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GEL PERMEATION CHROMATOGRAPHY OF MALTOOLIGOSACCHARIDES AT DIFFERENT TEMPERATURES

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SUMMARY

The separation of maltodextrins (glucose to maltoheptaose) on polyacrylamide gel has been studied at various temperatures. A negative temperature dependence of elution volume was established so that the distribution coefficients decreased with increasing temperature. This peculiarity gives rise to a negative enthalpy for the interacting effects between solute and the gel matrix.

INTRODUCTION

Gel permeation chromatography is a useful method for the separation of molecules according to their molecular size. This method has often been used to infer the molecular weight of an unknown substance from its elution volume. In most practical cases the logarithmic relation between elution volume and molecular weight is applied as reported by DETERMANN AND MICHEL[1]. This relation is based on experimental results with polysaccharides[2] and with polypeptides[3–5]. The theoretical background is the concept of restricted diffusion of solute molecules within the gel phase[6] and their exclusion from the gel matrix, respectively[7].

Both these models lead to the common expression

$$K = \text{const.} - \text{const.} \cdot \log M,$$

where K is the partition coefficient and M the molecular weight of the solute.

According to these concepts the elution volume of a given solute would be expected to be independent of temperature. This has, indeed, been established by MOORE AND HENDRICKSON[8], who studied the separation of polystyrols and polyethyleneglycols in a polystyrol gel with toluene, tetrahydrofuran and tetralin as solvents. In a previous paper we described the separation of low molecular weight maltooligosaccharides (maltodextrins) on polyacrylamide gel[9]. We also obtained a linear function between the logarithm of the molecular weight and the relative elution volume. This relation however only proved correct for maltodextrins with more than four glucose units, and the lower members did not fit on the straight line.

Furthermore we observed the influence of temperature on the elution volume, leading to an improvement of the separation effect at an elevated temperature[10]. In the meantime a set of experiments were therefore started in which the temperature effect was investigated and the results are described below.

Recently HJERTÉN[11] presented a calculation for partition experiments, based merely on thermodynamic considerations without any assumption about the separation mechanism. According to his conclusions non-coiled chains in homologous series of low molecular weight compounds should conform to an expression in which the logarithm of relative elution volume depends linearly on the molecular weight. He showed that our data fitted very well with his equation.

EXPERIMENTAL

Maltodextrins

The preparation of the maltodextrins from maltose using amylomaltase has been described earlier[9]. Dextran 2000 (Pharmacia) was added for the indication of V_o. 20 μl of a solution containing a total of 2 mg of maltodextrins were applied to the column for each run.

Column chromatography

Polyacrylamide gel (Bio-Gel P-2, *minus* 400 mesh, Lot. No. 3397) was fractionated by repeated settling and decanting the fines, and packed into a column (1.5 × 200 cm). The upper end of the column was sealed by inserting a flow adaptor, so that the total volume (V_t) was the same at all temperatures (= 362 ml). Distilled water was pumped through the column at a constant flow rate of 25.0 ml/h. The pressure rose to about 2 kp/cm^2. Application of the sample through an injection port and detection of the carbohydrates with orcinol sulphuric acid using an automated analyzer have been described in detail[9].

The column was preequilibrated for at least 12 h at the appropriate temperature prior to application of the sample. Each experiment was performed twice, the deviation of elution volume within two identical runs being in no case more than 1.5 ml.

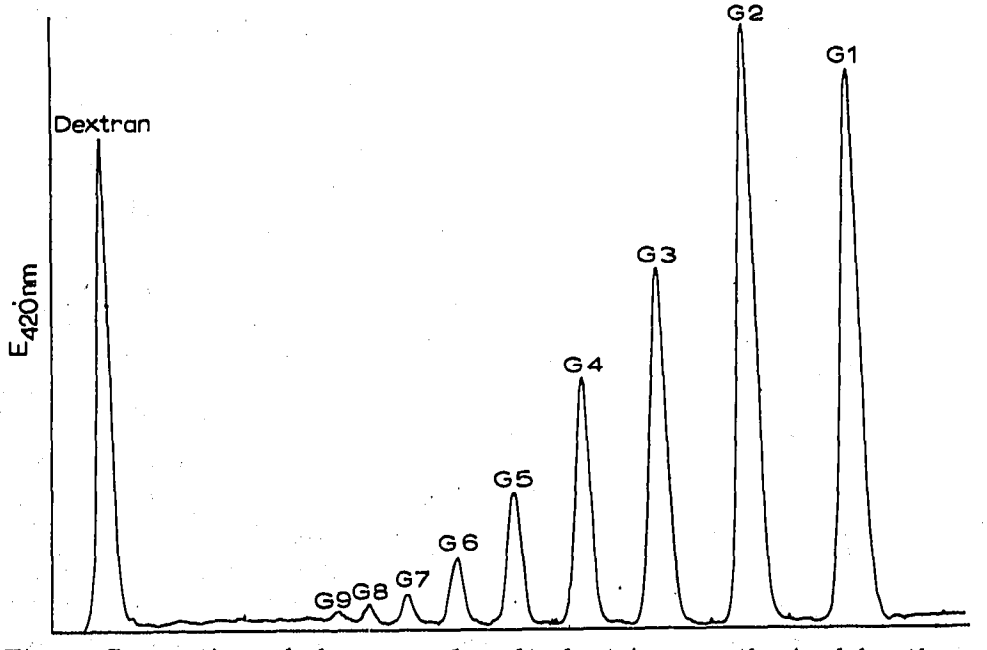

Fig. 1. Separation of glucose and maltodextrins, synthesized by the action of E. coli ML 30 on maltose. Bio-Gel P-2, minus 400 mesh, 65°; column 1.1 × 200 cm.

TABLE I

ELUTION VOLUMES V_e (ml) AND K VALUES FOR MALTODEXTRINS AT DIFFERENT TEMPERATURES

$V_t = 362$ ml.

Saccharide	*20°*		*30°*		*40°*		*50°*		*60°*		*70°*	
	V_e	K	V_e	K	V_e	K	V_e	K	V_e	K	V_e	K
Glucose	288.5	0.892	286.0	0.887	287.0	0.893	284.5	0.883	286.5	0.894	283.5	0.877
Maltose	272.0	0.800	268.5	0.789	268.5	0.790	265.0	0.775	266.3	0.784	263.0	0.766
Maltotriose	258.0	0.723	253.0	0.703	252.0	0.700	248.0	0.682	248.0	0.683	244.5	0.666
Maltotetraose	244.5	0.647	239.0	0.625	237.0	0.616	233.0	0.599	232.0	0.596	228.5	0.579
Maltopentaose	231.5	0.575	225.5	0.550	223.0	0.539	219.0	0.522	217.5	0.516	214.0	0.500
Maltohexaose	220.0	0.511	214.0	0.486	211.0	0.473	207.5	0.459	205.0	0.448	202.0	0.435
Maltoheptaose	210.0	0.455	204.0	0.430	—	—	196.5	0.398	194.5	0.391	191.5	0.378
Dextran: V_e	128.0		126.5		125.5		124.0		123.0		122.0	
V_i	180.0		180.0		181.0		182.0		183.0		184.0	

RESULTS

A typical elution diagram is shown in Fig. 1. The outer volume V_o is indicated by the peak of excluded dextran. The inner volume V_i was estimated by separate runs with ribose and erythrose, by water regain of the dry gel and by extrapolation. From these results the volume of the dry gel matrix V_m was estimated as 54 ml at 20° and 56 ml at 70°.

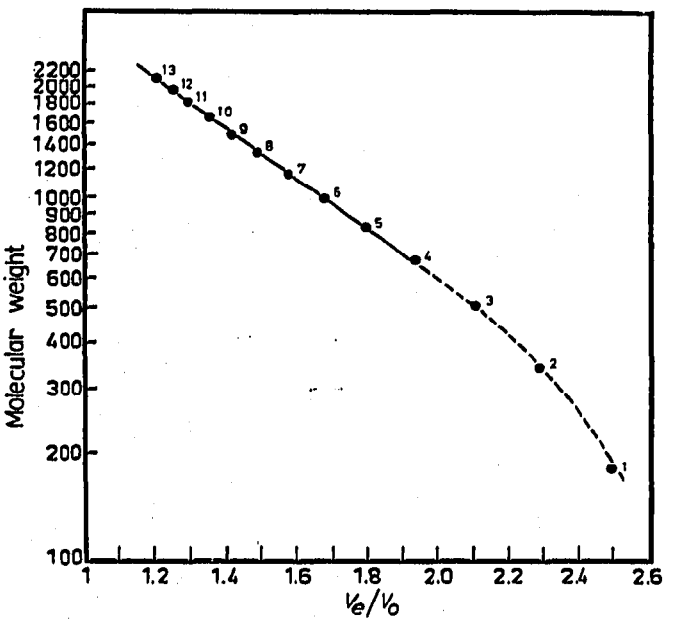

Fig. 2. Semi-logarithmic plot of molecular weight of maltodextrins *versus* relative elution volume (V_e/V_o). These results were obtained two years ago[9] with a much tighter packed gel column. So they are only qualitatively comparable to the results presented in this paper.

Fig. 2 shows a semi-logarithmic plot of molecular weight *versus* elution volumes at 60° from former experiments[9].

The elution volumes V_e at different temperatures are presented in Table I. The K values denote the portion of the inner volume, which is available for diffusion of the respective compound.

$$V_e = V_o + K \cdot V_i$$

$$K = \frac{V_e - V_o}{V_i}. \qquad (1)$$

As may be seen from Fig. 3 the values of $-\log K$ *versus* molecular weight are in quite good agreement with a linear function in the temperature range of 20° to 70°. Using the form of equation proposed by HJERTÉN[11]

$$-\log K = C_1 \cdot M + C_0 \qquad (2)$$

where M is the molecular weight, one can estimate the constant C_1 as the slope of the straight line and C_0 as the ordinate at the origin (Table II).

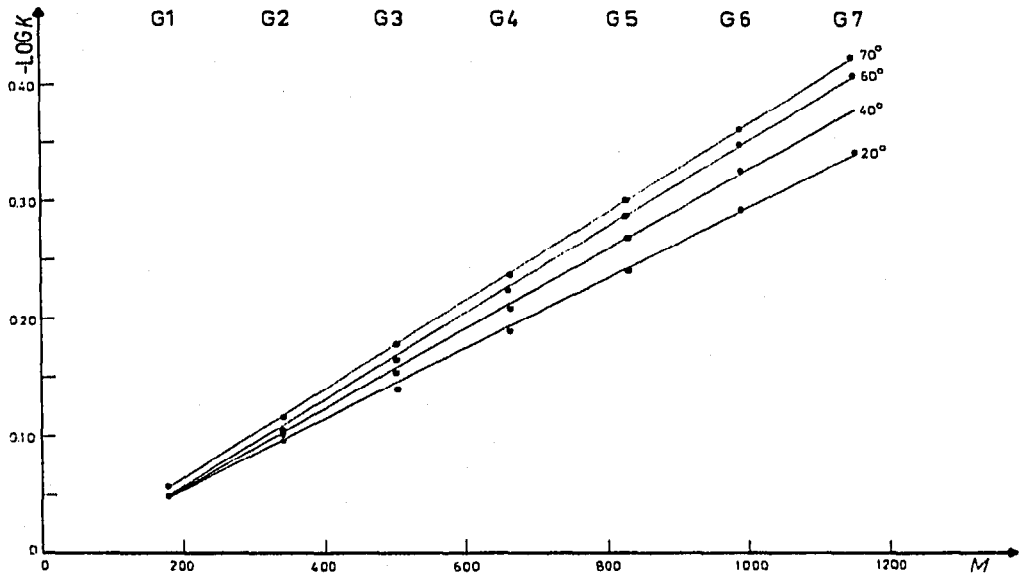

Fig. 3. Plots of — log K against the molecular weight M of maltooligosaccharides at various temperatures.

TABLE II

VALUES OF CONSTANTS C_1 AND C_0 FROM eqn. 2 FOR DIFFERENT TEMPERATURES AS CALCULATED BY THE METHOD OF LEAST SQUARES

Temperature	T	C_1	C_0	C_1/T
20°	293	$3.01 \cdot 10^{-4}$	−0.006	$1.026 \cdot 10^{-6}$
30°	303	$3.26 \cdot 10^{-4}$	−0.010	$1.077 \cdot 10^{-6}$
40°	313	$3.31 \cdot 10^{-4}$	−0.008	$1.057 \cdot 10^{-6}$
50°	323	$3.53 \cdot 10^{-4}$	−0.011	$1.091 \cdot 10^{-6}$
60°	333	$3.72 \cdot 10^{-4}$	−0.021	$1.117 \cdot 10^{-6}$
70°	343	$3.78 \cdot 10^{-4}$	−0.013	$1.100 \cdot 10^{-6}$

DISCUSSION

Several hypotheses have been proposed to explain the molecular sieving effect of polyacrylamide gels[12]. One concept would be a partition of the solute between the mobile phase and the entire gel phase as stationary phase. For this concept the BOLTZMANN equation can be applied according to BRÖNSTED[13]

$$K = \frac{C_s}{C_m} = e - \frac{\lambda}{kT} \tag{3}$$

in which K is the distribution coefficient, k the BOLTZMANN constant and λ an expression proportional to the molecular size. From eqn. 3 one would expect the constant C_0 (eqn. 2) to be the logarithm of the distribution coefficient for $M = 0$. K values are indeed very close to unity (1.01 to 1.05).

If the partition concept is to be valid, one should expect a temperature dependence of K, this means, V_e should be greater at higher temperatures. The opposite

effect is shown by our experiments however and the slope C_1 increases nearly proportionally with increasing absolute temperature (Table II). The change in free energy, ΔG^0, which occurs when one mole of the solute is transferred from the mobile phase to the solvent within the gel particles is

$$\Delta G^\circ = -RT \cdot \ln \frac{C_s}{C_m} = -RT \cdot \ln K \tag{4}$$

Taking into consideration some kind of interaction of the solute molecules with the gel matrix one has to expect an enthalpy effect which is related to free energy by the equation

$$\Delta G^\circ = \Delta H^\circ - T \cdot \Delta S^\circ \tag{5}$$

Combining eqns. 4 and 5 results in

$$-\ln K = \frac{\Delta H^\circ}{RT} - \frac{\Delta S^\circ}{R} \cdot \tag{6}$$

$-\log K$ should therefore be a linear function of the reciprocal of the absolute tem-

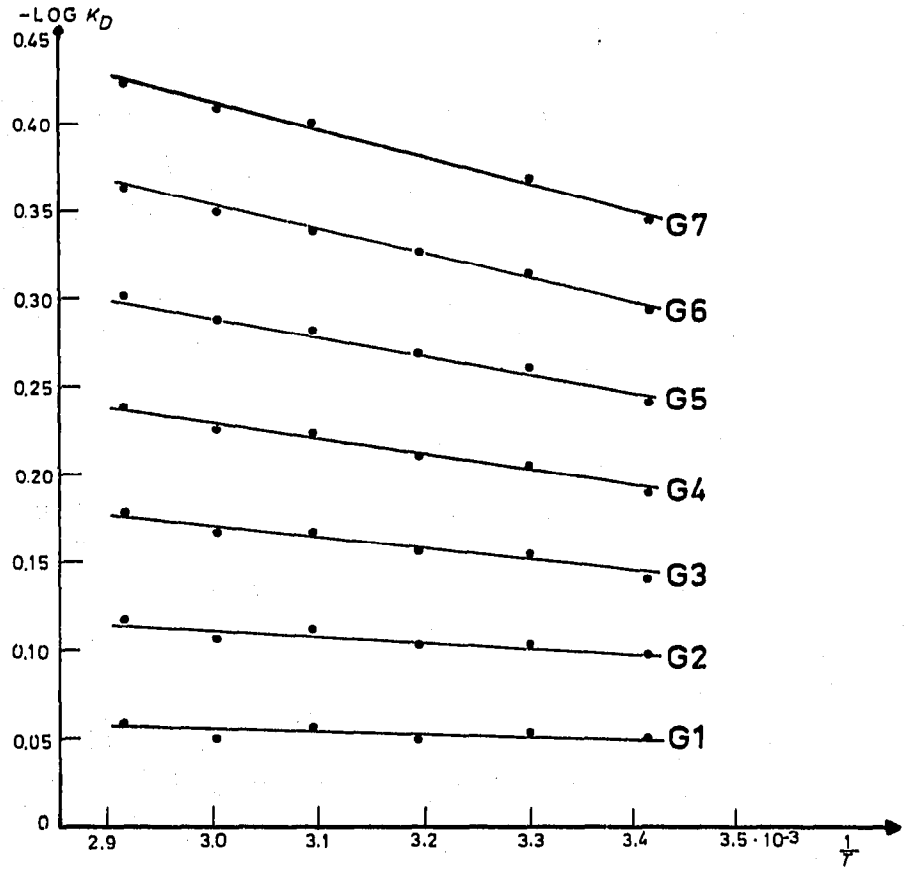

Fig. 4. Plots of $-\log K$ *versus* reciprocal temperature for maltooligosaccharides of different chain lengths.

perature. This is demonstrated in Fig. 4, in which the K values of Table I are plotted against $1/T$ in a semilogarithmic scale. The slopes of the curves,

$$\frac{\mathrm{d} \log K}{\mathrm{d} T}$$

were estimated by regression calculations with the aid of a Wang computer (Table III).

TABLE III

SLOPES OF THE CURVES FROM Fig. 4

Saccharide	*M*	$d(\log K)/dT$
Glucose	180	$-0.86 \cdot 10^{-4}$
Maltose	342	$-3.32 \cdot 10^{-4}$
Maltotriose	504	$-6.61 \cdot 10^{-4}$
Maltotetraose	666	$-9.09 \cdot 10^{-4}$
Maltopentaose	828	$-11.51 \cdot 10^{-4}$
Maltohexaose	990	$-13.52 \cdot 10^{-4}$
Maltoheptaose	1152	$-15.78 \cdot 10^{-4}$

The temperature dependence of $\log K$ points to a negative enthalpy or an interaction between solute and gel phase which increases with increasing molecular size.

$\Delta H°$ can be estimated from eqn. 6 by differentiation

$$\frac{\mathrm{d} (\ln K)}{\mathrm{d} T} = \frac{\Delta H°}{RT^2} \tag{7}$$

with $\Delta H° = \mathrm{d} (\log K)/\mathrm{d} T \cdot 4.075 \cdot 10^5$ (cal·mol^{-1}) at 25°. $\Delta G°$ can be found from eqn. 4 and $\Delta S°$ from eqn. 5 and the values are presented in Table IV.

TABLE IV

THERMODYNAMIC PARAMETERS OF MALTODEXTRINS ON BIOGEL P-2, AT 25°

	K[a] (25°)	$\Delta G°$ (cal·mol^{-1})	$\Delta G°/DP$[b]	$\Delta H°$ (cal·mol^{-1})	$\Delta H°/DP$[b]	$\Delta S°$ (cal·mol^{-1}·deg^{-1})
Glucose	0.892	+ 68	+68	− 35	−35	−0.35
Maltose	0.795	+134	+67	−135	−67	−0.90
Maltotriose	0.713	+200	+67	−269	−90	−1.57
Maltotetraose	0.635	+268	+67	−370	−92.5	−2.14
Maltopentaose	0.562	+341	+68	−469	−94	−2.72
Maltohexaose	0.499	+412	+69	−551	−92	−3.14
Maltoheptaose	0.442	+484	+69	−642	−92	−3.78

[a] K values for 25° have been calculated from Fig. 3.
[b] $\Delta G°$ or $\Delta H°$ divided by degree of polymerization.

Dividing $\Delta H°$ by the number of glucose units (DP degree of polymerization) within a maltodextrin chain we obtain increasing negative values for the first 3 members, indicating that the interaction with the gel matrix, which is brought about by the addition of a glucose unit first increases with growing chain length, but starting with maltotetraose remains constant. Furthermore the Δ log K values between consecutive members of the homologous series are nearly constant leading to a constant difference in $\Delta G°$ values. Table IV shows that the free energy for each glucose-unit added is almost constant. According to MARSDEN[14] this result is consistent with the assumption, that the free energy for the transport of a polymeric molecule from the mobile phase to the stationary phase is the sum of the free energies for the transport of the single units.

The linear relationship between log K and molecular weight is not usual in gel chromatography of proteins. However HOHN AND POLLMANN[15], working on separation of oligonucleotides of thymidylic acid on Sephadex G-25 have proved the validity of such a relationship. With nucleotides, however interactions with the gel phase do indeed exist. HJERTÉN[11] has pointed out that there are distinct differences between low molecular weight, non-coiled chain molecules such as maltodextrins and globular polymers such as proteins. The former should obey eqn. 2, where C_1 is a constant (at a given temperature) for isochemical substances in a homologous series. That this is only true for isochemical compounds was found in our former experiments[9], in which maltose (α-1,4-glucosidic) was clearly separated from isomaltose (α-1,6-) and maltotriose from isomaltotriose. Compounds with α-1,4-linkages are not isochemical with compounds containing α-1,6-linkages. On the other hand maltose and sucrose cannot be separated on Bio-Gel P-2.

The temperature dependence of the elution of maltodextrins from polyacrylamide gel is in contrast to the results of several authors, who have mentioned that the elution volume is essentially independent of temperature (*cf.* ref. 8). LEACH AND O'SHEA[5], separating high molecular weight proteins on Sephadex G-200 observed an increase of V_e with increasing temperature (25° and 40° respectively). Their values are not comparable however, because they had increased the ionic strength of the buffer which is also known to slow down the elution[3]. Furthermore these authors stated that "the peaks were broader at the higher temperature", in clear contrast to our observation[10]. On the other hand WHITAKER[3] has observed a decrease of elution volume for proteins on increasing temperature from 3.3 to 25°. BROWN[16] has recently published results on the separation of cellodextrins on polyacrylamide gel P-2 which are in very good accordance with our observations. This author would also obtain straight lines if log K is plotted against molecular weight. He also has observed a decrease of V_e with increasing temperature and he has drawn essentially the same conclusions as we did.

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