CHROM. 4918

THE SEPARATION OF CELLODEXTRINS BY GEL PERMEATION CHROMATOGRAPHY

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(Received July 3rd, 1970)

SUMMARY

The nature of the separation of the cellodextrin series, glucose–cellohexaose, on polyacrylamide and dextran gels has been investigated. It was found that the interaction between gel and solute increased with molecular weight, the effect being most pronounced with the dextran gel. One can anticipate that such adsorption effects will play an important role in all polar gel–solute systems when the solute has a conformation facilitating alignment with linear portions of the cross-linked network.

INTRODUCTION

The nature of the separation process for the cellodextrin series glucose–cellohexaose on polyacrylamide and dextrans gels has been studied as part of a general investigation of the structure and surface properties of water-swollen polymer networks. It was of interest to investigate the character of the interactive processes participating and the ability of such polar gels to separate rod-shaped molecules possessing little molecular flexibility[1].

Furthermore, the cellodextrins form an important homologous series useful in investigations concerned with the mechanism of carbohydrate depolymerization by enzymatic systems and by many chemical processes; they are also used extensively as model compounds in furthering our understanding of carbohydrate chemistry.

EXPERIMENTAL

Preparation of the cellodextrins

The cellodextrins were prepared by a modification of the method of MILLER *et al.*[2]. Whatman cellulose powder (CF-11), 90 g, was dissolved in 600 ml of fuming HCl at 0° and the solution allowed to warm to room temperature. It was then poured into 3600 ml of ice-cold distilled water and neutralized with 1260 g $NaHCO_3$. After standing for 24 h, the liquid was filtered to remove the fluffy, white precipitate

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and applied to a column of stearic acid-treated charcoal–Celite (dimensions 113 cm^2 × 800 cm). The stearic acid solution used was 2.5% in absolute ethanol. Gradient elution was performed with 20 l of water in one container connected through a siphon to 20 l of 50% absolute ethanol in a second container. Fractions were collected in an automatic collector (2100 × 18 ml). The carbohydrate contents of the fractions were measured using the orcinol reagent[3]. A typical fractionation is depicted in Fig. 1. The fractions were analyzed by thin-layer chromatography (TLC) using the system Kieselguhr-G buffered with 0.02 *M* sodium acetate and 65% isopropanol–ethyl acetate (1:1) as developer.

Fractions were combined according to the TLC results and the volume reduced in each case to about 200 ml by means of a rotary evaporator. The residues were

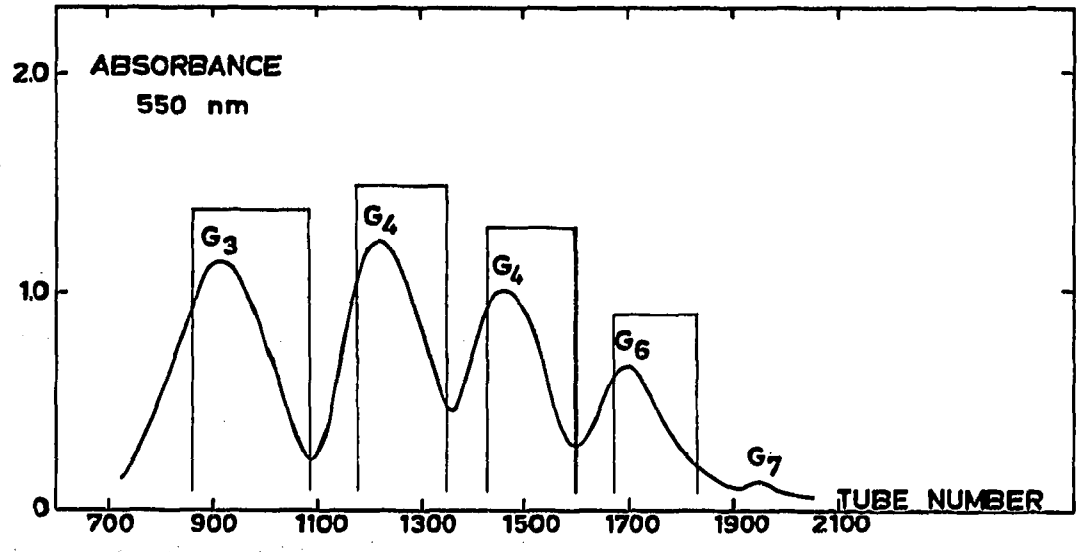

Fig. 1. Separation of cellodextrins, cellotriose–cellohexaose, on a carbon–Celite column obtained by ethanol–water gradient elution. The vertical axis represents the carbohydrate concentration determined by the orcinol method[3]. The rectangular zones represent the material isolated on the basis of the TLC analysis.

TABLE I

DIMENSIONS OF THE COLUMNS USED AT 25°

	Polyacrylamide P-2 (*ml*)	*Dextran G-15* (*ml*)
Settled bed volume	47.7	48.7
Void volume (Blue Dextran), V_0	17.1	19.25
Internal volume[a], V_I	28.7	17.0
Wt. of dry gel	15 g	~15 g
Column height	60 cm	
Cross-sectional area:	0.785 cm^2	
Flow-rate:	2 ml/h	
Sample volume:	0.1 ml	
Sample concentration:	1 mg/ml	

[a] Determined as the ordinate intercept of a plot of (V_e-V_0) *vs.* molecular weight for the cellodextrin series; this value agreed closely with that obtained from the elution of NaCl.

freeze-dried to yield approximately 4–5 g of each of the cellodextrins, cellotriose to cellohexaose.

Reagent-grade glucose and cellobiose were used to complete the series.

Column preparation

An accurately-weighed quantity of dried gel was allowed to swell in distilled water for 24 h. The slurry was de-gassed under high vacuum and the columns packed under gravity with continual addition of slurry to avoid layering defects in the columns. Subsequent to preparation each column was allowed to wash until a constant value of refractive index was obtained for the eluent. The columns were packed with polyacrylamide P-2, 200–400 mesh (Bio-Rad Laboratories, Richmond, Calif. U.S.A.) and Sephadex G-15, 40–120 μ (Pharmacia, Uppsala, Sweden). The final column dimensions are summarized in Table I. The pressure head (a Mariotte flask) was adjusted to provide a flow rate of about 2 ml/h at which it was judged, equilibrium conditions on the column would be approximated. All columns were thermostated to within $\pm$ 0.1° of the operating temperature required.

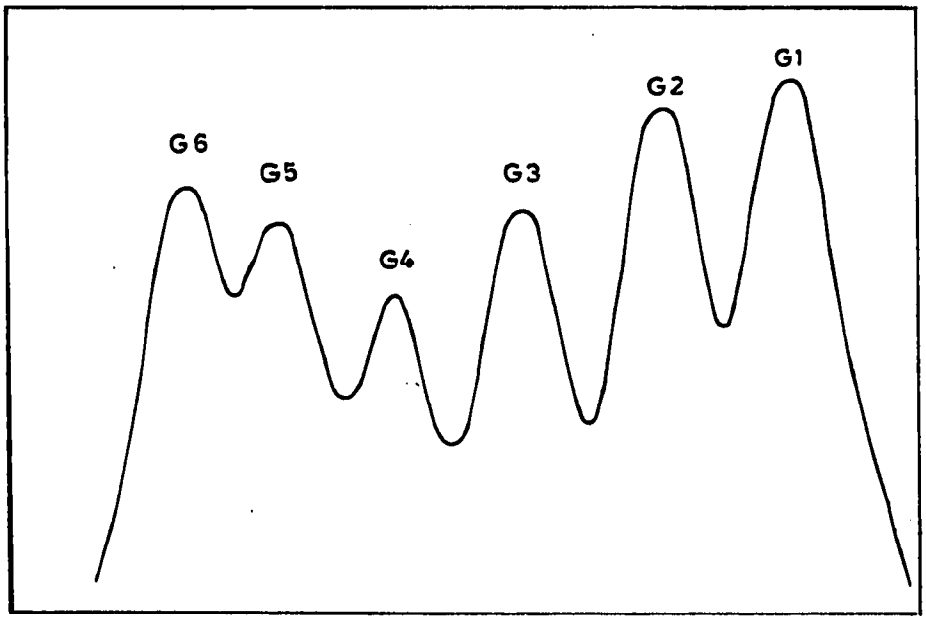

Fig. 2. Separation of cellodextrins, glucose–cellohexaose, on polyacrylamide P2 eluted with deionized water. The separation occurred within approximately one-third of the internal volume of the bed.

Sample application and detection

The sample solution (0.1 ml containing 0.1 mg solute) was applied to the gel surface with a micro pipette as a layer 1 mm deep. The solution was allowed to enter the bed, washed with successive portions of solvent and the column connected to the constant head device. The eluent was collected and the volume recorded to within $\pm$ 0.02 ml at regular intervals. A Waters Associates Model R4 differential, automatically-recording, refractometer was operated at $\times$ 8 attenuation. Under these conditions 90 % full-scale deflection was obtained at the sample maximum. The "dead"

volume between the end of the column and the refractive index cell was approximately 0.3 ml.

RESULTS AND DISCUSSION

Polyacrylamide gel

The separation of the cellodextrins on the P-2 column is illustrated in Fig. 2 for the mixture glucose–cellohexaose. By comparison with the total internal volume of the gel (V_I = 28.7 ml, 63% of settled bed volume) the separation of the six members of the series occurs over approximately 10 ml, *i.e.* about one-third of the internal volume.

The partition coefficient, K_D, is defined by:

$$K_D = \left(\frac{V_e - V_0}{V_t - V_0}\right)$$

where;

V_0 = void volume
V_t = total solvent volume in the column
V_e = elution volume for a given solute.

TABLE II

K_D AS A FUNCTION OF TEMPERATURE FOR THE CELLODEXTRINS ON POLYACRYLAMIDE P-2

Cellodextrin	*12° C*		*25° C*		*40° C*	
	K_D	ΔlnK_D	K_D	ΔlnK_D	K_D	ΔlnK_D
Glucose	0.92_6		0.92_1		0.91_5	
		0.080		0.084		0.088
Cellobiose	0.85_5		0.84_7		0.83_8	
		0.089		0.091		0.096
Cellotriose	0.78_2		0.77_3		0.76_1	
		0.097		0.099		0.105
Cellotetraose	0.71_0		0.70_0		0.68_5	
		0.090		0.093		0.098
Cellopentaose	0.64_9		0.63_8		0.62_1	
		0.079		0.078		0.082
Cellohexaose	0.60_0		0.59_0		0.57_2	

TABLE III

HYDRODYNAMIC PARAMETERS FOR CELLODEXTRINS IN AQUEOUS SOLUTION AT 25°

Cellodextrin	*Molecular weight (M)*	$[\eta]^a$ ($ml \cdot g^{-1}$)	$D \cdot 10^{6a}$	V^a ($ml \cdot mole^{-1}$)	L^a (Å)	r^b (Å)
Glucose	180	2.54	6.75	116	—	3.65
Cellobiose	342	2.74	5.16	222	14.6	4.85
Cellotriose	504	3.03	4.19	326	20.2	5.63
Cellotetraose	667	3.57	3.75	432	26.2	6.47
Cellopentaose	829	3.80	3.21	535	31.8	7.25
Cellohexaose	991	4.70	2.90	639	37.6	8.19

[a] Data of IHNAT AND GORING[1].
[b] Data of KURATH AND BUMP[10].

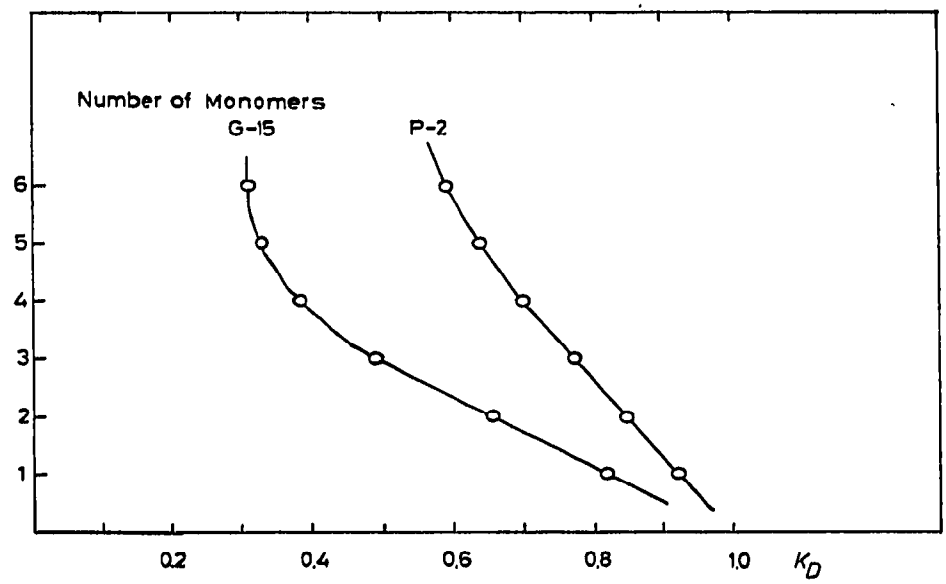

Fig. 3. Relationship between the number of chain units and K_D on polyacrylamide P2 and Sephadex G-15 with deionized water as eluant. The increasing curvature with increasing chain length indicates the influence of adsorption.

Values of K_D at various temperatures are summarized in Table II; Table III lists some pertinent structural and hydrodynamic parameters for the cellodextrins.

Fig. 3 shows the relationship between the number of chain units and the coefficient K_D. The relationship is linear for the compounds glucose–cellotetraose. Over the remaining part of the internal volume it appears that the separation may be only partly based on molecular size, there being a continuously varying relationship between chain length and K_D. It should be noted that plots of log (number of chain

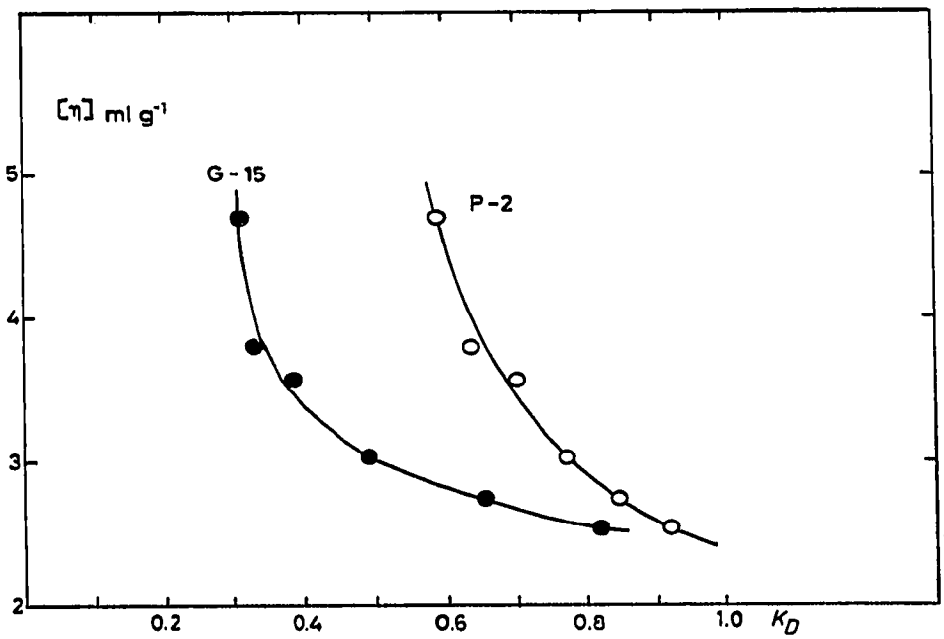

Fig. 4. Relationship between intrinsic viscosity and K_D for the cellodextrins in water.

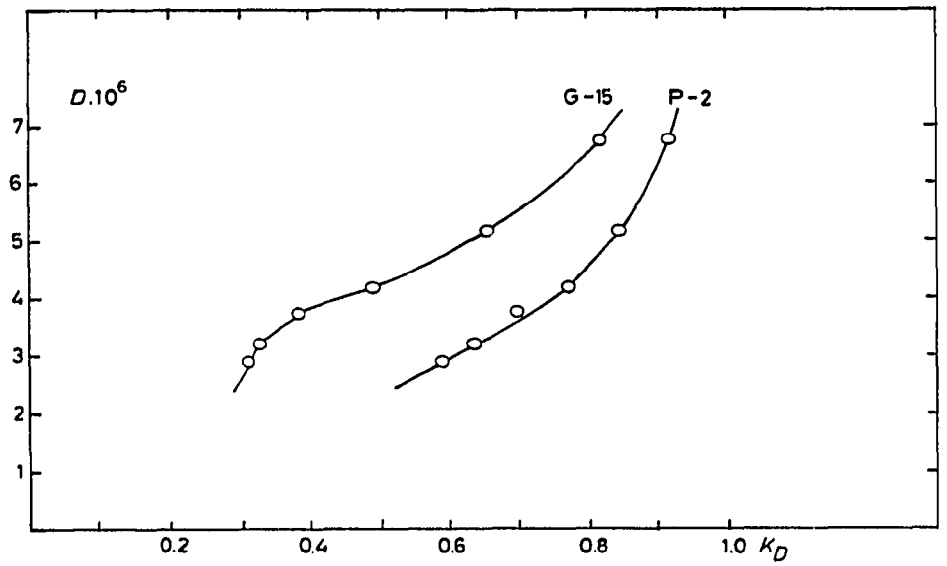

Fig. 5. Relationship between the free diffusion coefficient and K_D in water.

units) *versus* K_D have pronounced S-shaped forms. While the general shape of the curves in Fig. 3 is that expected from theory[6], the curvature is more pronounced and, as will be shown below, derives mainly from an increasing solute–gel interaction with increasing molecular weight; this phenomenon may be more important than is generally realised.

Plots of intrinsic viscosity and the free diffusion coefficient *versus* K_D are shown in Figs. 4 and 5, respectively. They illustrate that these hydrodynamic parameters are less suitable than the extended chain length for characterising the elution process. GIDDINGS *et al.*[6], using statistical mechanical methods with model systems, found that the mean external length of the molecule is more satisfactory than other molecular size paramenters for characterising partitioning in random, porous networks. However, the use of molecular weight or molar volume as a correlating parameter with K_D should be adequate for specifying partitioning within a fixed family of molecules.

Nature of the separation process

Much experimental evidence exists to show that sample migration in gel permeation is governed almost entirely by equilibrium considerations[4–6]. For example, as anticipated from theory[7], there is excellent agreement between the chromatographic partition coefficients and those derived from static equilibrium experiments; furthermore, the elution volumes normally show only a small dependence on flow rate for low molecular weight substances. Consequently one can assume that the elution volume of a given solute is a function of partition equilibrium and that diffusion is important only when considering the, usually small, peak dispersion (see below).

Assuming equilibrium, the free energy, $\Delta G°$, required to transport a molecule from the solvent surrounding the gel particles to the solvent within the voids in the gel may be written:

$$\Delta G° = -RT \ln \left(\frac{C_{\text{inside}}}{C_{\text{outside}}}\right) = -RT \ln K_D \qquad (1)$$

In the absence of enthalpy effects denoting interaction between gel and solute, we should expect:

$$\Delta G^\circ = -T\Delta S^\circ \qquad (2)$$

Here $\Delta G^\circ = R \ln \left(\frac{W_{\text{inside}}}{W_{\text{outside}}}\right)$,

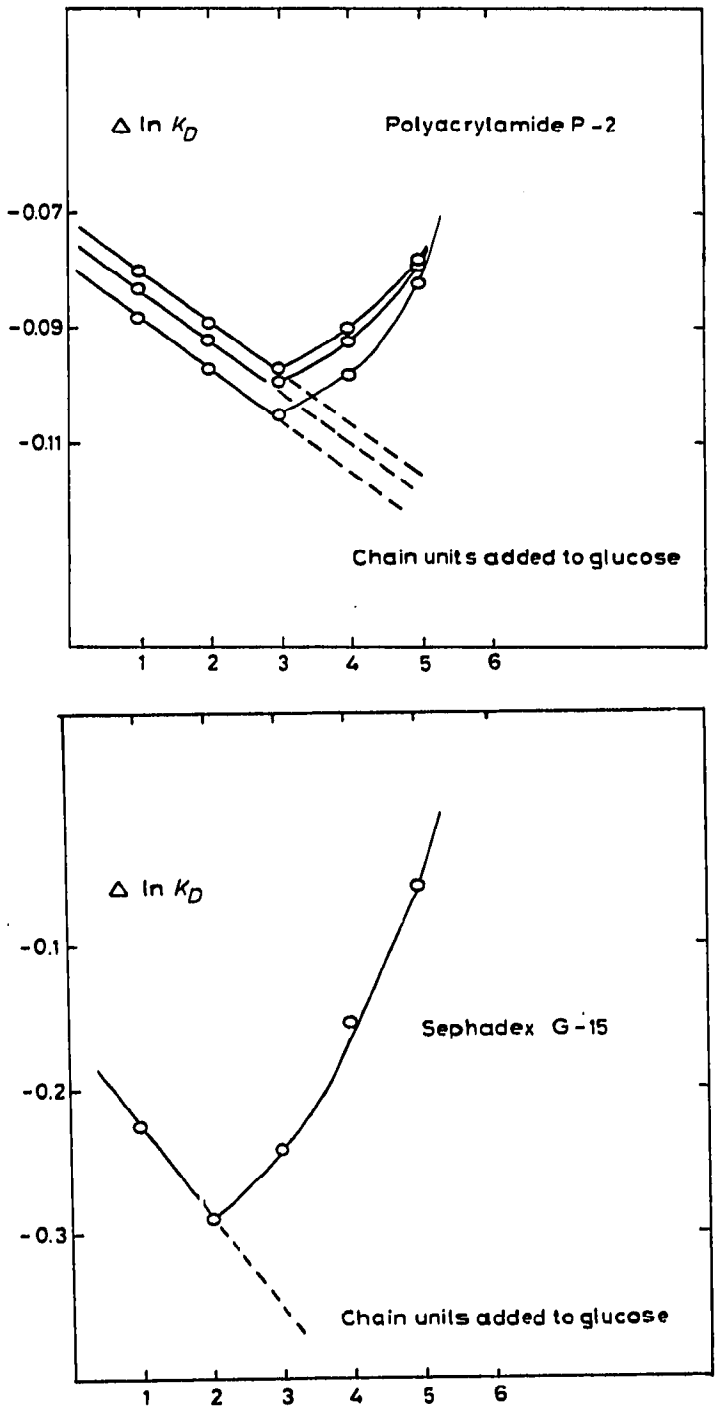

Fig. 6. $\Delta \ln K_D$ as a function of the number of chain units added to glucose in the cellodextrin series on: (a) polyacrylamide P2 at 40°, 25° and 12°; and (b) Sephadex G-15 at 25°.

where ($W_{inside}/W_{outside}$) is the ratio of possible configurations for the molecule within the pore compared with those in an equal volume of liquid bulk. This ratio is equal to K_D. A rod-shaped particle near a surface, for example, will be limited in rotational freedom and certain spatial configurations consequently forbidden; W_{inside} will thus be smaller than $W_{outside}$. Thus the partition function is proportional to the change in configurational entropy, as deduced by CASASSA[8] for randomly coiling chains.

As each anhydroglucose unit contributes a fixed amount to the molecular volume in an additive manner (see Table III), one would expect $\Delta \ln K_D$ to be constant for an homologous series such as the cellodextrins. Fig. 6 (a) shows that $\Delta \ln K_D$ is not constant, however, but at first decreases linearly with the addition of consecutive units and then increases rapidly; values of $\Delta \ln K_D$ are given in Table II. That $\Delta \ln K_D$ decreases rather than remaining constant is not entirely unexpected as the latter would only hold in the ideal case. The sharp upswing may be interpreted to mean that there are two opposing effects—on the one hand steric exclusion and on the other adsorption between gel and solute which occurs at higher molecular weight. The nature of the temperature dependence of K_D supports this view.

Temperature dependence of K_D

With interaction between gel and solute we have:

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \qquad (3)$$

ΔH° may be estimated from the dependence of $\ln K_D$ on temperature (Fig. 7);

$$\frac{d \ln K_D}{dT} = \frac{\Delta H^\circ}{RT^2}$$

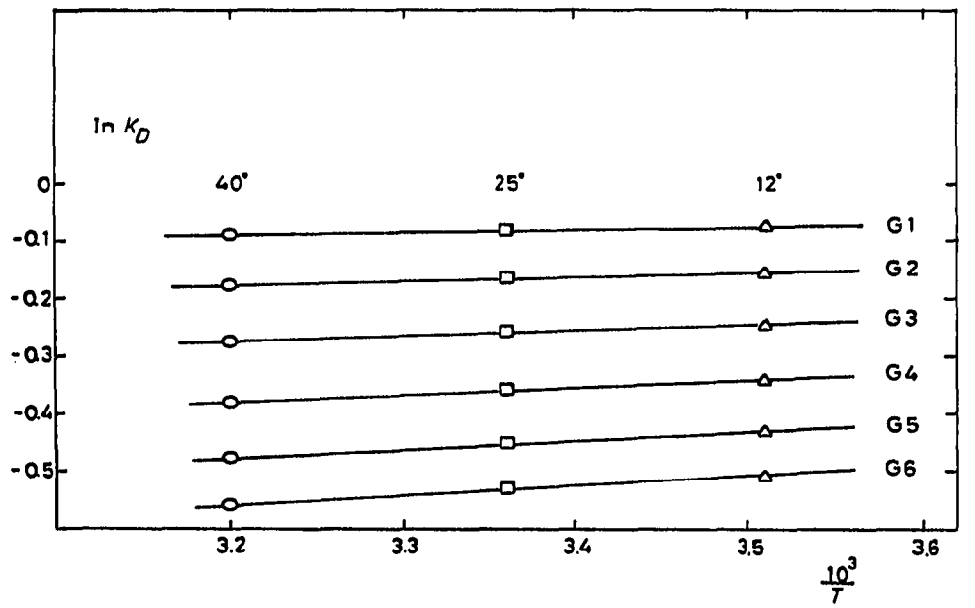

Fig. 7. The dependence of $\ln K_D$ on $1/T$ for the cellodextrin series on polyacrylamide P2 eluted with deionized water. The increasing slopes reflect negative enthalpies increasing as the series is ascended.

The entropy contribution follows from:

$$\Delta S^\circ = \left(\frac{\Delta H^\circ - \Delta G^\circ}{T}\right)$$

with ΔG° from Eqn. 1. Interpreting the changes in K_D with temperature in this way, the negative enthalpies (listed in Table IV) reflect an interaction with the gel which increases as the series is ascended. As the cellodextrins are rodlike, the frequency of interactive contacts will be proportional to the chain length; with increasing temperature the increased mobility results in elution at a smaller volume. One cannot exclude possible changes in the gel matrix with change in temperature. However, there was an insignificant change in V_I between 12° and 40°.

TABLE IV

ENTHALPY, FREE ENERGY AND ENTROPY PARAMETERS FOR CELLODEXTRINS ON POLYACRYLAMIDE P-2
ΔH° is calculated between 40° and 12°. The values of ΔG° and ΔS° are for 25°.

Cellodextrin	K_D (25°)	ΔH° ($cal \cdot mole^{-1}$)	ΔG° ($cal \cdot mole^{-1}$)	ΔS° ($cal \cdot mole^{-1}\ deg^{-1}$)
Glucose	0.92	−75	+50	−0.4
Cellobiose	0.85	−130	+100	−0.8
Cellotriose	0.77	−180	+155	−1.1
Cellotetraose	0.70	−230	+210	−1.5
Cellopentaose	0.64	−280	+265	−1.8
Cellohexaose	0.59	−305	+305	−2.0

The trend in K_D with increasing temperature is the opposite to that expected in comparison with the decreasing hydrodynamic volume as the temperature is increased. It has been established[9] that the cellodextrins behave hydrodynamically as rigid rods up to a temperature of at least 70°. There is, however, a progressive decrease in the degree of solvation which is manifested in the negative temperature coefficients of intrinsic viscosity. One would have anticipated an increase in K_D with increasing temperature and decreasing hydrodynamic volume if this change were responsible for the deviation from linearity in Fig. 3.

The hydrophilic character of polyacrylamide and cellodextrins leads one to expect such interactions with an increase in chain length. One can anticipate that in all polar gel–solute systems adsorption effects will play an important role when the solute has a conformation such that it can appropriately align with the linear portions of the cross linked-gel network.

The role of diffusion

The extent to which diffusion may influence the elution volume was examined. A known volume (50 ml) and concentration (0.3 $g \cdot dl^{-1}$) of a cellodextrin solution was added to a pre-swollen portion of gel (10 g in 50 ml water) and thoroughly mixed.

The decrease in cellodextrin concentration in the supernatant liquid was measured as a function of time employing the orcinol method[3] to measure the carbohydrate concentration. Equilibrium was rapid, final static concentrations being attained in less than 15 min for glucose, cellotetraose and cellohexaose. At the flow rate of the column (2 ml/h) this would correspond to steady state conditions

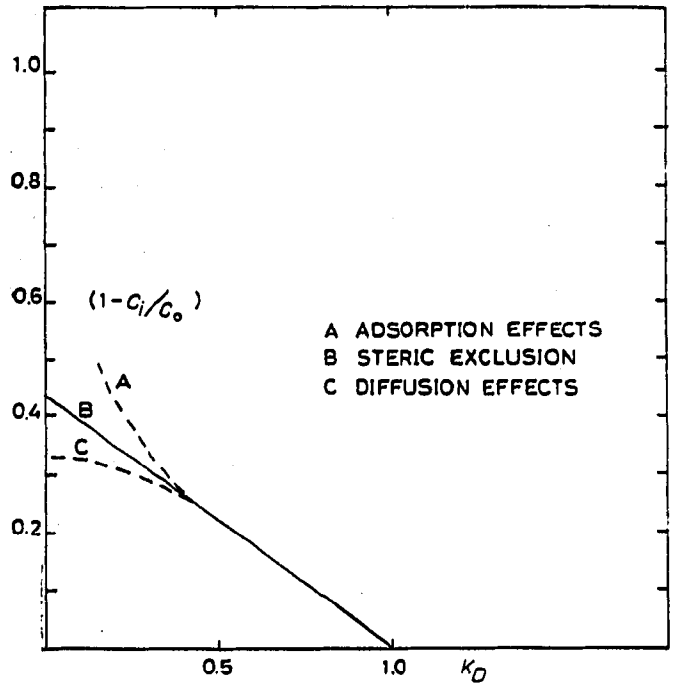

Fig. 8. Schematic diagram of the dependence of $(1-C_i/C_0)$ on K_D for static equilibrium experiments; C_i and C_0 are the initial and final concentrations of solution. Curve C indicates the behaviour when steady-state conditions are not attained when measuring K_D.

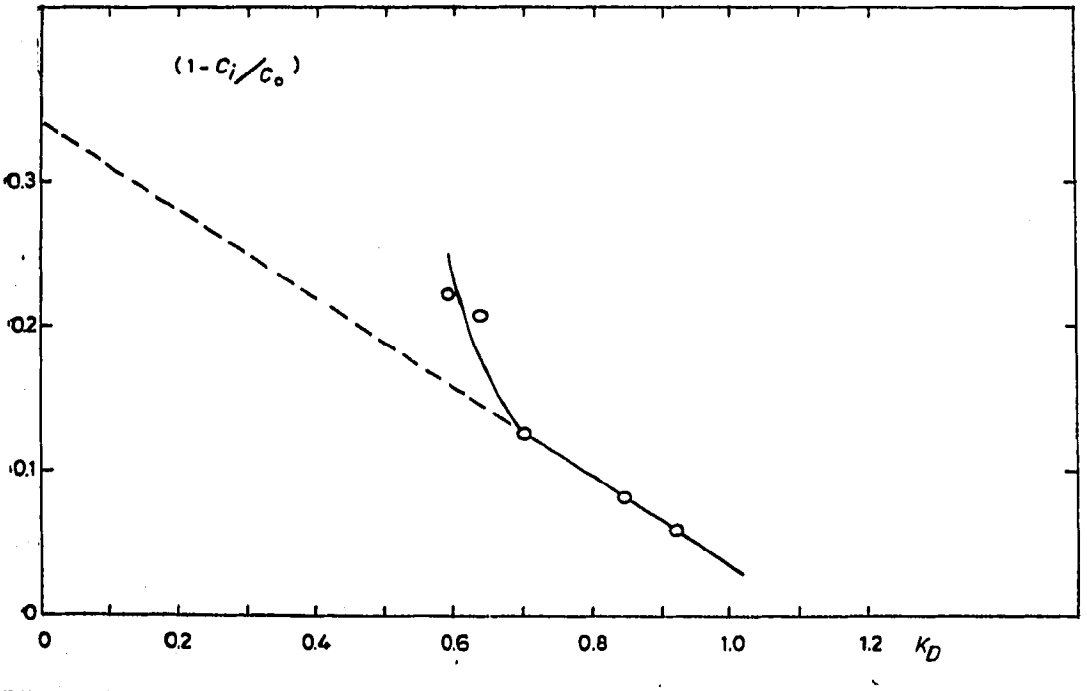

Fig. 9. Dependence of $(1-C_i/C_0)$ on K_D for the cellodextrin series in the system polyacrylamide P2/dionized water; *cf.* Fig. 8. The point for cellotriose is omitted.

subsequent to peak migration of less than 1.5% of the total bed volume. The coefficient K_D will thus approximate the equilibrium partition coefficient on elution.

Static equilibrium experiments

When a solution, volume V_i, concentration C_i, is mixed with a known weight of dry gel, there will be an increase in concentration of the supernatant solution at equilibrium which is proportional to the extent of steric exclusion of the solute from the swollen gel. Denoting the final concentration, C_0, we have[4]:

$$\left(1 - \frac{C_i}{C_0}\right) = \frac{V_g}{V_i}(1 - K_D)$$

where V_g is the internal volume of the gel; $K_D = C_g/C_0$ with C_g being the concentration inside the porous substrate. $(1-C_i/C_0)$ will be a linear function of K_D if diffusion effects are unimportant and the solute does not interact with the gel; these effects are depicted in Fig. 8.

TABLE V

K_D FOR CELLODEXTRINS–SEPHADEX G-15

Cellodextrin	K_D	$\Delta \ln K_D$
Glucose	0.82_0	
		0.225
Cellobiose	0.65_5	
		0.290
Cellotriose	0.49_0	
		0.241
Cellotetraose	0.38_5	
		0.154
Cellopentaose	0.33_0	
		0.059
Cellohexaose	0.31_1	

The initial and final concentrations were measured using a differential refractometer. 50 ml of a 0.3% solution of the cellodextrin were added to 10 g of the dry gel and allowed to equilibrate overnight. The results of these experiments are shown in Fig. 9 and again infer the influence of adsorption on K_D. The point for cellotriose is omitted as there was insufficient material for this measurement.

Dextran gel

The elution characteristics of the cellodextrins on Sephadex G-15 are similar to those for the polyacrylamide gel, except for the adsorption being much more pronounced. Although the bed volumes (Table I) of the gels were similar, the internal volume was substantially smaller (V_I = 17 ml; 37% of bed volume). The separation of the series, glucose–cellohexaose, occurred over approximately 9 ml, more than half of the internal volume, but separation as a linear function of chain length was restricted to the interval glucose–cellotriose (Fig. 3). Values of K_D are listed in Table V, and the behaviour of $\Delta \ln K_D$ as a function of increasing chain length is illustrated in Fig. 6(b).

ACKNOWLEDGEMENTS

This work forms part of a research programme financially supported by Billeruds AB, Mo och Domsjö AB, Stora Kopparbergs Bergslags AB, Svenska Cellulosa AB and Uddeholms AB. This support is gratefully acknowledged.

The author wishes to thank Professor STIG CLAESSON for stimulating discussions and the facilities placed at his disposal.

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