Gel permeation properties of decrystallized cotton cellulose

Cotton cellulose, decrystallized by ball milling[1], has been found to have gel permeation properties comparable to those of the most highly crosslinked dextran and polyacrylamide gels[2]. It is permeable to compounds of molecular weights below approximately 1500, and measurements of the retention of known compounds in this range are being used to obtain information regarding the structure of the amorphous polymer gel. The lower part of the range, below *circa* 700 molecular weight, has been explored with sugars that can be determined quantitatively from polarizations of fractions of the column eluate measured by the sensitive ETL-NPL automatic polarimeter*. Partial separation of two sugars may be detected readily by pairing a dextrorotatory sugar of high molecular weight, such as raffinose or stachyose, with either fructose or erythrose, which are levorotatory. Elution in the order of decreasing molecular weights is evident from the dextrorotations of the initial fractions collected, followed by levorotatory fractions containing a preponderance of the lower molecular weight sugar. Dependence of retention by the cellulose upon molecular weight was determined by experiments with individual sugars, for which the peak elution volumes were found to be inversely proportional to their molecular weights.

Relative elution volumes have been determined individually for the following sugars: erythrose (mol. wt. 120), fructose (mol. wt. 180), maltose monohydrate (mol. wt. 360), raffinose pentahydrate (mol. wt. 594), and stachyose tetrahydrate (mol. wt. 738). Measurements for each of the five sugars were made on comparable columns containing unmodified, decrystallized cellulose, and a sample of the same material that had been crosslinked to 5.2 % CH_2O content, by treatment with formaldehyde[3]. The effect of crosslinking was similar to that produced in other gels, the limit of permeability being reduced. Relative retentions do not differ sufficiently to make these materials efficient for separating sugars, but the results obtained with these columns, eluted at very slow rates of flow permitting local equilibrium to be approached closely, are of general interest in regard to the mechanism of separations effected by other gels having similar properties.

Experimental details will be described elsewhere[4] and only those results pertinent generally to techniques of gel permeation chromatography are reported here. The volumes characterizing each column, and the elution volumes of each of the five sugars on the unmodified cellulose and on the crosslinked cellulose column are assembled in Table I. These characteristic column volumes are defined as: V_t, total volume of column; V_0, void volume measured by the peak elution volume of blue dextran, mol. wt. *ca.* $2 \cdot 10^6$; V_s, volume occupied by dry solid, estimated from its weight and density; V_r, total internal volume, or regain, obtained by difference $V_t - (V_0 + V_s)$; and V_i, effective internal solvent volume determined by extrapolation of the curves of Fig. 1. The density of the solid was assumed to be 1.59, the theoretical value calculated from the unit-cell dimensions of crystalline cellulose[5]. Densities of the amorphous materials probably are slightly lower, and that of cross-

* ETL-NPL Automatic Polarimeter, Type 143A, manufactured by Bendix-Ericcson, U.K., Ltd., distributed in U.S. by Bendix Corp., Cincinnati Div., Cincinnati, Ohio. Mention of a company and/or product by the Department does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.

TABLE I

CHARACTERISTIC VOLUMES OF CELLULOSE COLUMNS AND ELUTION VOLUMES OF SUGARS

Column volumes	*Celluloses*			
	Unmodified		*Crosslinked*	
	Total	*Per g*	*Total*	*Per g*
Total volume, V_t (ml)	46.60	3.330	47.10	3.438
Void volume, V_0 (ml)	23.00	1.642	22.40	1.635
Solid volume, V_s (ml)	8.80	0.629	8.62	0.629
Regain volume, V_r (ml)	14.80	1.059	16.08	1.176
Internal volume, V_i (ml)	6.93	0.500	9.94	0.760
Weight of solid (g)	14.0	—	13.7	—
Sugars (mol.wt.)	*Elution volumes, V_e*			
	Ratio	*ml/g*	*Ratio*	*ml/g*
Dextran (reference)	(1.00)	1.642	(1.00)	1.636
Stachyose·4 H_2O (738)	1.18	1.937	1.23	2.012
Raffinose·5 H_2O (594)	1.20	1.970	1.27	2.079
Maltose·1 H_2O (360)	1.25	2.052	1.36	2.225
Fructose (180)	1.27	2.086	1.40	2.290
Erythrose (120)	1.27	2.086	1.43	2.340

linked cellulose may differ somewhat from the density of the unmodified sample. The probable magnitude of the difference in density is too small to account for the considerably larger effective internal volume found for the crosslinked cellulose for which V_i is about 50 % greater than that of the unmodified cellulose.

The elution volumes are plotted in Fig. 1 which shows that a simple inverse

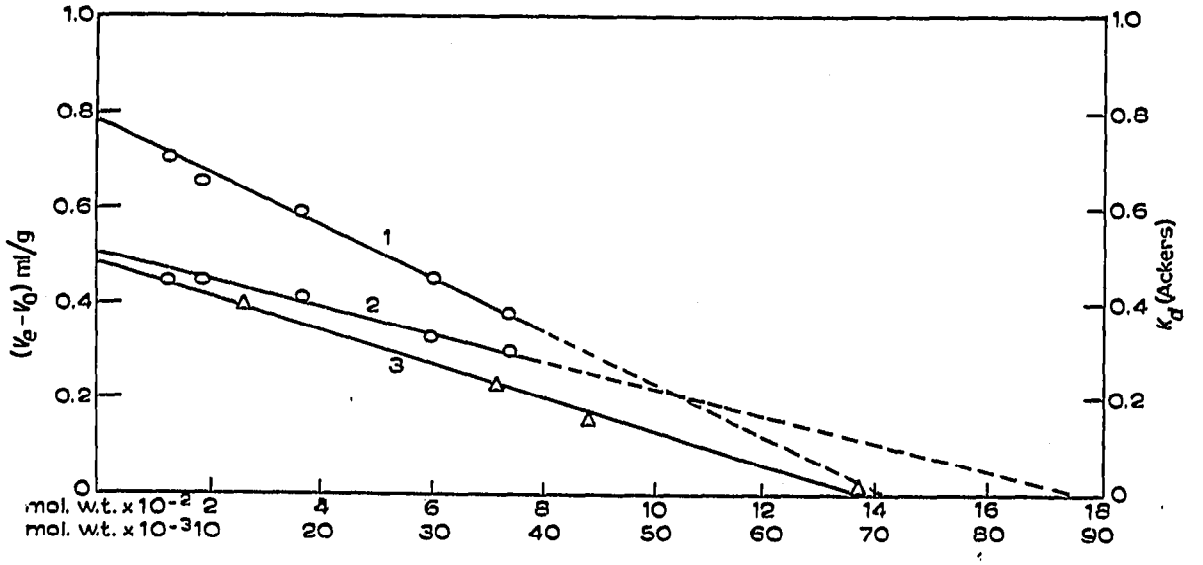

Fig. 1. Relation of elution volume, or distribution coefficient, to molecular weight. (1) Sugars on crosslinked cellulose; (2) sugars on unmodified cellulose (refer to left ordinate and upper abscissa scales); (3) ACKERS' data for cytochrome-*c*, β-lactoglobulin, ovalbumin and hemoglobin in equilibrium with Sephadex G-75 (refer to right ordinate and lower abscissa scales).

linear relationship to molecular weight is approximated closely, if molecular weights are taken to be those of the characteristic stable crystalline hydrates of the dextrorotatory sugars, maltose·1 H_2O, raffinose·5 H_2O, and stachyose·4 H_2O. Experiments with compounds of higher molecular weight will be necessary to determine whether linearity holds up to the limit of permeability; if extrapolation is justified, this limit is indicated to be approximately molecular weight 1750 for the unmodified cellulose, and approximately 1400 for the crosslinked sample. Extrapolation of these lines to their intercepts with the ordinate appears to provide the soundest basis for estimating the correct effective internal volumes of the columns. The total internal volumes, V_r, given in Table I are seen to be considerably larger than those obtained from Fig. 1 by extrapolation of curves 1 and 2. Binding of part of the total internal water by hydrophilic gel materials, which reduces the effective internal solvent volume, is known to occur[6]. Bound and free water have been distinguished in completely swollen amorphous regions of cotton fiber[7]. In the unmodified cellulose sample, approximately 53 % of the internal water appears to be bound. Substitution of hydroxyl groups by reaction with formaldehyde in the treated sample makes this material less hydrophilic, and only 38 % of the total water is bound, resulting in a larger effective internal solvent volume, V_i, for this column.

Solvent flow rates of only 1.0–1.5 ml/h/cm^2 were used in developing these columns. Rates above 4 ml/h/cm^2 were not possible because of the high resistance to flow of the finely divided cellulose. Chromatography of macromolecules on gel columns that can be eluted at relatively high flow rates has given excellent correlations of elution volumes with the logarithm of molecular weight in many cases[8]. Conditions are such that local equilibrium is never attained, and probably is not approached closely; the extent of permeation of the internal solvent volume depends largely upon the relative rates of diffusion of the solutes. Deviations from the logarithmic relationship have been reported, and may be accounted for by intermediate rate conditions under which permeation is controlled by partial equilibration as well as diffusion. MORRIS[9] found the logarithmic relationship to hold for a number of proteins on a column of Sephadex G-200, but results for the same proteins showed a deviation therefrom on a column of G-100 gel operated at the same flow rate, evidently because equilibrium could be approached more closely in the G-100 gel which is less permeable to molecules of a given size.

ACKERS[10] determined the distribution coefficients for a number of proteins, both in columns eluted by the usual procedures, and by equilibration of their solutions with the gels Sephadex G-75, G-100 and G-200. The coefficients, k_d, were calculated as $(V_e - V_0)/V_i$, in which the internal volume, V_i, was taken to be the elution volume of tritrium water (THO). Tritium is known to exchange readily with bound water and with hydrogens of the hydroxyl groups in cellulose, and undoubtedly exchanges with that in dextran also, resulting in a value at least equal to that of the total internal water rather than the effective internal solvent volume. Results of ACKERS' equilibrium measurements for four proteins with Sephadex G-75 are plotted as curve 3 of Fig. 1, showing a linear dependence of distribution coefficient on molecular weight which, by extrapolation, yields a value for the effective internal volume much smaller than that which he obtained with THO. Equilibrium measurements of distribution coefficients of a group of proteins with Sephadex G-200 deviated widely from those obtained by elution of the proteins from a column of

the same gel. A graph of data for the column experiment shows a smooth curve and, as closely as the coefficients can be estimated from this plot, they are related to the logarithm of the molecular weights of the proteins. Sephadex G-200 is a highly permeable gel with a large internal volume, and the difference between effective solvent volume and the total volume measured by THO is less than for the more highly crosslinked dextran gel. One report[11] of work in which the internal volume was determined with THO records values for the internal and external volumes, V_i and V_0, the sum of which exceeds the total volume calculated from dimensions given for one of the columns used. The empirical correlation of elution volumes with the logarithm of molecular weights, without reference to an estimated or independently determined internal volume, provides a sound basis for comparing the molecular weights of macromolecules by gel permeation through columns eluted at sufficiently high rates of flow. High rates of development, rather than equilibration with the internal solution, also should be more effective for chromatographic separations.

This work is being extended to develop a technique that can be used to provide a quantitative measure of differences in the cellulose polymer structure produced by chemical modifications[4].

Southern Regional Research Laboratory, Southern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture, New Orleans, La. (U.S.A.)

L. F. MARTIN
S. P. ROWLAND

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