Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1029 (2004) 239–247
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Capillary electrochromatography with monolithic silica columns III. Preparation of hydrophilic silica monoliths having surface-bound cyano groups: chromatographic characterization and application to the separation of carbohydrates, nucleosides, nucleic acid bases and other neutral polar species

Darin Allen, Ziad El Rassi*

Department of Chemistry, College of Arts and Sciences, 454A Physical Sciences, Oklahoma State University, Stillwater, OK 74078-3071, USA

Received 22 September 2003; received in revised form 25 November 2003; accepted 8 December 2003

Abstract

Two synthetic routes have been introduced and evaluated for the preparation of hydrophilic silica-based monoliths possessing surface-bound cyano functions. In one synthetic scheme, the silica monolith was reacted in a single step with 3-cyanopropyldimethylchlorosilane to yield a cyano phase referred to as CN-monolith. In a second synthetic route, the silica monolith was first reacted with γ-glycidoxypropyltrimethoxysilane (γ-GPTS), followed by a reaction with 3-hydroxypropionitrile (3-HPN) to give a stationary phase denoted CN–OH-monolith. Although the γ-GPTS was intended to play the role of a spacer arm to link the 3-HPN to the silica surface, this spacer arm became an integral part of the hydrophilic stationary phase. Thus, the CN–OH-monolith can be viewed as a double-layered stationary phase (i.e., stratified phase) with a hydroxy sub-layer and a cyano top layer. Due to its stronger hydrophilic character, the CN–OH-monolith yielded higher retention and better selectivity than the CN-monolith. The CN–OH-monolith was demonstrated in the normal-phase capillary electrochromatography (CEC) of various polar compounds including phenols and chloro-substituted phenols, nucleic acid bases, nucleosides, and nitrophenyl derivatives of mono- and oligosaccharides. The CN–OH-monolith yielded a relatively strong electroosmotic flow over a wide range of mobile phase composition, thus allowing rapid separation of the polar compounds studied.

Keywords: Electrochromatography; Monolithic columns; Carbohydrates; Nucleosides; Nucleic acid bases; Phenols; Chlorophenols

1. Introduction

Thus far, the vast majority of capillary electrochromatography (CEC) separations have been performed by a reversed-phase (RP) mode (for typical references see [1–11]) using non-polar stationary phases (e.g., C_{18} phases) and more polar hydro-organic mobile phases (e.g., acetonitrile–water, alcohol–water mixtures). Other modes of CEC separations such as ion exchange [12–14] and mixed mode (ionic/hydrophilic) [15,16] have been used but to a lesser extent than RP-CEC. This fact has often given the impression that CEC is simply a reversed-phase chromatography technique without moving parts (i.e., without pumps) whereby an electric field is applied to the capillary column to move the mobile phase across the capillary by the so-called electroosmotic flow (EOF). Although RP-CEC is the technique of choice for the separation of a wide range of hydrophobic to slightly polar compounds, the separation of very polar compounds (e.g., carbohydrates, nucleosides, amino acids) by RP-CEC requires the use of plain aqueous electrolyte solutions as the mobile phases to allow sufficient retention and selectivity for polar compounds. Unfortunately, the highly aqueous condition of the mobile phase combined with a non-wetted non-polar stationary phase usually leads to bubble formation in CEC and interruption of the separation process.

Normal-phase liquid chromatography (NPLC) employing polar stationary phases and less polar mobile phases

* Corresponding author. Tel.: +1-405-744-5931; fax: +1-405-744-6007.
E-mail address: zelrassi@biochem.okstate.edu (Z. El Rassi).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2003.12.015

(e.g., organic solvent mixtures) has proved to be very efficient for the separation of polar species by HPLC [17]. Since a wide variety of polar stationary phases (e.g., bare silica gels, silica bonded polar phases including amino, polyamine, cyano, amide, hydroxylic, poly(2-hydroxyethyl aspartamide) and cyclodextrin phases, ion-exchange resins, etc.) can be utilized to achieve NPLC, the more generic term "hydrophilic interaction chromatography" was proposed in 1990 by Alpert [18] to cover all forms of chromatography driven by polar (hydrophilic) interactions. As in NP-HPLC, normal-phase CEC (NP-CEC) is expected to yield better performance than RP-CEC for polar compounds. Despite this fact, only a few attempts have surfaced out including the use of bare silica microparticles [19–21], cellulose-based packing materials [20], amino and cyano phases consisting of a macroporous polymer-based monolith [22,23], strong cation-exchange silica based particles [e.g., poly(2-sulfoethyl-aspartamide)-silica] [15] and a hydrophilic macroporous weak anion-exchange polymer-based monolith [16]. All of these investigations are initial studies on NP-CEC and further research and development are needed in this area of CEC to enlarge the scope of applications of CEC.

Thus, it is the aim of this study to investigate NP-CEC with hydrophilic silica-based monoliths toward the separation of polar species. In this regard, two different synthetic schemes have been investigated to produce hydrophilic silica monoliths with surface bound cyano groups.

2. Materials and methods

2.1. Instrumentation

The instrument used for CEC was a P/ACE 5510 CE system from Beckman Instruments (Fullerton, CA, USA) equipped with a diode-array detector and a data handling system comprised of an IBM personal computer and P/ACE software. For column fabrication, temperature programming was carried out using a Sigma 3 gas chromatograph from Perkin-Elmer (Norwalk, CT, USA). Constant temperature processes were done using an Isotemp Oven from Fisher Scientific (Pittsburgh, PA, USA). In all cases, the model solutes were injected electrokinetically at 3 kV for 5 s.

2.2. Chemicals and materials

Sodium phosphate monobasic, phosphoric acid, thiourea and ammonium hydroxide were purchased from Mallinckrodt (Paris, KY, USA). Molecular biology-grade tris(hydroxymethyl)aminomethane (Tris), ammonium acetate, glacial acetic acid, hydrochloric acid, sulfuric acid, methylene chloride, phenol, resorcinol, catechol, hydroquinone, HPLC-grade methanol and HPLC-grade acetonitrile (ACN), were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Sodium hydroxide, formamide and *N*,*N*-dimethylformamide (DMF) were purchased from EM Science (Cherry Hill, NJ, USA). Toluene, triethylamine (TEA) (≥99%), 3-hydroxypropionitrile (3-HPN) (97%), 2,6-lutidine (≥99%), tetramethylorthosilicate (TMOS) (≥99%), boron trifluoride (BF_3) diethyl etherate, poly(ethylene glycol) (PEG) $M_W = 10{,}000$, and all of the chlorophenols including 2-chlorophenol (2-CP), 2,4-dichlorophenol (2,4-DCP), 3,4,5-trichlorophenol (3,4,5-TCP) and 2,4,5-TCP were from Aldrich (Milwaukee, WI, USA). Ethanol was purchased from Pharmco (Brookfield, CT, USA). Uracil, cytosine, adenine, uridine, adenosine, inosine and cytidine were from Sigma (St. Louis, MO, USA). The *p*-nitrophenyl (pNP) derivatives of mono- and oligosaccharides including pNP *N*-acetyl-β-D-glucosaminide (pNP-βGlcNAc), pNP *N*-acetyl-β-D-galactosaminide (pNP-βGalNAc), pNP *N*,*N*′-diacetyl-β-D-chitobioside (pNP-βchitobiose), pNP α-D-glucopyranoside (pNP-αGlc), pNP β-D-glucopyranoside (pNP-βGlc), pNP β-D-galactopyranoside (pNP-βGal), pNP α-D-maltoside (pNP-Mal), pNP α-D-maltotrioside, pNP α-D-maltotetraoside and pNP α-D-maltopentaoside as well as the *o*-nitrophenyl (oNP) derivatives of monosaccharides including oNP *N*-acetyl-α-D-glucosaminide (oNP-αGlcNAc) and oNP *N*-acetyl-β-D-galactosaminide (oNP-βGalNAc) were from Sigma. 3-Cyanopropyldimethylchlorosilane (3-CPDCS) and (γ-glycidoxypropyl)trimethoxysilane (γ-GPTS) were purchased from Huls America (Bristol, PA, USA). Pyrogallol was purchased from J.T. Baker (Phillipsburg, NJ, USA). Thymine was purchased from Nutritional Biochemicals (Cleveland, OH, USA). Fused-silica capillaries with an internal diameter of 100 μm and an outer diameter of 360 μm were purchased from Polymicro Technologies (Phoenix, AZ, USA).

2.3. Preparation of the silica backbone

Monolithic columns were prepared by a method described in previous papers [24,25]. This method was a modified version of the one previously described by Ishizuka et al. [26]. Briefly, a segment of 100 μm i.d. fused-silica capillary of a desired length was pre-treated hydro-thermally by rinsing with water and heating in a GC oven [27]. After pretreatment was complete, the capillary was injected with a polymerizing mixture containing TMOS. This mixture was prepared by adding 500 μl of TMOS to a solution that contained 0.1325 g of PEG in 1250 μl of 0.010 M acetic acid followed by stirring for 45 min at 0 °C. After the mixture was injected into the pre-treated capillary, the inlet and outlet ends of the capillary were formed into a circle by joining them together with a piece of PTFE tubing. The capillary was heated at 40 °C and allowed to react overnight. The resulting monolithic silica was rinsed with 0.010 M ammonium hydroxide then heated at 120 °C for 90 min. This 90 min ammonium hydroxide treatment was found optimum for pore tailoring whereby the micropores within the monolithic silica skeleton are converted to mesopores [25]. The capillary was then dried by rinsing the column with 200

proof ethanol followed by purging with helium at 160 p.s.i. (1 psi = 6894.76 Pa) for 60 min. The final heat treatment was done in a GC oven by ramping the temperature at a rate of 2.5 °C/min from 30 °C (2 min hold time) to 180 °C (60 min hold time), then again ramped at 2.5 °C/min to a final temperature of 330 °C where it was held for 21 h.

After the silica backbone was generated, stationary phase attachment was done by one of the procedures described in the following section. Columns 27 cm long were cut from the original 1 m capillary, thus yielding three columns per batch. All columns were completely filled with the monolith. Under this condition, the detection sensitivity is reduced due to light scattering by the silica monolith at the detection window [3,8,28]. The columns were rinsed with water and windows were formed at 7 cm from the outlet end by placing that portion of the capillary in fuming sulfuric acid until the polyimide coating was removed. After subsequent rinses of acetonitrile and water, the capillaries were installed into the P/ACE cartridges and were ready to be used.

2.4. Surface modification of the silica monoliths—formation of bonded phases

Fig. 1 shows a schematic of the two reaction pathways investigated for the generation of the desired cyano or cyano/hydroxy stationary phases. These steps were performed after the final heat treatment described in Section 2.3. For path A, 20 μl of 3-CPDCS and 10 μl of 2,6-lutidine were dissolved in 200 μl of methylene chloride. This solution was perfused through the column and reacted at 50 °C for 1 h. This step was repeated three times. The resulting stationary phase is denoted CN-monolith. For path B, 20 μl of γ-GPTS was dissolved in 200 μl of toluene. This solution was introduced into the column and reacted at 110 °C for 1 h. This step was repeated three times. After rinsing with toluene and then DMF, the column was filled with a solution containing 200 μl of DMF, 20 μl of 3-HPN and 3 μl of BF_3 etherate. The column was allowed to react at room temperature for 1 h. This step was repeated twice, and then the final reaction was done overnight. The resulting stationary phase is denoted CN–OH-monolith.

3. Results and discussion

3.1. Comparison of the cyano phases

Two different reaction schemes have been investigated for the preparation of cyano silica-based monolithic stationary phases. As can be seen in Fig. 1, path A yielded a cyano monolith (CN-monolith) in a single reaction step while path B gave a mixed ligand containing hydroxyl and cyano functions (i.e., CN–OH-monolith) in a two-step reaction. Although in path B, the treatment with γ-GPTS is aimed at providing a spacer arm for attaching the cyano ligand 3-HPN (i.e., the polar top layer), the spacer arm becomes a part of the interacting ligand, by providing a hydrophilic sublayer containing polar hydroxyl groups. In addition, epoxide moieties that do not react with 3-HPN will yield diol ligands (i.e., silylpropyl glyceryl ether ligands).

Table 1 summarizes the retention factor, k', separation efficiency and EOF velocity obtained on the two cyano phases with a series of model solutes. The mobile phase velocity on the CN-monolith is ∼17% higher than that on the CN–OH-monolith indicating more available silanol groups to support the EOF. This may reflect a lower phase ratio (i.e., lower surface coverage with polar ligands) for the CN-monolith or a better shielding of the silanols by the spacer arm (i.e., the polar sub-layer) in the CN–OH-monolith. Both reasoning could explain the difference in EOF velocity of the cyano monoliths under investigation as well as the higher k' values obtained with the CN–OH-monolith. The CN–OH-monolith

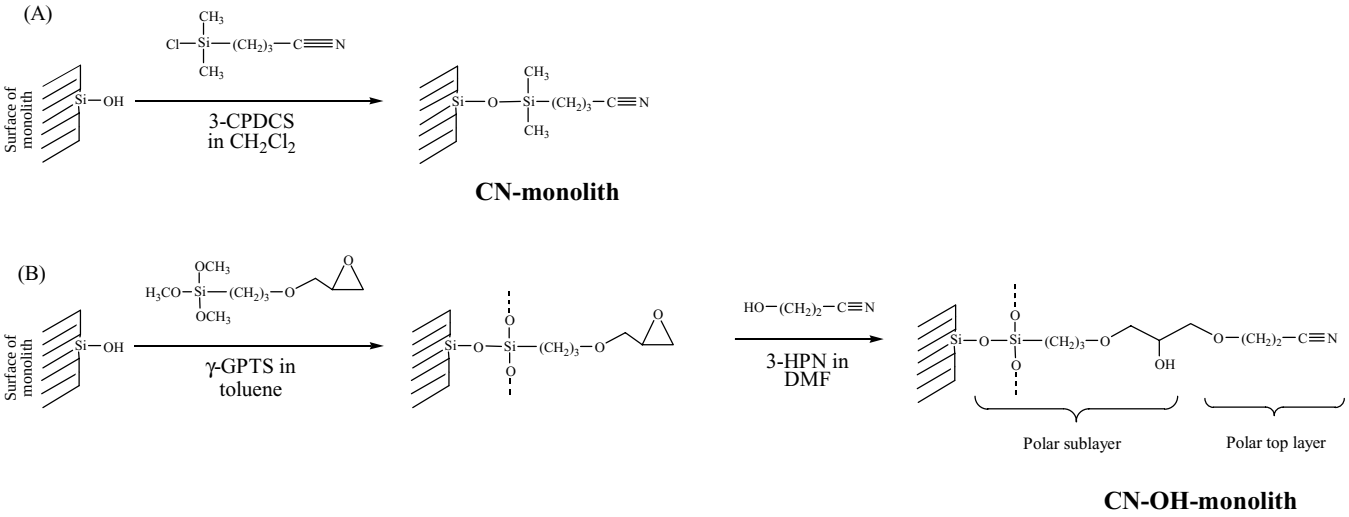


Fig. 1. Schematic of the two reaction pathways investigated. (A) Reaction of 3-CPDCS catalyzed by 2,6-lutidine in methylene chloride at 50 °C. (B) Reaction of γ-GPTS in toluene at 110 °C followed by reaction of 3-HPN in DMF catalyzed by BF_3 at room temperature.

Table 1
Comparison of CN-monolith and CN–OH monolith

Analytes	CN			CN–OH		
	k'	N_{avg} (plates/m)	u_{eo} (mm/s)	k'	N_{avg} (plates/m)	u_{eo} (mm/s)
DMF	~0		2.57	0.04		2.19
Formamide	0.018			0.11		
Thiourea	0.037			0.22		
2-CP	0.017			0.049		
2,4-DCP	0.042			0.091		
3,4,5-TCP	0.091	213 000		0.14	199 000	
2,4,5-TCP	0.39			0.29		

Conditions: monolithic capillary column, 27 cm (effective length 20 cm) × 100 μm i.d.; hydro-organic mobile phase, 5 mM phosphoric acid/TEA ($TEAPO_4$) (pH 6.5) at 95% (v/v) ACN; voltage, 20 kV; column temperature, 20 °C.

should yield a more polar stationary phase, thus providing a higher "polar" phase ratio and consequently, stronger retention under equivalent mobile phase compositions.

On the basis of the above results, the cyano monolith obtained via path B (i.e., CN–OH-monolith) shown in Fig. 1B, is a promising hydrophilic monolith for NP-CEC. In fact, a quick inspection of Fig. 2 reveals the superiority of CN–OH-monolith over CN-monolith in terms of retention and selectivity under the same elution conditions. The CN–OH-monolith was therefore used for the remainder of this investigation.

The CN–OH-monolithic columns showed stable chromatographic performance for more than three weeks of continuous use under the various mobile phase composition used in this study. The column-to-column reproducibility was evaluated in terms of RSD for the retention factor k' of DMF, formamide and thiourea and was found to be 8.46, 8.20 and 7.34% ($n = 5$), respectively.

3.2. Characterization of the CN–OH-monolith with polar solutes

3.2.1. Mobile phase composition

As in all CEC modes, the mobile phase composition not only impacts retention and selectivity, but also the mobile phase flow velocity due to the various effects of ions, organic solvent, pH and viscosity. Fig. 3 shows typical electrochromatograms obtained for toluene, DMF, formamide and thiourea under four different mobile phase compositions. The results are summarized in Table 2 in terms of k', average separation efficiencies and flow velocity. Toluene serves as the EOF marker [15]. While the mobile phase conditions in Fig. 3a, c and d (corresponding also to mobile phases **[a]**, **[c]** and **[d]** in Table 2) involve constant ACN concentration (i.e., 95%, (v/v)) but different nature of electrolytes, the conditions in Fig. 3b (mobile phase **[b]** in Table 2) calls for different nature and content of organic modifiers and electrolytes.

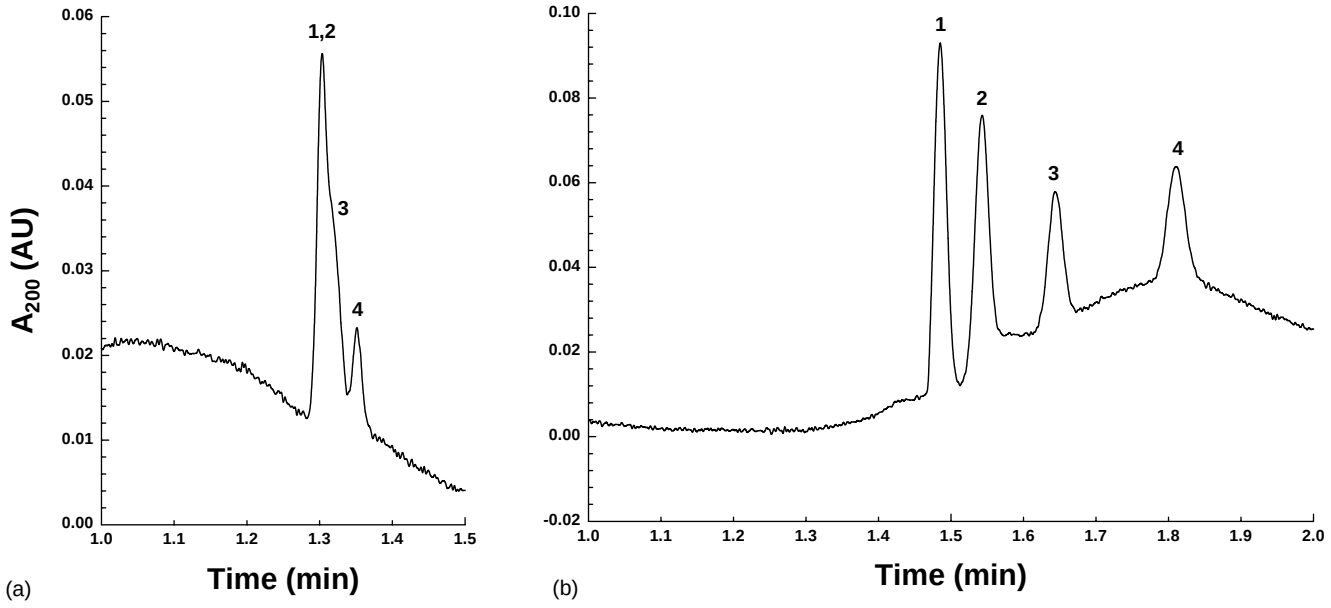


Fig. 2. Electrochromatograms of some model compounds on cyano columns made by two different pathways. Conditions: capillary column (a) CN-monolith and (b) CN–OH-monolith, 27 cm (effective length 20 cm) × 100 μm i.d.; hydro–organic mobile phase, 5 mM H_3PO_4/TEA ($TEAPO_4$), pH 6.5, at 95% (v/v) ACN; voltage, 20 kV; column temperature, 20 °C. Solutes: 1, toluene; 2, DMF; 3, formamide; 4, thiourea. Solute concentration, 2.0×10^{-4} M.

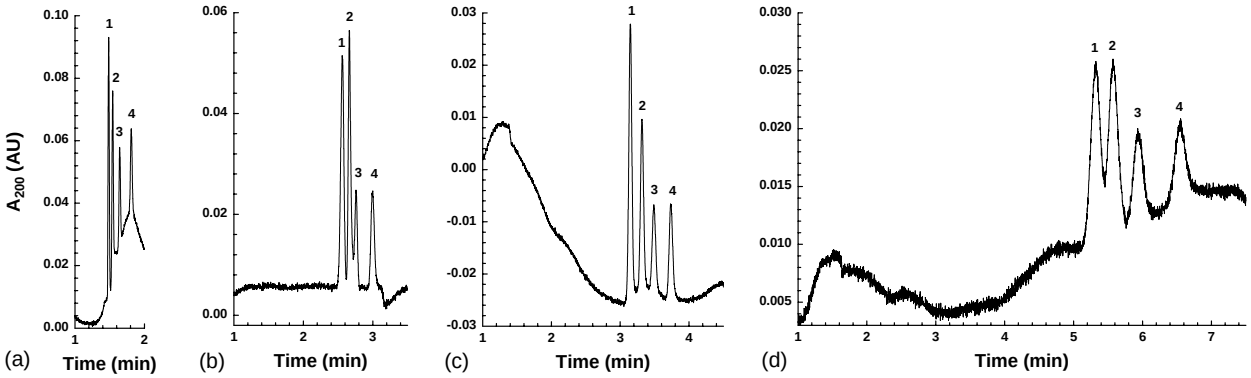


Fig. 3. Comparison of mobile phase conditions for the separation of model compounds on the CN–OH-monolithic column. Conditions: monolithic capillary column, 27 cm (effective length 20 cm) × 100 μm i.d.; hydro-organic mobile phase, (a) 5 mM $TEAPO_4$, pH 6.5, at 95% (v/v) ACN, (b) 92.65% ACN, 5% MeOH, 2.3% acetic acid, 0.05% (v/v) TEA, (c) 5 mM NH_4Ac pH 4.5 at 95% (v/v) ACN, (d) 5 mM Tris pH 7.8 at 95% (v/v) ACN; voltage, 20 kV; column temperature, 20 °C. Peak designations and solute concentration are the same as in Fig. 2.

Table 2
Effect of the mobile phase composition on the behavior of the CN–OH-monolith column

Number and mobile phase composition	k'			N_{avg} (plates/m)	u_{eo}(mm/s)
	DMF	Formamide	Thiourea		
[a]: 5 mM $TEAPO_4$, pH 6.5 at 95% (v/v) ACN	0.039	0.11	0.22	161 000	2.21
[b]: 92.65% (v/v) ACN, 5% (v/v) MeOH, 2.3% (v/v) acetic acid and 0.05% (v/v) TEA	0.040	0.077	0.17	117 000	1.30
[c]: 5 mM NH_4Ac, pH 4.5 at 95% (v/v) ACN	0.054	0.11	0.19	120 000	1.06
[d]: 5 mM Tris–HCl, pH 7.8 at 95% (v/v) ACN	0.049	0.11	0.23	36 000	0.62

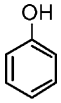
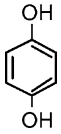
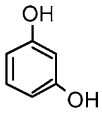
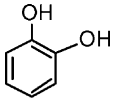
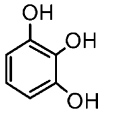
Conditions: monolithic capillary column, 20/27 cm × 100 μm i.d.; voltage, 20 kV; column temperature, 20 °C.

As can be seen in Table 2 small variations in k' values were observed for the four different mobile phases tested, with mobile phase **[b]** giving the lowest k' values. The change in mobile phase flow velocity is the most significant and noticeable. Mobile phase **[a]** yielded an EOF velocity 3.5 times greater than that exhibited by mobile phase **[d]**. This may explain, in part, the lowest separation efficiency observed with mobile phase **[d]**, whereby longitudinal molecular diffusion is contributing the most to band broadening. Another aspect of mobile phase composition is the contribution of the eluent component to baseline irregularities. Mobile phase irregularities in normal phase chromatography have been previously reported in HPLC [18] as well as in CEC [15]. Mobile phase **[a]** generated the best results in terms of separation efficiency and flow velocity, but produced the most irregular baseline, whereas mobile phase **[c]** ran cleaner and gave good separation efficiency and flow velocity.

3.2.2. Correlation of solute retention and structure

3.2.2.1. Phenols. To further characterize the CN–OH-monolith under investigation, a series of phenols were electrochromatographed using mobile phase **[a]**. The calculated k' values are shown in Table 3. As expected for a hydrophilic monolith, k' value increases with an increase in the number of hydroxyl groups within the molecule. Moreover, the

Table 3
Retention factor (k') for phenols obtained on CN–OH-monolith

Phenols	Structure	k'
Phenol		0.037
Hydroquinone		0.070
Resorcinol		0.089
Catechol		0.093
Pyrogallol		0.43

Conditions: monolithic capillary column, 27 (effective length 20 cm) × 100 μm i.d.; hydro-organic mobile phase, 5.0 mM $TEAPO_4$ (pH 6.5) at 95% (v/v) ACN; voltage, 20 kV; column temperature, 20 °C.

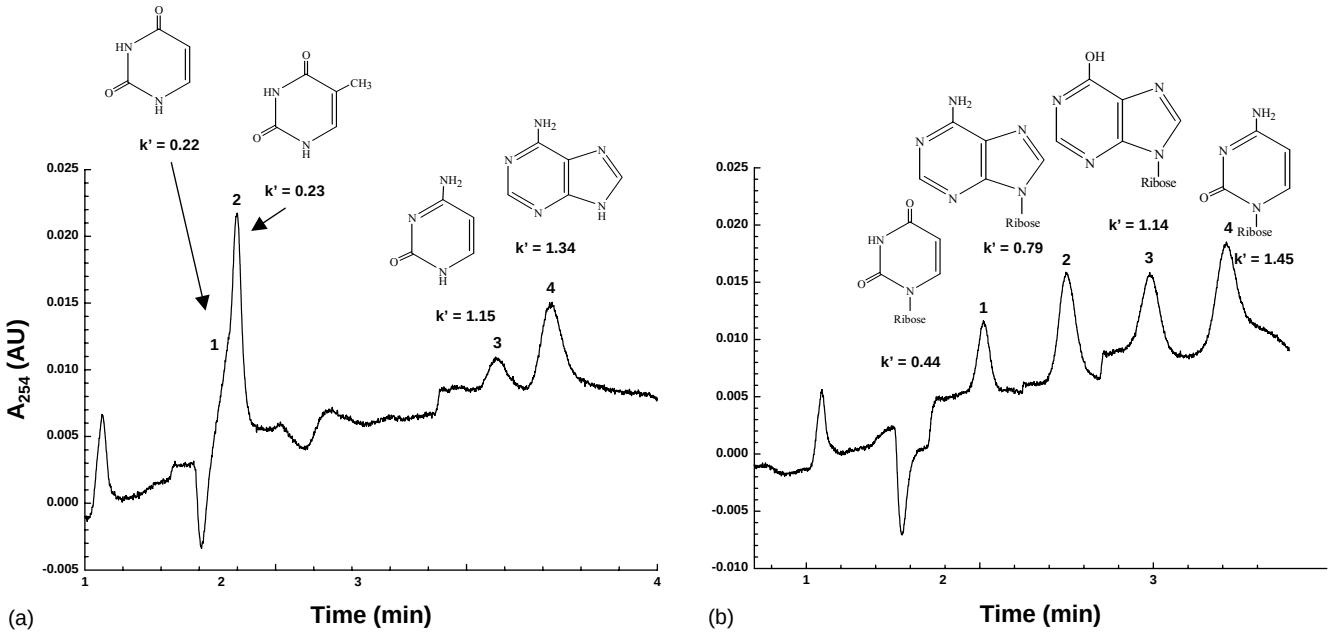


Fig. 4. Electrochromatograms of (a) nucleic acid bases and (b) nucleosides on the CN–OH-monolithic column. Conditions are the same as in Fig. 2 except wavelength is 254 nm. Solutes: (a) 1, uracil; 2, thymine; 3, cytosine; 4, adenine (b) 1, uridine; 2, adenosine; 3, inosine; 4, cytidine. Concentration for solute 1 is 5.5×10^{-4} M, for solute 3 is 7.5×10^{-4} M, and for the other solutes is 4.5×10^{-4} M.

extent of polar interactions depends on the position of the hydroxyl groups. This interaction is stronger for catechol (*ortho*-substituted phenol) and resorcinol (*meta*-substituted phenol) than for hydroquinone (*para*-substituted phenol). The hydroxyl group selectivity factor in the *para* position ($\alpha_{p\mathrm{OH}}$) can be obtained by taking the ratio between the retention factors for hydroquinone and phenol ($\alpha_{p\mathrm{OH}} = 1.89$). Similarly, the ratio of k' of resorcinol to that of phenol yields a value of 2.40 for the hydroxyl group selectivity factor in the *meta* position ($\alpha_{m\mathrm{OH}} = 2.40$). The ratio of k' value of catechol to that of phenol gives the hydroxyl group selectivity factor in the *ortho* position ($\alpha_{o\mathrm{OH}} = 2.51$). Thus, the positional group selectivity factors in the order of decreasing values are $\alpha_{o\mathrm{OH}} > \alpha_{m\mathrm{OH}} > \alpha_{p\mathrm{OH}}$.

3.2.2.2. Nucleic acid bases and their nucleosides. Due to the multiplicity of their polar groups, the nucleic acid bases and nucleosides are important probes for evaluating the polarity of the stationary phases under investigation, and in turn, the influence of functional groups on normal phase retention. As can be seen in Fig. 4a, the nucleic acid bases under investigation, namely uracil, thymine, cytosine and

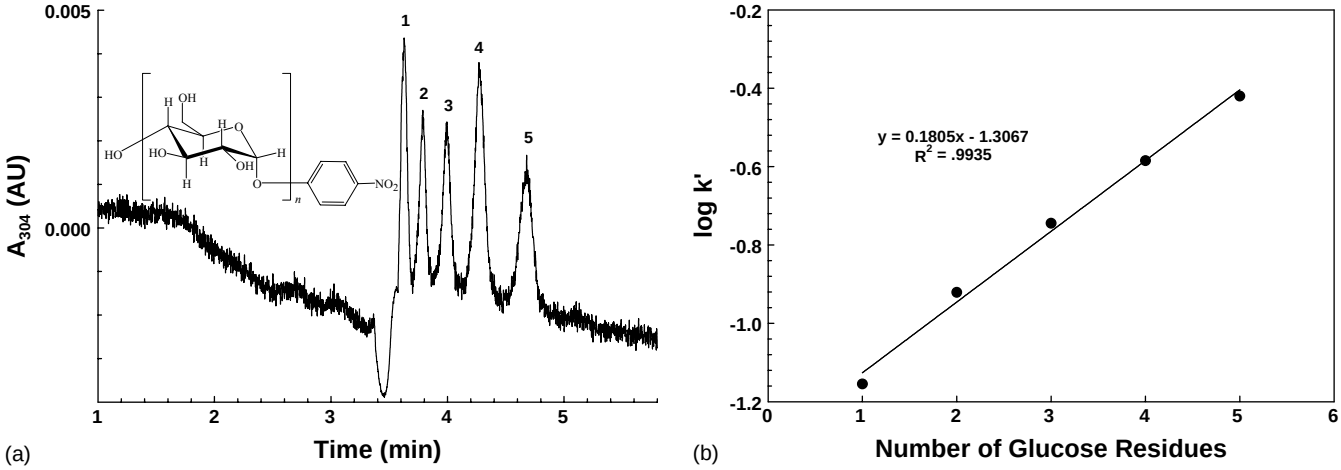


Fig. 5. (a) Electrochromatogram of pNP-αGlc and pNP maltooligosaccharides and (b) plot of $\log k'$ vs. number of glucose residues. Conditions: CN–OH-monolithic capillary column, 27 cm (effective length 20 cm) × 100 μm i.d.; hydro-organic mobile phase, 5 mM NH_4Ac (pH 4.5) at 80% acetonitrile (v/v); voltage, 20 kV; column temperature, 20 °C. Solutes: 1, pNP-αGlc; 2, pNP α-D-maltoside; 3, pNP α-D-maltotrioside; 4, pNP α-D-maltotetraoside; 5, pNP α-D-maltopentaoside. Solute concentration, 2.8×10^{-4} M.

adenine, elute in the order of increasing number of amine functions (particularly primary and secondary amines) and size. Upon conjugation of the bases with ribose to yield nucleosides, the interactions of the polar conjugates with the CN–OH-monolith increased for all analytes investigated except adenosine. The addition of ribose to uracil led to an increase in k' by a factor of ∼2.6, and the k' value of cytidine increased by a factor of 1.3 with respect to the base cytosine. On the other hand, the k' value of adenosine decreased by a factor of ∼1.7 when compared with the k' value for adenine. It appears from these data that the secondary amine group of adenine contributes much more to retention than the ribose moiety in adenosine whereby the conjugation with ribose transforms the secondary amine of the adenyl moiety to tertiary amine.

3.2.2.3. Nitrophenyl derivatives of mono- and oligosaccharides. The retention of some *ortho*- and *para*-nitrophenyl (oNP and pNP, respectively) derivatives of mono- and oligosaccharides was examined on the CN–OH-monolith. Fig. 5a shows the separation of pNP-maltooligosaccharides up to a degree of polymerization (DP) = 5 with a separation efficiency of 111 000 plates/m at a flow velocity of 1.0 mm/s. As expected for an NP-CEC retention mechanism, the oligomers retention increased with increasing DP since this results in an increase of the number of solute polar sites available for interaction with the fixed polar sites of the monolithic stationary phase. Also shown in Fig. 5b is the plot of $\log k'$ of the pNP-maltooligosaccharides versus the number of glucose residues in the sugar molecule. The plot is linear with an $R^2 = 0.9935$ and a slope, or

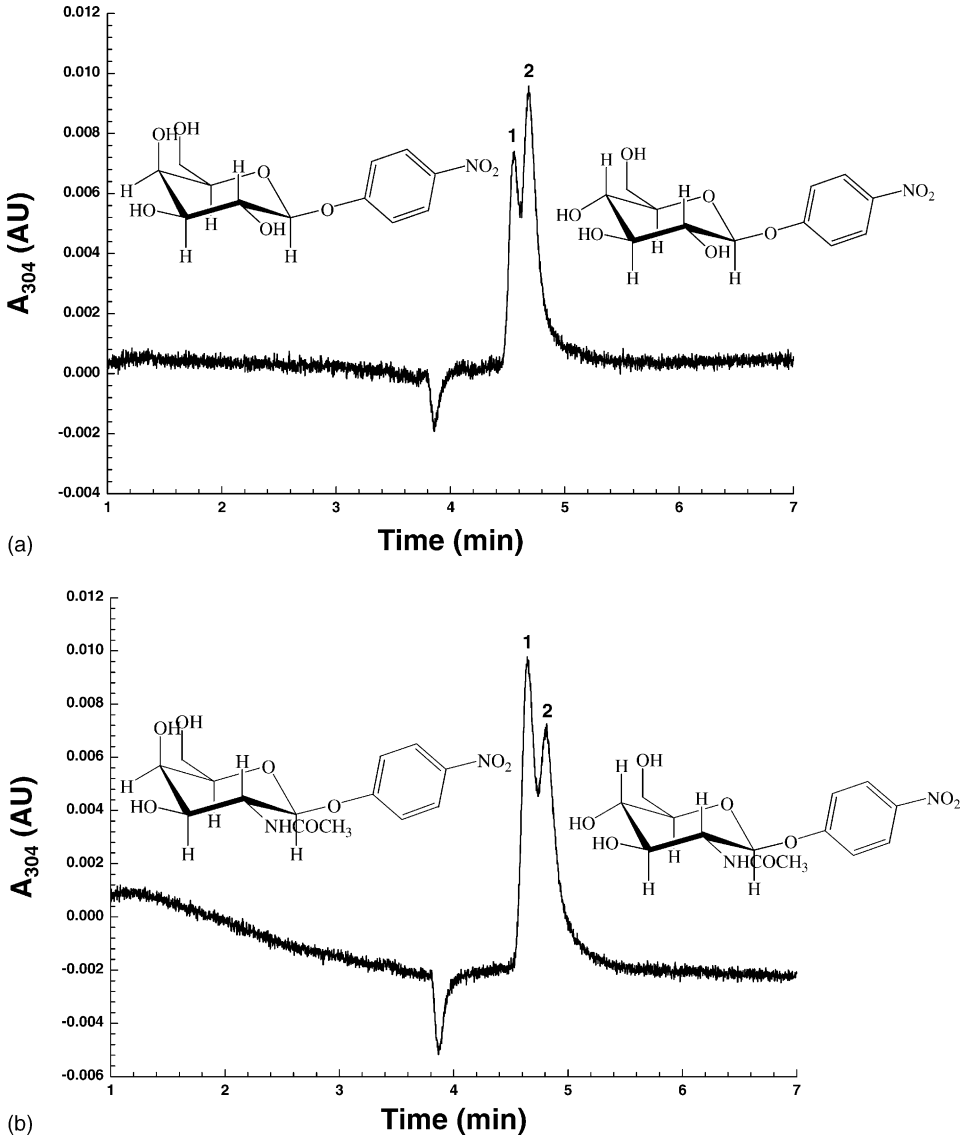


Fig. 6. Electrochromatograms of (a) pNP monosaccharides and (b) pNP *N*-acetyl monosaccharides obtained on the CN–OH-monolithic column. Conditions: monolithic capillary column, 27 cm (effective length 20 cm) × 100 μm i.d.; hydro-organic mobile phase, 5 mM NH_4Ac (pH 4.5) at 95% (v/v) ACN; voltage, 20 kV; column temperature, 30 °C. Solutes: (a) 1, pNP-βGal; 2, pNP-βGlc and (b) 1, pNP-βGalNAc; 2, pNP-βGlcNAc. Solute concentration, 2.8×10^{-4} M.

glucosyl group selectivity factor (α_{Glc}) of 0.18 in log units (i.e., an $\alpha_{Glc} = 1.51$). The *y*-intercept is equal to −1.3067 corresponding to a retention factor contribution for the pNP-residue of $k' = 0.049$. This interaction can be considered negligible since the pNP confers a relatively non-polar property to the sugar derivatives.

Upon increasing the acetonitrile content of the mobile phase from 80 to 95% (v/v), the glucosyl selectivity factor (α_{Glc}) increased from 1.51 to 3.94 as calculated from the ratio of retention factors between pNP α-D-maltoside and pNP-αGlc. Also, at 95% (v/v) ACN in the mobile phase, the α_{GlcNAc} (as calculated from the ratio of retention factors between pNP-βchitobiose and pNP-βGlcNAc) is relatively high, and equal to 8.35. These selectivity factors demonstrate the high discriminative power of the stationary phase under consideration.

Fig. 6 further illustrates the discriminative power of the CN–OH-monolith, namely the separation of pNP-βGal and pNP-βGlc (Fig. 6a) as well as that of pNP-βGalNAc and pNP-βGlcNAc. The solute pair pNP-βGal/pNP-βGlc and the solute pair pNP-βGalNAc/pNP-βGlcNAc only differ in the orientation of their hydroxyl groups at the C4 position.

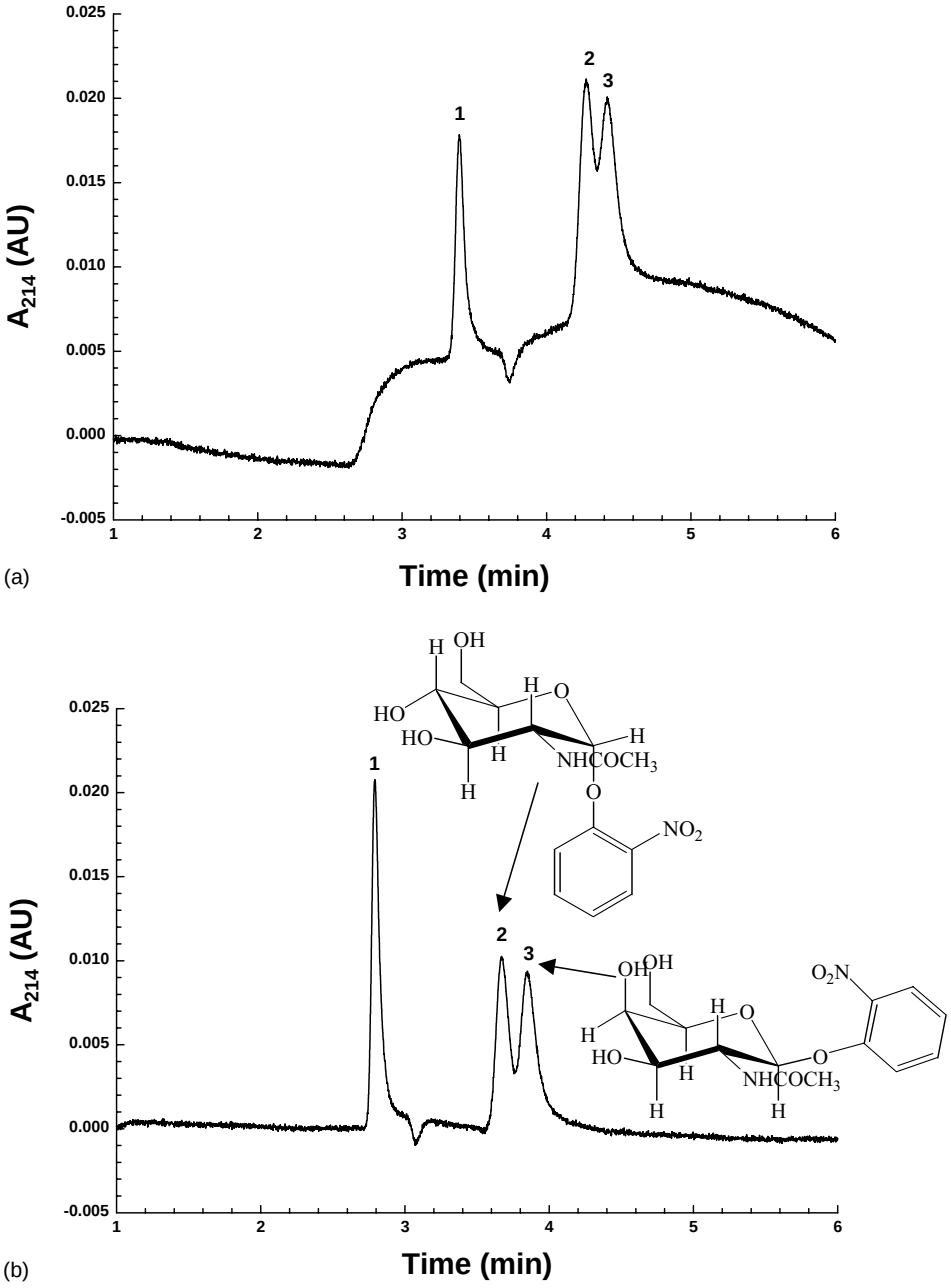


Fig. 7. Electrochromatograms of oNP derivatives of monosaccharides obtained on the CN–OH-monolithic column. Conditions: monolithic capillary column, 27 cm (effective length 20 cm) × 100 μm i.d.; hydro-organic mobile phase, (a) 5 mM and (b) 2.5 mM NH_4Ac (pH 4.5) at (a) 95% and (b) 97.5% (v/v) ACN; voltage, 20 kV; column temperature, 30 °C. Solutes: 1, toluene; 2, oNP-αGlcNAc; 3, oNP-βGalNAc. Solute concentration, 2.8×10^{-4} M.

These sugar pairs, which differ in configuration at just one of the several chiral centers, are called epimers. As shown in Fig. 6a and b pNP-βGal elutes before pNP-βGlc, and pNP-βGalNAc before pNP-βGlcNAc, respectively. This indicates that the hydroxyl group at the C4 position in the Gal residue (or GalNAc residue) engages in less interaction with the polar stationary phase than the hydroxyl group at the C4 position in the Glc residue (or GlcNAc residue).

Fig. 7 shows the separation of oNP-αGlcNAc and oNP-βGalNAc. In this case, the nitrophenyl GlcNAc derivative is less retained than the nitrophenyl GalNAc derivative. This may be due to the fact that in the β-anomer (i.e., oNP-βGalNAc) the equatorial glycosidic oNP residue interacts less strongly with the axial hydrogen atom on C5 than in the α-anomer (i.e., oNP-αGlcNAc) where the glycosidic oNP residue occupies an axial position. In addition, in the α-anomer solute the polar acetyl amine group is more sterically hindered by the glycosidic oNP residue than in the β-anomer solute. Both effects allow oNP-βGalNAc to interact stronger with the stationary phase than oNP-αGlcNAc. An improved resolution between oNP-αGlcNAc and oNP-βGalNAc (see Fig. 7b) is readily obtained by increasing the ACN content of the mobile phase from 95% (v/v) ACN (Fig. 7a) to 97.5% (v/v) ACN (Fig. 7b).

4. Conclusions

Silica-based monoliths with surface-bound stratified polar layers comprising a hydroxy sub-layer and a cyano top layer (i.e., CN–OH-monolith) proved very useful for NP-CEC of a wide range of polar compounds including phenols, chlorophenols, nucleic acid bases, nucleosides, mono- and oligosaccharides. The CN–OH-monolith yielded higher retention and better selectivity than the simple CN-monolith, probably due to the stronger polar character of the CN–OH-monolith. The extent of solute retention is readily adjusted by the acetonitrile content of the mobile phase, and the polar group (or residue) selectivity factor is strongly influenced by the group position within the solute and the organic content of the mobile phase. The CN–OH-monolith exhibited a relatively strong EOF over a wide range of mobile phase composition, which resulted in rapid analysis times of less than 5 min for most separations investigated.

Acknowledgements

The financial supports by the Environmental Institute's Centers for Water and Energy Research Programs at Oklahoma State University and in part by the seed grant from Oklahoma EPSCoR are greatly appreciated.

References

[1] J.H. Knox, I.H. Grant, Chromatographia 24 (1987) 135.
[2] H. Yamamoto, J. Baumann, F. Erni, J. Chromatogr. 593 (1992) 313.
[3] C. Yan, R. Dadoo, H. Zhao, R.N. Zare, Anal. Chem. 67 (1995) 2026.
[4] M. Dittmann, K. Wienand, F. Bek, G.P. Rozing, LC–GC 13 (1995) 800.
[5] M.M. Dittmann, K. Masuch, G.P. Rozing, J. Chromatogr. A 887 (2000) 209.
[6] A.S. Rathore, E. Wen, Cs. Horváth, Anal. Chem. 71 (1999) 2633.
[7] V. Pretorius, B.J. Hopkins, J.D. Schieke, J. Chromatogr. 99 (1974) 23.
[8] C. Yang, Z. El Rassi, Electrophoresis 19 (1998) 2061.
[9] C. Yang, Z. El Rassi, Electrophoresis 20 (1999) 2337.
[10] M. Zhang, Z. El Rassi, Electrophoresis 19 (1998) 2068.
[11] M. Zhang, G.K. Ostrander, Z. El Rassi, J. Chromatogr. A 887 (2000) 287.
[12] S. Zhang, W. Huang, J. Zhang, Cs. Horváth, J. Chromatogr. A 887 (2000) 465.
[13] M. Ye, H. Zou, Z. Liu, J. Ni, J. Chromatogr. A 869 (2000) 385.
[14] K. Walhagen, K.K. Unger, A.M. Olsson, M.T.W. Hearn, J. Chromatogr. A 853 (1999) 263.
[15] M. Ye, H. Zou, L. Kong, Z. Lei, R. Wu, J. Ni, LC–GC August (2002) 41.
[16] M. Lammerhofer, F. Svec, J.M.J. Frechet, W. Lindner, J. Chromatogr. A 925 (2001) 265.
[17] S.C. Churms, in: Z. El Rassi (Ed.), Carbohydrate Analysis by Modern Chromatography and Electrophoresis, Elsevier, Amsterdam, 2002, pp. 121–163.
[18] A.J. Alpert, J. Chromatogr. 499 (1990) 177.
[19] W. Wei, G.A. Luo, G.Y. Hua, C. Yan, J. Chromatogr. A 817 (1998) 65.
[20] A. Maruska, U. Pyell, J. Chromatogr. A 782 (1997) 167.
[21] E.P.C. Lai, E. Dabek-Zlotorzynska, Electrophoresis 20 (1999) 2366.
[22] A.H. Que, T. Konse, A.D. Baker, M.V. Novotny, Anal. Chem. 72 (2000) 2703.
[23] A.H. Que, M.V. Novotny, Anal. Chem. 74 (2002) 5184.
[24] D. Allen, Z. El Rassi, Analyst 128 (2003) 1249.
[25] D. Allen, Z. El Rassi, Electrophoresis 24 (2003) 408.
[26] N. Ishizuka, H. Minakuchi, K. Nakanishi, N. Soga, H. Nagayama, K. Hosoya, C.K. Tanaka, Anal. Chem. 72 (2000) 1275.
[27] J.D. Hayes, A. Malik, Anal. Chem. 72 (2000) 4090.
[28] B. Behnke, E. Grom, E. Bayer, J. Chromatogr. A 716 (1995) 207.