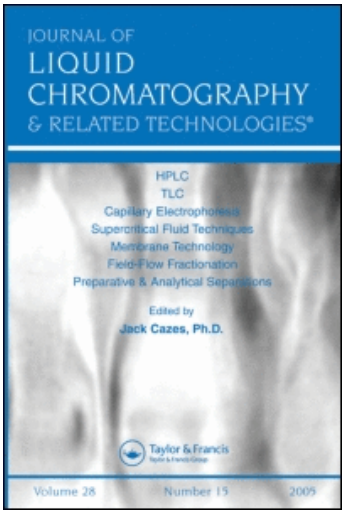

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Systematic Liquid Chromatographic Separation of Poly-, Oligo-, and Monosaccharides

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SYSTEMATIC LIQUID CHROMATOGRAPHIC SEPARATION OF POLY-, OLIGO-, AND MONOSACCHARIDES.

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ABSTRACT

High speed separations of poly-, oligo-, and monosaccharides were achieved on Chromosorb LC 9, Toyo Soda Starch Gel TSKLS170P5, and Hitachi 3013N packings. Post column reaction system with tetrazolium blue was used to obtain a low detection limit for oligo-, and monosaccharides. Polysaccharides were analysed within 20 min by gel permeation chromatography over TSKLS170P5 gel. DP 30 oligosaccharide was eluted in 30 min on Chromosorb LC 9 using a gradient of acetonitrile/water. Finally monosaccharides were separated in 25 min on Hitachi 3013N macroporous anion exchange resin.

INTRODUCTION

The analysis of poly-, oligo-, and monosaccharides is of the utmost importance in carbohydrate chemistry. Churms (1) published an excellent review on the separation of saccharides by gel permeation chromatography. Polysaccharides were analysed by GPC on Sephadex (2-4) or polyacrylamide gels (5-7); very long

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times were required for the separation. The newer macroporous resins, like the Shodex 10Npak (8) of the Toyo Soda gel (9) have reduced considerably the elution time. Controlled pore size macroporous ion exchange resin (10) as well as gel permeation (11-14) chromatography were also extensively used. Reversed phase liquid chromatography also served to separate homogeneous polymers (15), and saccharides have been separated by an adsorption mode (16-18). We previously reported (16) the use of a bonded NH_2 packing for the separation of oligosaccharides. Finally, ion exchange chromatography has often served for the separation of monosaccharides (12, 19-21). Generally, however, elution times are very long. Sinner and Puls (21) separated mono-, and disaccharides by ion-exchange chromatography with a high sensitivity post-column reaction system, the separation required 70 min.

In the present paper, we propose a high speed separation of poly-, oligo-, and monosaccharides by high performance liquid chromatography. A post-column reaction system based on the reduction of 3,3'-(3,3'-dimethoxy 1'1'-biphenyl-4,4'-diyl) bis 2,5-diphenyl-2H-tetrazolium dichloride, hereafter called tetrazolium blue (BT) was used for detection purposes (16,22).

EXPERIMENTAL

Apparatus

A modular liquid chromatograph was assembled from the following : three high pressure pumps (Waters Associates,

Model 6000A, and Altex, Model 110A), solvent programmer, Waters Associates, Model 660, a universal injector, Altex Model 905-19 or 905-42, a differential refractometer, Waters, Model R401 and a variable wavelength detector, Perkin-Elmer, Model LC-55. Chromatograms were recorded with a Brinkman, Model 2541, or Linear Instruments, Model 915, recorder . The reactor for the reduction of tetrazolium blue consisted of a 4m long PTFE tubing, 0.5 mm i.d., rolled on itself to make a 5 cm coil. The reactor was connected to the outlet of the chromatographic column, to an auxiliary pump, and to a detector through an Omnifit three port valve, Unimetrics Co., Model 10009.

MATERIALS

Chemicals serving as standards were analytical reagents used without further purification. Glucose and lactose were BDH chemicals; galactose, stachyose tetrahydrate, and maltotriose came from Aldrich Chemical Inc., fructose, inulin, xylan, and mannose were from Nutritional Biochemical Corp. Xylose and phosphoric acid were Fisher's reagents. Polysaccharides and the wood extract were kindly supplied by Dr. H. Morikawa from the Polymer Laboratory, University of Montreal. Dextran-T 2000, 500, 70, 40, and 10 were purchased from Pharmacia, tetrazolium blue came from Sigma Chemical and hydrolyzed starch was from Plastistarch Corp. (St. Laurent, Quebec). Acetonitrile (ACN) was Fisher's HPLC grade solvent. Water was first distilled in glass and then treated with the Milli-Q system of the Millipore Corp.

Procedures

All columns were prepared in our laboratory. Toyo Soda starch gel TSKLS170P5, 5 microns average diameter, was packed in a stainless steel tubing, 25 cm long x 0.7 cm i.d. The column was equiped with Swagelok fittings and a 2 micron (Altex) SS filter. Water was used for packing purposes. Similarly, Johns-Manville LC 9, a bonded NH_2 packing, was packed in a tubing 25 cm long x 0.43 cm i.d. using isopropyl alcohol and methanol. Hitachi 3013N, an anion exchange resin, was converted to the phosphate form and then packed by a wet slurry method in a 25 cm long x 0.43 cm i.d. tubing using acetonitrile in water as solvent.

The detection of sugars was accomplished as previously described (16) by reducing tetrazolium blue. The first 3m of the 4m reactor were immersed in a water bath kept at 85° C; the last meter was at room temperature. The reactor was collecting the effluent from the analytical column and the tetrazolium blue solution as supplied by an auxiliary pump. The tetrazolium blue was added as a 2% solution prepared in 50% ethanol/water. The solution was also 0.18 M in sodium hydroxide. The chromatograms were measured at 530 nm, the absorption maximum for tetrazolium blue monoformazan, the product of the reaction.

RESULTS AND DISCUSSION

Polysaccharides were separated by gel permeation chromatography. A differential refractometer was used for detection

purposes. The calibration curve of dextrans on starch gel TSKLS170P5 was obtained and is shown in Figure 1. The column had 4450 plates /25 cm for glucose. The exclusion limit was around M.W. 2 x 10^6. Although the fractionnation range is quite large, the packing allows a short elution time for glucose (20 min.) which, in itself, is a major advantage and renders the system attractive for the analysis of degraded products such as hydrolyzed starch, hydrolyzed xylan , wood extract... In Figure 2, chromatogram A represents the separation of hydrolyzed xylan chromatogram B, the separation of a wood extract, and chromatogram C, the separation of hydrolyzed starch. The traces A,B, and C show that the various samples are mixtures of poly-, oligo-, and mono-

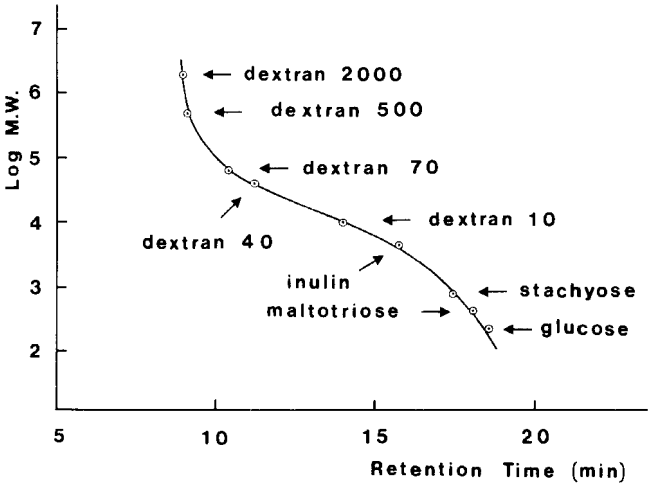

Figure 1: Calibration curve for TSKLS170P5 starch gel with Dextrans. Column 25 cm x 0.7 cm i.d. Elution: Water at 0.5 ml min^{-1}.

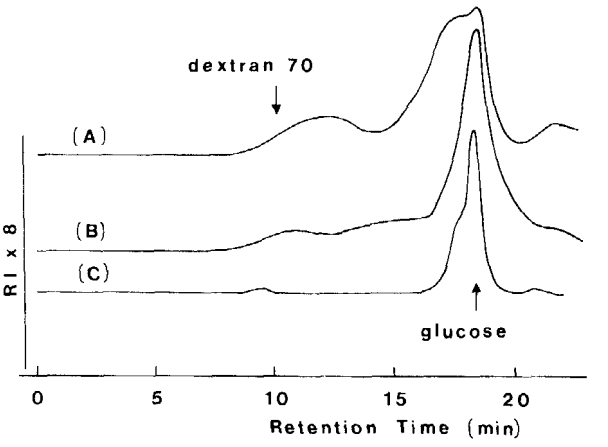

Figure 2: Gel Permeation chromatograms for hydrolyzed xylan (A), wood extract (B), and hydrolyzed starch (C). Column: 25 cm x 0.7 cm i.d. of TSKLS170P5 starch gel. Elution: Water at 0.5 ml min^{-1}. Detection: refractive Index: RI x 8.

saccharides. Representative fractions of oligosaccharides and monosaccharides were collected for further characterization.

The sample of oligosaccharides collected after fractionation by GPC was injected over the Chromosorb LC 9 column and separated in a short time with an acetonitrile/water gradient. The chromatograph obtained when a wood extract is injected is shown in Figure 3, conditions of elution are given with the chromatogram. DP 30 oligosaccharide (M.W. 5405) was eluted in 30 min. Figure 4-A shows the chromatogram for hydrolyzed starch and 4-B represents the trace obtained when an impure cellotriose sample was injected for identification purposes in chromatogram A. Similarly, the separation of the hydro-

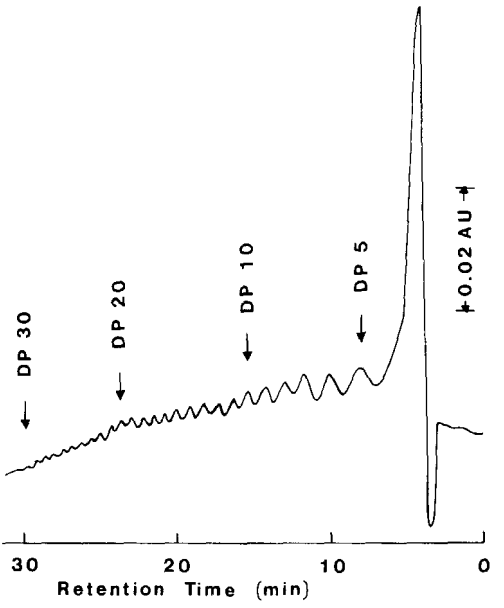

Figure 3: Chromatogram of a wood extract fractionated by GPC using reversed phase chromatography. Column: 25 cm x 0.43 cm i.d. filled with Chromosorb LC 9. Elution: Linear gradient from 70% ACN to 62.5% ACN in water in 30 min. Flowrate: 1.0 ml min^{-1}. Detection: Visible detection of the tetrazolium blue monoformazan at 530 nm. Flowrate for tetrazolium blue: 1.5 ml min^{-1}. Temperature of reactor: 85° C

lization products of xylan illustrates an interesting application of the method. The chromatogram of this separation is shown in Figure 5; along the trace, around DP 10 and DP 25, two reproducible patterns, A and B, were always observed. We suggest that these are related to the mechanisms of degradation and could be useful in the study of these mechanisms.

Finally, the separation of various monosaccharides, namely xylose, fructose, mannose, galactose, and glucose was achieved

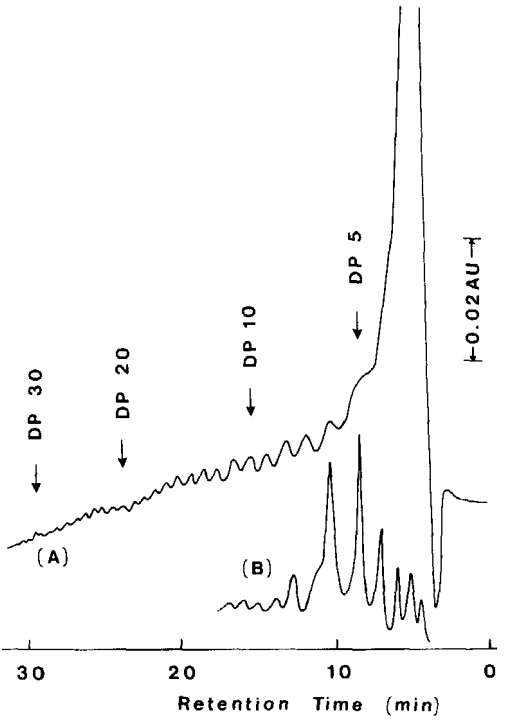

Figure 4: Reversed phase chromatography of hydrolyzed starch (A) and impure cellotriose (B) on Chromosorb LC 9. Conditions: same as in Figure 3.

through a Hitachi 3013N anion exchange resin. The results are shown in Figure 6. The resin was studied as the borate, sulfate, phosphate, and chloride forms. Of these ions, only the phosphate permitted separation in a reasonable time, the capacity ratios, k', decreasing in the order:

borate > phosphate > sulfate > chloride.

Acetonitrile in water was the eluent. The separation was achieved in 25 min. Chromatogram A in Figure 6 represents various standards of monosaccharides and chromatograms B and

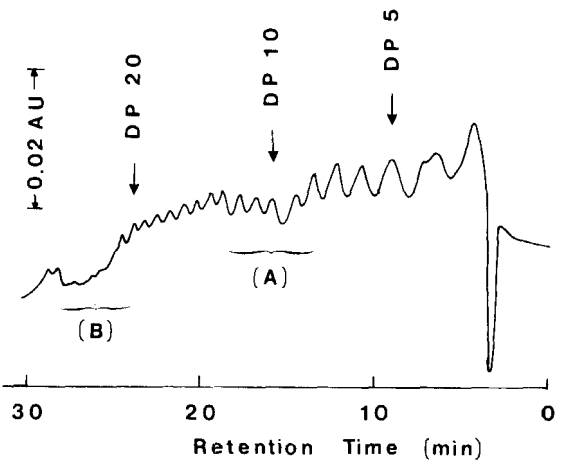

Figure 5: Reversed phase chromatography of hydrolyzed xylan on Chromosorb LC 9. Conditions: same as in Figure 3.

C represent respectively the separation of a monomeric fraction of hydrolyzed starch and wood extract. It is noteworthy to observe that the starch fraction gives only glucose and that the wood fraction shows many monosaccharides; xylose and glucose being the main constituents. The other peaks found correspond to galactose, mannose, and fructose. The peaks preceding xylose could be due to reducing substances such as arabinose and the uronic acids (23) likely to be present in a wood extract.

CONCLUSION

The method was found to give fast and reproducible results for the separation of poly-, oligo-, and monosaccharides. The limit of detection, as established previously, (16) is very low, typically less than 10 ng for monosaccharides.

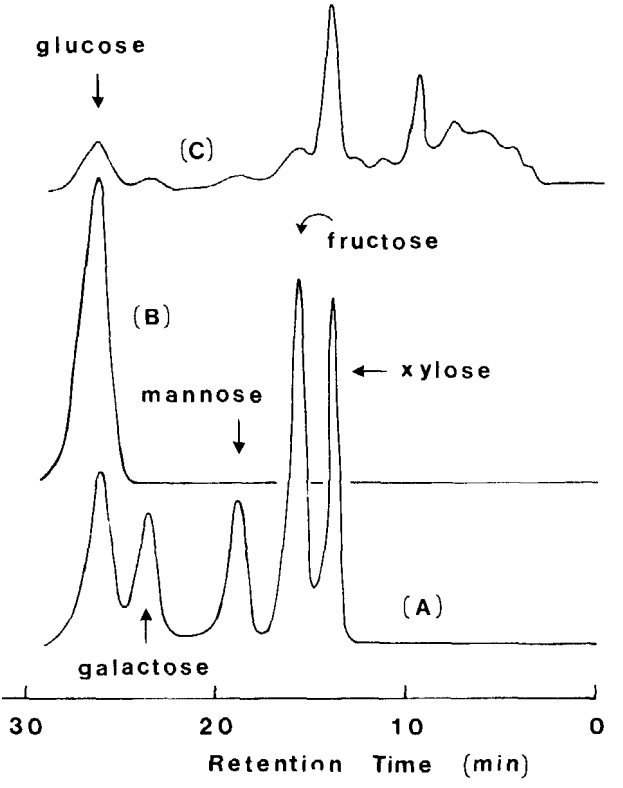

Figure 6: Separation of various monosaccharides on Hitachi 3013N anion exchange resin. Column: 25 cm x 0.43 cm i.d. Elution: isocratic, 80% ACN in water at 1.0 ml min^{-1}. Detection: same as in Figure 3.

Thus the method should be quite useful in clinical, food, and carbohydrate chemistry.

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