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GEL FILTRATION OF OLIGOSACCHARIDES ON CROSS-LINKED STARCH AND CELLULOSE

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SUMMARY

Cross-linking of microcrystalline cellulose and starch with epichlorohydrin under alkaline conditions afforded gels suitable for gel permeation chromatography of oligosaccharides. Successful separation of a mixture of mono-, di-, and trisaccharides using water as a mobile phase showed that starch modified in the described manner is an excellent gel permeation material. Although the chromatographic properties of the cellulose gel under investigation were comparable with those of other cellulose gels used for gel permeation chromatography, a complete separation of a monosaccharide from a trisaccharide could not be obtained.

INTRODUCTION

Ever since PORATH AND FLODIN[1,2] carried out, for the first time, gel filtration on polysaccharides using dextran cross-linked with epichlorohydrin, this technique has been widely used as an important means of fractionation of various substances.

Several types of cross-linked dextran, with different degrees of swelling, both in water and organic solvents are on the market under the trade name Sephadex.

DETERMAN *et al.*[3] tried to use cellulose gel for gel permeation chromatography (GPC). He precipitated cellulose from a Schweizer's solution with benzoic and acetic acid and obtained a solid, chemically stable gel in the form of spherical particles, diameter 0.03–0.3 mm. However, this gel with a pore diameter, in water, of some hundreds of nanometers, is only suitable for GPC of substances with a molecular weight in the range 10^4–10^7.

MARTIN AND ROWLAND[4] observed that the cross-linking of cellulose with formaldehyde decreases the permeability of large particles and, at the same time, improves the separation of low molecular weight substances. They cross-linked decrystallized cellulose with formaldehyde and obtained a gel suitable for GPC of oligosaccharides of molecular weight up to 10^3.

Oligosaccharides in the molecular weight range of 10^2–10^3 were separated on starch gel for the first time by LATHE AND RUTHVEN[5] who used a column packing of maize starch.

The most advantageous way in which cellulose and starch may be modified in order to acquire gel permeation properties is obviously cross-linking. Epichlorohydrin proves to be a suitable cross-linking agent.

The purpose of the present work is to compare the gel permeation properties of cross-linked microcrystalline cellulose, cross-linked starch and Sephadex G 15 with one another.

EXPERIMENTAL

Materials

Two supports used in this work, C_E (cellulose gel) and S_E (starch gel) have been prepared by cross-linking microcrystalline cellulose and potato starch, respectively.

C_E: the cellulose gel. The cellulose gel was made from microcrystalline cellulose according to ref. 6. Microcrystalline cellulose was made alkaline in an inert solvent and treated, under stirring, with epichlorohydrin. The molar ratio of the components was cellulose–sodium hydroxide–epichlorohydrin, 1:1:0.5. The cellulose modified in this manner contained approximately 0.4–0.45 hydroxypropyl bridges per one D-glucose unit. The most suitable fraction (0.06–0.1 mesh) was obtained by sieving. The volume of gel swollen in water was 3.4 ml/g (ref. 7). The flow rate through a layer of 10 cm, at a pressure of 500 mm H_2O, was 150 ml/h cm².

S_E: the starch gel. This gel was prepared by cross-linking potato starch under alkaline conditions according to ref. 8. The volume of gel swollen in water was 2.5 ml/g and the flow rate through a 10 cm layer was 200 ml/h cm², at 500 mm H_2O pressure.
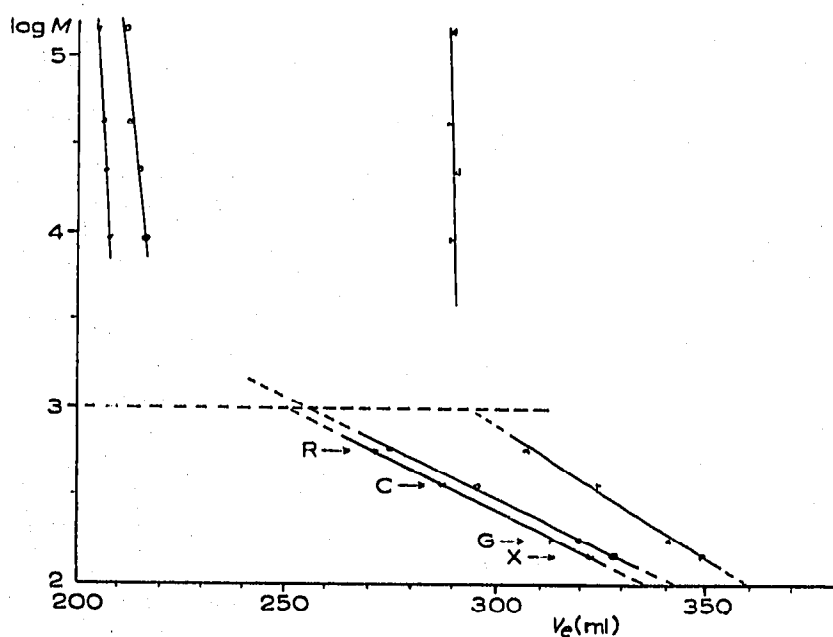

Fig. 1.Dependence of the molecular weight of oligosaccharides and dextrans upon elution volumes of various gels. Column 6 cm² × 78 cm. X = D-xylose; G = D-glucose; R = raffinose; C = cellobiose; + = Sephadex G-15; ○ = S_E; △ = C_E.
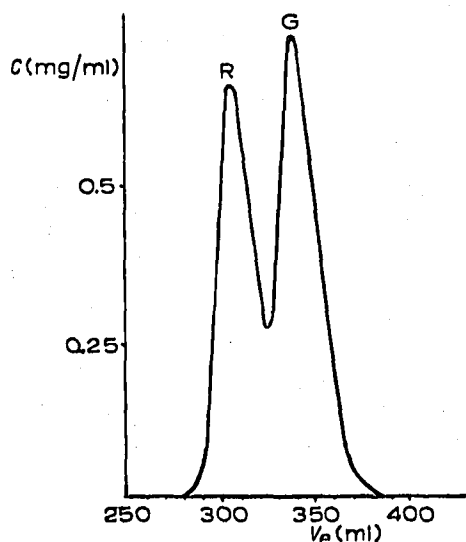
Fig. 2. Elution curve of a mixture of raffinose and D-glucose on C_E. Column 6 cm² × 78 cm. R = raffinose; G = D-glucose.

Sephadex G-15. A commercially available product of bed volume 3 ml/g, 0.04–0.12 mesh was used.

GPC equipment and the separated mixtures

The gels were tested in a simple apparatus[9] which comprised a glass column closed with a teflon piston. The concentration of the effluent was determined using a differential refractometer (Waters, Model R-4). The solvent was supplied by means of a Waters' pumping system. Column dimensions were 6 cm² × 78 cm and 6 cm² × 156 cm, respectively.

The columns were packed by allowing the swollen gel to settle at an absolute
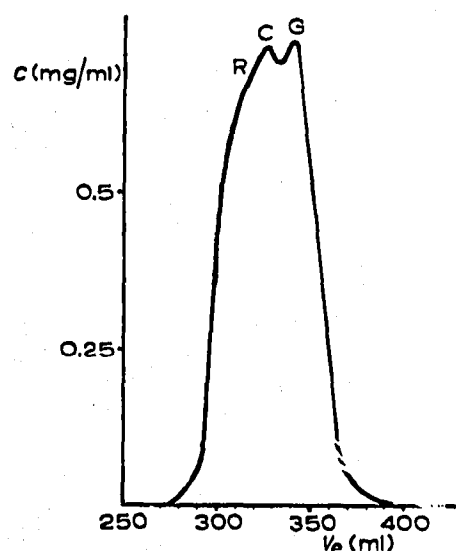

Fig. 3. Elution curve of a mixture of raffinose, cellobiose and D-glucose on C_E. Column 6 cm² × 78 cm. R = raffinose; C = cellobiose, G = D-glucose.

effluent flow rate two times larger than that at elution. Several series of experiments were carried out using D-glucose, D-xylose, cellobiose, raffinose and four fractions of dextran of mol. wt. 9300–167 000 (determined viscosimetrically). When the shorter column (length 78 cm and the total bed volume 478 cm³) was used the sample was injected at a concentration of each of the components of 4 mg/ml either separately or in various combinations (Figs. 2–5). In experiments on the longer column (total bed volume 935 cm³) the sample concentration was doubled. The volume of the injected sample was 2 ml in every case.

RESULTS AND DISCUSSION

In all experiments symmetrical elution curves were obtained. The gels were tested by plotting log M against elution volume V_e which was linear over a certain range (Fig. 1). As elution volume the one was taken at which the elution curve reached its maximum. The elution volumes remained constant regardless of the
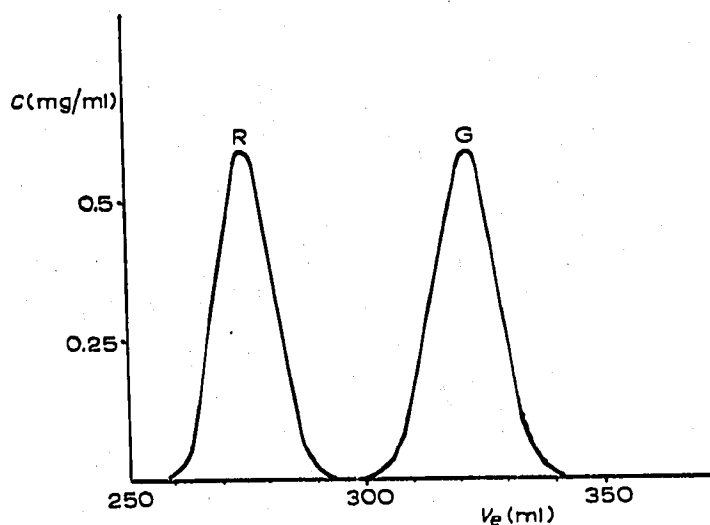

Fig. 4. Elution curve of a mixture of raffinose and D-glucose on S_E. Column 6 cm² × 78 cm. R = raffinose; G = D-glucose.

combination of components in the sample injected, e.g., individual components, a mixture of two (raffinose and D-glucose, Figs. 2 and 4) or three components (raffinose, cellobiose and D-glucose, Figs. 3 and 5). These experiments were carried out on the 78 cm column.

It follows from the above that modified starch has the best separation properties over the range of molecular weight under investigation and obviously, also the most advantageous distribution of pores. As standard oligosaccharides of molecular weight in the range $7 \cdot 10^2 - 9 \cdot 10^3$ were not available, no conclusion as to the range over which the dependance log M vs. V_e is linear could be made. We presume, however, that extrapolation up to log $M \cong 3.2$ may be done; i.e., a satisfactory separation of oligosaccharides up to molecular weight $M \cong 1200$ should well be possible.

The elution volume of high molecular weight dextrans, which can be considered virtually excluded from the gel pores, is given by the interstitial volume V_0, and thus it is useless for separation purposes. Its value depends both on the conditions of
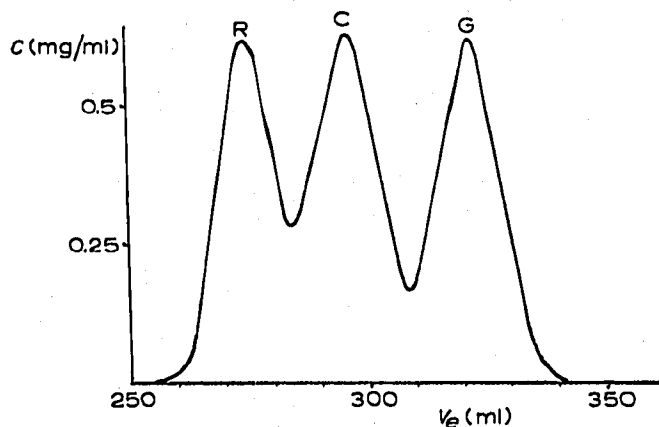
Fig. 5. Elution curve of a mixture of raffinose, cellobiose and D-glucose on S_E. Column 6 cm² × 78 cm. R = raffinose; C = cellobiose; G = D-glucose.

column packing and on the size and shape of the gel particles. As in the course of our work the experimental conditions remained unchanged, the shape of the particles turned out to be the determining factor. The comparative data are summarized in Table I where V_0 is expressed in per cent of the total bed volume V_t. It is obvious that the spherical particles of Sephadex G-15 give the lowest and rod-shaped cellulose the highest V_0.

Let us now compare the slopes of the linear parts of the calibration dependence log M vs. V_e for three different gels (Fig. 1). Lowest separation efficiency is shown by the cellulose gel while best results can be obtained on starch gel (cf. also Figs. 2–5).

The shape of the calibration dependence log M vs. V_e for starch, in the region of 4 < log M < 5, shows that here the large pore volume is not quite negligible.

TABLE I

INTERSTITIAL VOLUME OF GELS WITH DIFFERENTLY SHAPED PARTICLES

Gel	Shape	V_0 (ml)	V_0 (%)
Sephadex G-15	Spherical	204	43.6
Starch cross-linked with epichlorohydrin (C$_E$)	Irregularly oval, "potato-shaped"	212	45.3
Cellulose cross-linked with epichlorohydrin (C$_E$)	Rod-shaped	290	62.0

However, the selectivity of the gel over this range of molecular weights is very low. On the other hand, over the lower range of molecular weights, using an adequately long column, complete separation of the components under investigation was obtained (Fig. 6).

In Table II, some of our results are compared with those of MARTIN AND ROWLAND[4] obtained on non-modified decrystallized cellulose and on cellulose cross-linked with formaldehyde containing up to 5.2% of formaldehyde. Here we give the differences expressed in terms of reduced elution volume $V_{red} = V_e/V_t$ of two
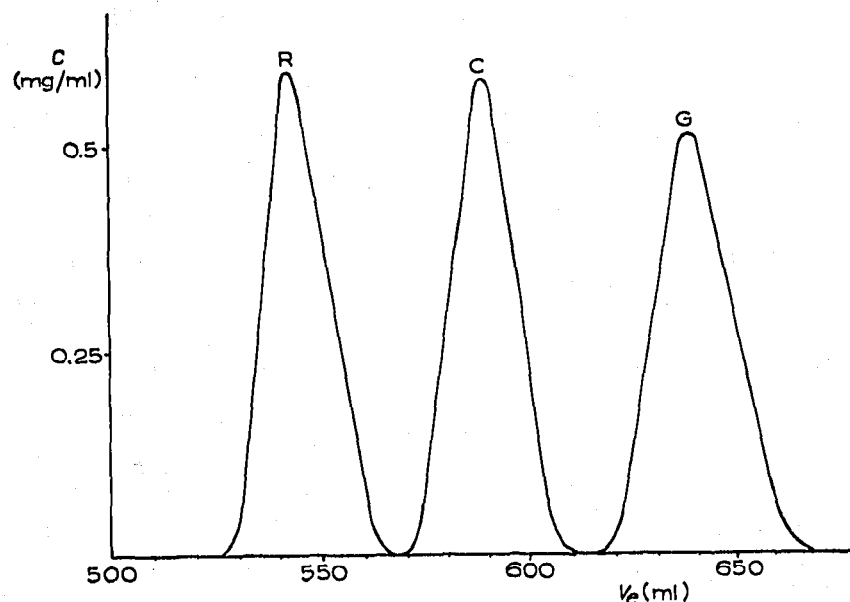
Fig. 6. Elution curve of a mixture of raffinose, cellobiose and D-glucose on S_E. Column 6 cm² × 156 cm. R = raffinose; C = cellobiose; G = D-glucose.

fictitious substances of mol. wt. 100 and 1000 obtained by extrapolation. Reduced elution volume, since it is independent of column size (as long as this is not extremely small) can be a useful criterion of comparison of the separation efficiency of individual gels.

Concluding, starch cross-linked with epichlorohydrin is well suited for sepa-

TABLE II

THE DIFFERENCES IN REDUCED ELUTION VOLUMES (V_{red})[a] OF SUBSTANCES WITH MOL. WT. = 100 AND 1000 AS FOUND FOR VARIOUS GELS.

Gel	$(V_{red})_{1000}^{100}$
Sephadex G-15	0.182
Starch cross-linked with epichlorohydrin (S_E)	0.188
Cellulose cross-linked with epichlorohydrin (C_E)	0.141
Cellulose cross-linked with formaldehyde[b]	0.138
Non-modified powdered cellulose[b]	0.076

[a] $V_{red} = V_e/V_t$, where V_e is elution volume and V_t the total volume.
[b] Calculation according to results of MARTIN AND ROWLAND[4].

ration of oligosaccharides, on the other hand the separation properties of a gel based on modified cellulose is not very good. This may, however, be associated with the larger interstitial volume V_0 caused by rod-shaped gel particles (Table I). The properties of C_E and those of cellulose cross-linked with formaldehyde[4], as shown in Table II, are approximately the same.

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