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GEL CHROMATOGRAPHY OF GLUCOSE OLIGOMERS

A STUDY OF OPERATIONAL PARAMETERS*

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SUMMARY

The effects of solute concentration, column length, flow-rate and temperature upon the elution behavior of glucose oligomers are discussed. A linear relationship between peak height and concentration was established for polymers up to maltoheptaose. Resolution increased considerably by increasing column length. Elution behavior of the homologous series of glucose oligomers was flow and temperature dependent. Gel bed parameters and theoretical data as influenced by operational parameters are presented.

INTRODUCTION

Gel chromatography (GPC) has been used as a technique for the separation of carbohydrates and carbohydrate polymers[1–6]. Dextran (Sephadex) and polyacrylamide (Bio-Gel P) gels have been the most extensively used packing materials for such separations. Polar solvents, particularly water, have been used as eluents.

The effects of operational parameters upon resolution and time of analysis in GPC of carbohydrates is of utmost importance. The present paper deals with the effects of sample concentration, column length, flow-rate temperature upon the elution behavior and resolution of glucose oligomers in a 42° dextrose equivalent (D.E.) corn syrup on Bio-Gel-P. The effect of fractionation range and exclusion limit of the gel upon resolution of glucose oligomers has been previously reported[7].

EXPERIMENTAL

The gel chromatograph was assembled in the laboratory from commercially available components. Polyacrylamide Bio-Gel P-2, 400 mesh (Bio-Rad Labs.,

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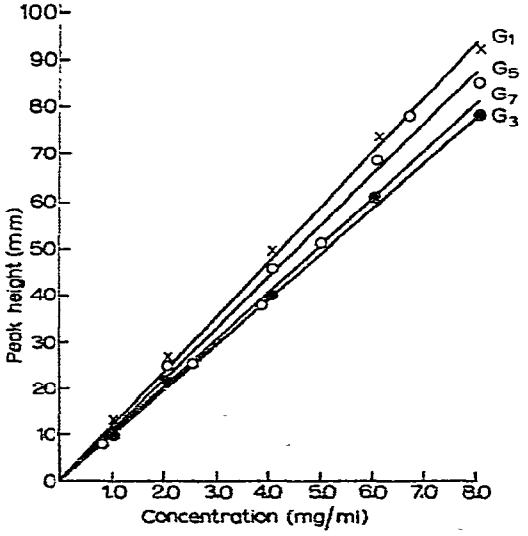
Richmond, Calif., U.S.A.) was used as a packing material throughout the course of this investigation. Distilled water was used as the sole eluent and detection was accomplished via a differential refractive index monitor. The preparation of the gel, the column packing procedure, as well as the experimental conditions, have been previously described[7].

RESULTS AND DISCUSSION

Peak height versus concentration

GPC differs from other forms of liquid chromatography in that peak widths of a solute are equal and independent of concentration. This being true, calibration curves can be obtained by plotting peak heights against concentration. These curves can be used for quantitative estimations of solute concentrations. The use of precision pumps, however, is necessary to maintain constant flow-rates.

Aqueous standard solutions of glucose (G_1), maltose (G_2) (Fischer Scientific, Pittsburgh, Pa., U.S.A.), maltotriose (G_3) maltotetraose (G_4), maltopentaose (G_5) (Regis, Chicago, Ill., U.S.A.), maltohexaose (G_6) and maltoheptaose (G_7) (Pierce, Rockford, Ill., U.S.A.) were made containing concentrations of 1–8 mg/ml. Three chromatograms were obtained for each concentration where peak heights and peak widths for each solute were measured. The elution of these standards were later used to identify the peaks in a 42° D.E. corn syrup. Figs. 1 and 2 show plots of peak heights *versus* concentration for the authentic solutes. A linear relationship was obtained for all the polymers up to the maximum concentrations used (8 mg/ml) illustrating the usefulness of the GPC technique for quantitative measurements of solutes where calibration curves can be obtained based only on peak height measurements.



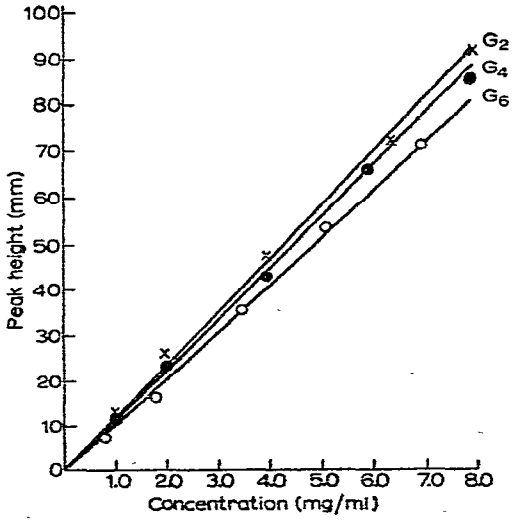


Fig. 1. Peak height *versus* concentration of glucose polymers.

Fig. 2. Peak height *versus* concentration of glucose polymers.

Effect of column length on elution behavior

Column efficiency is normally expressed numerically by the number of theoretical plates, N, which is a function of elution volume and peak width of a solute. Doubling the length of a column doubles the elution volume and zone separations of solutes. Resolution is improved and is proportional to the square root of column length because zone spreading also increases with increase in column length.

In order to evaluate the effect of column length on elution behavior of glucose polymers, two columns were packed and operated under the same conditions. The height of the longer column was adjusted so that it was double that of the shorter one. The longer column consisted of two 1-m columns in series and the shorter one of a single 1-m column, the I.D. of both columns being 1.6 cm. Figs. 3 and 4 show chromatograms of glucose polymers on both columns. Oligomers up to 12 glucose units were resolved. An interesting result was the separation of the iso isomers of the low-molecular-weight polymers. Isomaltose was separated from maltose, isomaltotriose was separated from maltotriose and isomaltotetraose was separated from maltotetraose. The three iso isomers appear as separate peaks in Fig. 4 demonstrating the effectiveness of the improved resolution with increasing column length. It is likely that the iso isomers of glucose polymers with more than 4 glucose units are also present in the syrup hydrolysate. These are not seen as separate peaks either because of their low concentrations or because the number of theoretical plates in the column is still

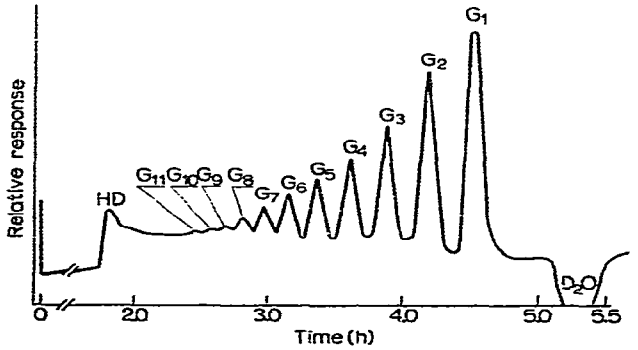


Fig. 3. Gel chromatogram of glucose polymers on Bio-Gel P-2 with a 1-m column.

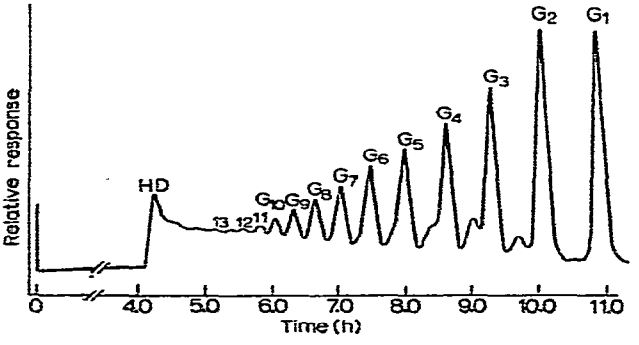


Fig. 4. Gel chromatogram of glucose polymers on Bio-Gel P-2 with two 1-m columns in series.

not sufficient. To increase the column length further would be impractical because of the long analysis time and the possible collapse of the gel due to the high column back pressures. Another alternative to achieve improved resolution without increasing column length, would be to use a recycling technique[8–11]. This technique has been used with polymeric porous beads for the separations of hydrocarbons, alcohols, triglycerides and surfactants[12].

Another aspect of this experiment relates to the operating mechanism or mechanisms in GPC separations. It is generally believed that the exclusion mechanism is the main one responsible for separation of chemical species by GPC and that separation occurs on the basis of relative molecular size[13]. In the present study maltose, maltotriose and maltotetraose have the same molecular weights as their iso isomers and elute later than these. The only structural difference between these isomers is the location of the linkage. If exclusion were the only operating mechanism, the iso isomers should have different mean cross-sections as compared with their isomers in solution and thus lower elution volumes. It may, in fact, be true that the 1–6-linkage is responsible for such elution behavior, but other factors such as hydrogen bonding, adsorption, degree of hydration and partial specific volumes should all be considered in accounting for such behavior[1,11,13].

Theoretical plate data for both columns were calculated and compared. Tables I and II show the values found for the short and long columns, respectively.

TABLE I

THEORETICAL PLATE DATA FOR GLUCOSE POLYMERS SEPARATED ON BIO-GEL P-2 USING A 1-m COLUMN

V_e = elution volume; W = peak width; K_{av} = distribution coefficient; N_{req} = number of theoretical plates required for separation with $R = 1$; N = number of theoretical plates; R = resolution; V_0 (void volume) = 42.4 ml; V_t (total volume) = 164.0 ml (for definitions see ref. 15).

Compound	V_e *(ml)*	W *(ml)*	K_{av}	N_{req} $(\times 10^3)$	N $(\times 10^3)$	R
G_1	115.8	5.67	0.604		6.67	
				2.6		1.67
G_2	106.8	5.10	0.530		7.02	
				2.5		1.69
G_3	98.3	4.93	0.460		6.36	
				2.5		1.62
G_4	90.4	4.82	0.395		5.63	
				2.4		1.56
G_5	83.1	4.54	0.335		5.36	
				3.4		1.30
G_6	77.4	4.25	0.288		5.31	
				4.7		1.09
G_7	72.9	3.97	0.251		5.40	
				4.2		1.18
G_8	68.4	3.68	0.214		5.53	
				6.5		0.96
G_9	65.0	3.40	0.186		5.85	
				5.8		1.00
G_{10}	61.6	3.40	0.158		5.25	
				11		0.74
G_{11}	59.3	2.84	0 139		6.98	

TABLE II

THEORETICAL PLATE DATA FOR GLUCOSE POLYMERS SEPARATED ON BIO-GEL P-2 USING TWO 1-m COLUMNS IN SERIES

For definition of terms see Table I. $V_0 = 93.8$ ml; $V_t = 328.0$ ml.

Compound	V_e *(ml)*	W *(ml)*	K_{av}	N_{req} $(\times 10^3)$	N $(\times 10^3)$	R
G_1	241.2	7.94	0.629		14.8	
				2.7		2.43
G_2	222.6	7.37	0.550		14.6	
				2.9		2.24
G_3	206.1	7.37	0.480		12.5	
				3.2		1.98
G_4	191.5	7.37	0.417		10.8	
				2.9		1.93
G_5	177.3	7.37	0.356		9.26	
				3.6		1.60
G_6	165.5	7.37	0.306		8.07	
				4.8		1.30
G_7	155.9	7.37	0.265		7.16	
				5.4		1.20
G_8	147.4	6.80	0.229		7.52	
				6.5		1.12
G_9	140.1	6.24	0.198		8.06	
				8.2		1.04
G_{10}	133.9	5.67	0 171		8.92	
				8.8		1.06
G_{11}	128.2	5.10	0.147		10.1	

The elution volume of the homologous series of glucose polymers is doubled on the long column. Peak widths are greater on the long column but are not double those of the shorter and thus resolution is improved on the longer column. Distribution coefficients are approximately the same on both columns, as expected, since the void volume also increases with increase in column length. The number of theoretical plates is doubled on the long column while the number of theoretical plates required is about the same for both columns.

Effect of flow-rate on elution behavior

Table III shows the gel bed parameters and experimental conditions used to separate glucose polymers on Bio-Gel P-2 at various flow-rates. Theoretical data for the polymers at the various flow-rates were calculated and Table IV shows the values obtained for elution volume, partition coefficient and resolution.

It is generally believed that elution volumes of chemical species are independent of the flow-rate[14,15]. Most of the work demonstrating this behavior has been done with rigid packing materials where there is little or no compressibility of the gels with increase in operating pressure. There have been few reports on the behavior of soft gels as packing materials which is the case in aqueous GPC. The results shown here demonstrate that elution volume is flow-rate dependent and increases with increase in flow-rate.

TABLE III

GEL BED PARAMETERS AND EXPERIMENTAL CONDITIONS OF BIO-GEL P-2 COLUMN AT VARIOUS FLOW-RATES

Parameter	*Flow-rate (ml)*		
	19.0	*24.6*	*30.0*
Total volume (ml)	180.0	180.0	180.0
Void volume (ml)	51.1	51.6	52.4
Internal volume (ml)	101.5	102.5	103.5
Polymer volume (ml)	27.4	25.9	24.1
Elution volume D_2O (ml)	152.6	154.1	155.9
Sample volume (μl)	200	200	200
Temperature (°C)	45	45	45
Detector attenuation	4×	4×	4×

TABLE IV

VARIATION IN ELUTION VOLUME V_e, PARTITION COEFFICIENT K_D AND RESOLUTION R OF GLUCOSE POLYMERS SEPARATED ON BIO-GEL P-2 AT DIFFERENT FLOW-RATES

Compound	*Flow-rate (ml/min)*								
	19.0			*24.6*			*30.0*		
	V_e	K_D	R	V_e	K_D	R	V_e	K_D	R
G_1	129.1	0.768		129.8	0.763		131.7	0.766	
			1.96			1.87			1.86
G_2	119.3	0.672		120.3	0.670		121.4	0.667	
			1.89			1.84			1.61
G_3	110.3	0.583		111.5	0.584		113.0	0.586	
			1.60			1.62			1.53
G_4	102.8	0.509		103.7	0.508		105.1	0.509	
			1.61			1.47			1.35
G_5	95.4	0.436		96.6	0.439		98.0	0.440	
			1.34			1.41			1.33
G_6	89.5	0.378		90.1	0.376		91.4	0.377	
			1.26			1.18			1.06
G_7	84.2	0.326		84.9	0.325		86.6	0.330	
			1.16			1.02			1.13
G_8	79.7	0.282		80.7	0.284		81.8	0.284	
			1.07			1.07			0.98
G_9	76.0	0.245		76.8	0.246		78.1	0.248	
			1.06			1.08			1.10
G_{10}	72.7	0.213		73.4	0.213		74.3	0.212	

Effect of temperature on elution behavior

Table V shows the gel bed parameters of Bio-Gel P-2 column at the different temperatures. Elution volume, partition coefficient and resolution values for the polymers at the different temperatures are shown in Table VI.

The elution volumes and partition coefficients for all the glucose polymers consistently decreased with increase in temperature. Resolution was markedly increased with increase in temperature but could be considered satisfactory for all practical purposes at all the temperatures used.

TABLE V

GEL BED PARAMETERS OF BIO-GEL P-2 COLUMN AT DIFFERENT TEMPERATURES

Parameter	*Temperature*		
	35°	*45°*	*55°*
Total volume (ml)	164.0	164.0	164.0
Void volume (ml)	43.2	42.0	41.4
Internal volume (ml)*	85.8	86.5	87.3
Polymer volume (ml)	35.0	35.5	35.3
Column height (cm)	82.0	82.0	82.0
Cross-sectional area (cm^2)	2.0	2.0	2.0
Flow-rate (ml/h)	28.8	28.8	28.8

* Determined as the ordinate intercept of a plot of ($V_e - V_0$) *versus* molecular weight for the maltodextrin series at the different temperatures.

TABLE VI

VARIATION IN ELUTION VOLUME V_e, PARTITION COEFFICIENT K_D AND RESOLUTION R OF GLUCOSE POLYMERS SEPARATED ON BIO-GEL P-2 AT DIFFERENT TEMPERATURES

Compound	*Temperature (°C)*								
	35			*45*			*55*		
	V_e	K_D	R	V_e	K_D	R	V_e	K_D	R
G_1	120.1	0.896		118.2	0.881		116.2	0.857	
			1.52			2.02			1.94
G_2	110.8	0.788		107.6	0.758		106.0	0.740	
			1.34			1.66			1.64
G_3	103.2	0.699		99.6	0.666		98.1	0.649	
			1.37			1.58			1.63
G_4	95.6	0.611		92.2	0.580		90.7	0.565	
			1.27			1.45			1.47
G_5	88.4	0.527		85.2	0.499		83.8	0.486	
			1.04			1.28			1.34
G_6	82.8	0.462		79.2	0 430		77.7	0.416	
			1.06			1.15			1.26
G_7	77.4	0.399		74.3	0.373		72.7	0.358	
			0.97			1.10			1.16
G_8	73.0	0.347		70.1	0.325		68.6	0.312	
Void volume (ml)			43.2			42.0			41.4
Internal volume (ml)			85.8			86.5			87.3
Total volume (ml)			164.0			164.0			164.0

There are many hypotheses to explain the molecular sieving effect of polyacrylamide gel[16]. If the exclusion theory is considered, then the elution volume of a solute should be temperature-independent. Moor and Hendrickson[17] determined the temperature-independence of elution volume of polystyrols and polyethylene glycols separated on polystyrol gels with toluene, tetrahydrofuran and tetralin as solvents. Little *et al.*[18], however, found that elution volume of polystyrene standards, acetonitrile and *o*-dichlorobenzene decreased with increase in temperature. They attributed this behavior to the relaxing and uncoiling of the solutes at high temperature thus

causing them to occupy larger volumes. This would indicate a greater portion of the pores in the gel matrix may exclude the compounds and cause them to elute earlier from the column. This may be the case for some coiled compounds which in fact uncoil with increase in temperature. The same reasoning can not, however, be applied to maltodextrin polymers that are known to be rod-like and behave like rigid rods up to a temperature of at least 70° [19,20]. The exclusion mechanism is, however, present in the separation process since there is a linear relationship between $-\log K_D$ and molecular weight of malto-oligosaccharides as shown in Fig. 5. Another concept used in explaining the sieving effect is based on partitioning. In this case, elution volume is expected

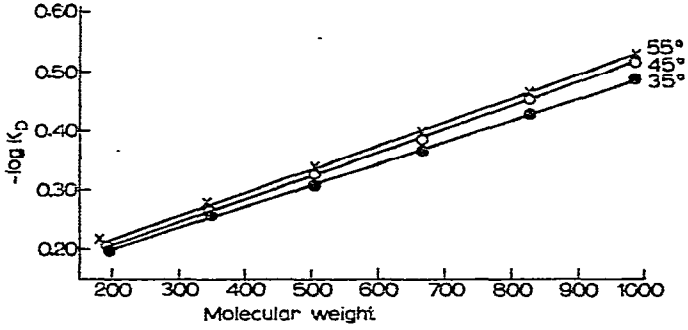


Fig. 5. Relationship between $-\log K_D$ and molecular weight of glucose polymers at different temperatures.

to be temperature dependent and would increase with temperature. This was not observed here as is shown in Table V. Still another hypothesis proposed to explain the polyacrylamide sieving effect is based on thermodynamic considerations. Brown[1] and Dellweg *et al.*[21] studied the nature of separations of cellodextrins and maltodextrins, respectively, on GPC. Contributions of free energy, enthalpy and entropy terms were calculated for a homologous series of the polymers. In both studies it was established that a negative temperature dependence of elution volume existed so that distribution coefficients decreased with increase in temperature. Their results are in agreement with our findings as shown in Table V.

It is probably safe to assume that there are several mechanisms operating simultaneously in the separation process of solutes by GPC. Experimental data should not be interpreted on the basis of a single mechanism as the only one involved in the separations. Interactions such as solvent–gel, solute–solvent and solute–gel must all be considered, since all of them apparently contribute to the patterns observed in these polymer separations.

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