AUTOMATED CHROMATOGRAPHY OF MONOSACCHARIDES ON ANION EXCHANGE RESINS

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Partition chromatography on anion exchange resins in water–ethanol is a useful tool in the separation of various monosaccharides[1]. In previous work strongly basic resins with a styrene–divinylbenzene matrix were employed. The chief purpose of the present work is to study the application of strongly basic anion exchangers with a more polar resin matrix. In all the experiments carried out in this work cross-linked dextran containing quaternary ammonium ions was employed. The eluate was analyzed automatically using the anthrone method.

RESULTS AND DISCUSSION

A typical chromatogram obtained from a run with four pentoses is reproduced in Fig. 1. It is seen that four peaks were obtained in the order: ribose, lyxose, arabinose, xylose. Under these working conditions some overlapping occurred between the curves corresponding to lyxose and arabinose. The chromatogram can be evaluated for quantitative purposes, however, using conventional methods[2]. The run was completed in less than 4 h.

The separation of some monosaccharides, including those present in common plant materials, is demonstrated in Fig. 2. This run was completed in less than 7 h.

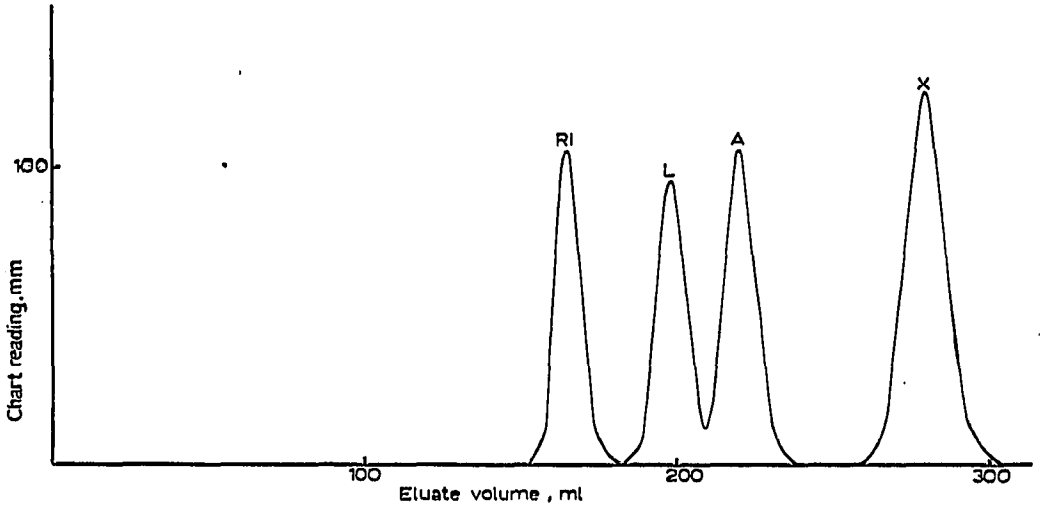

Fig. 1. Separation of four pentoses in 95% ethanol at 90°. Resin bed 840 × 6.0 mm, SO_4^{2-}. Flow rate 5.0 ml cm^{-2} min^{-1}. Ri = ribose, 0.50 mg; L = lyxose, 0.60 mg; A = arabinose, 0.50 mg; X = xylose, 0.50 mg.

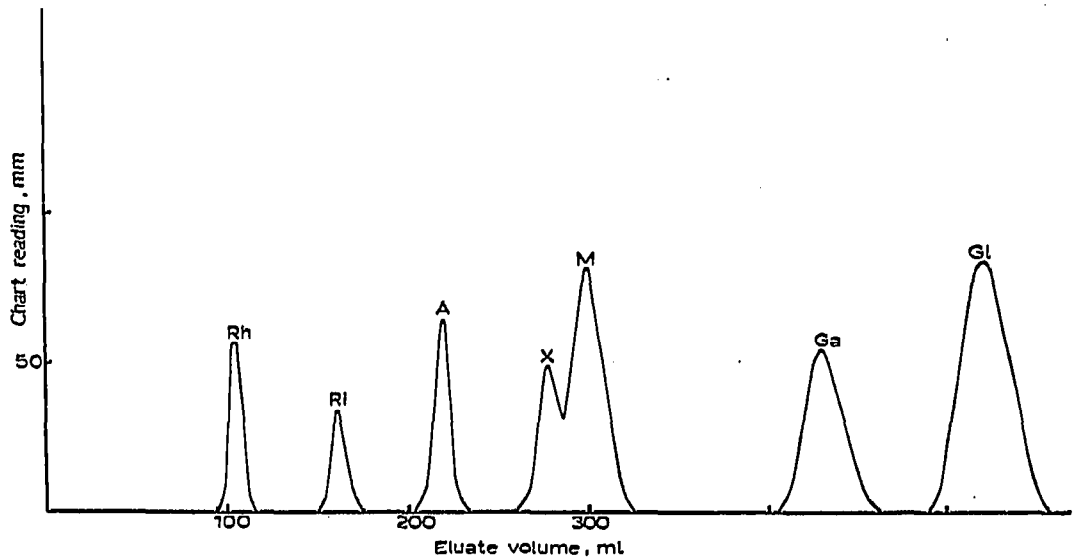

Fig. 2. Separation of some common monosaccharides in 95% ethanol at 90°. Resin bed 840 × 6.0 mm, SO_4^{2-}. Flow rate 5.0 ml cm^{-2} min^{-1}. Rh = rhamnose, 0.15 mg; Ri = ribose, 0.15 mg; A = arabinose, 0.30 mg; X = xylose, 0.15 mg; M = mannose, 0.45 mg; Ga = galactose, 0.45 mg; Gl = glucose, 0.45 mg.

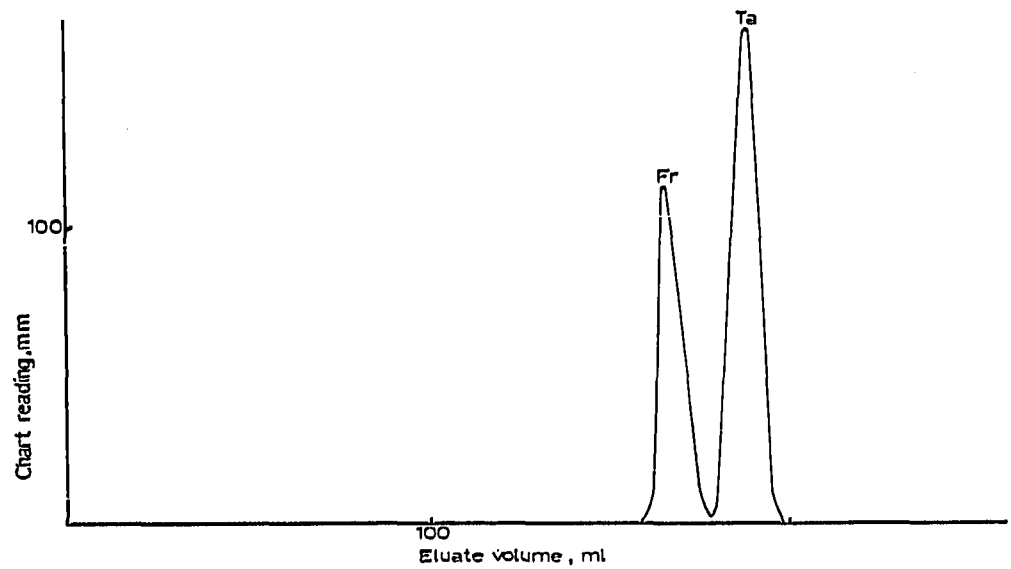

Fig. 3. Separation of 0.15 mg fructose (Fr) and 0.30 mg tagatose (Ta) in 95% ethanol at 65°. Resin bed 890 × 4.3 mm, SO_4^{2-}. Flow rate 2.3 ml cm^{-2} min^{-1}.

It can be seen that the separation of most species was excellent. The separation of xylose from mannose was, however, less effective than that obtained with anion exchange resins with a styrene–divinylbenzene matrix, whereas the separations of arabinose from xylose and of galactose from glucose were better than those reported previously[3]. It is worth mentioning that the separation of xylose from mannose was markedly improved when the chromatogram was run at 110°. At this temperature the position of the base line increased slightly during the run which indicates that some interfering reactions occurred. This chromatogram was completed within 3 h without applying a higher pressure than 40 atm. At 110° and a higher ethanol concentration (97 %) serious side reactions were observed in this system.

A rule valid for all the monosaccharides studied, is that the peak elution volume decreases with increased temperature. Furthermore, the width of the elution curves decreases. In some systems, however, the separation factors (*i.e.* the ratio between the distribution coefficients) decrease to such an extent that the separation is jeopardized at very high temperature. A typical example is the separation of fructose and tagatose. At 90°, fructose exhibited a peak elution volume only slightly lower than that of tagatose, and when these ketoses were run in a mixture, serious overlapping was observed. At 65° (Fig. 3) the separation was satisfactory. With styrene–divinylbenzene resins these sugars could not be distinguished from one another[3].

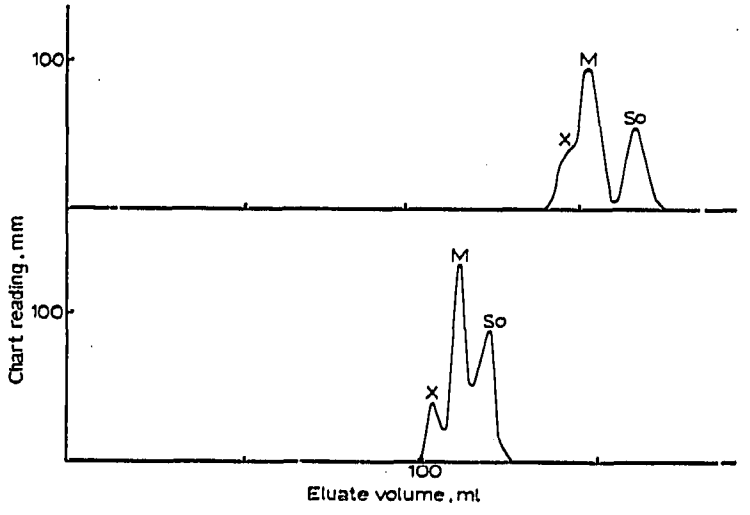

Fig. 4. Influence of temperature on the separation of xylose (X), mannose (M), and sorbose (So) in 95% ethanol. Resin bed 890 × 4.3 mm, SO_4^{2-}. Flow rate 2.3 ml cm^{-2} min^{-1}. Upper chromatogram 75°. Lower chromatogram 95°.

Another striking example of the influence of temperature is given in Fig. 4. The upper chromatogram shows that at 75° serious overlapping occurred between xylose and mannose whereas the separation from sorbose was satisfactory, with only a slight overlapping with mannose. At 95° the separation of xylose from mannose improved to such an extent that a rough quantitative determination of xylose could be made. The separation of mannose from sorbose on the other hand became so much worse that only approximate determinations could be made.

From the peak elution volumes recorded in all chromatographic runs on the

6 mm columns the volume distribution coefficients (D_v) were calculated as usual[4]. The average values are given in Table I. This table also includes the runs carried out with a lower degree of crosslinking. It is seen that the order of elution was the same with both resins and that the distribution coefficients were lower with the resin having lower crosslinking. With this resin the influence of the ethanol concentration was studied. As expected, this factor had an influence in the same direction as that with styrene–divinylbenzene resins. It should be mentioned that in 99.3 % ethanol the monosaccharides appeared much later than at the concentrations reported in the table. At this ethanol concentration the curves were too broad for any practical separation purposes.

TABLE I

VOLUME DISTRIBUTION COEFFICIENTS IN 95 % ETHANOL AT DIFFERENT TEMPERATURES

Values in parentheses refer to experiments in 92.4 % ethanol.

Resin	*A*, SO_4^{2-}		*B*, SO_4^{2-}				*B*, Cl^-
Temperature	*75°*		*75°*	*90°*	*97°*	*110°*	*90°*
Digitoxose		(0.7)	0.8	0.7			0.4
2-Deoxyribose	1.4	(1.3)	1.8	1.4			0.7
2-Deoxygalactose			3.7	3.1			1.4
Rhamnose			4.8	4.0	3.6	3.2	1.4
2-Deoxyglucose				4.1			1.6
Fucose	5.4		4.4	3.9			
Ribose	5.1	(4.3)	6.9	6.5	5.8	4.9	2.0
Lyxose			9.7	7.9	6.9		
Arabinose	6.9	(6.1)	10.5	8.9	7.8	6.5	2.7
Xylose	8.2	(7.1)	14.0	11.5	9.9	8.0	2.9
Fructose			14.6	12.1	10.6		
Mannose	9.3	(7.9)	15.2	12.4	10.7	8.8	3.7
Tagatose			16.7	13.1	11.1		
Sorbose			16.9	13.7	11.3		
Galactose	12.6	(11.0)	23.0	18.1	15.3	12.1	4.2
Glucose	14.7	(12.1)	29.7	22.0	18.3	14.3	4.4

In a comparison of the distribution coefficients with those observed with the sulfate form of the styrene–divinylbenzene resins studied in earlier work it was shown that, under comparable condition, the sugars are held much more strongly by the latter type of resin. This is explained by the higher ionic concentration inside the styrene–divinylbenzene resins. It is interesting that the order of elution for most sugars is the same with both types of resin. Exceptions are the ketoses and mannose which on a styrene–divinylbenzene resin were eluted in the following order: tagatose (10.0), fructose (10.3), sorbose (11.0), mannose (11.8). The values within parentheses are the distribution coefficients observed with Dowex 1-X8 in 86 % ethanol at 90°. It can be concluded that with monosaccharides the interaction forces between the resin matrix and the solutes exhibit a specificity and have a marked influence upon the separability of some of these strongly polar solutes.

All separations discussed above were carried out with the sulfate form of the resin. With styrene–divinylbenzene resins it was found that glucose was held much more effectively with the sulfate form than with the chloride form[5]. As shown in the

last column of Table I this also holds true with the dextran resin. Moreover, the distribution coefficients of all the other sugars investigated in this work were lower with the chloride form. No reversal in the order of elution was observed, but with most sugars the separation factors were less favorable with the chloride form. An interesting exception, which can be taken advantage of in certain practical analyses, is the separation factor xylose: mannose which was more favorable with the chloride resin than with the sulfate form. Two chromatograms are given in Fig. 5. As shown

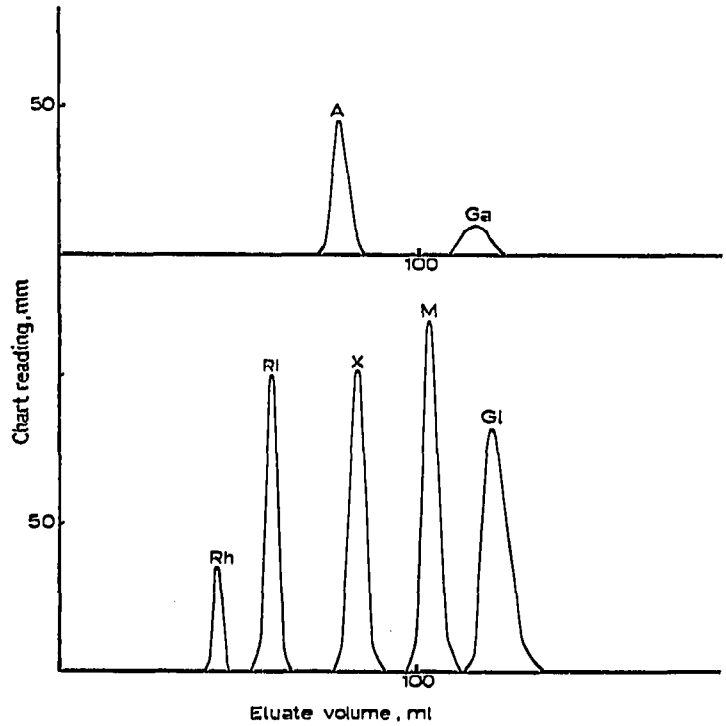

Fig. 5. Separation of some common monosaccharides in 95% ethanol at 90°. Resin bed 890 × 6.0 mm, Ci^-. Flow rate 2.8 ml cm^{-2} min^{-1}. Rh = rhamnose, 0.05 mg; Ri = ribose, 0.15 mg; A = arabinose, 0.15 mg; X = xylose, 0.15 mg; M = mannose, 0.25 mg; Ga = galactose, 0.15 mg; Gl = glucose, 0.25 mg.

in the lower chromatogram a mixture of rhamnose, ribose, xylose, mannose, and glucose was completely separated in less than 3 h. Arabinose showed a behavior similar to that of xylose. Similarly, the position of galactose (upper chromatogram) differed only slightly from that of glucose. Hence, it is obvious that the chloride form can be used in some systems whereas the sulfate form is to be preferred in other analyses.

With 2-deoxysugars which are known to be very reactive[6], reactions which seriously interfered with the analyses were observed in runs carried out at 90° and at higher temperatures. These complications occurred with both the sulfate and chloride forms. In parallel runs with the sulfate form of a styrene–divinylbenzene resin no decomposition could be detected. This effect is demonstrated by a chromatogram from a run of 2-deoxyglucose and rhamnose (Fig. 6), which is stable under the conditions used. This example was chosen because these two deoxysugars could not be separated from each other on a styrene–divinylbenzene resin. Serious overlapping also occurred with the sulfate form of the dextran resin, but as can be seen from

Fig. 6, the separation on the chloride resin is good enough to permit a rough quantitative estimation. Calibration runs must be made with similar amounts under identical conditions.

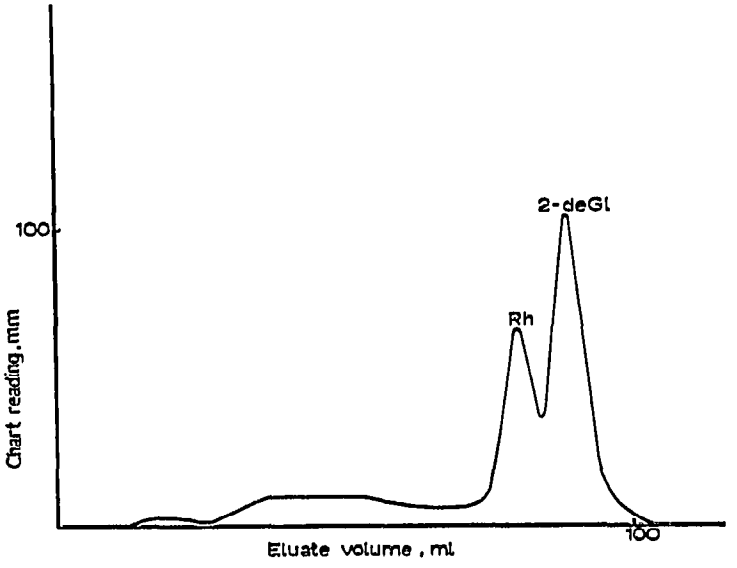

Fig. 6. Separation of 0.25 mg rhamnose (Rh) and 0.25 mg 2-deoxyglucose (2-deGl) in 95% ethanol at 100°. Resin bed 890 × 6.0 mm, Cl^-. Flow rate 2.8 ml cm^{-2} min^{-1}.

From the results presented above it is evident that in several separations anion exchangers with a dextran matrix can compete favorably with styrene–divinylbenzene resins. In some separations, for example that of tagatose and fructose, superior results were achieved with the dextran resin.

EXPERIMENTAL

Chromatographic system

The columns, which were made from Pyrex glass tubes with an inner diameter of 6 mm, were jacketed and furnished with teflon fittings and a porous teflon bottom. Water, or at higher temperatures, polyglycol was used as the heating medium and circulated from an ultra-thermostat. Anion exchangers of strongly basic type, specially prepared for our purposes by Pharmacia Fine Chemicals, Uppsala, Sweden, were used throughout this work. The resins were made from dextran polymers crosslinked with epichlorhydrin by the introduction of quaternary ammonium groups. The exchange capacity was 3 milliequiv. per g of dry resin (chloride form). Two batches of resin with different degrees of crosslinking were used: (A) with a low degree of crosslinking and (B) with a higher crosslinking. Before the introduction of the ion exchange groups the water uptake of the resins determined according to FLODIN[7] was: (A) 2.5 and (B) 1.5 g water per g dry resin. The particle size was: A 10–40 μ and B 5–30 μ. These figures refer to determinations in 90% ethanol. All chromatograms reproduced in this paper were obtained in runs with resin B.

The column was conditioned with ethanol of the concentration and temperature to be used in the subsequent run. When working above 75° the water circulation through the jacket was stopped and the column allowed to cool below the boiling

point of the eluant before loading the column. The eluant was boiled continuously before it was fed into the column by means of a piston pump of stainless steel (Lewa). The pressure drop in the column was about 40 atm. in most runs reproduced in this paper.

Analysing system

The eluate from the column was mixed directly with anthrone reagent (2 g anthrone per 1 sulfuric acid, *puriss.*) in the ratio 1:2. The reagent was fed into the analysing system with another Lewa pump. The original piston became corroded, probably arising from the uptake of atmospheric water, but this difficulty was circumvented by substituting the original piston for one which was covered with a teflon tubing. After eluate and reagent were mixed, the color was developed by passing the solution through a teflon tubing (I.D. 1.2 mm) submerged in a heating bath kept at 100°. The time of reaction was about 1 min. The transmittance was measured at 625 mμ in a 2 mm flow cell using a three channel absorptiometer from LKB-Produkter, Stockholm, and recorded automatically. The recorder, which was purchased from the same firm, was run with a scale expansion of 5.

Reproducibility

The reproducibility was very good in repeated experiments with resin B (as well as in experiments with Dowex 1-X8; 10–14 μ). The peak elution volumes differed by 1 % or less from the mean. The drift in the base line was negligible (less than 2 mm in an 8-h run). The areas under the elution curves, from which the quantities of various monosaccharides present in an unknown sample can be calculated by comparison with runs with known amounts of the same species, showed somewhat greater variations than the peak elution volumes. In duplicate runs with well separated sugars the deviation from the mean was 5 % or less. By applying suitable internal standards the accuracy can be increased. Further improvements are no doubt possible.

The more lightly crosslinked resin gave very broad elution bands and hence the accuracy in the determinations was not as good. This can be explained by the relatively large particle size (10–40 μ). When a second ion exchanger of the same type and with the same degree of crosslinking, but with a smaller particle size (1–25 μ) was studied, much sharper elution curves were obtained. Because of the compressibility of this resin, however, it was impossible to avoid fluctuations in flow of the eluate. These fluctuations resulted in a noisy base line and discontinuities in the elution curves.

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SUMMARY

An automated method for the separation of various monosaccharides by partition chromatography on strongly basic anion exchangers with a dextran matrix is

described. Elution is carried out with water–ethanol mixtures. In some systems anion exchangers of this type give better separations than styrene–divinylbenzene resins. Resins in the sulfate form are more versatile than in the chloride form.

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