THE EFFECTS OF SORPTION AND MOLECULAR SIZE OF SOLUTES UPON ELUTION FROM POLYHYDROXYLIC GELS

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(First received May 24th, 1971; revised manuscript received August 10th, 1971)

SUMMARY

A study is reported of the elution of a variety of sugars, methylated sugars, polyethylene glycols, and derivatives of 2-imidazolidinone (ethyleneurea) from columns of the dextran gel Sephadex G-15**. Low-molecular-weight polyethylene glycols were eluted in much smaller volumes than sugars having comparable molecular weights. While the homologous series exhibited the usual inverse relation of logarithms of the relative elution volumes to molecular weights, sorption appears to be a factor in the greater retention of sugars, which have more numerous hydroxyl groups capable of hydrogen bonding with the polyhydroxylic gel. The 2-imidazolidinone derivatives show no simple relationship of elution volumes to molecular weights, but sorption *via* hydrogens on ring nitrogen atoms appears to be a factor in determining their relative elution volumes.

INTRODUCTION

Gel permeation chromatography using solutes of known, discrete molecular weights has been useful as a technique for investigation of changes of the internal pore structures resulting from physical[1,2] or chemical modification[3,4] of fibrous cotton cellulose. In this application the finely divided samples of cellulose constitute the gel packing of the columns through which the solutes are eluted individually by water at low flow rates. Measurements of the elution volumes provide data from which the apparent internal pore volumes and limits of permeability of the cellulose may be derived. A series of saccharides of increasing molecular weights, from the mono-saccharide, glucose, to the tetrasaccharide, stachyose, is employed in this technique. The advantage of using these sugars is that, if molecular sizes in solution are assumed to be proportional to molecular weights of the characteristic crystalline hydrates, an inverse, linear relation of elution volume to molecular weight is obtained. The relative sizes of the solute molecules are: glucose·H_2O (198), maltose·H_2O (360), raffinose·5

H_2O (594), and stachyose·$4H_2O$ (738). The linear relationship may be extrapolated to the apparent total internal pore volume at zero molecular weight and the molecular weight corresponding to zero internal volume, *i.e.*, the void volumes of the columns.

The small internal, permeable volume of fibrous cellulose limits the accuracy with which relative elution volumes of the solutes can be determined. The permeable volume of Sephadex G-15 is several times larger than that of cellulose, and this gel was used to provide more accurate and reproducible measurements of the elution volumes of the sugars and of their completely methylated derivatives. The results indicate that the elution volumes of various solutes depend upon sorption as well as difference of molecular size in solution. In addition, experiments were carried out with the Sephadex gel employing polyethylene glycols, and methyl-terminated polymers and oligomers of ethylene glycol as solutes, covering the range of molecular weight from 62 to 1000. A group of 2-imidazolidinone and substituted imidazolidinones (ethyleneureas) were also investigated because of their commercial importance as reagents for modifying cotton. Polyethyleneimines, also used for modifying cotton, were so strongly adsorbed by the dextran that they could not be eluted with water.

EXPERIMENTAL

Materials

Sephadex G-15, spherical bead form, was used as supplied by, and in accordance with recommendations of Pharmacia Fine Chemicals, Inc. Most of the solutes were obtained in sufficiently high purity from various commercial sources. D-Erythrose, D-ribose, and stachyose tetrahydrate were from Nutritional Biochemical Corp., Cleveland, Ohio. D-Mannose, D-maltose, raffinose pentahydrate, melezitose, and cellobiose were from Calbiochem, Los Angeles, Calif. D-Lyxose, D-arabinose, D-xylose, and D-galactose were from the Pfanstiehl Laboratories, Inc., Phillipsburg, N.J. Isomaltose was from Pierce Chemical Co., Rockford, Ill. Sucrose was a National Bureau of Standards sample. Oligomers of the ethylene glycol series and corresponding methyl-terminated compounds were from Eastman Organic Chemicals, Rochester, N.Y., or from J. T. Baker Chemical Co., Phillipsburg, N.J. High-molecular-weight polyethylene glycols were samples from Union Carbide Corp., New York, N.Y. Completely methylated glucose, sucrose, raffinose, and stachyose were prepared by the method of GILLIS[5] and purified by thick-layer chromatography.

Methods

The procedure described by FLODIN AND KUPKE[6] was used for packing the columns. An extension tube equal in length and diameter to those of the columns was employed, through which the swollen Sephadex was settled uniformly into the columns from an agitated suspension. The columns were Chromatronix precision bore, 2.54 cm (1 in.) diameter by approx. 45 cm length of packing between the top and bottom bed supports. Solutes were introduced as separate 4% solutions in water through a 0.1-ml sample loop. Injection of individual samples was spaced to prevent overlapping of elution curves. Elution was carried out with distilled water at a linear flow rate of approx. 8.5 cm/h, estimated from the void volume and volumetric flow. The eluate was monitored continuously by a Waters Associates R4 recording differential refracto-

meter. Volumes were determined gravimetrically by collecting approx. 10-ml fractions in tared test tubes, then summing the weights and proportional parts of fractions between injection and the peak of the elution curve for each solute. As the three columns used in the course of the experiments differed slightly in total packed volumes the data are expressed as ratios, R_g, of the elution volumes to that of glucose. A sample of glucose was included with four or five other solutes applied in each experiment. Nine measurements of the elution volume of glucose on one column averaged 158.2 ml, with a standard deviation of 0.16. R_g values of the solutes tested ranged from approx. 0.560 for the void volume measured by high-molecular-weight dextran to 1.270 for the solute with the highest permeability, and are accurate to about ± 0.003.

RESULTS

Saccharides and permethylated saccharides

The results obtained for three groups of isomeric saccharides are presented in Table I. In addition to these groups, measurements were made for the pair of isomeric

TABLE I

RELATIVE ELUTION VOLUMES AND MOLECULAR WEIGHTS OF GROUPS OF ISOMERIC SACCHARIDES

Pentoses mol. wt. 150	R_g values[a]	Hexoses mol. wt. 180	R_g values[a]	Disaccharides mol. wt. 342	R_g values[a]
Ribose	1.065	Psicose	1.040	Sucrose	0.926
Lyxose	1.062	Mannose	1.029	Maltose	0.922
Xylose	1.047	Sorbose	1.026	Turanose	0.920
Arabinose	1.024	Fructose	1.018	Cellobiose	0.907
		Glucose[b]	1.000	Trehalose	0.902
		Galactose	0.990	Lactose	0.895
				Isomaltose	0.889
				Melibiose	0.877
				Gentiobiose	0.870
Total range	0.041		0.050		0.056
Total range (ml)	6.7		8.2		9.1

[a] R_g = ratio of elution volume to that of glucose in the same test.
[b] Average elution volumes for glucose: Col. S-5, 162.13 ml; Col. S-6, 158.02 ml; Col. S-7, 164.13 ml. Each average from nine measurements on one column.

trisaccharides, raffinose and melezitose, which had R_g values of 0.828 and 0.866, respectively. The one tetrasaccharide tested, stachyose, had an R_g value of 0.767, while the tetrose, erythrose, had an R_g value of 1.086. Employing the relationship developed by HJERTÉN[7], values of $-\log (R_g - 0.560)$, in which $0.560 = R_g$ for a totally excluded solute, are plotted in relation to molecular weights of glucose, sucrose, raffinose, and stachyose as shown by curve A of Fig. 1. Although these sugars are not homologous, the values appear to fit the logarithmic relationship quite satisfactorily. If the sugars are assigned molecular sizes proportional to the molecular weights of their characteristic hydrates, a simple inverse, linear relationship is obtained for the
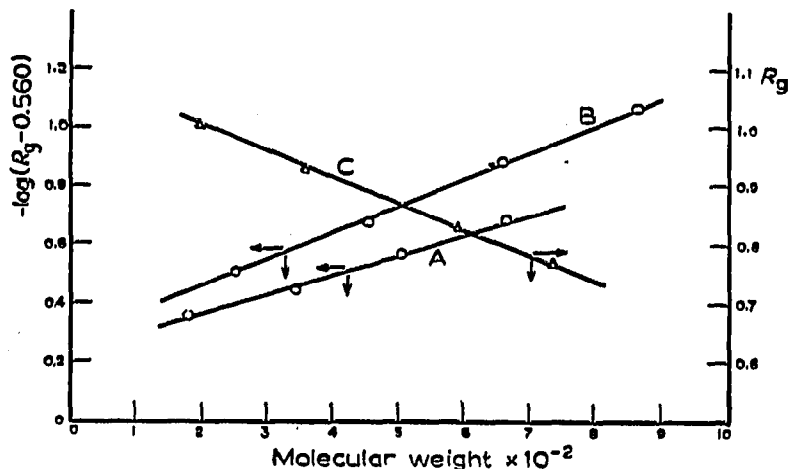
Fig. 1. Relative elution volumes of sugars as $- \log (R_g - 0.560)$ (refer to left ordinate) vs. molecular weights; curve A = glucose, sucrose, raffinose, and stachyose; curve B = pentamethyl glucose, octamethyl sucrose, undecamethyl raffinose, and tetradecamethyl stachyose (permethylated derivatives of A). Linear relation on curve C of relative elution volumes as R_g (refer to right ordinate) to molecular weights of the characteristic hydrates: glucose $\cdot H_2O$, maltose $\cdot H_2O$, raffinose $\cdot 5H_2O$, and stachyose $\cdot 4H_2O$.

relative elution volumes. This is shown by curve C of Fig. 1, which is fitted quite accurately by least squares with the equation:

$$R_g = 1.086 - 0.434 \cdot 10^{-3} \cdot MW$$

where MW is the molecular weight of the sugar hydrate.

To determine the effect of sorption upon retention of the sugars through hydroxyl groups, permethylated derivatives were prepared. These solutes, with molecular weights (MW) and corresponding relative elution volumes were: pentamethyl glucose, MW 250, R_g 0.875; octamethyl sucrose, MW 454, R_g 0.770; undecamethyl raffinose, MW 658, R_g 0.690; and tetradecamethyl stachyose, MW 862, R_g 0.645. These data are plotted as $-\log (R_g - 0.560)$ vs. molecular weights in curve B, Fig. 1. It must be noted that higher values are given by the negative logarithmic relation for solutes which are retained less by the gel. It is evident that blocking of the hydroxyl groups caused a marked decrease in retention of these solutes, out of proportion to the increased molecular weights due to permethylation. Sorption has an effect superimposed upon that of molecular size in determining relative elution volumes of the unsubstituted sugars.

Polyethylene glycols

Low-molecular-weight members of the polyethylene glycol homologous series are available as molecularly homogeneous compounds only up to the tetramer. Corresponding methyl-terminated oligomers are available as the glymes, and were included in the experiments. Higher members of both series were represented by molecularly heterogeneous fractions of these polymers. Table II lists the molecular weights of this series, together with the R_g values obtained. The available fractions having $\overline{M}_w = 350$, 550, and 750 were methyl-terminated polymers, whereas the sample having $\overline{M}_w = 1000$ was terminated by free-hydroxyl groups. The extended chain

TABLE II

RELATIVE ELUTION VOLUMES AND MOLECULAR WEIGHTS OF POLYETHYLENEGLYCOLS (PEG's AND PEG DIMETHYL ETHERS)

Solutes	Mol. wt.	Relative elution volumes (R_g values)		
		Run 1	Run 2	Average
Ethylene glycol	62	1.089	1.090	1.090
Diethylene glycol	106	1.008	1.010	1.009
Triethylene glycol	150	0.938	0.942	0.940
Tetraethylene glycol	194	0.882	0.883	0.883
EG dimethyl ether	90	1.004	1.001'	1.003
DiEG dimethyl ether	134	0.935	0.937	0.936
TriEG dimethyl ether	178	0.878	0.881	0.880
TetraEG dimethyl ether	222	0.834	0.838	0.836
PEG[a] 350 methyl ether	350	0.721		
PEG 550 methyl ether	550	0.653		
PEG 750 methyl ether	750	0.609		
PEG 1000	1000	0.585		
PEG 6000	6000	0.560[b]		

[a] Commercial "Carbowax" fractions; nominal weight average mol. wt.
[b] Void volume of column; limit of permeability is ca 1200–1500 mol. wt.

oligomers and polymers of this series are all eluted in much smaller volumes than sugars having equal molecular weights.

Fig. 2 shows the relation of $-\log (R_g - 0.560)$ to molecular weights of these solutes. In this graph the methyl-terminated series has larger values than the free hydroxyl compounds which are retained to a greater extent. The lines are essentially parallel for the two series of solutes with molecular weights below 300; however, the high-molecular-weight members of the series lie on a line with a significantly different
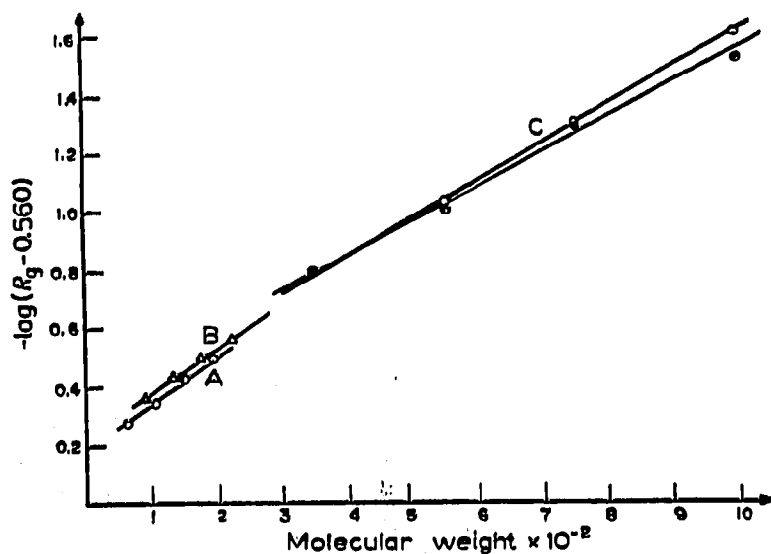

Fig. 2. Relative elution volumes of polyethylene glycols as $-\log (R_g - 0.560)$ vs. molecular weights. Curve A = hydroxyl-terminated oligomers; curve B = methoxyl-terminated oligomers; curve C = higher polymers, methoxyl-terminated except polymer of $MW = 1000$.

slope. The individual lines are fitted satisfactorily by least squares, from which the slopes are $0.1634 \cdot 10^{-2}$ for the low-molecular-weight, free-hydroxyl-terminated oligomers, $0.1589 \cdot 10^{-2}$ for the methyl-terminated oligomers, and $0.1257 \cdot 10^{-2}$ for the higher-molecular-weight polymers. The hydroxyl-terminated polymer with $\bar{M}_w = 1000$ lies on the same line as the three methyl-terminated polymers with $\bar{M}_w = 350$, 550, and 750, within experimental error. The influence of the terminal free-hydroxyl groups is not significant at high molecular weights.

2-Imidazolidinones and polyethyleneimines

The measurements obtained for 2-imidazolidinone and fourteen of its derivatives are assembled in Table III. These solutes have generally higher R_g values than the sugars. As in the case of the sugar series, isomeric members of this group have

TABLE III

RELATIVE ELUTION VOLUMES AND MOLECULAR WEIGHTS OF 2-IMIDAZOLIDINONE AND DERIVATIVES

Substituents[a] to 2-imidazolidinone				Mol. wt.	Relative elution volumes (R_g values)
R_1	R_2	R_3	R_4		
H	H	H	H	86	1.276
H	H	H	CH_3	100	1.274
H	H	OH	OH	118	1.270
H	H	OCH_3	OCH_3	146	1.230
H	CH_3	H	H	100	1.218
H	CH_2OH	H	H	116	1.193
CH_3	CH_2OH	H	H	130	1.124
CH_3	CH_2OCH_3	H	H	144	1.106
CH_2OH	CH_2OH	H	H	146	1.106
CH_3	CH_3	OH	OH	146	1.114
CH_2OCH_3	CH_2OCH_3	H	H	174	1.097
CH_3	CH_3	OCH_3	OCH_3	174	1.050
CH_2OH	CH_2OH	OH	OH	178	1.101
CH_3	CH_2OCH_3	OCH_3	OCH_3	204	0.999
CH_2OCH_3	CH_2OCH_3	OCH_3	OCH_3	234	0.960

[a] Substituent groups:
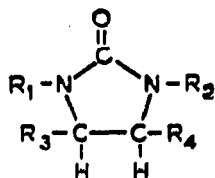
significantly different R_g values. Elution volumes are largest for those derivatives having both nitrogens of the imidazolidinone ring carrying hydrogens. There is a general trend toward lower relative elution volumes (R_g values) as molecular weights increase; for a given molecular weight lower values are found for derivatives carrying methyl ether groups than for those having free-hydroxyl groups.

The monomer of the ethyleneimine series had a large R_g value indicative of sorption. Oligomers of this type of nitrogen compound could not be eluted from the gel by water.

DISCUSSION

The results obtained with all of these varied types of solutes make it evident that their relative elution volumes, R_g values, cannot be functions of their molecular weights alone. The data obtained for each of the groups of related compounds provides strong evidence that sorption by the dextran gel is a significant factor. It is not possible to determine from these measurements the magnitude of the effects of sorption and of molecular size independently. Methylation of hydroxyl groups, or other alterations of structure which tend to reduce sorption are accompanied by simultaneous changes of molecular size. In experiments with sugar derivatives, reported elsewhere[8], it was found that R_g values depend significantly upon stereochemical configurations for variously modified pentoses, hexoses and disaccharides; however, the values were equal within experimental error for D- and L-enantiomers tested.

Data for the three groups of isomeric compounds (Table I) show that the ranges of measured R_g values are 0.041 for the pentoses, 0.050 for the hexoses, and 0.056 for disaccharides. Psicose, mannose, and sorbose have larger R_g values than the arabinose which has a lower molecular weight. The differences are significant, and may be attributed to variations in the orientations of hydroxyl groups, some of which may be more favorable for hydrogen bonding than others. In the case of raffinose (R_g 0.828) and melezitose (R_g 0.866) it is possible that the former occupies 20% more space in solution that the latter, because of differences in flexibility of the linkages of the hexose moieties. It is equally possible that the orientation of the numerous hydroxyl groups in melezitose is more favorable to hydrogen bonding than that in raffinose.

Obviously, all of the saccharides may be fitted to a linear equation if they are arbitrarily assigned appropriate relative molecular sizes. It was fortuitous that readily available sugars have relative elution volumes, resulting from the combined effects of sorption and molecular size, which are linearly related to molecular weights of hydrates. This relation is still valid, and can be used empirically to detect changes in gel pore structures.

Polyethylene glycols have even smaller elution volumes than the permethylated saccharides of equal molecular weights, although the oxygens of both classes of compounds (with the exception of the terminal hydroxyl groups of the glycols) are in ether form. Sorption due to these terminal proton-donor-groups is measured by the decrease in R_g values when the terminal groups are methylated, as will be seen by comparing curve A with curve B of Fig. 2. Reduction of R_g values for the methylated glycols is significant. The configuration of polyethylene glycols in various solvents has been investigated extensively by ELIAS[9] who employed viscometric and other techniques. The PEG's were found to be not associated in water, and to be in the form of highly extended random coils in this solvent. This would explain the difference from permethylated saccharides on the basis of steric exclusion due to molecular size. The saccharides with free-hydroxyl groups have larger R_g values than either of these two classes of solutes, due principally to increased retention attributable to sorption. The 2-imidazolidinone and its derivatives as a group have larger elution volumes and R_g values than any of the other classes of solutes tested. As in the case of saccharides, isomeric derivatives have significantly different R_g values. The data in Table III provide evidence of the effect of sorption. The first four compounds listed have hydrogens on the ring nitrogen atoms, which apparently form stronger hydrogen

bonds than those attached to ring oxygen atoms. In general, the R_g values decrease with increasing molecular weights. Complete substitution of the four available ring atoms resulted in the lowest R_g values in the series. There is evidence of some sorption through hydrogen bonding of hydroxyls or of methylol groups attached to the imidazolidinone nucleus.

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