High-performance liquid chromatographic separation of carbohydrates on a stationary phase prepared from polystyrene-based resin and novel amines

Takako Masuda[a,*], Kei-ichi Kitahara[a], Yoshihiro Aikawa[b], Sadao Arai[a]

[a]*Department of Chemistry, Tokyo Medical University, 6-1-1 Shinjuku, Shinjuku-ku, Tokyo 160-8402, Japan*
[b]*Faculty of Human Life, Ochanomizu University, 2-1-1 Otuka, Bunkyou-ku, Tokyo 112-0012, Japan*

Abstract

New anion-exchange stationary phases O_n ($n=1$, 2 and 3) with a dimethylamino terminal functional group, where n is the number of oxyethylene units $[-(CH_2CH_2O)_n-]$, were prepared by the reaction of chloromethylated porous styrene–divinylbenzene copolymer beads and amines $[(CH_3)_2N-(CH_2CH_2O)_nCH_2CH_2-N(CH_3)_2]$. HPLC separations of monosaccharides (sorbitol, fucose, glucosamine, mannose, glucose, galactose, fructose, allose and altrose) and disaccharides (trehalose, lactose, cellobiose and maltose) were performed successfully on these stationary phases. The ether group of the stationary phases O_n was found to affect the separation of carbohydrates. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Carbohydrates are very important species involved in many biological processes. The understanding of the structure–function relationships and the development of new synthetic methodologies for carbohydrates are receiving considerable interest [1]. Although the qualitative and quantitative analysis of carbohydrates is of great importance, the structural diversity of carbohydrates makes their analysis difficult. Under highly alkaline conditions, the hydroxyl groups of carbohydrates are either partially or completely ionized, thus the anodic response of ionized carbohydrates can be detected electrochemically, which has led to the high-performance liquid chromatographic (HPLC) separation of carbohydrates on

anion-exchange sorbents, followed by their electrochemical detection (ED) [2]. HPLC–ED with a variety of electrode materials is a powerful tool for the separation of small amounts of nonderivatized carbohydrates [3–6].

Most of the anion-exchange columns currently employed for the separations of carbohydrates are packed with electrostatically latex-coated, pellicular, polymer-based anion-exchange sorbents [7–9], which are prepared by multi-step or complex procedures, or macroporous poly(styrene–divinylbenzene) sorbents with trimethylammonium functional groups [10,11]. Recently, Ohkubo et al. reported a monodisperse polymer-based anion exchanger prepared via two-step swelling and polymerization followed by quaternization with triethylamine [12]. Analytical conditions, such as temperature, mobile phase pH, the addition of divalent cations to the mobile phase, etc., have been optimized to achieve

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*Corresponding author. Tel./fax: +81-3-3351-9069.
E-mail address: m-chem@tokyo-med.ac.jp (T. Masuda).

sensitive detection [11,13]. However, no systematic studies on anion-exchange sorbents with quaternary ammonium functional groups have been published.

We reported the synthesis of anion-exchange stationary phases M_n and D_n with quaternary nitrogen atoms prepared by the reaction of chloromethylated styrene–divinylbenzene copolymers with *N,N*-dimethylalkylamines (monoamines) and *N,N,N′,N′*-tetramethyldiaminoalkanes (diamines) (Fig. 1). HPLC separations of monosaccharides and disaccharides have been achieved by these stationary phases using ED with a Ni–Ti alloy electrode. The capacity factors (k') of various monosaccharides increases as the number of methylene units in the stationary phases M_n increased. The k' values obtained with stationary phases D_n which contained terminal dimethylamino groups increased dramatically as the number of methylene units increased [14,15], indicating that carbohydrate separations were greatly affected by the functional methylene groups of the sorbents.

In this paper, we describe the synthesis of a new type of anion-exchange stationary phase that contains oxyethylene units, and report the effects of the structure on the separation of carbohydrates.

2. Experimental

2.1. Materials

All carbohydrates were purchased from Sigma (St. Louis, MO, USA), Wako (Osaka, Japan) and Tokyo Kasei (Tokyo, Japan). Bis(2-chloroethyl)ether, 1,2-bis(2-chloroethoxy)ethane, tetraethyleneglycol, 1,1-dimethylhydrazine and other solvents were from Tokyo Kasei. 1,11-Dibromo-3,6,9-trioxaundecane was prepared according to a published procedure [16]. The porous beads of chloromethylated styrene–divinylbenzene copolymer (diameter, 5 μm; pore size, 270 Å; divinylbenzene content, 54%) were supplied by Nishio Industry (Tokyo, Japan).

2.2. Equipment

The HPLC experiments were performed using a Shimadzu LC-10AD pump (Kyoto, Japan) with a Chratec VI-501PS electrochemical 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
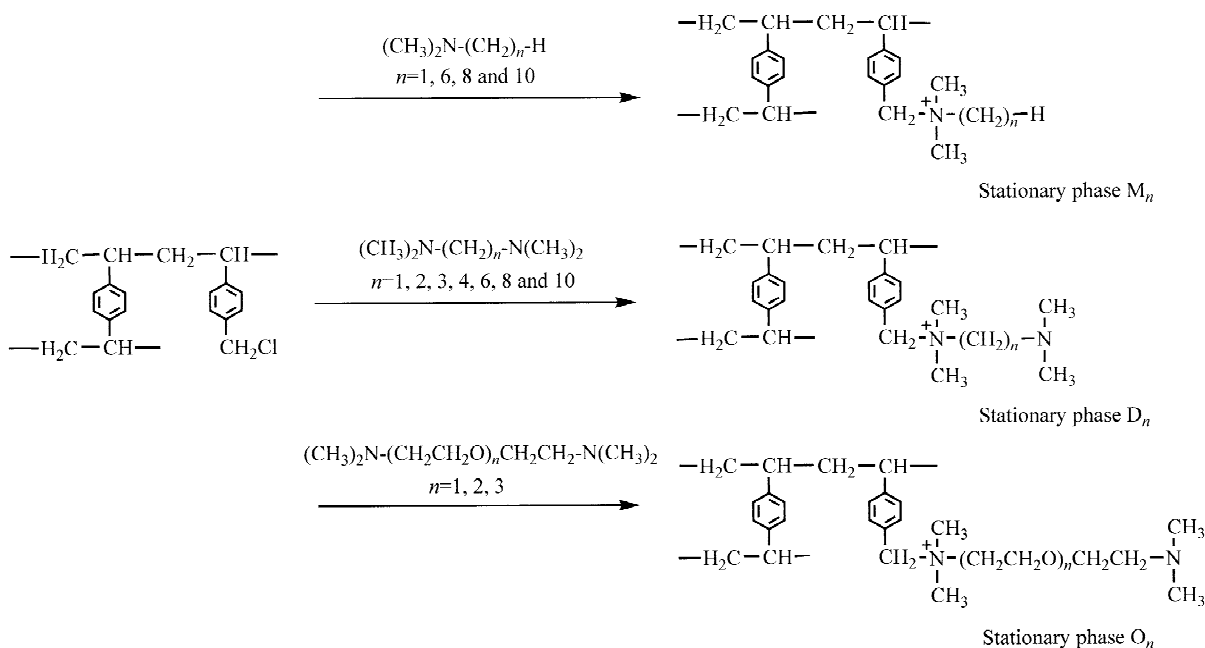

Fig. 1. Synthesis of anion-exchange stationary phases M_n, D_n and O_n.

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 (Kyoto, Japan) flow cell. The optimal detection potential for the Ni–Ti electrode in 100 mM NaOH mobile phase is 500 mV [4]. Samples were injected using a non-metal Rheodyne (Cotati, CA, USA) Model 9125 injection valve.

^{1}H NMR (300 MHz, C[2]HCl$_3$) and ^{13}C NMR (75 MHz, C[2]HCl$_3$) spectra were recorded on a JOEL JNM-ECP 300. Chemical shifts are reported as δ values with respect to tetramethylsilane (TMS) as internal standard. Atmospheric pressure chemical ionization (APCI) mass spectra were obtained on an Hitachi M-1000 mass spectrometer. The amines were distilled on a Kugelrohr apparatus (Sibata glass tube oven GTO 350RG), and the reported temperatures are uncorrected.

2.3. Preparation of $(CH_3)_2N(CH_2CH_2O)_nCH_2CH_2N(CH_3)_2$

The synthetic route for the preparation of tertiary amines is shown in Fig. 2 [17]. A mixture of the appropriate halide (0.05 mol) and 1,1-dimethylhydrazine (0.15 mol) in acetonitrile (100 ml) was refluxed for 6 h. The solvent was removed under reduced pressure to give a white solid, which was washed with diethyl ether (100 ml) and dried. The hydrazinium salt thus obtained was used fo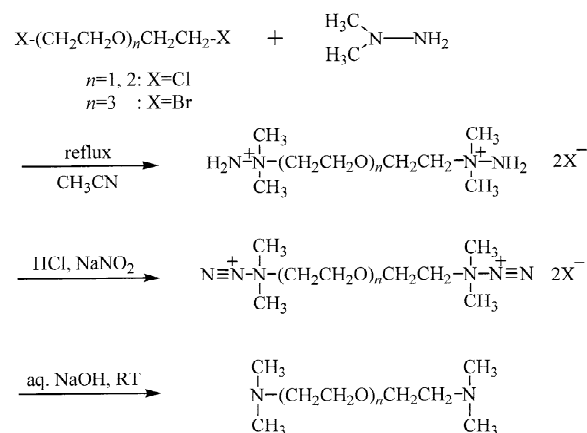r the following reaction without further purification. A suspension of the salt in 100 ml of 4 M HCl was stirred until the salt dissolved. A solution of NaNO$_2$ (0.72 mol) in 250 ml water was added from a dropping funnel to the ice-cooled, magnetically stirred aqueous solution over a period of 30 min. Vigorous gas evolution was observed, and stirring was continued overnight. The reaction mixture was made strongly basic by the addition of 50% NaOH (30 ml) to the cooled solution. The mixture was extracted with diethyl ether (2×100 ml) and evaporated. The residue was purified by distillation with a Kugelrohr apparatus.

2.3.1. $(CH_3)_2N–CH_2CH_2OCH_2CH_2–N(CH_3)_2$
Yield 5.9 g (73%), b.p. 120 °C/2 mmHg (76 °C/10 mmHg [18]; 1 mmHg=133.322 Pa). APCI–MS: 161 (MH[+]). ^{1}H NMR (300 MHz, C[2]HCl$_3$): δ=3.54 (t, J = 6 Hz, 4H), 2.50 (t, J = 6 Hz, 4H), 2.26 (s, 12H). ^{13}C NMR (75 MHz, C[2]HCl$_3$): δ = 69.07, 58.78, 45.81.

2.3.2. $(CH_3)_2N–(CH_2CH_2O)_2CH_2CH_2–N(CH_3)_2$
Yield 6.0 g (58%), b.p. 125 °C/2 mmHg (83 °C/2 mmHg [19]). APCI–MS: 205 (MH[+]). ^{1}H NMR (300 MHz, C[2]HCl$_3$): δ=3.62 (s, 4H), 3.58 (t, J = 6 Hz, 4H), 2.51 (t, J = 6 Hz, 4H), 2.26 (s, 12H). ^{13}C NMR (75 MHz, C[2]HCl$_3$): δ=70.34, 69.32, 58.79, 45.85.

2.3.3. $(CH_3)_2N–(CH_2CH_2O)_3CH_2CH_2–N(CH_3)_2$
Yield 6.3 g (72%), b.p. 170 °C/2 mmHg (92 °C/0.17 mmHg [20]). APCI–MS: 249 (MH[+]). ^{1}H NMR (300 MHz, C[2]HCl$_3$): δ=3.64–3.62 (m, 8H), 3.57 (t, J = 6 Hz, 4H), 2.50 (t, J = 6 Hz, 4H), 2.26 (s, 12H). ^{13}C NMR (75 MHz, C[2]HCl$_3$): δ=70.57, 70.34, 69.34, 58.82, 45.86.

2.4. Synthesis of stationary phases

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

Fig. 2. Synthesis of tertiary amines.

under vacuum, stationary phases O_1, O_2 and O_3 were obtained. Elemental analyses were performed by the Microanalysis Department at Tohoku University and yielded the following results: stationary phase O_1 [found: C, 72.63; H, 8.00; N, 2.55%; calculated for the diamine: 0.91 mmol/g (based on N)]; stationary phase O_2 [found: C, 71.17; H, 8.20; N, 2.20%; calculated for the diamine: 0.79 mmol/g (based on N)]; stationary phase O_3 [found: C, 67.36; H, 7.95; N, 2.04%; calculated for the diamine: 0.73 mmol/g (based on N)].

2.5. HPLC columns and procedures

The stationary phases were suspended in 50 ml 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 ketone (PEEK) column using 100 mM NaOH as the mobile phase at a constant pressure of 200 kg/cm^2 by a Shimadzu LC-10AD pump (Kyoto, Japan).

The sodium hydroxide solutions 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.

After each run, the columns were cleaned by elution with 100 mM NaOH at 1.0 ml/min for 20 min, and re-equilibrated to the starting conditions.

3. Results and discussion

3.1. Characterization of columns

The new anion-exchange stationary phases O_n ($n = 1$, 2 and 3) with terminal dimethylamino groups, where n is the number of oxyethylene units [$-(CH_2CH_2O)_n-$], were obtained from the reaction of chloromethylated styrene–divinylbenzene copolymer and amines [$(CH_3)_2N-(CH_2CH_2O)_nCH_2CH_2-N(CH_3)_2$] (Fig. 1). The chloromethyl group of the copolymer reacted readily with the amines affording the corresponding quaternary ammonium salts. Elemental analysis showed that the nitrogen content of the sorbents (O_1, O_2 and O_3) was 0.73–0.91 mmol/g.

Columns packed with these stationary phases could be used for over 6 months with strong alkaline

eluents without any loss of sensitivity or reproducibility.

3.2. Separations of carbohydrates

As shown in Table 1, carbohydrates have pK_a values in the range 12–14. Because of these subtle differences in the pK_a values of the hydroxyl groups, the oxyanions of carbohydrates generated under highly alkaline conditions interact with the positively charged stationary phase and facilitate their separation by anion-exchange mechanisms.

Fig. 3 shows a typical chromatogram of eight common carbohydrates using stationary phase O_1 and 100 mM NaOH as the eluent. The capacity factors (k') of the various monosaccharides (sorbitol, fucose, glucosamine, mannose, glucose, galactose, fructose, allose and altrose) and disaccharides (trehalose, lactose, cellobiose and maltose) on stationary phases O_1, O_2 and O_3 are shown in Table 1, along with the previously reported k' values on stationary phases M_6–D_{10} [21].

The elution orders of carbohydrates with stationary phases O_1, O_2 and O_3 are similar to stationary phases M_6–D_{10}. The k' values for these stationary phases are related to the pK_a values of the analytes. The reduced k' value of sorbitol (pK_a 13.60) compared to glucose (pK_a 12.28) can be readily understood on the basis of their acidities. Likewise, the loss of hydroxyl groups, such as in fucose, reduces the k' value. This also applies to amino sugars (e.g., glucosamine), in which an OH group is replaced by an amino group.

Glucose was eluted from stationary phases O_1, O_2 and O_3 with mobile phases at various alkaline concentrations, from 20 to 100 mM. As shown in Fig. 4, the k' values of these stationary phases decreased with increasing sodium hydroxide concentration. For stationary phases M_8, D_8 and O_2, which have the same chain length, the NaOH dependence of the k' values was similar (Fig. 5). These results indicate that, at higher pH, the carbohydrates become more ionized and their interaction with the stationary phases increases. However, the elution sequence (in the order mannose, glucose, galactose) is different from the pK_a sequence (in the order galactose, glucose, mannose), indicating that

Table 1
Capacity factors (k') for various monosaccharides and disaccharides on stationary phases M, D and O[a,b]

Carbohydrate	pK_a [9]	Stationary phase								
		O_1	O_2	O_3	M_6 [15]	M_8 [15]	M_{10} [15]	D_6 [15]	D_8 [21]	D_{10} [21]
Sorbitol	13.60	0.88	1.05	0.99	0.54	0.55	0.63	0.74	1.05	1.25
Fucose		1.96	2.19	2.12	1.15	1.21	1.49	1.41	2.20	2.59
Glucosamine		2.39	2.81	2.69	1.73	1.75	1.94	1.88	2.95	3.45
Mannose	12.08	3.32	3.81	3.66	1.98	2.04	2.36	2.58	3.48	4.15
Glucose	12.28	3.63	4.28	4.05	2.16	2.31	2.61	2.84	4.19	4.79
Galactose	12.39	3.81	4.41	4.22	2.24	2.40	2.70	3.08	4.35	5.04
Fructose	12.03	4.49	5.27	5.37	2.73	2.78	3.24	3.86	5.50	6.24
Allose		5.68	6.66	6.40	3.21	3.23	3.87	4.18	6.52	7.61
Altrose		8.17	9.63	9.12	4.60	4.70	5.45	6.26	9.56	11.30
Trehalose		1.20	1.42	1.20	0.84	0.93	1.16	0.89	1.45	1.74
Lactose	11.98	6.30	7.34	8.12	4.13	4.59	6.89	4.25	7.69	8.47
Cellobiose		10.42	12.38	11.44	5.09	7.60		7.01	13.16	14.79
Maltose	11.94	18.30	21.86	19.83	6.86			11.86	22.96	26.26

[a] HPLC conditions: eluent, 100 mM NaOH; flow-rate, 1.0 ml/min; detection potential, $+0.50$ V vs. Ag/AgCl.

[b] The capacity factor is defined as $k' = (V_A - V_0)/V_0$, where V_A is the retention volume of analyte A and V_0 is the void volume of the column. The k' values are normalized to an ion-exchange capacity of 1.00 mmol/g (based on the N content).
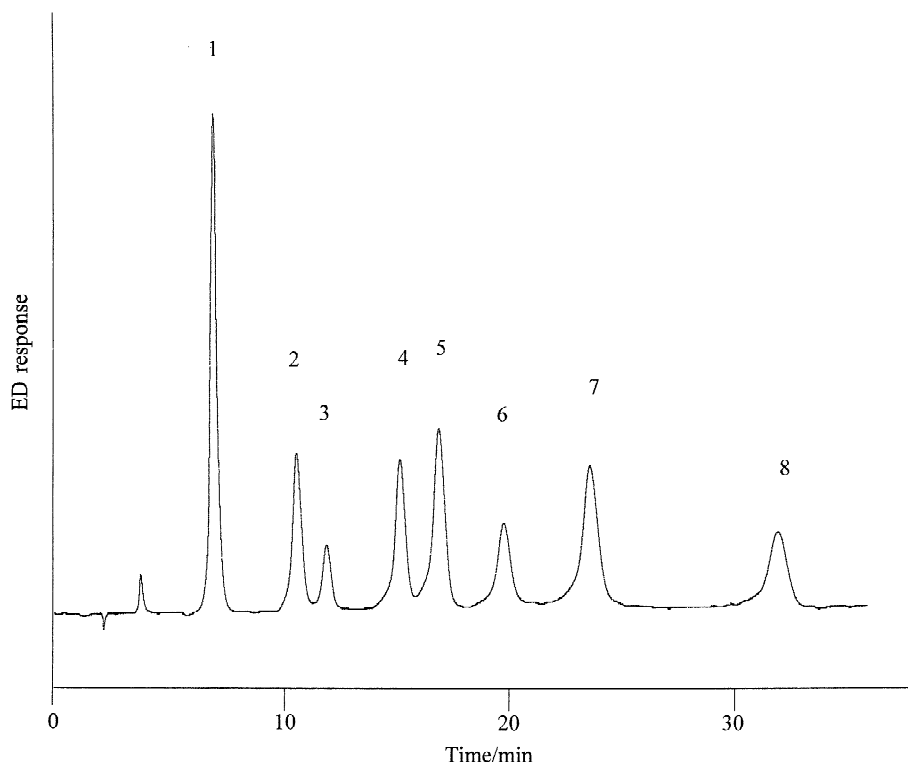

Fig. 3. Separation of monosaccharides on stationary phase O. Sample: (1) sorbitiol, (2) fucose, (3) glucosamine, (4) mannose, (5) galactose, (6) fructose, (7) allose, (8) altrose. Concentration, 0.28 mM each; eluent, 100 mM NaOH; flow-rate, 1.0 ml/min.
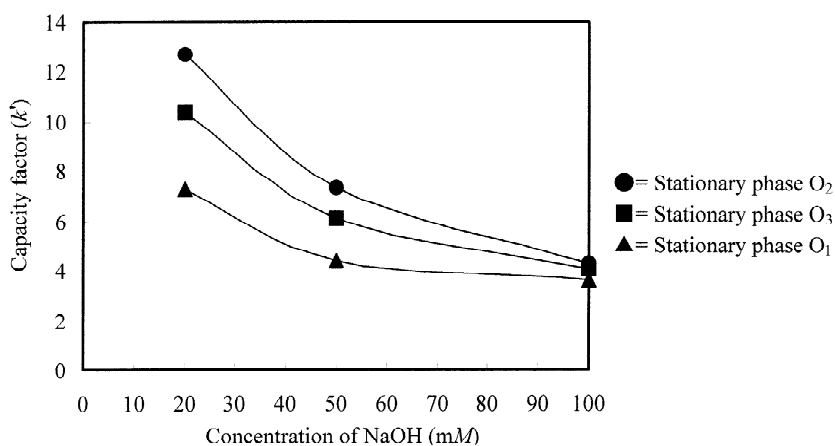
Fig. 4. Plots of glucose k' values as a function of the concentration of sodium hydroxide in the eluent on stationary phases O_1, O_2 and O_3. Flow-rate, 1.0 ml/min.

other factors probably also play a role in this separation process [2].

As shown in Fig. 6, k' values on stationary phases M_n gradually increase as the chain length increases. In the case of stationary phases D_n which carry terminal dimethylamino groups, k' values increase dramatically. On the other hand, on stationary phase O_1, which has both a terminal dimethylamino group and an ether group, k' values are larger than on stationary phase D_6 (Table 1, Fig. 6). Interestingly,

k' values are largest on stationary phase O_2 and smaller on stationary phase O_3, indicating that interactions between the carbohydrates and stationary phases O_n are affected by the presence of the ether group.

It has been reported that polyethyleneglycol dimethyl ethers form coordination complexes with sodium ions and the complexation constants increase with increasing number of oxygen atoms in the chain [22]. Although stationary phase O_3 has only three
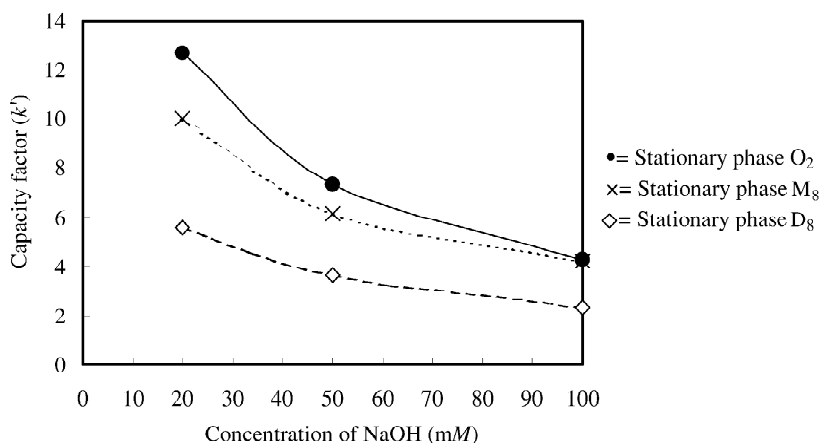

Fig. 5. Plots of glucose k' values as a function of the concentration of sodium hydroxide in the eluent on stationary phases O_2, M_8 and D_8. Flow-rate, 1.0 ml/min.
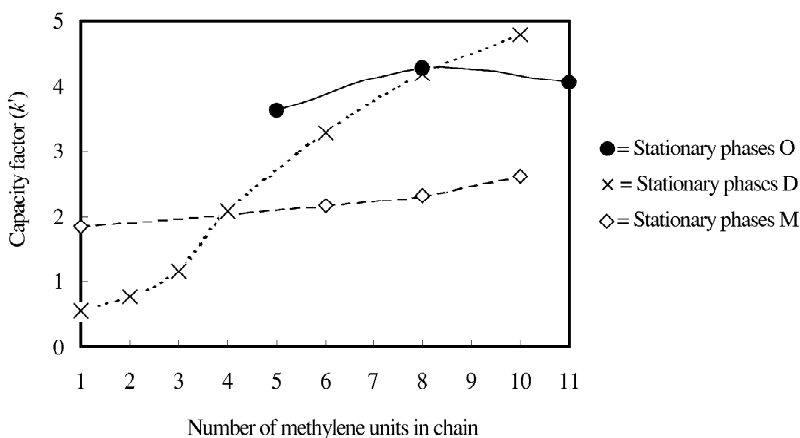
Fig. 6. Plots of glucose k' values as a function of the number of methylene units in the chain on stationary phases O_1, O_2 and O_3. Eluent, 100 mM NaOH; flow-rate, 1.0 ml/min.

oxygen atoms and a terminal tertiary nitrogen atom, it might be possible that, under high sodium hydroxide concentrations, stationary phase O_3 interacts with a sodium ion causing k' values on stationary phase O_3 to be slightly smaller than on stationary phase O_2.

4. Conclusions

In conclusion, we have prepared new anion-exchange stationary phases O_n which contain oxyethylene units and terminal dimethylamino groups by reacting porous chloromethylated styrene–divinylbenzene copolymer beads and amines $[(CH_3)_2N-(CH_2CH_2O)_nCH_2CH_2-N(CH_3)_2]$. HPLC separations of carbohydrates were successfully performed on these stationary phases. Contrary to our previously reported stationary phases M_n and D_n, stationary phases O_n with oxyethylene units show that the unique k' values depended on the chain length of the stationary phases.

Acknowledgements

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