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CHROMATOGRAPHY OF MIXTURES OF OLIGO- AND MONOSACCHARIDES ON DEAE-SPHERON

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SUMMARY

Chromatography of borate complexes of oligosaccharides was carried out on a diethylaminoethyl derivative of Spheron™ (glycol methacrylate macroporous gel) of nominal capacity 2.25 mequiv./g. The effect of borate concentration, temperature and pH on retention of six oligosaccharides, and the effect of the flow-rate on the resolution of two oligosaccharides, was investigated. The suitability of the optimal conditions was demonstrated by the separation of a mixture of twelve mono- and oligosaccharides. The retention volumes of 26 different saccharides are tabulated. The possibilities of a practical utilization of this method are illustrated by the separation of saccharides from samples of sugar-beet and sugar-cane molasses.

INTRODUCTION

Ion exchangers are widely used in column chromatography of saccharides[1], including high-performance liquid chromatography (HPLC)[2]. They are used for purification of natural samples[3], as packings for chromatographic columns and recently in detection systems[4].

For the separation of complex mixtures of natural sugars, the procedures based on the separation of borate complexes of saccharides were found to give the best results in ion-exchange chromatography. Chytilová *et al.*[5] showed that strongly basic anion exchangers based on a polystyrene microporous matrix can be replaced by medium basic ion exchangers carrying diethylaminoethyl groups on the macroporous glycol methacrylate matrix, known under the name Spheron™, in the separation of

borate complexes of saccharides. In contrast to classical anion exchangers with 8% cross-linkages, these macroporous semi-rigid column packings are characterized by low compressibility and higher efficiency for substances with low retention volumes. In this paper the possibilities of the use of DEAE-Spheron in the separation of more complex mixtures of saccharides are demonstrated.

MATERIAL AND METHODS

DEAE-Spheron anion exchangers of medium basicity have been described in previous papers[5–7]. The DEAE-Spheron (lot 6/C/16) used in this paper was prepared in the laboratory[6,7]. Its capacity was 2.25 mequiv./g and its mean particle diameter 27.4 μm. The variation coefficient of the particle diameter was 14.4%. Chemicals, apparatus and the preparation of the column were as described[5].

During the development of the method, the following were investigated: effect of concentration of borate within the range 0.025–0.100 *M* in the elution buffer of pH 8.52, effect of elution temperature in the 30–60° interval with 0.1 *M* borate buffer of pH 8.52; and effect of pH of a 0.1 *M* borate buffer in the 7.5–9.0 range at 50° on the retention of oligosaccharides (saccharose, raffinose, cellobiose, maltose, lactose and stachyose) on the column. The effect of these variables and of the flow-rate of the eluent in the 30–110 ml/h interval on the column efficiency for saccharose and raffinose and on the resolution of these two sugars was also determined. The evaluation of the column efficiency and the resolution and of their dependence on experimental conditions by the analysis of scattering was carried out on a HP 9820 computer, using the program elaborated earlier[8].

RESULTS AND DISCUSSION

The observed effects of the elution conditions on the chromatographic behaviour of oligosaccharides can be summarized as follows.

The oligosaccharides tested are eluted with 0.1 *M* borate buffer of pH 8.5 at 50° in the order saccharose, raffinose, cellobiose, maltose, stachyose and lactose. The last two saccharides could not be separated by any of the tested combinations of conditions. However, in nature these two sugars do not occur simultaneously. When the eluent was diluted with water, stachyose lagged slightly behind lactose, while the order of other components remained unchanged. The resolution of the pair saccharose-raffinose distinctly improved (probability level $P = 0.99$) when the buffer was diluted owing to the combined effects of several factors: increase in the difference of retention volumes, increase in the column efficiency (Fig. 1) and decrease of the participation of spreading in the detector.

Changes of pH in the studied range did not lead to changes in the elution order. An increase of pH favoured the resolution of raffinose and cellobiose, while the separations of maltose from stachyose and raffinose from saccharose deteriorated. The effect of pH on column efficiency for saccharose and raffinose could not be proved, and the observed decrease in the resolution of these two sugars on the column was caused by a decrease in the difference of retention volumes.

In contrast, an increase in column temperature improves the resolution of

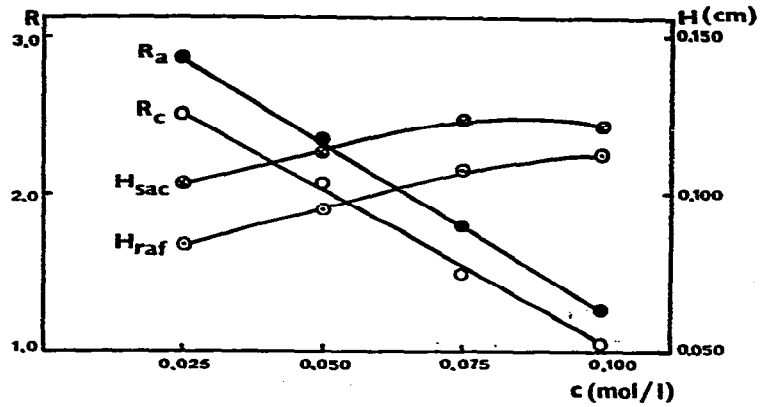

Fig. 1. Dependence of the resolution of the saccharose–raffinose pair and of column efficiency on the concentration of the borate elution buffer. Chromatographic conditions: pH of the eluent, 8.5; flow-rate, 50 ml/h; temperature, 50°; column dimensions, 48.8 × 0.6 cm. Symbols: R_a = resolution of the saccharose–raffinose pair recorded on the chromatogram; R_c = resolution of this pair of peaks, corrected for the spreading in the reactor; H_{sac} = plate height for saccharose; H_{raf} = plate height for raffinose.

these two saccharides by an increase in column efficiency (demonstrated at $P = 0.99$), without any effect on selectivity. The plate heights found for saccharose and raffinose at 60° were only 60% (for saccharose) or 55% (for raffinose) of the values measured at 30°.

The effect of the flow-rate on column efficiency was also highly significant. Linear regression analysis of the experimental values is shown in Fig. 2. DEAE-Spheron retained its low compressibility when the flow through the column was increased. At a flow-rate of 30 ml/h the resistance of a 52.7 × 0.6 cm column was

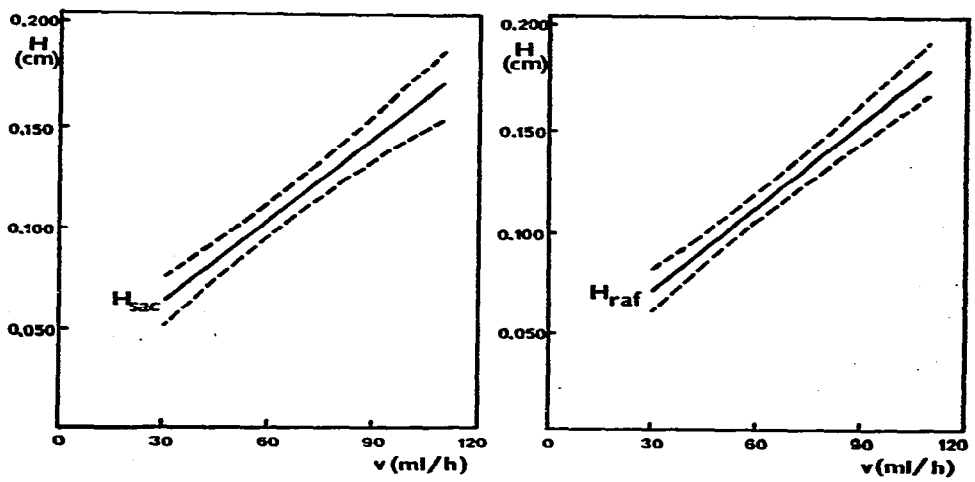

Fig. 2. Comparison of the dependence of column efficiency on flow-rate for saccharose (on the left) and raffinose (on the right) after linear regression analysis of experimental results. Chromatographic conditions: column temperature, 50°; eluent, 0.03 M borate of pH 7.50. Symbols as in Fig. 1. The dashed line indicates the limits of reliability for a 95% significance level.

1.1–2.5 atm; at 110 ml/h, the ion-exchange column was compressed by 0.8% and the resistance increased to 4.7–8.4 atm.

From the experiments it follows that the best resolution of the poorly resolvable pair saccharose–raffinose may be achieved at low concentration and pH of the eluent, low flow-rate and high column temperature. Under these conditions, optimum for the separation of weakly retained saccharides, other saccharides cannot be determined by isocratic elution, owing to excessively long retention times. Therefore various variants of stepwise elution with buffers of increasing pH and borate concentration were tested. The separation of mixtures of saccharides achieved under the conditions representing an optimum compromise between the requirements of high resolution and low retention volumes is shown in Fig. 3. Oligosaccharides are eluted with 0.03 *M* borate buffer of pH 7.5 (buffer A). The effect of the next eluent (0.100 *M* borate of pH 8.85, buffer B) which was introduced into the column after the elution of saccharose was manifested by the elution of a sharp peak of rhamnose. After elution of maltose, the third buffer (C) was applied to elute strongly retained borate complexes of monosaccharides. Buffer (C) »ad an almost identical pH to buffer B, but an increased borate concentration (0.250 *M*).

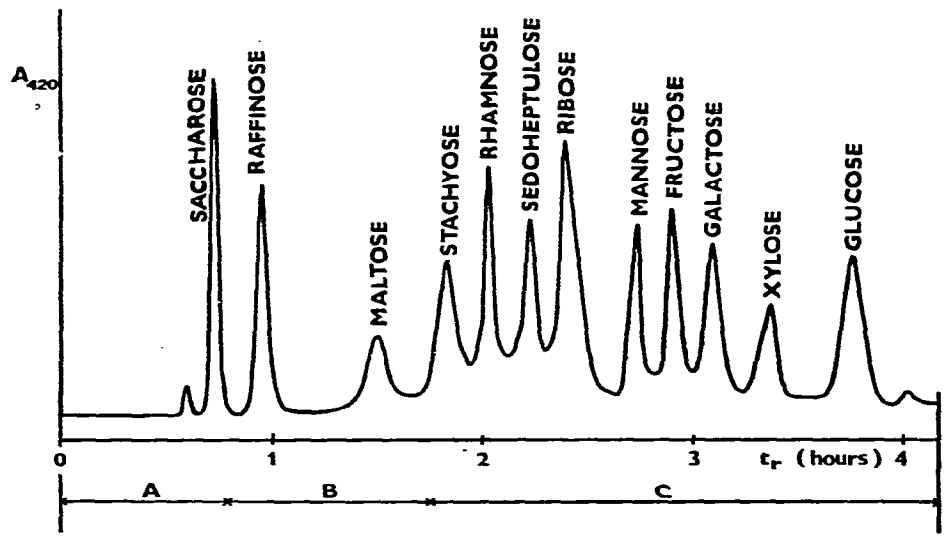

Fig. 3. Separation of a standard mixture of saccharides. Chromatographic conditions: flow-rate, 50 ml/h; elution temperature, 60°: column dimensions, 48.7 × 0.6 cm. Stepwise elution with the buffers A (0.03 *M* borate of pH 7.50), B (0.100 *M* borate of pH 8.85) and C (0.250 *M* borate of pH 8.88). Weights of saccharides: saccharose, raffinose, stachyose, sedoheptulose, glucose and fructose, 40.0 μg each; mannose and galactose, 36.0 μg each; ribose, 16.0 μg; xylose, 24.0 μg; rhamnose, 65.6 μg; and maltose, 80.0 μg.

The separation was carried out on a column (60 × 0.6 cm) packed to a height of 48.7 cm with DEAE-Spheron. The flow-rate at 60° was 50 ml/h. The procedure described can be used to separate natural mixtures of oligo- and monosaccharides. Examples of its application in the sugar industry are shown in Figs. 4 and 5. The good separation of raffinose from an excess of saccharose on the chromatogram of sugar-beet molasses and the resolution of fructose from psicose

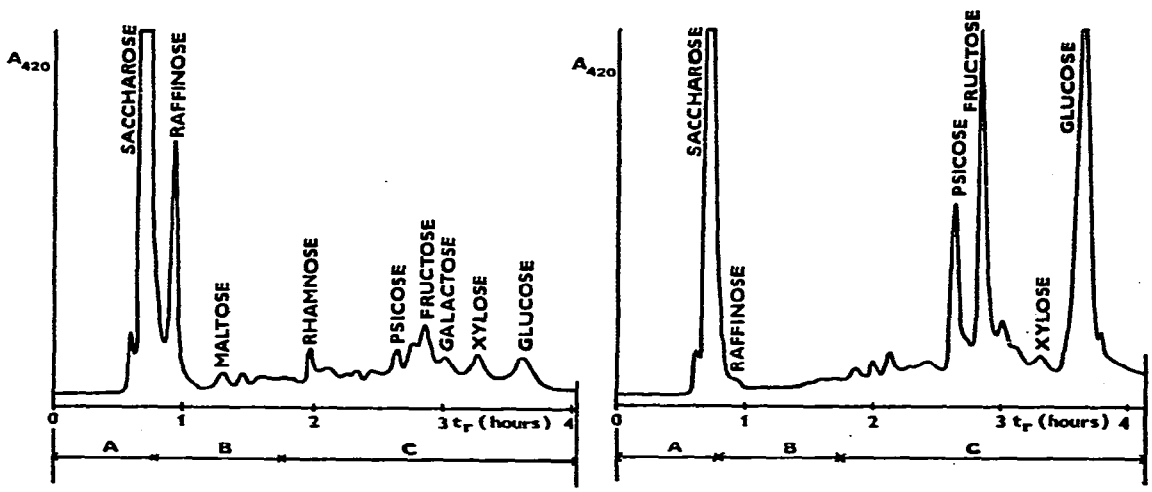

Fig. 4. Separation of saccharides in a sample of sugar-beet molasses. Chromatographic conditions as in Fig. 3. Weight of the molasses sample, 0.60 mg.

Fig. 5. Separation of saccharides in a sample of sugar-cane molasses. Chromatographic conditions as in Fig. 3. Weight of the molasses sample, 0.30 mg.

TABLE I

RETENTION VOLUMES OF SACCHARIDES

Chromatographic conditions: eluent flow, 50 ml/h; column length, 48.7 cm; elution temperature, 60°. Eluents: for saccharides 1–8, 0.03 *M* borate of pH 7.50; for saccharides 9–12, 0.100 *M* borate of pH 8.85; for saccharides 13–26, 0.250 *M* borate of pH 8.88.

No.	*Saccharide*	V_r *(ml)*	*No.*	*Saccharide*	V_r *(ml)*
1	Saccharose	18.9	14	Psicose	122
2	Trehalose	20.3	15	Turanose	125
3	Melezitose	28.6	16	Fructose	126
4	Raffinose	30.6	17	Arabinose	131
5	Cellobiose	38.7	18	Fucose	134
6	Maltose	56.2	19	Allose	139
7	Stachyose	75.5	20	Galactose	139
8	Lactose	80.8	21	Tagatose	143
9	Rhamnose	84.9	22	Altrose	149
10	Sedoheptulose	96.0	23	Xylose	152
11	Lyxose	98.5	24	Sorbose	157
12	Ribose	103	25	Glucose	174
13	Mannose	121	26	Melibiose	181

(formed during the industrial processing) deserve special attention. Table I lists retention volumes of 26 saccharides, and can be used to find other possible applications of this method.

The sample of DEAE-Spheron used in our study is not identical with the ion exchangers used by Chytilová *et al.*[5]. It not only had a somewhat higher capacity, but the procedure used for its preparation also permitted a partial quaternization of the functional group. Therefore it also displayed a distinctly higher

affinity for borate complexes of saccharides, which cannot be explained merely by the slightly higher capacity in comparison with the ion exchangers used earlier. To achieve retention times of monosaccharides identical with those in ref. [5], for a column packed with a more basic DEAE-Spheron we had to use three times the concentration of eluent.

The efficiency of the column packed with our DEAE-Spheron was similar to that achieved by Chytilová *et al.*[5] with another DEAE-Spheron of similar granulation. For monosaccharides eluted with 0.3 *M* borate at 50° and 50 ml/h flow-rate, the heights equivalent to a theoretical plate ranged from 0.053 to 0.126 cm (in the paper cited[5] the plate heights for a 0.1 *M* eluent at the same temperature and flow-rate were within the range 0.065–0.104 cm). For oligosaccharides, efficiencies close to those found for monosaccharides (see Fig. 1) were achieved in our study. Hence, DEAE-Spheron may be considered, even from the point of view of the kinetics of the chromatographic separation, as a suitable material for the separation of complex mixtures of saccharides, and in view of its different selectivity for the separation of sugars is a suitable alternative or complementary procedure to the HPLC methods for saccharides, which take advantage of the chemically bonded alkylamine phases.

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