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Determination of xylose oligomers and monosaccharides by anion-exchange chromatography with pulsed amperometric detection

SHIFEN MOU*, QUN SUN and DEPEI LU
Research Centre for Eco-Environmental Sciences, Academia Sinica, P.O. Box 934, Beijing 100083 (China)

ABSTRACT

Anion-exchange chromatography followed by pulsed amperometric detection (AE–PAD) was applied to the determination of xylose oligomers and some monosaccharides. Under alkaline conditions, underivatized xylose oligomers (DP1–DP7; DP = degree of polymerization) were readily separated within 12 min. Underivatized monosaccharides (fucose, galactose, glucose, mannose, arabinose, xylose, galactosamine and glucosamine) were separated within 30 min at an eluent flow-rate of 1 ml min^{-1}. The limits of determination of xylose oligomers were from 0.08 to 0.01 nmol. Results for both xylose oligomers and monosaccharide determination by AE–PAD were more sensitive than those obtained by conventional high-performance liquid chromatography. This method was also employed to determine the monosaccharides in pretreated peat samples.

INTRODUCTION

Compared with thin-layer chromatography [1–3], liquid chromatographic methods [4–10] for the determination of carbohydrates are faster and give higher resolution capacities, but they have often suffered from both poor column performance with respect to selectivity and efficiency and detector insensitivity. Gradient incompatibility is also a problem associated with refractive index (RI) (and occasionally UV) detectors. Rocklin and Pohl [11] first applied high-performance anion-exchange chromatography with pulsed amperometric detection (AE–PAD) to the determination of carbohydrates. Not only does this method provide more efficient separation and detection than high-performance liquid chromatography (HPLC), it also minimizes sample preparation. Larew and Johnson [12] used AE–PAD to separate and determine maltooligosaccharides found in corn syrup. Chatterton and Harrison [13] determined the kestose in plants. Hardy and Townsend [14–17] studied the determination of mono- and oligosaccharides in glycoconjugates extracted from biological samples.

This paper reports the separation and detection of eight monosaccharides (fucose, galactosamine, arabinose, glucosamine, galactose, glucose, xylose and mannose) and a series of β-1–4-linked xylose oligomers (from xylose to xyloheptaose) using AE–PAD.

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EXPERIMENTAL

Materials

Xylose oligomers were kindly provide by C. C. Tu [18]. They were obtained through partial acid hydrolysis of the xylan from corncob holocellulose.

L-Fucose and D-mannose were purchased form Serva, D-galactosamine and D-glucosamine from Sigma, L-arabinose and D-xylose from Shanghai Chemical Factory and D-galactose, D-glucose, sodium hydroxide and sodium acetate from Beijing Chemical Factory. All the reagents were of analytical-reagent grade.

Sodium hydroxide solution (50% w/w) was prepared uing 10 MΩ deionized water and handled according to ref. 19.

Chromatographic apparatus

All chromatography was performed on a Dionex Series 4500i IC system at 25°C. A Dionex GPM pump and a Model PAD II pulsed amperometric detector with a gold working electrode were used. The potentials and durations used for detecting xylose oligomers were as follows: $E_1 = 0.08$ V, $t_1 = 300$ ms; $E_2 = 0.6$ V, $t_2 = 120$ ms; $E_3 = -0.8$ V, $t_3 = 300$ ms. For monosaccharides, the detection conditions by PAD were as follows: $E_1 = 0.1$ V, $t_1 = 480$ ms; $E_2 = 0.6$ V, $t_2 = 120$ ms; $E_3 = -0.8$ V, $t_3 = 60$ ms.

The carbohydrates were separated on a column (250 × 4 mm I.D.) of Dionex CarboPac PA1 pellicular anion-exchange resin coupled with a Dionex CarboPac guard column. A Dionex post-column reactor was used to provide a flow-rate of 0.300 *M* sodium hydroxide solution of 0.8 ml min^{-1}. The chromatographic data were collected and plotted by using a Spectra-Physics Model SP4270 integrator.

Four eluents were used: (1) 0.100 *M* sodium hydroxide solution, (2) deionized water, (3) 0.150 *M* sodium hydroxide solution and (4) 0.150 *M* sodium hydroxide–0.500 *M* sodium acetate. In order to remove dissolved carbon dioxide from the eluents, they were degassed by a sonicator coupled with a vacuum aspirator pump for 30 min prior to use.

Procedure

Fig. 1 shows the chromatographic configuration for detecting carbohydrates. The gradient programme used to elute xylose oligomers was 4 min of isocratic elution

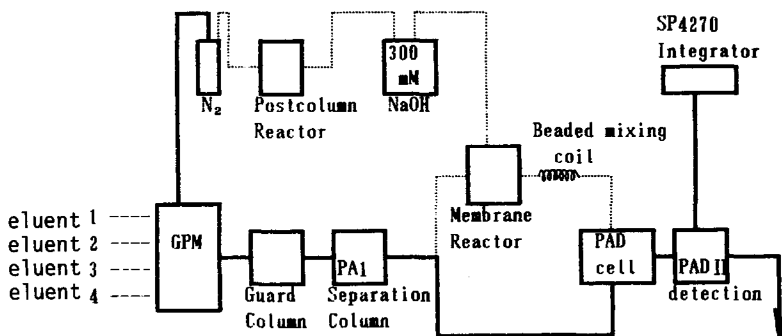

Fig. 1. Chromatographic configuration for analysis of carbohydrates by AE–PAD.

with a mixture of eluents 3 and 4 (85:15), followed by a linear increase to 20% eluent 4 from 4 to 6 min.

For the separation of monosaccharides, two gradient programmes were used. In programme A, the sample was introduced into the system equilibrated with a mixture of eluents 1 and 2 (10:90) and the proportion of eluent 2 was increased linearly to 100% from 0 to 2.5 min, the latter condition being maintained until the end of the analysis. Programme B stated with a mixture of eluents 1 and 2 (7:93) with a linear increase to 100% eluent 2 from 0 to 2.5 min. In order to optimize the PAD sensitivity and minimize baseline drift, post-column addition of 0.300 *M* sodium hydroxide solution was required. As shown by the dotted line in Fig. 1, a post-column reactor, instead of a DQP-1 pump, was used to provide a flow-rate of 0.8 ml min^{-1} of 0.300 *M* sodium hydroxide solution; the eluent and 0.300 *M* sodium hydroxide solution were mixed in a membrane reactor and a beaded mixing coil 80 cm long, then passed to the PAD cell.

In order to remove carbonate and other strongly retained contaminants, 0.200 *M* sodium hydroxide solution should be run for 15 min between injections, thus ensuring reproducibility. The peak heights of the sugars have a tendency to vary with time. Frequent calibration could minimize the errors in concentration measurements.

RESULTS AND DISCUSSION

Optimum conditions for detection of xylose oligomers (X_1–X_7)

In general, the observed anion-exchange affinity follows the trend [11] sugar alcohols < monosaccharides < disaccharides < oligosaccharides. Hence the eluent for the separation of xylose oligomers should be strong enough to push the retained components out of the column. With 0.150 *M* sodium hydroxide solution as the eluent, the retention times of X_4, X_5, X_6 and X_7 were more than 50 min, the peaks were tailed and the detection sensitivity was very low. With 0.075 *M* sodium acetate

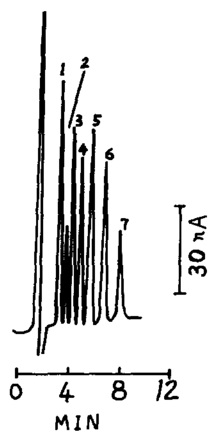

Fig. 2. Chromatogram of β-1–4-linked xylose oligomers. Peaks: 1 = 0.2 ppm xylose; 2 = 4.0 ppm xylotriose; 3 = 1.0 ppm xylobiose; 4 = 4.0 ppm xylotetraose; 5 = 5.0 ppm xylopentaose; 6 = 5.0 ppm xylohexaose; 7 = 5.0 ppm xyloheptaose.

TABLE I

LINEARITY, RELATIVE STANDARD DEVIATION (R.S.D.) AND DETECTION LIMITS FOR XYLOSE OLIGOMERS

Compound[a]	Linearity correlation coefficient	R.S.D. (%) (n = 5)	Detection limit (mg l^{-1})
X_1	0.9997 (range 0.01–0.24 ppm)	4.47 (at 0.08 ppm level)	0.01
X_2	0.9980 (range 0.06–1.20 ppm)	3.72 (at 0.40 ppm level)	0.06
X_3	0.9985 (range 0.40–5.00 ppm)	4.51 (at 1.60 ppm level)	0.40
X_4	0.9997 (range 0.30–5.00 ppm)	3.72 (at 1.60 ppm level)	0.30
X_5	0.9981 (range 0.30–6.00 ppm)	4.60 (at 2.00 ppm level)	0.30
X_6	0.9986 (range 0.30–6.00 ppm)	3.82 (at 2.00 ppm level)	0.30
X_7	0.9992 (range 0.50–6.00 ppm)	2.89 (at 2.00 ppm level)	0.50

[a] X_1–X_7 are xylose oligomers DP1–DP7.

as the eluent for the xylose oligomers in a gradient programme, a good separation and detection within 12 min were achieved (see Fig. 2).

From Fig. 2, the retention time of xylobiose was longer than that of xylotriose. This phenomenon has often been observed in the separation of oligosaccharides by HPAE–PAD [11,15–17].

Hardy and Townsend [15] deduced that the retention time of oligosaccharides was related to the accessibility of oxyanions in the latter to the functional groups on the stationary phase of the HPAE column. Hence the retention time of oligosaccharides is not proportional to the degree of polymerization (DP).

The detection limits for xylose oligomers ranged from 0.01 to 0.08 nmol (see Table I); however, the detection limits of xylose oligomers by HPLC with RI detection was only 4.0 nmol [10].

The results also showed good linearity and reproducibility (see Table I). All the linearity correlation coefficients for the xylose oligomers were between 0.9998 and 0.9980, the R.S.D.s were less than 4.6%.

Optimum conditions for detection of monosaccharides

Fucose, galactosamine, glucosamine, galactose, glucose, mannose, xylose and arabinose are common monosaccharides in glycoconjugates of biological samples. The determination of these sugars is of fundamental importance in nutrition, medical

TABLE II

DISSOCIATION CONSTANTS OF MONOSACCHARIDES (IN WATER AT 25°C)[a]

Sugar	pK_a
Arabinose	12.34
Galactose	12.35
Glucose	12.28
Xylose	12.15
Mannose	12.08

[a] From *Lange's Handbook of Chemistry*, 13th ed.

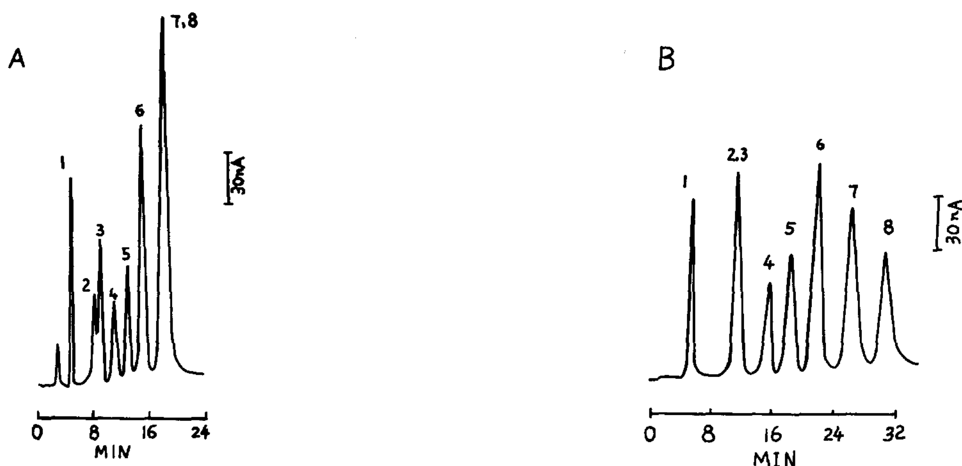

Fig. 3. Chromatogram of the monosaccharides. Peaks: 1 = 0.3 ppm fucose 2 = 0.9 ppm galactosamine; 3 = 1.2 ppm arabinose; 4 = 1.2 ppm glucosamine; 5 = 1.2 ppm galactose; 6 = 3.0 ppm glucose; 7 = 3.0 ppm xylose; 8 = 3.0 ppm mannose. (A) Gradient programme A; (B) gradient programme B.

cell biology and biotechnology research. These sugars have similar structures, some of which are epimers. It has been difficult to separate them by conventional HPLC.

The hydroxyl groups of the monosaccharides have pK_a values in the range 12–14 [19], allowing ionization at alkaline pH and potential separation by anion-exchange chromatography (see Table II). The experiments showed that, with gradient programme A, the sugars were well resolved except mannose, which overlapped with xylose (see Fig. 3A). With gradient programme B, mannose was well separated from xylose, but galactosamine overlapped with arabinose (see Fig. 3B).

Fig. 3 and Table II show that the retention times of the separated monosaccharides increase as their pK_a values decrease. This suggests that the pK_a values have a high potential for chromatographic selectivity. Galactosamine and glucosamine are amino sugars, and the different functional groups between galN and galactose gave a good resolution. Glucosamine and glucose were of the same character.

TABLE III

LINEARITY, RELATIVE STANDARD DEVIATION AND DETECTION LIMITS FOR MONOSACCHARIDES

Compound	Linearity correlation coefficient	R.S.D. (%) (n = 5)	Detection limit (mg l^{-1})
Fucose	0.9998 (range 0.05–0.6 ppm)	2.22 (at 0.1 ppm level)	0.01
Galactosamine	0.9966 (range 0.06–1.0 ppm)	4.89 (at 0.3 ppm level)	0.06
Arabinose	0.9990 (range 0.02–0.8 ppm)	4.67 (at 0.4 ppm level)	0.05
Glucosamine	0.9958 (range 0.06–0.8 ppm)	4.00 (at 0.4 ppm level)	0.06
Galactose	0.9995 (range 0.03–1.2 ppm)	2.36 (at 0.4 ppm level)	0.05
Glucose	0.9993 (range 0.08–2.2 ppm)	4.48 (at 1.0 ppm level)	0.10
Xylose	0.9989 (range 0.08–2.5 ppm)	4.49 (at 1.0 ppm level)	0.15
Mannose	0.9998 (range 0.10–2.5 ppm)	4.00 (at 1.0 ppm level)	0.20

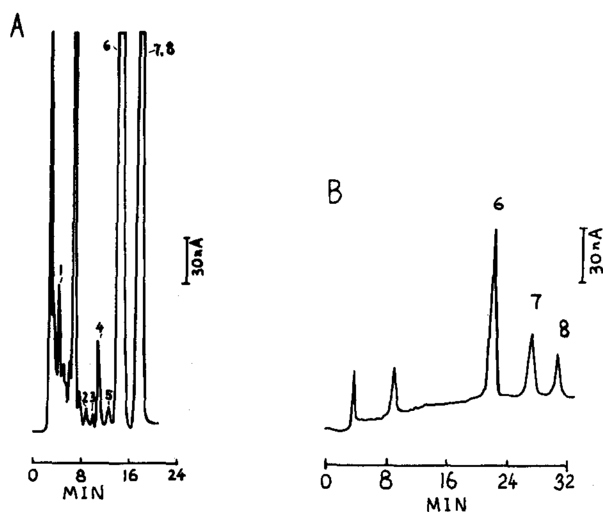

Fig. 4. Chromatogram for the analysis of a peat sample. Peaks: 1 = fucose; 2 = galactosamine; 3 = arabinose; 4 = glucosamine; 5 = galactose; 6 = glucose; 7 = xylose; 8 = mannose. (A) Gradient programme A; (B) gradient programme B.

Arabinose and galactose have the same pK_a values, but the ionic radius of arabinose is smaller that that of galactose. Is is clear that the latter has a greater affinity tot the resin than arabinose, so that its retention time was longer.

This experiment for detecting the sugars improved the detection limits by one to two orders of magnitude compared with HPLC [9], and ranged from 0.01 to 0.20 mg l^{-1} (see Table III). Table III also shows good linearity and reproducibility for the determination of the sugars.

Sample analysis

Peat is a new culture medium for cultivating bacteria, and measuring the sugar content of peat is important. Fig. 4 shows the chromatogram of the pretreated peat sample. Table IV gives the detection results. The sugars in the peat sample were numerous with large amounts of glucose, xylose and mannose.

TABLE IV

RESULTS FOR ANALYSIS OF PRETREATED PEAT SAMPLE

Compound	Concentration (mg l^{-1})	Compound	Concentration (mg l^{-1})
Fucose	0.36	Galactose	0.19
Galactosamine	0.20	Glucose	36.67
Arabinose	0.13	Xylose	34.00
Glucosamine	1.73	Mannose	38.57

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