GEL CHROMATOGRAPHIC SEPARATION OF OLIGOSACCHARIDES AT ELEVATED TEMPERATURE

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1. Introduction

The separation of carbohydrates on Sephadex-gels has been described in several papers [1–4]. Preliminary studies on the chromatographic behavior of polyacrylamide gels indicate the usefulness of this support in the separation of sugars [5–7].

In our work polyacrylamide gel was found to be superior to gels based on dextran because it does not give off carbohydrates [8] and is not attacked by bacteria.

A survey of the literature showed that the effect of elevated temperatures on the resolution of carbohydrates by gel chromatography has not been described. Hough, Jones and Wadman reported that the separation of sugars on paper chromatograms is markedly improved at elevated temperatures [9]. Thoma, Wright and French separated maltose homologs on a heated cellulose column and found that high temperatures increase both the resolution and mobility of saccharides [10].

In this paper we discuss the effect of elevated temperatures on the chromatographic separation of oligosaccharides on polyacrylamide gel.

2. Materials and methods

All chemicals, reagent grade, used in this work were obtained from Merck A.G., Darmstadt, Germany, and were used without further purification. A glass-jacketed 2.5 × 100 cm column from Pharmacia, Uppsala, Sweden, was used. Bio-Gel P-2 (200–400 mesh) was obtained from Bio-Rad Laboratories, München, Germany. A Milton-Roy Minipump was used to pump the eluent (degased water) through the column at a fixed rate of 58 ml/h. The samples were injected with a 100 µl Hamilton syringe through a Swagelok-Union Tee covered with a rubber septum. In a typical run 20 µl of a solution containing 1.12 mg of a sugar-mixture (mono- to tetrasaccharides) were applied to the column.

For the determination of carbohydrates we used an automated system based on the orcinol colorimetric method described previously by Kesler [11]. The absorbance at 420 nm was measured in a Zeiss colorimeter (Elko III) equipped with a 1 cm flow cell and a Sargent recorder Model SRL.

E. coli ML 30 was kindly supplied by Dr. H.Schwinn.

3. Results

The resolving power for the separation of oligosaccharides by high-temperature gel chromatography

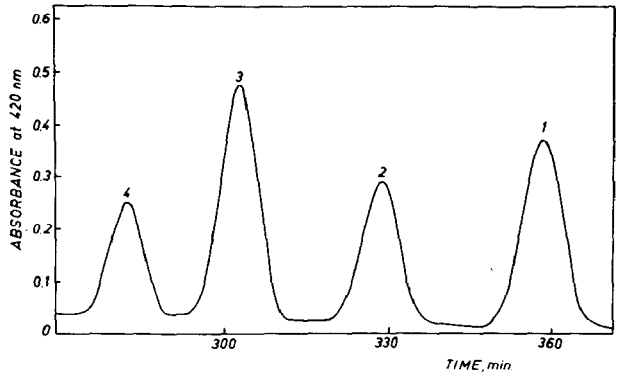

Fig. 1. Chromatography of glucose (1), lactose (2), raffinose (3) and stachyose (4) on Bio-Gel P-2, at 65°C. Column 2.5 × 100 cm. Flow rate, 58 ml per hour. Eluent, water. 20 µl of a solution containing glucose, 320 µg; lactose, 240 µg; raffinose, 360 µg; stachyose, 200 µg, was applied to the column.

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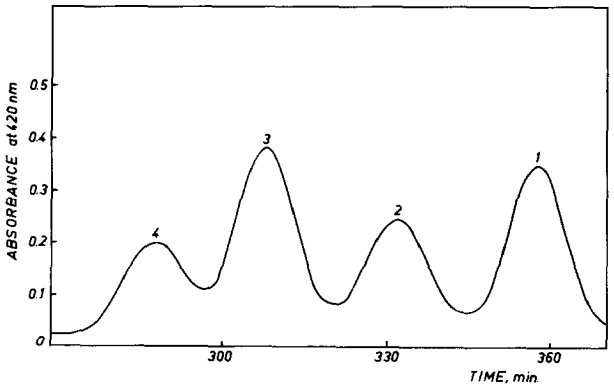

Fig. 2. Chromatography of glucose (1), lactose (2), raffinose (3) and stachyose (4) on Bio-Gel P-2, at 10°C. Column, flow rate and load were the same as in fig. 1.

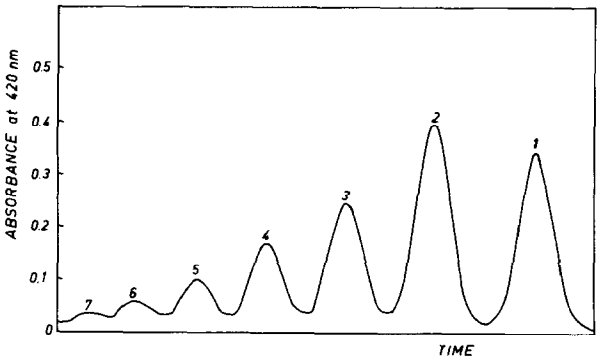

Fig. 3. Chromatogram of maltooligosaccharides produced by the action of amylomaltase on maltose. Flow rate, 70 ml per hour. Bio-Gel P-2, at 65°C. Column 2.5 X 100 cm. Eluent, water. Peaks 1–7 = glucose, maltose, maltotriose, maltotetraose, maltopentaose, maltohexaose and maltoheptaose.

is illustrated in the chromatogram of fig. 1. Glucose (1), lactose (2), raffinose (3) and stachyose (4) are clearly separated according to their molecular weights. The column temperature was 65°C.

Fig. 2 shows a run at 10°C of the same sugar mixture.

Column performance can be expressed by theoretical plate number or high equivalent to a theoretical plate (HETP) as adopted directly from gas-chromatographic practice. The number of theoretical plates is defined by the following equation [12]:

$$N = (4V_e/w)^2 ,$$

where V_e is the elution volume and w the peak width measured from the base line. The equation for the HETP can be expressed in the form:

$$\text{HETP} = \frac{H}{N} \text{ mm} ,$$

where H is the length of the gel bed.

Decreasing HETP values result in a better separation of the gel bed. The HETP for raffinose at 65 and 10°C was 0.1 and 0.3 mm respectively.

Fig. 3 shows a chromatogram of maltooligosaccharides synthesized by the action of amylomaltase (E.C.2.4.1.3) from maltose-induced cells of *Escherichia coli* ML 30 according to Barker and Bourne [13]. The results indicate that linear saccharides in a range of glucose up to maltoheptaose were completely separated.

Thus, gel chromatography of carbohydrates on polyacrylamide provides a useful extension of older methods for separating such mixtures. The procedure is rapid and economical since Bio-Gel in contrast to ion-exchange methods [11] can be used without prior equilibration. No deterioration of the column properties was observed during a period of several months.

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