Journal of Chromatography, 366 (1986) 412–416
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 760

Note

Separation of carbohydrates by high-performance liquid chromatography on porous pyridinium polymer columns

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(Received April 24th, 1986)

In the analysis of carbohydrates by partition high-performance liquid chromatography (HPLC), anion/cation-exchange resins[1–11] and silica gel bearing low-polarity functional groups[12–15] have been used as column packings. Previously, we have investigated the applicability of porous vinylpyridine and pyridinium polymers as column packings for reversed-phase and/or ion-exchange HPLC[16,17].

In this paper, an application of the vinylpyridinium polymers to the separation of carbohydrates by HPLC is described. The effects of counter ions and of the length of the alkyl chain in the pyridinium polymer were examined. The difference in functional groups between conventional anion-exchange resins and pyridinium polymers is expected to improve the retention behaviour of carbohydrates.

EXPERIMENTAL

Apparatus and materials

An Hitachi 633A high-performance liquid chromatograph was used together with a SF-1107 refractive index detector (Atto Co.). The specific surface area was measured with a Monosorb (Yuasa Battery Co.) surface area analyzer. All chemicals were of analytical or reagent grade and were used without further purification.

Preparation of column packings

Macroporous 4-vinylpyridine polymer cross-linked with 20% divinylbenzene (4VP) and N-methyl-4-vinylpyridinium bromide polymer [4VP-Me(I)] were prepared as described previously[17].

N-Butylpyridinium bromide polymer [4VP-Bu(I)]. A 25-ml volume of *n*-butyl bromide was added to 5 g of 4VP (particle size, 10–15 μm) and the mixture was refluxed for 18 h, then filtered and washed with methanol. This polymer contained 4.51% of nitrogen and 24.67% of bromine.

The bromide polymers were packed into stainless-steel columns (25 cm × 4 mm I.D.) and converted into the phosphate, sulphate or nitrate forms by pumping 1000 ml of 0.5 *M* phosphate buffer, sodium sulphate or sodium nitrate through the polymer bed, respectively. Unless stated otherwise, the phosphate forms were prepared with 0.5 *M* sodium dihydrogenphosphate (pH 4.3). The bed was washed with 2000 ml of water. The polymers converted into different ionic forms were packed into the analytical column (50 cm × 2.6 mm I.D.) using acetonitrile–water (4:1).

0021-9673/86/$03.50

Determination of water regain

The polymer particles (0.2–0.5 mm) were used in this experiment. The polymer was weighed after wetting thoroughly with water and centrifuging according to the procedure of Dieter and Walton[18]. The centrifuged polymer was dried to constant weight at 50°C under reduced pressure. The water regain was calculated from the difference in weight between the swollen and dried polymer.

Chromatography

Unless specified otherwise, the following conditions were used throughout. Acetonitrile–water (4:1) was used as the eluent at a flow-rate of 1 ml/min. The column was maintained at 70°C with external circulation. The sample solutions (200 mg/ml) were prepared in water and 2.5–10 μl were injected. The void volume of the system was determined from the peak due to water.

RESULTS AND DISCUSSION

The physical properties of the polymers used are summarized in Table I. The specific surface area and the water regain varied with the ionic forms. The retentions of carbohydrates on the various columns are shown in Table II. Although the capacity factors of the 4VP column are also shown, the retention is weaker than on the quaternized polymer columns. Among the different ionic forms of 4VP-Me polymer, the order of retention of carbohydrates is phosphate > sulphate > bromide > nitrate.

TABLE I

CHARACTERIZATION OF POLYMER COLUMNS

Polymer	*Ionic form*	*Specific surface area (m^2/g)*	*Water regain (g/g dry polymer)*	*Weight of polymer packed into a column (g)**
4VP	–	17.9	0.963	0.840
4VP-Me(I)	Bromide	11.0	0.990	1.137
4VP-Me(II)	Phosphate	12.6	1.101	1.055
4VP-Me(III)	Nitrate	14.3	1.002	1.057
4VP-Me(IV)	Sulphate	12.4	1.085	1.019
4VP-Bu(I)	Bromide	13.8	0.870	1.107
4VP-Bu(II)	Phosphate	14.2	1.005	1.090

* Column: 50 cm × 2.6 mm I.D.

Smaller k' values for the carbohydrates were observed on the 4VP-Bu polymer than on the 4VP-Me polymer in both bromide and phosphate forms. The retention of carbohydrates on 4VP-Bu(I) is very weak and size-exclusion behaviour was observed; the disaccharides were eluted before the monosaccharides. In a comparison of the bromide form and the phosphate form, the effect of the counter ion on 4VP-Bu is stronger than that on 4VP-Me.

The elution order of carbohydrates on the several ionic forms of the polymers, except for 4VP-Bu(I), is deoxyhexose < aldopentose $\leqslant$ ketohexose < aldohexose

TABLE II

EFFECT OF COUNTER IONS IN ALKYLPYRIDINIUM POLYMERS ON THE CAPACITY FACTOR, k', OF VARIOUS CARBOHYDRATES

Compound	*4VP-Me*				*4VP-Bu*		*4VP*
	I	*II*	*III*	*IV*	*I*	*II*	
Fucose	0.90	1.74	0.64	1.69	0.28	1.71	0.35
Ribose	1.03	2.56	0.73	2.26	0.43	2.45	0.39
Arabinose	1.42	3.89	0.96	3.41	0.49	3.21	0.39
Fructose	1.43	4.71	0.89	3.93	0.46	3.92	0.43
Xylose	1.47	4.96	0.98	4.40	0.57	4.49	0.46
Sorbose	1.52	5.57	0.97	4.82	0.50	4.47	0.44
Mannose	1.89	6.38	1.12	5.60	0.59	4.91	0.43
Galactose	2.10	8.33	1.28	7.25	0.62	6.20	0.41
Glucose	2.19	10.04	1.28	8.28	0.64	7.29	0.46
Sucrose	2.19	13.62	1.13	10.20	*	8.83	0.45
Lactose	2.59	13.62	1.40	10.68	*	7.93	0.39
Maltose	2.55	18.06	1.39	14.00	*	11.50	0.53

* The peak of the compound overlapped with the solvent peak.

< disaccharide. D'Amboise *et al.*[7] reported the elution order fucose = ribose < xylose < arabinose < fructoise < galactose in HPLC on an anion-exchange column (Hitachi 3013N, phosphate form). No significant difference in the order of elution of aldopentose, ketohexose and aldohexose is observed between the present columns and the Hitachi 3013N column, but the elution order of the aldopentoses was different. Although the peaks of fucose and ribose were not separated on the Hitachi 3013N column, complete separation of these compounds was achieved on the phosphate form of the 4VP-Me polymer.

Among the various ionic forms of the 4VP-Me polymers, the phosphate form showed the best resolution of carbohydrates (Table III). The effect of the pH of the

TABLE III

PEAK RESOLUTION (R_s) OF SELECTED CARBOHYDRATES ON VARIOUS COLUMNS

R_s is defined as the ratio of the distance between the maxima of two adjacent peaks and the arithmetic mean of their base widths: $R_s = 2(t_{R2} - t_{R1})/(w_1 + w_2)$.

Compounds	*4VP-Me*				*4VP-Bu*		*4VP*
	I	*II*	*III*	*IV*	*I*	*II*	
Fucose–ribose	0.24	1.46	0.23	0.46	0.31	0.89	0.09
Ribose–arabinose	0.86	2.30	0.60	1.51	0.16	0.88	0.02
Arabinose–xylose	0.15	1.55	0.05	0.96	0.17	1.39	0.16
Xylose–mannose	0.73	1.63	0.31	1.07	0.06	0.37	0.08
Mannose–galactose	0.33	1.80	0.29	1.06	0.05	0.90	0
Fructose–glucose	1.14	4.89	0.79	3.00	0.34	2.39	0.04
Glucose–sucrose	0.05	1.36	0.22	0.63	–	0.72	0.02

TABLE IV

EFFECT OF pH OF PHOSPHATE BUFFER USED FOR CONVERSION OF 4VP-Me INTO THE PHOSPHATE FORM ON k' OF SELECTED CARBOHYDRATES

Compound	*pH*			
	4.3	*6.0*	*7.0*	*8.0*
Fucose	1.74	1.82	2.34	3.03
Ribose	2.56	2.59	3.12	4.10
Arabinose	3.89	3.90	4.83	6.50
Xylose	4.96	5.07	6.30	8.18
Mannose	6.38	6.44	7.89	11.24
Galactose	8.33	8.43	10.91	14.88

phosphate buffer used for conditioning the 4VP-Me polymer on the k' of carbohydrates was investigated (Table IV). Increasing pH resulted in an increase in k' and good peak resolution. Sufficient peak resolutions and rapid elution of carbohydrates were obtained at pH 4.3. Mopper and Degens[4] stated that the chromatographic separation of carbohydrates was attributable to the partition of water between the polymer and the mobile phase. This statement shows that the hydrophilicity of the polymer may affect the retention of carbohydrates. Among the different ionic forms of the same pyridinium polymer, a linear relationship exists between the water regain of the polymer and the k' value per gram of packing material (Fig. 1).

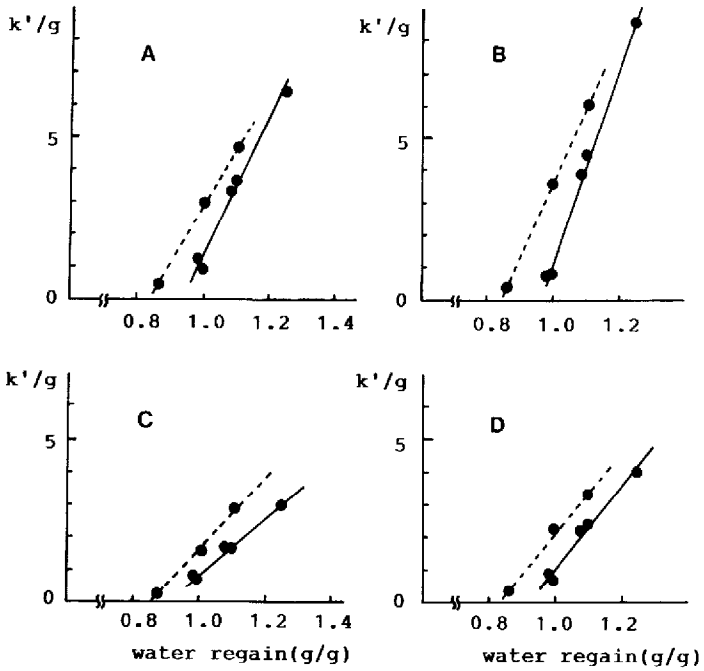

Fig. 1. Relationship between the water regain of the polymers and k' per gram of polymer. Polymers: ●—●, 4VP-Me; ●---●, 4VP-Bu. Carbohydrates: (A) arabinose; (B) fructose; (C) fucose; (D) ribose.

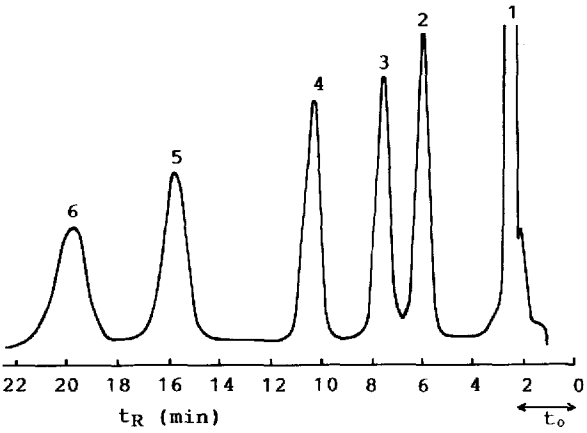

Fig. 2. A typical chromatogram of carbohydrates on a column of 4VP-Me(II) polymer. Peaks: 1 = solvent; 2 = fucose; 3 = ribose; 4 = arabinose; 5 = mannose; 6 = galactose.

Fig. 2 shows the separation of a mixture of several carbohydrates by use of the 4VP-Me(II) column. The carbohydrates were eluted rapidly as on the Hitachi 3013N column, and the good resolution of fucose and ribose was characteristic.

It is concluded that the length of the alkyl group and the ionic forms of the pyridinium polymer affect the retention of carbohydrates. Among the columns tested, the best separation was attained with the phosphate form of the 4VP-Me polymer.

ACKNOWLEDGEMENT

The authors thank Miss E. Sekiguchi for technical assistance.

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