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GEL PERMEATION CHROMATOGRAPHY OF GLUCOSE OLIGOMERS ON POLYACRYLAMIDE GELS

THERMODYNAMIC AND STERIC PARTITION MECHANISMS

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SUMMARY

The nature of the separation of the maltodextrin, cyclodextrin, cellodextrin and gentiodextrin series has been investigated. The dependence of the partition coefficient K_D on temperature and on the nature of the oligomers is discussed. The partition coefficient is determined by both energetic and steric factors, so a two-step mechanism is proposed. Moreover, the experimental data at 65° can be interpreted on the basis of an exclusion mechanism.

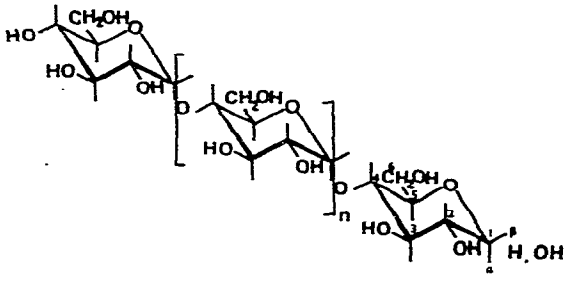
INTRODUCTION

Many experimental results are now available concerning the gel permeation chromatography (GPC) of malto-oligosaccharides and cello-oligosaccharides. Generally, a thermodynamic approach is employed based on the dependence of the apparent distribution coefficient K_D on temperature[1–5]. However, the steric exclusion process has never been considered in this connection.

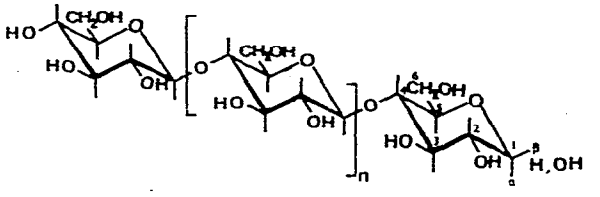
In this work, we have tested the behaviour of four series of oligosaccharides whose monomeric unit is D-glucose. The only difference between the series is in the intermonomeric bond (Fig. 1). The variation of the partition coefficient K_D as a function of the degree of polymerization (N) and of the temperature is examined taking into account the hydrodynamic volume expressed by the product $[\eta]M$, where $[\eta]$ is the intrinsic viscosity and M the molecular weight.

EXPERIMENTAL

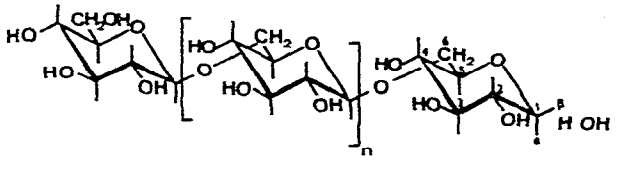
The gel chromatograph was assembled in the laboratory from commercially available components. Polyacrylamide Bio-Gel P-2 (200–400 mesh) was used as the packing material. The gel was hydrated overnight and the gel suspension was poured into the water-jacketed column. A Milton-Roy minipump was used to pump deionized distilled water through the column at a fixed rate of 14 ml/h, and detection was accomplished via a differential refractive index monitor (R401; Waters Assoc., Milford, Mass., U.S.A.). Samples were prepared by making a 10% (w/v) solution of the oligosaccharides; 100–200-μl samples were injected via a septum injector.



(A) MALTOOLIGOSACCHARIDES



(B) CELLOOLIGOSACCHARIDES



(C) GENTIOOLIGOSACCHARIDES

Fig. 1. The chemical structure of the oligomers investigated: A = malto-oligosaccharides [α (1 → 4)]; B = cello-oligosaccharides [β (1 → 4)]; C = gentio-oligosaccharides [β (1 → 6)].

The malto-oligosaccharides and cello-oligosaccharides were obtained by preparative GPC after acetolysis of the corresponding polymer[6]. The gentio-oligosaccharides were prepared by step addition according to Vignon[7]. The cyclodextrins were commercially available materials from the Corn Products (Englewood Cliffs, N.J., U.S.A.). A typical fractionation is depicted in Fig. 2.

RESULTS AND DISCUSSION

Calibration

The distribution of a solute is generally given by a partition coefficient

$$K_D = \frac{(V_e - V_o)}{V_p} \tag{1}$$

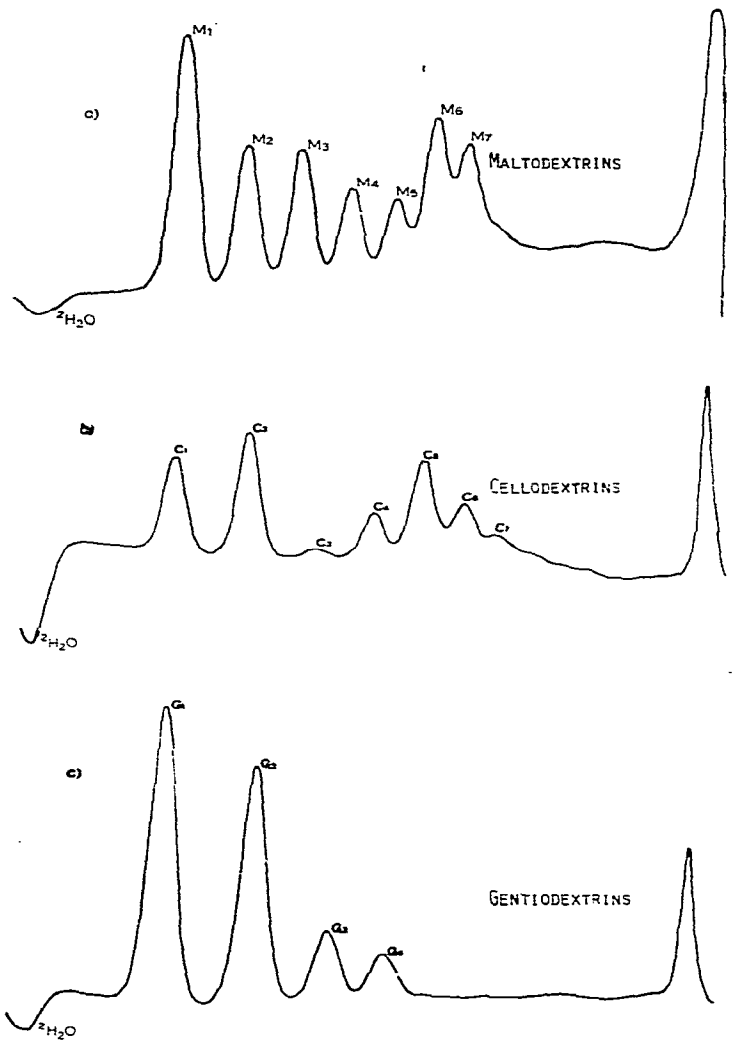


Fig. 2. Chromatograms obtained at 65°. The references are ^2H_2O and a high-molecular-weight dextran.

where V_e is the elution volume of the considered solute, V_o the outer volume and V_p the inner volume of the gel. The value of V_o is accurately determined from the peak due to the high-molecular-weight polymer fraction. Note that, as indicated in Table I, the value of V_o decreases when the temperature increases, owing to an increase in the swelling of the gel. The value of V_p is difficult to determine. Low-molecular-weight solutes for which the diffusion in the gel must be unrestricted (and then $V_e = V_o + V_p$) gave unsatisfactory results. To overcome this problem, we have determined the total volume of the column, the volume occupied by the gel ($V_g = \bar{V} \cdot P$, where P is the mass of dried gel and $\bar{V}$ its specific volume). The results are summarized in Table I. From these data, no solute would seem to be ideal, nevertheless, since D-glucose is the common monomeric unit we have used this as a ref-

TABLE I

EXPERIMENTAL CONDITIONS AND CALIBRATION

Bed volume of gel, $V_T = 238$ ml (at 25°); mass of dry gel = 70 g; volume of the matrix ($\bar{V} = 0.69$), $V_g = 48.3$ ml; total volume of solvent ($V_o + V_p$) = 190 ml.

Characteristic determined	*Solute*	*Temp.*	V_e *(ml)*	σ *(ml)*	*Temp.*	*Bed volume, V(ml/g)*
V_o	Dextran	25°	74.5	1.20	25°	3.22
		65°	70.5	1.20	65°	3.44
	PVP*	25°	74.5			
		65°	70			
						V_p *(ml)*
$V_o + V_p$	^2H_2O	25°	210.5	3.12		136
		65°	208			137.5
	Acetone	25°	167			92.5
		65°	170			99.5
	Ethanol	25°	167.5			
		65°	175.5			
	Glucose	25°	184.5	2.6		110
		65°	180	2.14		109.5

* Polyvinylpyrrolidone.

erence. The coefficient K_G, calculated by introducing $V_p = V_e^{\text{glucose}} - V_o$, will be directly connected with an intrinsic K_D if K_G is multiplied by $k = (V_e^{\text{glucose}} - V_o)/(V_e^{\text{reference}} - V_o)$. The constant k depends only on the experimental conditions (temperature, solvent).

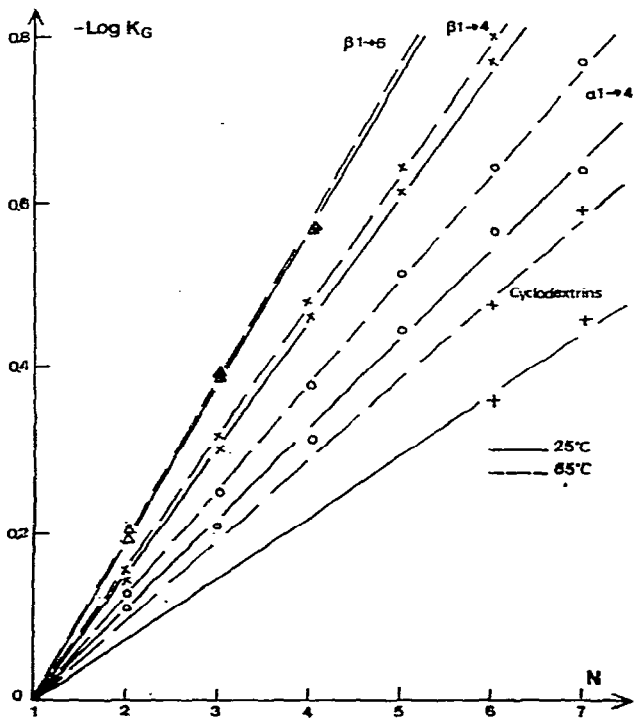


Fig. 3. Dependence of $-\log K_G$ on the degree of polymerization N and on temperature.

For low-molecular-weight solutes, the dispersion of the peak, σ, is much larger than with high-molecular-weight solutes. This dispersion increase generally coincides with an increase in V_e when the solute interacts loosely with the gel.

Thermodynamic approach

Fig. 3 shows the linear dependence of log K_G as a function of N as previously reported[1-5]; K_G decreases with increasing temperature for each oligosaccharide. Assuming the establishment of an equilibrium between a solute in the mobile phase and the solute inside the gel, the free-energy change between the two states, $\Delta G^0 = -RT \log K_G$, can be considered as a sum of the different terms corresponding to each step involved[8].

The value of K_G has been studied as a function of the temperature for each oligomer; from the relation

$$-\ln K_G = \frac{\Delta H^0}{RT} - \frac{\Delta S^0}{R} \tag{2}$$

a graph of $-\ln K_G$ against $1/T$ provides the value of ΔH^0 from the slope and the value of ΔS^0 from the intercept. The values of ΔG^0, ΔH^0 and ΔS^0 are given in Table II. These values are plotted as a function of N in Fig. 4; the fractionation is characterized by well defined increments per monomeric unit as shown in Table III. Note that the width of each peak is independent of N but depends on the oligomer and on the temperature, probably owing to further interactions in addition to the normal axial dispersion (Table IV).

TABLE II

THERMODYNAMIC PARAMETERS OF FRACTIONATION

Oligosaccharides	ΔG^0 (25°) (cal/mol)	ΔG^0 (65°) (cal/mol)	$-\Delta H^0$ (cal/mol)	$-\Delta S^0$ (cal/mol·K)
Maltodextrins				
Maltose	44	86	80	0.49
Maltotriose	83	168	208	1.12
Maltotetraose	125	257	327	1.73
Maltopentaose	175	347	365	2.10
Maltohexaose	223	434	406	2.48
Maltoheptaose	253	521	667	3.52
Cellodextrins				
Cellobiose	55	103	91	0.58
Cellotriose	119	214	89	0.89
Cellotetraose	182	324	102	1.26
Cellopentaose	242	434	142	1.70
Cellohexaose	305	541	156	2.06
Gentiodextrins				
Gentiobiose	78	139	34	0.50
Gentiotriose	153	266	24	0.84
Gentiotetraose	223	388	40	1.25

From these results (Tables III, IV) we conclude that the interaction with the gel depends on the type of oligomer (*e.g.*, the existence of a primary hydroxyl group, the flexibility of the chain, etc.); as shown by the ΔH^0 and ΔS^0 values, the strength of the interaction decreases in the order cyclodextrins > maltodextrins > cello-

TABLE III

THERMODYNAMIC INCREMENTS OF THE DIFFERENT SERIES OF OLIGOSACCHARIDES

Oligosaccharides	$-\Delta H^0$ *per monomeric unit [from* $\Delta H^0(N)$*]*	$-\Delta S^0$ *per monomeric unit [from* $\Delta S^0(N)$*]*
$\beta(1 \rightarrow 6)$	12.4	0.42
$\beta(1 \rightarrow 4)$	33.2	0.42
$\alpha(1 \rightarrow 4)$	111.4	0.57

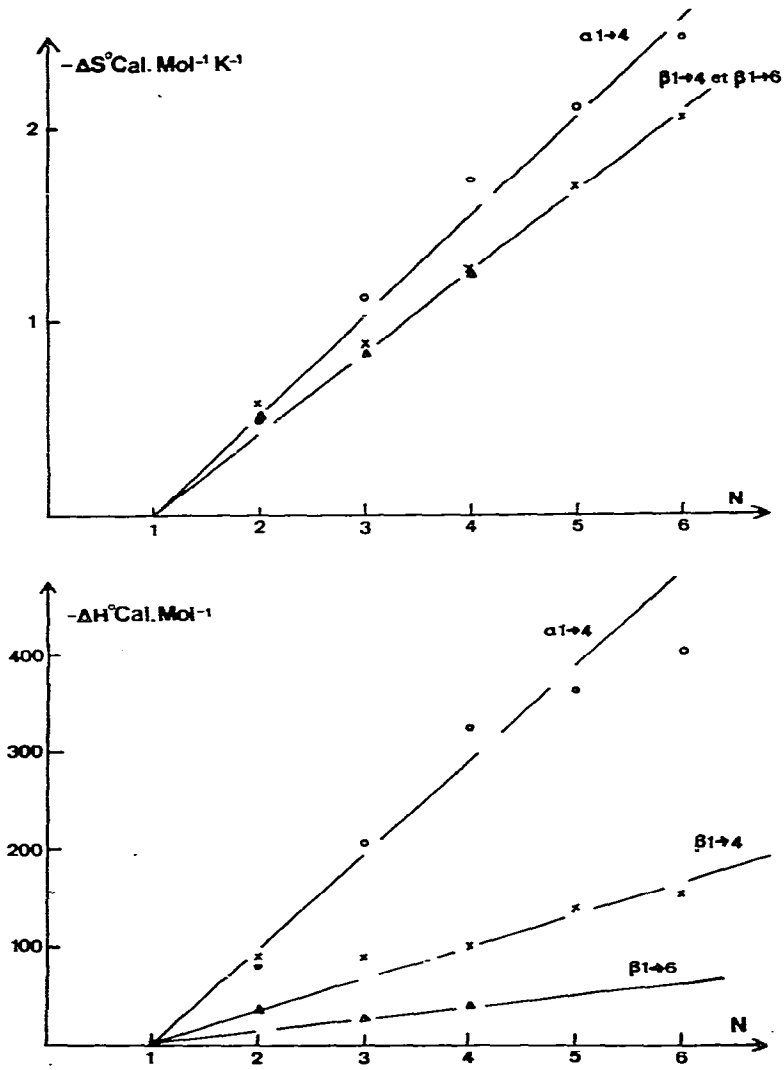


Fig. 4. Dependence of the thermodynamic parameters ΔH^0 and ΔS^0 on N.

TABLE IV

VARIATION OF THE WIDTH OF THE ELUTION PEAK AS A FUNCTION OF THE TEMPERATURE AND OF THE CHEMICAL STRUCTURE

	σ (ml)	
	25°	65°
Dextrans	1.20	1.20
Glucose	2.60	2.14
Maltodextrins	—	2.19
Cellodextrins	2.68	2.14
Gentiodextrins	2.66	2.08

TABLE V

INTRINSIC VISCOSITIES OF CELLO-OLIGOSACCHARIDES AND MALTO-OLIGOSACCHARIDES

Oligosaccharides	$[\eta]^*$ (ml/g)	
	25°	65°
Cellodextrins		
Glucose	1.95	1.63
Cellobiose	2.18	1.87
Cellotriose	2.62	2.27
Cellotetraose	2.95	2.72
Cellopentaose	3.37	2.94
Cellohexaose	3.60	—
Maltodextrins		
Glucose	1.95	1.63
Maltose	2.09	1.73
Maltotriose	2.30	1.91
Maltotetraose	2.64	2.22
Maltopentaose	2.75	2.41
Maltohexaose	2.90	2.55
Maltoheptaose	3.01	2.74

* Determined with an Ubbelohde automatic viscometer from FICA (Le Mesnil, Saint Denis. France).

dextrins > gentiodextrins. In particular, the large decrease in the value of ΔS^0 for maltodextrins must correspond to a large change in the state of hydration of the molecules on the gel. The selectivity of the fractionation increases at higher temperatures by reducing this interaction. The higher the temperature the better are the results as shown by an increase in the slope of the $-\ln K_G(N)$ plot and a decrease in the width of the peak (σ).

Steric approach

The intrinsic viscosity has been measured at 25° and 65° for $\alpha\,(1 \rightarrow 4)$ and $\beta\,(1 \rightarrow 4)$ oligomers to determine the hydrodynamic volume which is generally accepted as the fundamental parameter for GPC fractionation[9]. These values are given in Table V and plotted using D-glucose as reference in Fig. 5. Experimental data should not be interpreted on the basis of a single exclusion mechanism because:

(1) For the same degree of swelling at a constant temperature, 25°, the curves corresponding to both series of oligomers do not coincide.

(2) When the temperature increases the swelling of the gel increases and if the product $[\eta]M$ decreases K_G should increase too; but as shown in Fig. 5, we observe a decrease of V_e with increasing temperature.

So, to explain the experimental results, specific gel–solute interactions must be introduced. These interactions decrease when the temperature increases and disappear at 65° when both sets of data expressed in terms of $[\eta]M$ lie practically on the same curve. Steric exclusion is then the only mechanism for fractionation.

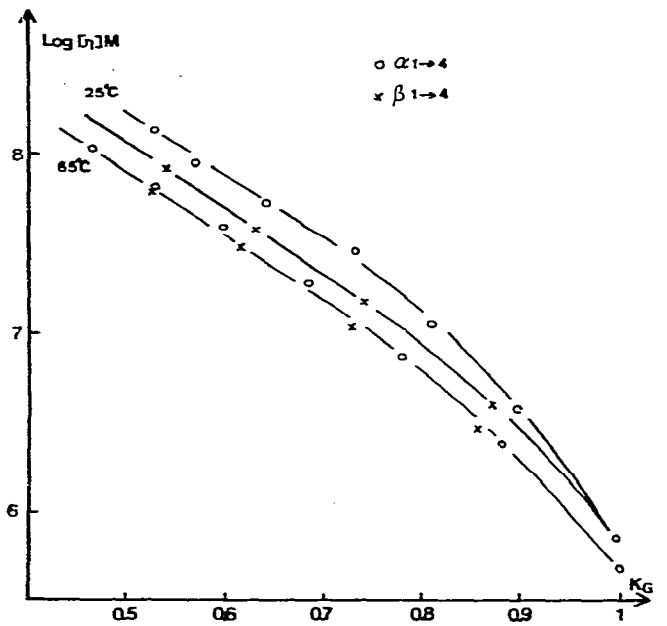


Fig. 5. Hydrodynamic calibration and its dependence on temperature.

Formulation proposed

To take into account both the steric exclusion process and the adsorption on the gel, a double equilibrium is considered:

solute in the mobile phase $\rightleftarrows$ solute in the pores $\rightleftarrows$ solute adsorbed in the gel phase

Consequently, the elution volume takes the form:

$$V_e = V_o + K_1 V_p + K_1 K_2 V_g$$

where K_1 and K_2 characterize the fractionation by the steric exclusion effect and by the "adsorption" effect respectively and V_p and V_g are the porous volume and

the volume of the swollen gel matrix. Since V_g and V_p are proportional to the dry-weight of the gel, we find that:

$$V_e = V_o + \left(K_1 + K_2 \frac{\overline{V}}{\tau}\right) V_p \tag{3}$$

$$\text{with } K_D = K_1 + K_2 \frac{\overline{V}}{\tau} \text{ and } K_G = \frac{K_D}{k} = K_1' + K_2' \frac{\overline{V}}{\tau} \tag{3'}$$

In this relation, V_g must be taken into account if a "dissolution" effect is considered[10]. The surface area S which is proportional to the weight of the gel must be included if the "adsorption" is a surface process; τ is the degree of swelling of the gel.

The values of both the constants K_1 and K_2 (divided by the constant k) can be independently deduced as follows. First, we can determine the constant K_1 (25°) for the exclusion mechanism from the curve $\log [\eta]M(K_G)$ established at 65° if $[\eta]$ (25°) is known. A theoretical approach for exclusion in a simplified model in which the pores of the gel are cylindrical and the solute is a spherical molecule leads to the relation:

$$K_1 = \left(1 - \frac{r}{R}\right)^2 \tag{4}$$

where r, the radius of solute, varies as $[\eta]^{1/3}$ and R, the pore radius, varies as $\tau^{1/2}$. From eqn. 4, the values of K_1' (25°) can be calculated assuming K_1' (65°) = K_G (65°). Both sets of values obtained for K_1' (25°) agree well (Table VI). Secondly, K_2' can be estimated from eqn. 3'; again, a higher affinity is found for the malto-oligosaccharide than for the cello-oligosaccharide (Table VI).

TABLE VI

PARTITION COEFFICIENTS K_1' AND K_2' AT 25°

Oligosaccharide	K_G (25°) *(exptl.)*	K_1' *deduced from the curve* $[\eta]M$ *at 65°*	K_1' (25°) *(calc.) (eqn. 4)*	K_2' (25°)
Maltose	0.895	0.835	0.865	0.073
Maltotriose	0.811	0.740	0.752	0.166
Maltotetraose	0.730	0.635	0.647	0.271
Maltopentaose	0.642	0.555	0.558	0.318
Maltohexaose	0.569	0.490	0.482	0.382
Cellobiose	0.870	0.825	0.841	0.073
Cellotriose	0.741	0.700	0.699	0.127
Cellotetraose	0.632	0.595	0.587	0.162
Cellopentaose	0.542	0.500	0.480	0.273

CONCLUSIONS

The nature of the separation process for D-glucose oligomers on polyacrylamide gels has been studied. The values of $-\log K_D$ and molecular weight are in

quite good agreement with a linear function in the temperature range of 25–65°. In addition, the temperature dependence of the elution of oligomers from polyacrylamide gels agrees well with Brown's and Dellweg's observations.

The classical thermodynamic interpretation demonstrated the additivity of the effect per monomeric unit. The fractionation is characterized by a set of parameters $\Delta H^0/N$ and $\Delta S^0/N$ which depend on the nature of the oligosaccharide.

From hydrodynamic investigations, steric exclusion has been discussed; at 65° the fractionation is based on $[\eta]M$, but at lower temperatures there is a solute–gel interaction characteristic of the oligomer series. The affinity follows the sequence: cyclo-oligosaccharides > malto-oligosaccharides > cello-oligosaccharides > gentio-oligosaccharides.

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