\bar{M}_n$ of hyaluronate fractions in 0.5 *M* NaCl were determined by use of a Mechrolab Model 501 membrane osmometer.

Batch Adsorption and Elution. In all cases *adsorbent* refers to DEAE-cellulose. A slurry of the acid- and alkali-washed adsorbent was prepared in water (or dilute NaCl) for rapid volumetric transfer. Weighed portions of polymer solution and adsorbent slurry were mixed. After a suitable adsorption period the system was centrifuged, the supernatant withdrawn, and eluting solvent of the desired NaCl concentration added. The tube contents were allowed to equilibrate for at least 24 hr with mechanical agitation and were again centrifuged. This cycle was repeated at each NaCl concentration until polymer concentration dropped to 0.01–0.02 g/l. (exhaustive elution). Adsorbent capacity was determined by adding an excess of polymer in salt-free solution to the adsorbent slurry.

Analytical Column Fractionations. A 1.3-cm i.d. column, packed with about 9 g of adsorbent, was loaded with tumor hyaluronate in aqueous solution at the top of the column. In fractionation a, a sample of 48 mg was eluted at a flow rate of 4.5 ml/hr by the gradient-elution method. The polymer appeared in the eluate at 0.29 *M* NaCl and was eluted at nearly constant concentration until 70% elution had occurred. Elution was complete at a volume of 265 ml, or 0.8 *M* NaCl. In fractionation b, 195 mg of tumor hyaluronate was added in 0.05 *M* NaCl and eluted in 0.5 *M* NaCl at the same flow rate.

Two further fractionations were carried out in a small column, 9-mm i.d. × 15 cm long. The polymer was adsorbed by mixing with adsorbent slurry as described above. The slurry was poured into the column; packing occurred by gravity flow. Stepwise elution was carried out at 6.5–8.5° with successively increasing concentrations of NaCl. In fractionation c, 58 mg of crude BVH, $[\eta]$ 740 ml/g, was mixed with 0.76 g of adsorbent. Fractions were eluted in about 200 ml of each solvent, usually over a 4–6-hr period. A refractionation of fraction 18 (Preparative Fractionation) was performed on 10.3 mg adsorbed on 45 mg of adsorbent. Elution was carried out at 0.6 ml/hr for about 24 hr for each fraction.

Preparative Fractionation. A solution of crude BVH containing 16.2 g of hyaluronate and 2.3 g of protein was partially purified by precipitation with CPC and acetone. The product containing 14.7 g of hyaluronate was adsorbed on 75.5 g of adsorbent. All but 0.2 g of hyaluronate and no more than 0.2 g of protein were adsorbed from 0.05 *M* NaCl. The slurry was poured into a column 5-cm i.d. × 50 cm long. Fractions were removed by stepwise elution, which was continued until polymer concentration dropped to about 0.01 g/l. This required from 10 to 13 l. of solvent, collected over a period of at least 24 hr. Polymer fractions were recovered by precipitation with CPC, followed by continuous-flow centrifugation. Recovery in the latter step was 70–80%; losses were due chiefly to solubility of cetylpyridinium hyaluronate. Final recovery was effected by twice redissolving the precipitate in NaCl and reprecipitating with acetone.

Results and Discussion

Approach to Equilibrium. When a salt-free solution of sodium hyaluronate is stirred with excess DEAE-cellulose, no trace of polymer is found after a few minutes of stirring. When polymer and adsorbent were mixed at 0.27 *M* NaCl, approximate equilibrium with respect to polymer concentration was reached in about 1 hr. The value of $[\eta]$ decreased over a much longer period as indicated by the following data [given as time (hr), $[\eta]$ (ml/g)]: 0.25, 1330; 1.3, 1213; 5.0, 1226; 7.3, 1068; 22.7, 983; 240, 936. Evidently equilibrium with respect to molecular weight, as indicated by $[\eta]$, required at least 24 hr. The conclusion is that, when NaCl is present during adsorption, all molecular weights are adsorbed, followed by an exchange between adsorbed and solution phases, during which the solution phase is enriched in small molecules and the adsorbed phase in large ones.

A similar study of desorption was made by addition of NaCl to preadsorbed hyaluronate to give a concentration of 0.28 *M* NaCl. A steady polymer concentration was reached within 6 hr as indicated by the following data [given as time (hr), concn (g/l.)]: 0.33, 0.68; 1.17, 0.79; 6.0, 0.96; 47, 0.94; 169, 0.98. In the desorption case, however, the equilibrium molecular weight was established immediately. Values of $[\eta]$ were [time (hr), $[\eta]$ (ml/g)] 0.33, 1147; 1.17, 1078; 6.0, 1100; 169, 1121. The larger molecules appear not to be desorbed.

Equilibrium Properties. The equilibrium of the system, water–ionic polysaccharide–NaCl–DEAE-cellulose, was studied for the sodium salts of hyaluronic acid, chondroitin sulfuric acid, and heparin. Analysis of batch and column elution data indicated that the fractional capacity θ of adsorbent occupied by the polymer species was an important parameter. The capacity C was defined operationally as the weight adsorbed per gram of adsorbent from an excess of polymer in salt-free solution, so that θ = weight adsorbed per gram of adsorbent/C. The value of θ after *exhaustive* elution at a given molarity M of the NaCl eluent, indicated by θ^*, was found to depend to a good approximation only on M and the temperature. By exhaustive elution is meant that the polymer concentration fell to about 0.01 g/l. The relation of θ^* to M is shown in Figure 1. While the complete curves have a sigmoidal shape, the experimental points at most NaCl concentrations define a reasonably straight line of the form: $\theta^* = aM + b$. If the value of M at the intercept of this line at $\theta^* = 1$ is called M_1 and that at $\theta^* = 0$ is called M_0, $a = 1/(M_0 - M_1)$, and

$$\theta^* = \frac{M_0 - M}{M_0 - M_1} \tag{1}$$

The constants for each polymer are given in Table I. Equivalent capacities were calculated with the assumption of two and three and one-half ionizable groups per disaccharide unit for chondroitin sulfate and heparin, respectively.[16] The equivalent adsorbent capacity by base titration is 0.74 mequiv/g, so that 80–90% of base equivalents of polymer can be adsorbed. The relationship of θ^* to M appears to be independent of molecular weight, since the points in Figure 1 corresponding to the refractionation of fraction 18 (Preparative Fractionation) represent material of substantially

Table I. Parameters for Elution from DEAE-cellulose

Polymer	Temp, °C	C, g/g	C, mequiv/g	M_1	M_0
Hyaluronate	25	0.255	0.59	0.015	0.292
Hyaluronate	9			0.015	0.267
Chondroitin sulfate	25	0.17	0.68	0.097	0.55
Heparin	25	0.11	0.62	0.27	0.65

(14) B. H. Zimm, *J. Chem. Phys.*, **16**, 1099 (1948).
(15) E. F. Casassa and H. Eisenberg, *J. Phys. Chem.*, **64**, 753 (1960).
(16) M. Stacey and S. A. Barker, "Carbohydrates of Living Tissues," D. Van Nostrand Co., London, 1962, pp 65, 97.

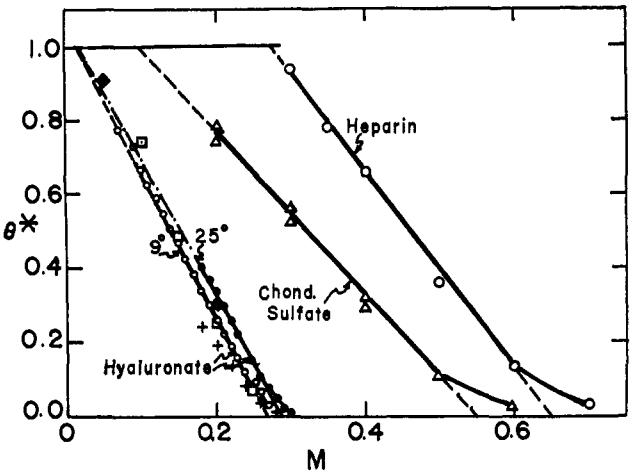

Figure 1. Exhaustive polymer elution as a function of the NaCl molarity, M. Dashed lines are extrapolations of linear sections of curves. Heparin and chondroitin sulfate points were obtained from batch adsorption experiments. Hyaluronate points represent: ○, preparative column at 9°; +, analytical column at 8.5°; ●, analytical column at 25°; ◆, batch adsorption at 25°; □, column refractionation of fraction 18 at 6.5°.

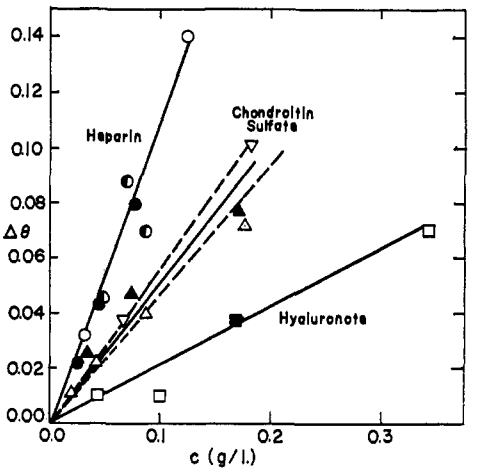

Figure 2. Polymer equilibrium data. Solid lines represent attempts to fit all data for a given polymer. The dashed lines for chondroitin sulfate show possible effect of molecular weight; the lower dashed line represents a rough linear fit of data at 0.2 M NaCl, upper that at 0.4 M. Symbols correspond to elution at different values of M (in parentheses): hyaluronate, □ (0.2 M), ■ (0.25 M); chondroitin sulfate, △ (0.2 M), ▲ (0.3 M), ▽ (0.4 M); heparin, ● (0.35 M), ◐ (0.4 M), ○ (0.5 M).

higher $[\eta]$ at a given θ^* than do those for BVH (*cf.* Tables V and VI).

Decreasing temperature causes a decrease in θ^* at a given value of M in the column elutions of hyaluronate, according to Figure 1. Direct confirmation of this result was obtained in a batch experiment, in which exhaustive elution in 0.2 M NaCl at 25° gave $\theta^* = 0.33$. A decrease in the temperature to 2° caused desorption until θ reached 0.21. The polymer was readsorbed at 25°, indicating reversibility. The desorption reaction may be written

$$n\mathrm{Cl}^- + \mathrm{R}_n\mathrm{P} \longrightarrow \mathrm{P}^{n-} + n\mathrm{RCl} \qquad (2)$$

where R represents an amine group of the adsorbent and P^{n-} is a polyanion bearing n negative charges. The desorption is exothermal, since reaction 2 goes to the right with decreasing temperature. This result agrees with the finding[17] that the adsorption of the sodium salt of poly(methacrylic acid) on DEAE-Sephadex is endothermal. We have also found that cetylpyridinium hyaluronate increases in solubility in NaCl solution with decreasing temperature.

The equilibrium with respect to polymer in solution may also be estimated. Experiment indicates that a volume (cc) about five times the dry weight (g) of adsorbent is unavailable to unadsorbed hyaluronate. The polymer mass balance was calculated with the assumption that all other volume is supernatant. This assumption affects principally the hyaluronate calculation, since adsorption of that species is weakest. The calculated results, plotted in Figure 2, indicate proportionality of $\Delta\theta = \theta - \theta^*$ and the polymer concentration c in solution. Estimated values of the adsorption coefficient $\Delta\theta/c$ are 0.2 for hyaluronate, 0.5 for chondroitin sulfate, and 1.1 for heparin; these values are roughly proportional to the linear charge densities based on proposed structures:[16] 1, 2, and 3.5 charges per disaccharide unit, respectively.

The most attractive theoretical models are those which assume an ion-exchanging surface[18] or an adsorbed liquid solution phase.[17] The predictions of equilibrium deduced from these models do not differ importantly; both represent essentially mass-action formulations of reaction 2. The result of Semenza, who used a Langmuir adsorption treatment, may be written

$$\frac{1}{N}\log\frac{\theta}{f_{\mathrm{P}}[\mathrm{P}]} = \log K + \log\frac{1-\theta}{f_{\mathrm{Cl}}[\mathrm{Cl}^-]} \qquad (3)$$

where [P] and $[\mathrm{Cl}^-]$ represent (molar) concentrations, N is the number of adsorbent sites associated with a polymer molecule, f_{P} and f_{Cl} are activity coefficients, and K is an equilibrium constant. In the derivation of eq 3, the site fraction $1 - \theta$ not associated with polymer was assumed to be associated with chloride ions. The appropriate value of N is, in general, not known. However, when N may be assumed large, say 100 or more, the left-hand side of eq 3 changes only slightly with [P], so that the last term in eq 3 is nearly constant. This prediction may be compared with experiment by rewriting eq 1 in the form

$$1 - \theta^* = M_{\mathrm{eff}}/(M_0 - M_1) \qquad (4)$$

where $M_{\mathrm{eff}} = M - M_1$, the molarity of NaCl in excess of the threshold concentration M_1 at which desorption begins. The ratio $(1 - \theta^*)/M_{\mathrm{eff}}$ is seen to be constant for the linear sections of the curves of Figure 1. The resemblance of this result to the prediction of eq 3 for large N is evident.

When activity coefficients are ignored, eq 3 predicts approximate linearity of log [P] with $\Delta\theta$, however, which is incompatible with the data of Figure 2. A theory which properly accounts for activity coefficients in each phase will undoubtedly be required for adequate treatment of this system. The fact that significant salt concentrations are required to reach appreciable equilibrium polymer concentrations in solution is ascribable, as in coacervate phases,[19] to elementary effects of ionic strength on solubility.

(17) J. Feitelson and R. Josephs, *Biopolymers*, **1**, 331 (1963).
(18) G. Semenza, *J. Chromatog.*, **18**, 359 (1965).
(19) M. J. Voorn, *Fortschr. Hochpolymer.-Forsch.*, **1**, 192 (1959).

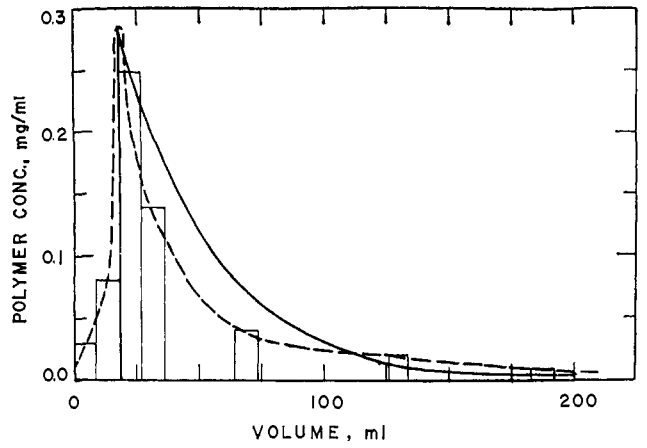

Figure 3. Change of hyaluronate concentration with elution volume. The data show elution by 0.2 *M* NaCl during analytical fractionation c. Block heights represent polymer concentration (areas give the polymer weight) in a subfraction. The dotted line represents fit of experimental data and the solid line, a hypothetical exponential decrease in concentration.

Polymer Fractionation. Fractionations of sodium hyaluronate from two sources have been performed to demonstrate the utility of the ion-exchange adsorption technique and to prepare a series of fractions for physical studies. In two preliminary column fractionations (a and b), polymer was loaded at the top of a column previously packed with adsorbent. This technique was practicable, but tended to clog the column. In fractionation a, mesothelioma tumor hyaluronate was eluted by a gradient-elution technique. Analytical data at selected values of eluent NaCl molarity *M* gave [*M*, $[\eta]$ (ml/g in 1 *M* NaCl)]: 0.29, 770; 0.34, 830; 0.38, 1040; 0.40, 1170; 0.45, 1180; 0.50, 840; 0.54, 950; 0.59, 825. The value of $[\eta]$ for the unfractionated polymer was 980 ml/g in 1 *M* NaCl. Some fractionation by molecular weight occurred until the eluent reached about 0.40 *M*, at which concentration all hyaluronate is soluble. No evidence of fractionation occurred thereafter.

Tumor hyaluronate was eluted from the same column with 0.5 *M* NaCl in fractionation b. The concentration of polymer in the eluate increased initially to a maximum of nearly 0.5 mg/ml and then decreased exponentially to 10% of the maximum after passage of 1 l. of eluent. Little or no fractionation by molecular weight occurred. Recovery in both fractionations a and b exceeded 90%.

A batch fractionation of the tumor hyaluronate led to the results given in Table II. Each fraction was obtained experimentally as a set of three or four subfractions at the same NaCl concentration. Values of $[\eta]$ within a set of subfractions always showed approximate constancy or small increases as extraction was repeated. The values of $[\eta]$ reported are weight averages of the values for the subfractions. The average $[\eta]$ for all fractions recovered is 897 ml/g, substantially less than the value of 1310 ml/g for the unfractionated material. Recovery was only 80%; some high-molecular-weight polymer was evidently not recovered or some degradation occurred during the elution process.

The preliminary experiments demonstrate that molecular-weight fractionation occurs essentially as an equilibrium extraction. The superior separation in the batch fractionation to that in the gradient-elution experiment suggested that stepwise elution combined with the practical advantages of the column would prove the most efficient technique.

Table II. Batch Fractionation of Tumor Hyaluronate at 5°

Fraction	Eluent (NaCl), *M*	Polymer recovered, mg	$[\eta]$, ml/g
5	0.18	0.5	...
6	0.20	26.4	410
7	0.22	22.4	600
8	0.24	36.3	770
9	0.26	24.1	980
10	0.28	21.9	1190
11	0.30	15.4	1640
12	0.32	4.2	1920
13	0.34	0.4	...
14	0.40	0.0	
		151.6	

Stepwise column elutions of BVH adsorbed on DEAE-cellulose prior to column packing have been performed. Elution was continued at a given NaCl concentration until the polymer concentration fell to 0.01–0.02 g/l. The data for fractionation c of 58 mg of BVH given in Table III indicate quantitative recovery of polymer, as well as efficient fractionation.

Table III. Analytical Column Fraction of Hyaluronate at 8.5°

Eluent (NaCl), *M*	Hyaluronate recovery, mg	Protein recovery, mg	$[\eta]$,[a] ml/g
0.05	...	2.5	
0.15	..	2.5	
0.18	11.7	1.3	299
0.20	9.9	...	353
0.22	11.1	...	573
0.24	10.1	...	950
0.26	8.8	...	1370
0.28	4.9	...	1511
0.30	1.6	...	(1800)[b]
0.50	0.7	...	(2000)[b]
	58.8		

[a] Calculated from single viscosity measurements. [b] Assumed value.

No evidence of degradation appears; the weight-average value of $[\eta]$ for the fractions is 795 compared to 740 for the unfractionated material. The discrepancy may be due to experimental error introduced by the method of determining $[\eta]$ for the fractions from single measurements. A typical plot of hyaluronate concentration against eluent volume is given in Figure 3. The unbroken curve represents a hypothetical instantaneous increase to the peak concentration, followed by an exponential decrease. The curve is drawn to enclose the same area as the experimental curve. A better fit could be achieved by a more rapidly decreasing exponential followed by a linear section.

A practical advantage of the elution technique is purification from protein, as noted previously by Berman.[6] About 65% of the original protein content of 7.7 mg was eluted in 0.01 and 0.15 *M* NaCl, where negligible hyaluronate was eluted. Another 17% came out in

0.18 M NaCl, so that protein contamination of the remaining fractions was about 3% compared to 13% in the original material.

A large-scale preparative fractionation designed to provide fractions large enough for extensive physical characterization was also carried out. Adsorption of the polymer from 0.05 M NaCl supernatant to give $\theta = 0.78$ left all but a negligible portion of the proteins present unadsorbed. Fractionation data are presented in Table IV. Polymer recovery is quantitative within experimental error. The remarkable uniformity in size of fractions reflects the linearity of θ^* with M discussed previously. Values of $[\eta]$ again show a steady increase with the single exception at fraction 12. Fragmentary measurements on two or three subfractions of fractions 18 and 19 showed a slight tendency for $[\eta]$ to decrease during elution, perhaps due to a limited chromatographic action of the gel permeation type. The final dried fractions had protein contents of from 0.2 to 0.5%.

Table IV. Preparative Fractionation of Hyaluronate (BVH) at 9°

Fraction	Eluent (NaCl), M	Wt recovered, g	$[\eta]^a$
1	0.07	0.18	...
2	0.085	0.43	80
3	0.09	0.36	...
4	0.10	0.83	180
5	0.11	0.84	...
6	0.12	0.73	
7	0.13	0.85	290
8	0.14	0.86	...
9	0.15	0.78	...
10	0.16	0.86	434
11	0.17	0.81	493
12	0.18	0.86	457
13	0.19	0.76	556
14	0.20	0.81	585
15	0.21	0.80	637
16	0.22	0.68	760
17	0.23	0.65	935
18	0.24	0.72	1097
19	0.25	0.59	1350
20	0.26	0.51	1450
21	0.27	0.55	1590
22	0.30	0.48	2200
23	0.35	0.06	...
		15.00	

[a] Calculated from single viscosity measurements, with the exception of fractions 4, 7, 10, 13, 15, 18, and 21, where extrapolations were made.

Characterization of Fractions. A useful measure of the breadth of distribution in polymer fractions is the ratio $\bar{M}_w/\bar{M}_n$ of the weight- to number-average molecular weight. For an unfractionated sample of BVH similar to that used in this work, this ratio was found[20] to be 1.9, which resembles the value for many unfractionated synthetic polymers. Data obtained for selected fractions are given in Table V. The values of $\bar{M}_n$ were obtained by osmotic pressure measurements in 0.5 M NaCl and those of $\bar{M}_w$ by light-scattering measurements in the same solvent. The ratio $\bar{M}_w/\bar{M}_n$ is seen to be about 1.2, on the average, for the fractions reported. This value is comparable to those obtained by the usual precipitation technique.

(20) R. L. Cleland, unpublished results.

Table V. Molecular Weight Data for Hyaluronate Fractions

Fraction	$10^{-5}\bar{M}_n$	$10^{-5}\bar{M}_w$	$\bar{M}_w/\bar{M}_n$
7	0.95	1.10	1.16
15	2.7	3.4	1.26
18	4.6	5.3	1.15

A more thorough characterization of fraction 18 was made by a refractionation on DEAE-cellulose of a sample of 10.3 mg. Stepwise column elution was used successfully with a bed volume of about 0.5 ml. Data for the experiment appear in Table VI. The weight-average $[\eta]$ for the fractions was 1077, compared to 1097 for the original fraction.

Table VI. Refractionation of Fraction 18 on DEAE-cellulose at 6.5°

Eluent (NaCl), M	Polymer recovery, mg	$[\eta]$, ml/g	$10^{-5}M_w$ [a]
0.10	2.78	770	3.29
0.15	2.70	1060	4.96
0.20	2.50	1135	5.42
0.25	1.98	1290	6.79
0.30	0.70	1530	7.97
	10.66		

[a] Calculated from the equation: $[\eta] = 0.0403M_w^{0.775}$ (ref 9).

The refractionation data were treated by the method of Tung,[21] which is suitable when the integral weight distribution function is of sigmoid shape. The function

$$I(x) = 1 - \exp(-0.0465x^{3.77}) \quad (5)$$

fitted the data well, where $I(x)$ is the cumulative weight fraction, whose value was assumed to occur at the middle of each subfraction of molecular weight x. The Tung function leads to the expression

$$\bar{M}_w/\bar{M}_n = \Gamma(1 + 1/b')\Gamma(1 - 1/b') \quad (6)$$

where b' is the exponent of x in eq 5. For $b' = 3.77$, $\bar{M}_w/\bar{M}_n = 1.13$, which agrees within experimental error with the value from direct measurement.

With the exception of the entropy of mixing contribution, nearly all terms in the free-energy change upon adsorption of a polyelectrolyte should be proportional either to charge or to degree of polymerization.[17,19] The expression relating the polymer volume fraction ϕ_P'' in the adsorbed phase to that (ϕ_P') in the solution phase is, then, for a Flory–Huggins entropy of mixing[19]

$$\phi_P''/\phi_P' = \exp(\sigma x) \quad (7)$$

where σ is a parameter independent of x, but strongly dependent on linear charge density. Flory[22] has discussed the implications of eq 7 for precipitation fractionation of nonionic polymers. The resemblance of $\bar{M}_w/\bar{M}_n$ values in this work to those in other two-phase fractionation systems is therefore reasonable.

Conclusions

Cross-linked, ion-exchange materials of polysaccharide origin are suitable adsorbents for molecular-weight fractionation of a polyelectrolyte of fixed charge

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density. Stepwise elution is superior for this purpose to gradient elution, although a very slowly changing gradient might be a useful modification of the technique. The approximate linearity of θ^* with M assures fractions of approximately equal weight when equal increments in eluent concentration are used. Reproducibility of the θ^*–M relation facilitates prediction of elution conditions for a desired fractionation. Fractions obtained are comparable in sharpness to those obtained by other techniques depending on distribution between two phases. Characterization of the breadth of distribution in fractions obtained permits the use of this method for the estimation of molecular weight distribution curves for unfractionated materials on the basis of analytical fractionation results. Fractionation by charge density is also indicated by our data and has been previously demonstrated[4] experimentally for the ionic polysaccharides studied here. Fractionation should, in fact, be much more sensitive to variation in charge density than to molecular weight variation at a single charge density.

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Low-Temperature Microwave Absorption in Insulating Materials

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Abstract: Measurements of the dielectric loss of crystalline and vitreous quartz, polyethylenes of different degree of crystallinity, and one polar polymer at 32 GHz between 4.2 and 300°K are presented and discusssed. In the higher temperature range the loss is interpreted as background loss due to photon–phonon interaction by means of polar impurities in the structure. At low temperatures additional loss is found that yields the same temperature behavior as the mechanical loss at comparable frequencies and is tentatively ascribed to the same intrinsic lattice absorption, coupled over anharmonicity terms to the electromagnetic field.

In the course of our investigations to separate relaxation- and resonance-type dielectric loss at microwave frequencies, temperature-dependent measurements were carried out down to 4.2°K. Here our first experimental results on organic and inorganic polymers shall be presented and discussed. The results give evidence that the dielectric loss, seen at microwave frequencies, is mainly background loss, *i.e.*, loss due to photon–phonon interaction. Additional loss was found at very low temperatures which to a high degree depends on the quality of the lattice. With increasing temperature it reaches a maximum or plateau and then merges into the background loss. It seems to us that here, for the first time, we found evidence for electromagnetic absorption rising directly from the thermal lattice phonons, coupled to the electric field by lattice anharmonicity.

Experimental Section

Measurements were performed on solid disk-shaped samples at 32 GHz in a H_{01n} cavity resonator placed in a liquid helium dewar, which is described in detail elsewhere.[1,2] The resonator plunger, holding the sample, is fixed to the bottom of the mounting tube, which is screwed into the cryostate flange; the resonator itself and the waveguide coupling systems are movable over a micrometer drive on top of the kryostate flange by means of an elastic vacuum connection. The resonance position is read at the micrometer, but the half-width is determined by changing the frequency and reading Δf at half-power points directly on the "Schomandl" frequency stabilization device. Measurements were performed, after evacuating the whole system and cooling down to 4.2°K, with He contact gas at a partial pressure of 10^{-3} torr within and around the resonator. The heating rate is approximately 1°/min, adjusted first by the He boiling rate, later by additional heating. Q of the empty cavity varied from 2.4 to 1.5×10^4 between 4 and 300°K, fluctuating about ±2%. The least measurable loss therefore should have the order of magnitude of 5×10^{-6}, which is confirmed by our experimental results.

Results

The measured values of dielectric loss between 4 and 280°K at 32 GHz are shown for quartz (synthetic quartz from Steeg & Reuter), quartz glass, polyethylene (density 0.918 and 0.960, respectively), and polyoxymethylene (Delrin) in Figures 1–4. Dielectric constant values simply follow the density variation, except for the polar polymer, for which they are included in Figure 4 (no significance can be given to the absolute value of ϵ', which is inaccurate up to 10% because of the special resonator design).

The values on quartz show the accuracy and reproducibility of our measurements: no structure of the loss curves can be resolved below 5×10^{-6} (≅70°K). No structure or relaxation peaks are seen in the continuously rising loss curve above 70°K either. This might be surprising, compared with mechanical loss–temperature curves[3,4] and high-frequency measure-

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