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High-performance liquid chromatographic separation of carbohydrates on stationary phases prepared from polystyrene-based resin and tertiary amines
Effect of chemical structure of anion-exchange sorbents

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Abstract

Two types of new anion-exchange stationary phases were readily prepared by the reaction of porous particles of chloromethylated styrene–divinylbenzene copolymer with various amines. The stationary phases M_n, where n is the length of alkyl chain, were obtained by the reaction with *N*,*N*-dimethylalkylamines. The reaction with *N*,*N*,*N*′,*N*′-tetramethyldiaminoalkanes also afforded the stationary phases D_n with a terminal functional group, where n is the length of methylene bridge. The HPLC separations of monosaccharides, disaccharides and oligosaccharides were successfully performed on these stationary phases using electrochemical detection with a Ni–Ti alloy working electrode in alkaline eluents. The effects of chemical structures of stationary phases on the separation of carbohydrates are described.

Keywords: Stationary phases, LC; Carbohydrates

1. Introduction

The separation and sensitive detection of carbohydrates and their derivatives has become a subject of great interest because of their important role in a wide range of biological processes [1]. However, many of the analytical methodologies developed for determining carbohydrates by high-performance liquid chromatography (HPLC) have difficulties due to the complexity and physical properties of the analytes [2]. For example, carbohydrates lack chromophoric groups absorbing in the normal UV–visible range, and thus it is necessary to derivatize carbohydrates in order to use optical methods. Carbohydrates also exhibit relatively low sensitivity on a differential refractive index detector. On the other hand, electrochemical detection (ED) of carbohydrates following liquid chromatographic separations has significant advantages over other detection methods.

The hydroxyl groups of carbohydrates are either partially or completely ionized under highly alkaline conditions, and the anodic response of the ionized carbohydrates is electrochemically detected. There-

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fore, derivatization with fluorometric or UV-absorbing reagents is not required when using electrochemical detection. Thus carbohydrates can be separated and detected by anion-exchange mechanisms and electroanalytical techniques with a variety of electrode materials [3–12]. Pulsed amperometric detection (PAD) with an Au working electrode has been used extensively in combination with several anion-exchange stationary phases [13], such as electrostatically latex-coated pellicular polymeric-based anion-exchange sorbents [14–17], macroporous poly(vinylbenzylchloride–divinylbenzene) with quaternary ammonium groups [14], a macroporous poly(*N*,*N*,*N*-trimethylammoniummethylstyrene–divinylbenzene) strong anion exchanger [18,19], and cross-linked poly(styrene–divinylbenzene) with trimethylammonium group [20]. In addition, ED with a Ni–Ti alloy working electrode has been recently reported as an effective and highly sensitive detector by Luo and Kuwana [9]. They applied this detector to the analysis of carbohydrate using electrostatically latex-coated pellicular polymeric-based anion-exchange sorbents.

However, these anion-exchange sorbents have been prepared by multi-step or complex procedures and therefore a convenient access to the anion-exchange sorbents has been required. Furthermore, as the anion-exchange sites trimethylammonium group has mainly be used and no systematic studies on quaternary ammonium anion-exchange sorbents have been published. In a recent communication we have reported that novel anion-exchange stationary phases with a terminal functional group were easily prepared by the reaction of chloromethylated styrene–divinylbenzene copolymer with diamines [$(CH_3)_2N–(CH_2)_n–N(CH_3)_2$], and HPLC separations of monosaccharides and disaccharides have been achieved using the stationary phases coupled with PAD [21].

Here we detail the separation of carbohydrates with two types of new anion-exchange resins prepared by quaternization of a series of monoamines and diamines with porous particles of chloromethylated styrene–divinylbenzene copolymer using ED with a Ni–Ti alloy electrode. The effect of the chemical structures of the stationary phases on the separation of carbohydrates is described.

2. Experimental

2.1. Materials and methods

All amines for the preparation of stationary phases were purchased from Tokyo Kasei (Tokyo, Japan) and used without further purification. Porous particles of chloromethylated styrene–divinylbenzene copolymer (diameter, 5 μm; pore size, 270 Å; divinylbenzene, 54%) were supplied by Nishio Industry (Tokyo, Japan). Carbohydrates were purchased from Sigma (St. Louis, MO, USA), Wako (Osaka, Japan) and Tokyo Kasei.

Stationary phase structural information was obtained using solid-state cross polarization magic angle spinning (CP-MAS) ^{13}C nuclear magnetic resonance (NMR) spectrometry. Spectra were measured with a JEOL EX 270 spectrometer operating at 270 MHz with a spinning rate of 5.95 KHz, a ^{1}H 90° pulse of 4 μs, a contact time of 2 ms, a repetition time of 10 s and 1000 scans.

The HPLC experiments were performed on a Shimadzu LC-10AD Pump (Kyoto, Japan) with a Chratec VI-501PS Electro Chemical Detector (Kyoto, Japan) consisting of an amperometric flow-through cell with a Ni–Ti alloy working electrode and a silver–silver chloride reference electrode. The Ni–Ti alloy wires [Ni–Ti: NI205100 (55:45, w/w), 0.8 mm diameter] were purchased from Goodfellow (Cambridge, UK). The Ni–Ti working electrode was made by embedding the Ni–Ti wire into a PTFE block fitted to a Chratec flow cell. The optimal detection potential for the Ni–Ti electrode is 500 mV in 0.1 *M* NaOH mobile phase [9]. Samples were injected by using a non-metal Rheodyne (Cotati, CA, USA) Model 9125 injection valve.

2.2. Preparation of stationary phases

The syntheses of anion-exchange stationary phases are shown in Fig. 1. The porous particles of the polymer were dispersed in 5-fold (v/w) of 20% aqueous solution of the corresponding amine by sonication for about 10 min. The mixture was heated at 60°C for 4 h under swirling, and then slowly cooled to room temperature. The product was filtered on a sintered-glass filter and washed with water, 6 *M*

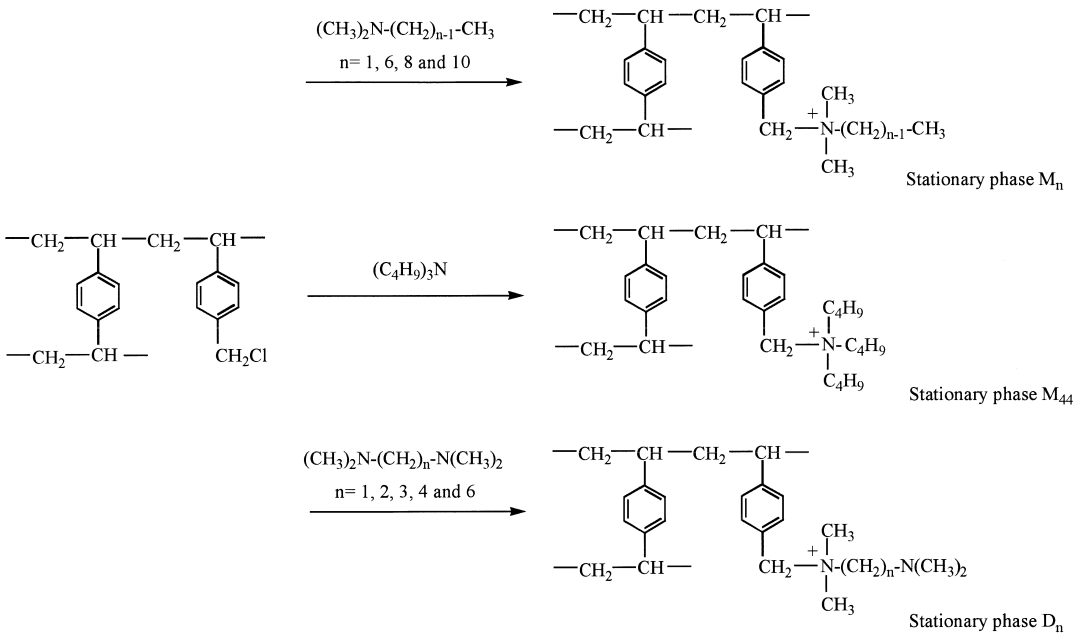

Fig. 1. Synthesis of anion-exchange stationary phases M_n~D_n.

HCl and water. After drying under vacuum, stationary phases M_1~D_6 were obtained. The results of elemental analysis and the nitrogen content of stationary phases M_1~D_6 are shown in Table 1. Elemental analysis were performed by the Microanalysis Department at Tohoku University.

2.3. HPLC columns and procedures

The stationary phases were suspended in 50 ml of water obtained from a Millipore Milli-Q system (Millipore, Bedford, MA, USA), sonicated for 5 min and packed into a 250×4.6 mm I.D. polyether ether

Table 1
Elemental analysis of stationary phases M_1~D_6

Stationary phase	Amine	Elemental analysis			Calculations for the amine (based on N) (mmol/g)
		C (%)	H (%)	N (%)	
M_1	$N(CH_3)_3$	71.35	8.42	2.61	1.86
M_6	$N(CH_3)_2$–$(CH_2)_5$–CH_3	74.68	8.25	1.83	1.31
M_8	$N(CH_3)_2$–$(CH_2)_7$–CH_3	74.23	8.65	1.87	1.33
M_{10}	$N(CH_3)_2$–$(CH_2)_9$–CH_3	75.11	8.46	1.61	1.15
M_{44}	$N(C_4H_9)_3$	77.30	7.90	0.77	0.55
D_1	$N(CH_3)_2$–CH_2–$N(CH_3)_2$	77.26	8.52	2.70	0.96
D_2	$N(CH_3)_2$–$(CH_2)_2$–$N(CH_3)_2$	74.49	8.19	4.02	1.44
D_3	$N(CH_3)_2$–$(CH_2)_3$–$N(CH_3)_2$	75.63	8.60	3.16	1.13
D_4	$N(CH_3)_2$–$(CH_2)_4$–$N(CH_3)_2$	72.07	8.85	2.98	1.06
D_6	$N(CH_3)_2$–$(CH_2)_6$–$N(CH_3)_2$	70.43	8.72	2.69	0.96

ketone (PEEK) columns (Nishio Industry) using 0.1 *M* NaOH as the mobile phase at a constant pressure of 100 kg/cm^2 by a Shimadzu LC-10AD Pump.

Eluents of 0.1 *M* NaOH were prepared by the dilution of a 50% (w/w) stock NaOH solution with Milli-Q water. All mobile phases were deaerated by dispersed helium. This prevents absorption of carbon dioxide and subsequent formation of carbonate ion which would act as displacing ion and shorten retention times.

After each run these columns were eluted with 0.1 *M* NaOH at 1.0 ml/min for 20 min for cleaning and reequilibrated to the starting conditions.

3. Results and discussion

3.1. Characterization of columns

We have prepared two types of new anion-exchange stationary phases M_n and D_n. The stationary phases M_n, where *n* is the length of alkyl chain, were prepared by quaternization with *N*,*N*-dimethylalkylamine. The stationary phase M_{44} was also prepared from tributylamine. The stationary phases D_n with a terminal functional group, where *n* is the length of methylene bridge, were obtained from *N*,*N*,*N*′,*N*′-tetramethyldiaminoalkanes. The chloromethyl group of the copolymer was easily reacted with the amines to afford the corresponding quaternary ammonium salts.

The presence of ammonium groups in stationary phases were confirmed by elemental analysis and solid-state ^{13}C CP-MAS NMR spectrometry. The ^{13}C spectrum of the stationary phase D_6 clearly shows the appearance of new peaks between 20 and 70 ppm compared with the starting copolymer, as indicated in Fig. 2. The ^{13}C NMR spectra of benzyldimethylalkyl ammonium chloride in C^2HCl_3 also shows peaks for aliphatic carbons between 20 and 70 ppm [22]. These results support the presence of quaternary ammonium groups in the stationary phase D_6.

The columns packed with these stationary phases can be used for over six months with 0.1 *M* NaOH without loss of performance.

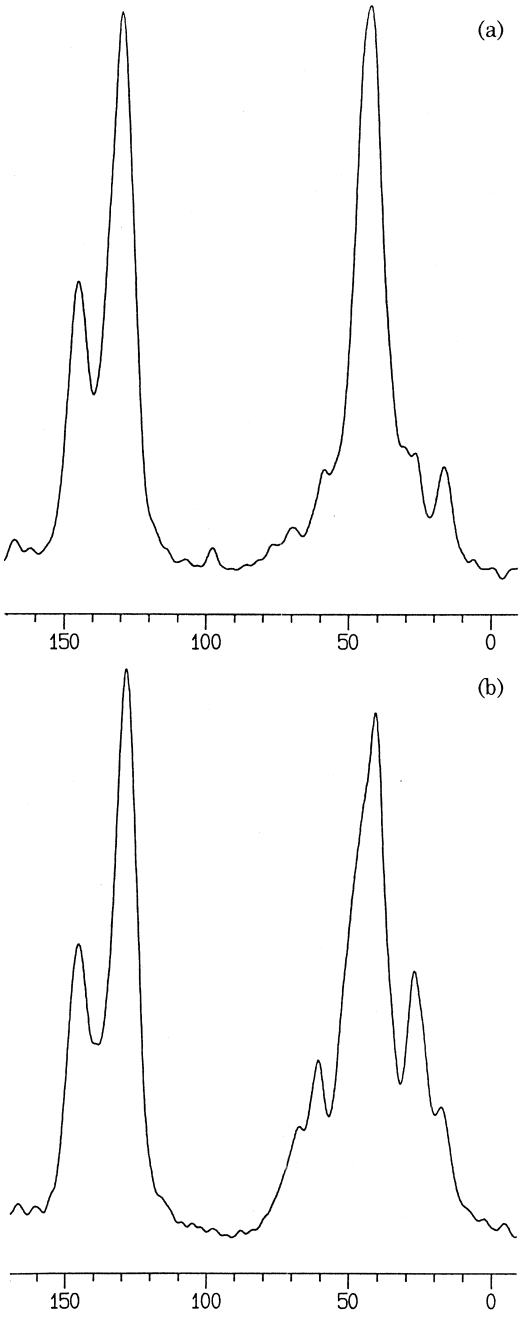

Fig. 2. ^{13}C CP-MAS NMR spectra of starting copolymer (a) and the stationary phase D_6 (b).

3.2. Separations of carbohydrates

The HPLC separations of standard monosac-

charides (sorbitol, fucose, mannose, glucose, galactose, fructose, allose, altrose and glucosamine) and disaccharides (trehalose, lactose, cellobiose and maltose) were successfully performed on these stationary phases using ED with Ni–Ti alloy electrode in 0.1 *M* NaOH. A typical chromatogram illustrating the separation of these monosaccharides is shown in Fig. 3. The capacity factors (k') of various monosaccharides and disaccharides on stationary phases M_n~D_n are shown in Table 2.

At high pH, carbohydrates are at least partially ionized and thus can be separated by anion-exchange mechanisms. Table 2 shows that k' values of glucose (pK_a 12.28 [14]) are smaller than those of fructose (pK_a 12.03). The reduced sugar such as sorbitol (pK_a 13.60) resulted in less retention time due to the lack of acidic anomeric hydroxyl group. Carbohydrates with fewer hydroxyl groups, such as fucose, also reduces k' values. Similar results were found in the amino sugar (glucosamine), in which an OH group of glucose is replaced with an amino group. These retention times are closely related to the pK_a values of carbohydrates [3,14,20]. However, other factors may also play a role in the separation process since k' values of mannose (pK_a 12.08) are smaller than those of glucose (pK_a 12.28).

As shown in Table 2 and Fig. 4, the k' values of all carbohydrates tend to increase as the length of carbon chains of stationary phases M_1~M_{10} increases. These results probably indicate that the increase of the hydrophobicity of the ion-exchange site resulted in the increase of k' values. Similar trends were found in the separation of monovalent anions by quaternary ammonium resins [23]. The k' values with the stationary phase M_{44} prepared from tributylamine, however, are found to be smaller than those with the stationary phase M_1 prepared from trimethylamine, probably because of steric effect of bulky tributylammonium group.

In contrast, the k' values obtained by using the stationary phases D_n with a terminal dimethylamino group dramatically increased as the length of methylene bridge increased (Table 2 and Fig. 5). However, the k' values with the stationary phases D_1, D_2 and D_3 were smaller than those with the stationary phase M_1. This result would be ascribable to the presence

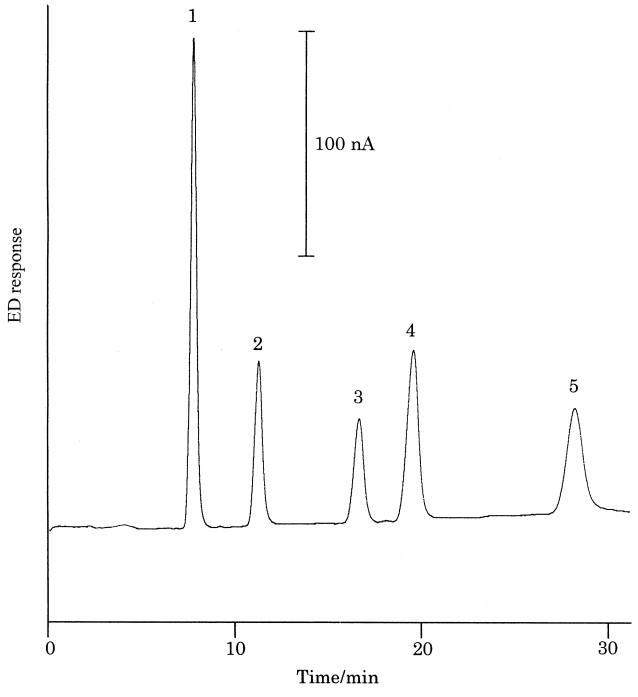

Fig. 3. Separation of monosaccharides on the stationary phase D_6. Sample: (1) sorbitol, (2) fucose, (3) mannose, (4) galactose, (5) allose; concentration, 1 nmol each; eluent, 0.1 *M* NaOH; flow-rate, 1.0 ml/min.

Table 2
Capacity factor (k') for various monosaccharides and disaccharides on stationary phases M_1~D_6 [a]

Carbohydrate	Stationary phase									
	M_1	M_6	M_8	M_{10}	M_{44}	D_1	D_2	D_3	D_4	D_6
Sorbitol	0.44	0.54	0.55	0.63	0.38	0.11	0.22	0.32	0.52	0.74
Fucose	0.99	1.15	1.21	1.49	1.60	0.50	0.70	0.72	1.11	1.41
Mannose	1.75	1.98	2.04	2.36	1.25	0.52	0.73	1.12	2.08	2.58
Glucose	1.84	2.16	2.31	2.61	1.40	0.55	0.78	1.15	2.09	2.84
Galactose	1.90	2.24	2.40	2.70	1.47	0.47	0.82	1.20	2.36	3.08
Fructose	2.21	2.73	2.78	3.24	2.02	0.74	1.02	1.49	2.87	3.86
Allose	2.72	3.21	3.23	3.87	2.15		1.17		3.88	4.18
Altrose	3.82	4.60	4.70	5.45	3.02		1.60		5.61	6.26
Glucosamine	1.57	1.73	1.75	1.94	1.37	0.48	0.72	0.98	1.60	1.88
Trehalose	0.67	0.84	0.93	1.16	0.49	0.20		0.32	0.80	0.89
Lactose	3.66	4.13	4.59	6.89	2.09	0.52		2.19	3.31	4.25
Cellobiose	4.13	5.09	7.60		3.24	1.17		2.99	5.80	7.01
Maltose		6.86			5.02	1.80		4.93	7.51	11.86

[a] HPLC conditions: eluent, 0.1 *M* NaOH; flow-rate, 1.0 ml/min; detection potential, +0.50 V vs. Ag/AgCl. The capacity factor is defined as follows: $k'=(V_A-V_0)/V_0$, where V_A is the retention volume of the analyte A and V_0 is the void volume of the column. The k' values are normalized to ion-exchange capacity 1.00 mmol/g (based on N).

of terminal dimethylamino group with high electron density, which would suppress the interaction between quaternary nitrogen and ionized carbohydrates. On the other hand, the effect of dimethylamino group would decrease as the spacer length between the quaternary nitrogen atom and terminal dimethylamino group increases and therefore, the k' values of the stationary phase D_4 turned out to be comparable to the values with the stationary phase M_6. It is interesting to note that the k'

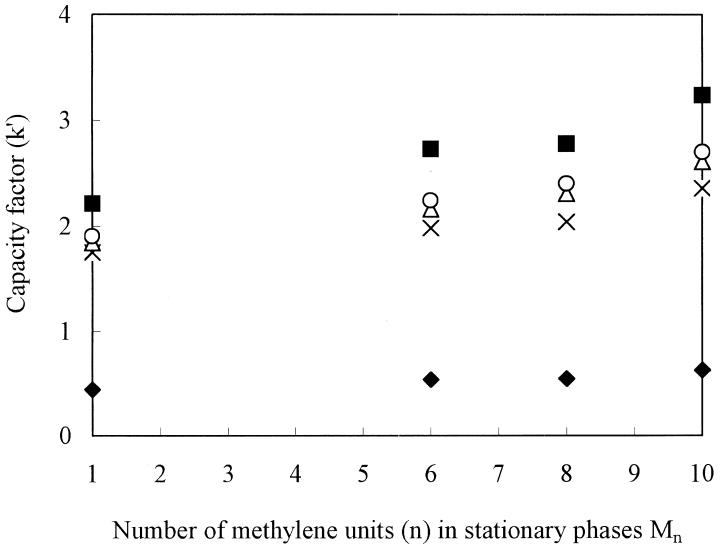

Fig. 4. Plots of capacity factors (k') as a function of the number of methylene units in stationary phases M_n. Eluent, 0.1 *M* NaOH; flow-rate, 1.0 ml/min. Samples: ♦=sorbitol; ×=mannose; △=glucose; ○=galactose; ■=fructose.

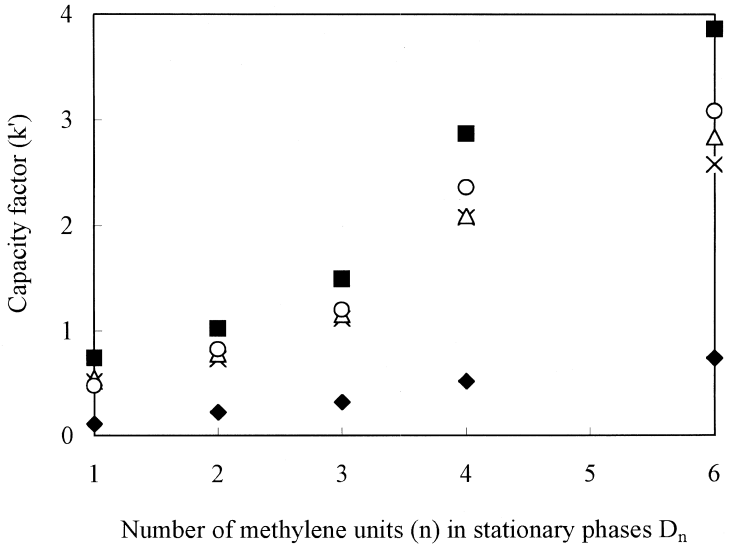

Fig. 5. Plots of capacity factors (k') as a function of the number of methylene units in stationary phases D_n. Eluent, 0.1 *M* NaOH; flow-rate, 1.0 ml/min. Samples: ♦=sorbitol; ×=mannose; △=glucose; ○=galactose; ■=fructose.

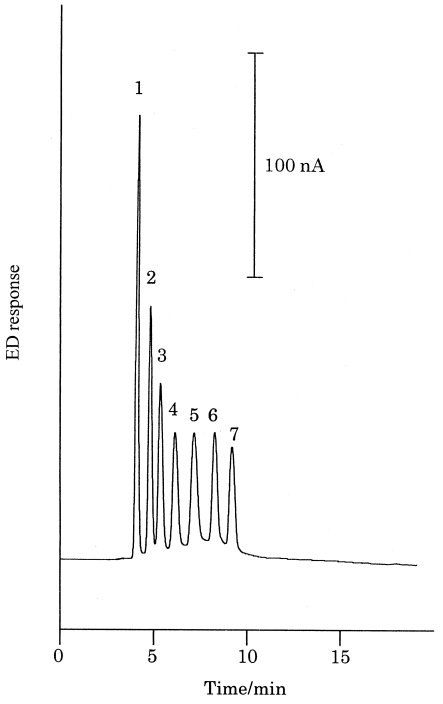

Fig. 6. Separation of oligosaccharides on the stationary phase D_6. Sample: (1) glucose, (2) maltose, (3) maltotriose, (4) maltotetraose, (5) maltopentaose, (6) maltohexaose, (7) maltoheptaose; concentration, 1 nmol each; eluent, A: 0.1 *M* NaOH–0.1 *M* AcONa, B: 0.1 *M* NaOH–0.5 *M* AcONa, 20–100% B in 10 min; flow-rate, 1.0 ml/min.

values of monosaccharides with the stationary phase D_6 are larger than those with the stationary phases M_8 and M_{10}.

Figs. 4 and 5 clearly show that the stationary phase D_6 is the most effective one out of ten stationary phases we have prepared. Therefore, the stationary phase D_6 would be a good candidate for separating maltooligosaccharides. Fig. 6 shows a good separation of maltooligosaccharides using linear gradient of sodium acetate in 0.1 *M* NaOH. The k' values for an homologous series of oligosaccharides increase in a regular manner with the number of glucose residue as follows: glucose (0.31), maltose (0.56), maltotriose (0.75), maltotetraose (1.02), maltopentaose (1.36), maltohexaose (1.72), maltoheptaose (2.03).

4. Conclusions

In conclusion, we have prepared a series of new anion-exchange stationary phases by the reaction of chloromethylated styrene–divinylbenzene copolymer with monoamines and diamines. The HPLC separation of carbohydrates were successfully performed on these readily prepared stationary phases. Further-

more, the chemical structures of stationary phases, especially terminal functional group, were found to affect the separation of carbohydrates.

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