ELSEVIER

Journal of Chromatography A, 914 (2001) 211–222

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Acrylamide-based monoliths as robust stationary phases for capillary electrochromatography

Daniela Hoegger, Ruth Freitag*

Center of Biotechnology, Department of Chemistry, Swiss Federal Institute of Technology, 1015 Lausanne, Switzerland

Abstract

A method is described for the synthesis of rigid, macroporous polymers (monoliths) to be used as stationary phases in capillary electrochromatography (CEC). The procedure reproducibly results in columns with good mechanical and chemical stability. Once the procedure was optimized, it yielded the desired CEC columns in nearly 100% of the cases. The batch-to-batch standard deviation of the migration of the electroosmotic flow (EOF) marker for nine randomly chosen columns was 5%. The polymerization is carried out inside the capillary, an aqueous phase is used as solvent. Monomers based on acrylamides with varying hydrophilicity were used to introduce the interactive moieties together with piperazine diacrylamide as cross-linker and vinylsulfonic acid as provider of the charged, EOF-producing moieties. The pore size of the monoliths was adjusted by adding varying amounts of ammonium sulfate to the reaction mixture. In this manner, the average pore size of a given monolith could be reproducibly adjusted to values ranging from 50 nm to 1.3 μm. The procedure was optimized for four particular types of monoliths, which differed in hydrophobicity. The latter was adjusted by introducing suitable co-monomers, such as alkyl chain-bearing molecules, into the monolithic structure. Attempts to systematically investigate the chromatographic behavior of the monolithic stationary phases were made, using a model mixture of aromatic compounds as sample. The standard deviations for the run-to-run reproducibility of the retention times for unretained and retained analytes were <1.5%. Flat Van Deemter curves were measured even at elevated flow-rates (2 mm/s). Plate heights between 10 and 15 μm were measured in this range. The retention order was taken as the principal indication for the chromatographic mode. The separation was found to be governed neither by pure reversed-phase nor by pure normal-phase chromatography, even on monoliths, where large amounts of C_6 ligands had been introduced.

Keywords: Stationary phases, electrochromatography; Monolithic columns; Electrochromatography; Nonsteroidal anti-inflammatory drugs; Aromatic compounds

1. Introduction

Capillary electrochromatography (CEC) can be envisioned as a hybrid technique between capillary electrophoresis (CE) and high-performance liquid chromatography (HPLC). Just as in CE, the mobile phase in CEC is driven by the electroosmotic flow (EOF). The result is a very flat flow profile and therefore band broadening by dispersion is usually less pronounced in CEC than in hydrodynamically driven conventional chromatography [1–5]. Other than in CE, where only a rather restricted number of molecular parameters can be exploited to bring about separation, in CEC the separation takes place in a ''column'' filled with an interactive stationary phase. The corresponding increase in the variety of putative exploitable separation mechanisms makes CEC more

*Corresponding author. Tel.: +41-21-6936-162.
E-mail address: ruth.freitag@epfl.ch (R. Freitag).

0021-9673/01/$ – see front matter
PII: S0021-9673(00)01119-5

selective and versatile than CE. Since the separation distances (column length) are much shorter in CEC than in CE, CEC may in the future also become a very interesting option for chip-based microseparation techniques [6].

At present, most CEC applications use columns packed with traditional HPLC beads, most often composite silica particles. Extremely good separations have been achieved with such columns, hence demonstrating the potential of the technique [7,8]. However, the packing procedure for particle-based capillary columns is relatively delicate, especially as far as the introduction of supporting frits is concerned. Commercially available packed CEC columns are therefore still extremely expensive and sometimes awkward to use. The frits, for instance, tend to generate bubbles, which inevitably lead to current (and system) breakdown. Bed instabilities and column clogging have also been reported. Especially in the case of microanalytical devices, packed columns appear to be highly unsuitable.

The fact that a typical CEC column today is packed with a stationary phase originally developed for HPLC creates other, more principal, problems as well. The chromatographic separation mechanisms most suitable to HPLC, for example reversed phase, ion exchange, and hydrophobic interaction, may not necessarily be equally successful in CEC. In order to generate the endoosmotic flow, i.e., the pump, it is in CEC necessary to always incorporate some charged functions into a stationary phase. This leads inevitably to a mixed-mode type of separation in the case of charged analytes and hydrophobic stationary phases with the ensuing adsorption problems and undesirable peak shapes. This is, of course, an important reason why CEC has to date mostly been investigated for the separation of neutral compounds. Efforts to extend the application of CEC to other types of analytes are clearly needed. However, the systematic investigation of possible interaction and separation principles is handicapped by the fact that the surface chemistry of the current stationary phases cannot be predetermined in a controlled manner.

It has been suggested that other stationary phase morphologies, such as monoliths, are more suited to the needs of CEC than packed beds [9–12]. Various approaches for the production of monoliths as stationary phases for CEC have been published [11–19]. Thus, the possibility of creating such stationary phases can be considered as established. However, as stated in a recent review on this subject, an in-depth investigation of the chromatographic behavior of such stationary phases is at present still lacking [20]. A first attempt to relate surface functionality and pore structure with the efficiency of electrochromatographic separations has been presented by Peters et al. [21,22]. However, the development of robust algorithms for the optimization of CEC separations and ultimately a selection of assured separation conditions for a whole range of potential analytes would doubtlessly benefit from the availability of a wide variety of stationary phases with different polarities. In addition, any scientifically sound investigation would require stationary phases robust enough to be used repeatedly for many applications perhaps even under varied conditions without a significant change in performance.

In this context we would like to report on our recent investigation of monolithic stationary phases for CEC based on acrylamide derivatives. Attempts to systematically investigate the chromatographic behavior of such stationary phases are described as well.

2. Experimental

2.1. Materials and equipment

Piperazine diacrylamide (PDA) was purchased from Bio-Rad (Hercules, CA, USA). *N*,*N*,*N′*,*N′*-Tetramethylethylenediamine (TEMED), *N*,*N*-dimethylacrylamide (DMAA), methacrylamide (MA), phosphate buffer (50 m*M*, pH 7) and vinylsulfonic acid (VSA, as a 30% aqueous solution of its sodium salt), as well as solvents like acetonitrile and methanol were from Fluka (Buchs, Switzerland). Butylacrylate (C_4), hexylacrylate (C_6), formamide, ammonium persulfate (APS), ammonium sulfate and 3-(trimethoxysilyl)propyl methacrylate were from Sigma (St. Louis, MO, USA). The aromatic test compounds for the separation studies were from Fluka, the pharmaceuticals were from Sigma. All chemicals have been used in the highest available purity grade, but without further purification.

The fused-silica capillaries (75 μm I.D.×360 μm

O.D.) were from Polymicro Technologies (Phoenix, AZ, USA). A commercial packed capillary column (ODS C_{18}, 3 μm particle diameter, 25 cm×100 μm I.D.) from Hypersil (Runcorn, UK) was used as a reference for reversed-phase CEC.

A Hewlett-Packard ^{3D}CE system (Hewlett-Packard, Waldbronn, Germany), upgraded to be used for CEC (possibility to apply pressures of up to 12 bar) was used for all CEC measurements. An isocratic HPLC pump Model 422 from Kontron (Watford, UK) was used to flush the columns after polymerization. Scanning electron microscopy (SEM) pictures were taken with a XLF 30 FEG instrument from Phillips.

2.2. Monolith synthesis

Prior to the introduction of the monolith, capillary walls were pretreated with 3-(trimethoxysilyl)propyl methacrylate (bind-silane), using a method described by Ericson et al. [12] in slightly modified form. The capillary was flushed with 0.2 *M* NaOH (30 min), followed by 0.2 *M* HCl (30 min) and water (30 min). Then a 30% (v/v) solution of bind-silane in acetone was pumped through for 15 min. Afterwards the capillary ends were sealed with GC septa and kept overnight at room temperature. Just before introduction of the polymerization mixture (see below), the capillary was flushed with methanol and water. For the polymerization, monomers, cross-linker and ammonium sulfate were dissolved in 1 ml of a 50 m*M* phosphate buffer (pH 7). In the case of C_4 or C_6 monomers, a mixture of the buffer–formamide (1:1, v/v) was used instead. The mixture was degassed by ultrasonication for 10 min, then 10 μl of a 10% ammonium persulfate and 10 μl of a 10% TEMED solution were added and briefly mixed (5 μl of each solution in the case of methacrylamide as a monomer). Subsequently, the mixture was directly filled into the capillary, as the polymerization started spontaneously soon after. The ends of the capillary were again plugged with the GC septa and the polymerization was allowed to proceed overnight at room temperature. Afterwards, the detection window was created in the capillary by burning off a section of the outer polyimide coating and locally pyrrolyzing the monolith inside. Before, during and after the creation of the detection widow, the capillaries were flushed with pure water [or an acetonitrile–water (8:2, v/v) mixture in the case of the C_6 columns in order to avoid collapse of the alkyl chains] for a total of about 30 min, applying a pressure of up to 150 bar. This treatment was found to be most efficient in removing the ashes produced during pyrrolization. Before use, every column was inspected under the microscope for uniformity of the stationary phase. Columns, which showed irregularities were discarded.

In addition, selected polymers were prepared in ''bulk'' quantities of about 5 g. These polymers were Soxhlet extracted for 24 h in methanol, i.e., a solvent in which the monomers and short polymer chains are well soluble. Extraction was followed by drying in vacuum. Afterwards the porosities of the polymers were determined by mercury intrusion porosimetry (Porosimeter 2000 WS; Carlo-Erba, Milan, Italy). Their appearance was investigated by SEM.

2.3. Electrochromatography

A mixture of nine neutral, aromatic compounds, namely hydroquinone monomethyl ether, phenol, 2-naphthol, catechol, hydroquinone, resorcinol, naphthalene, phenanthrene, and pyrene was used as standard mixture for the described investigations. Samples were dissolved in pure acetonitrile. In the demonstration of a putative application, a mixture of eight acidic pharmaceuticals was used, namely baclofen, indoprofen, ibuprofen, fenoprofen, ketoprofen, suprofen, diclofenac, and mefenamic acid. These compounds were dissolved in methanol. Stock solutions of all compounds were prepared to have a concentration of 60 m*M*. Immediately before use, the mixtures were diluted to reach a final concentration between 5 and 15 m*M* for each compound.

As indicated in the individual experiments, mobile phases for the CEC separations consisted of secondary, sometimes even ternary mixtures of acetonitrile, methanol and aqueous buffers at various pH. Sample injection was performed electrokinetically (10 kV for 3 s). Pressure injection (10 bar for 3 s) was also used occasionally. The effective length of the capillary columns was 27 cm (inlet-to-detector, corresponding to a total length of 35.5 cm). Dimethylformamide (DMF) was used as unretained EOF marker. Prior to each set of experiments, the

column was preconditioned by flushing with the new mobile phase at 11 bar for about 30 min, while applying a voltage of 7 kV, followed by pure voltage equilibration (25 kV) for another 10 min. In between runs under identical chromatographic conditions, no conditioning was performed. The elution order in the electrochromatograms was determined by spiking the sample with the individual compounds.

3. Results and discussion

3.1. Monolith synthesis

Like any packed column, the monoliths under investigation here, have to satisfy certain criteria in order to be useful as stationary phases for electrochromatographic separations. They should be uniform over all the column length and show good mechanical and chemical stability. They should be macroporous with a controllable porosity and ideally show little tendency for swelling in any type of solvent. In addition, the final monolithic stationary phase should bear interactive sites for chromatographic retention/separation, but also some charged moieties in order to assure the EOF. Last but not least, true chromatographic monoliths should give reproducible results over several runs in order to allow a systematic investigation and separation method development.

Most polymerizations of monoliths reported to date in the pertinent literature were performed in organic solvents [11,23–26]. The resulting monoliths were usually rather hydrophobic in nature. Instead of following these examples, we opted for a polymerization using water (respectively a hydro–organic mixture) as solvent, an approach first suggested by Hjertén and co-workers [27–29]. It is known that this approach can be used with more hydrophilic/polar monomers. It should therefore result in more hydrophilic stationary phases, whose properties should be somewhat complementary to those of the hydrophobic ones. The porosity of a monolith prepared in water is assured by the addition of sufficient amounts of a salt such as ammonium sulfate, i.e., by a hydrophobic (''salting out'') effect [14,15,28,30,31].

The first part of our experiments was dedicated to the development of a robust synthesis procedure for CEC-compatible monoliths. Since an investigation of the chromatographic performance in relation to the monolith composition was planned, continuous beds of different hydrophobicities were prepared by using methacrylamide, dimethylacrylamide, butylacrylate and hexylacrylate, respectively to introduce the interactive sites. The preparation of the corresponding monoliths was a complex problem, since several highly interdependent parameters were found to determine the result of such a synthesis. The total monomer concentration together with the cross-linking degree determines, for example, the optical and mechanical properties of the resulting polymer. The concentration of the ammonium sulfate determines the pore structure (size). If one of these parameters is changed, all the others have to be adjusted in relation. Furthermore other factors like the chemical nature of the monomers or the initiator concentration also play an important role.

Different compositions of polymerization mixtures were tested first in a bulk approach, in order to obtain some information concerning polymer properties and porosity as a function of the synthesis conditions. The nomenclature introduced by Hjertén was used to describe the monoliths in terms of the total monomer content (%T) and the cross-linking degree (%C) [32]. In a first step the cross-linking degree (%C) and the total monomer concentration (%T) were varied, and the polymerization and precipitation kinetics observed, respectively. The resulting polymers were qualitatively evaluated for their rigidity, consistency and optical aspects. In a second step the effect of increasing the ammonium sulfate concentrations under otherwise identical polymerization conditions was investigated. The results are summarized in Table 1.

As expected, a gel type domain and a macro-

Table 1
Qualitative comparison of polymer compositions[a]

%T	%C	Consistency	Optical properties
35	10	Hard, brittle	White
35	55	Very hard	White
5	10	Gel like	Translucid
5	55	Soft, granulous	White

[a] Monomer: DMAA, cross-linker: PDA, no ammonium sulfate added.

porous domain could be observed for the various combinations depending on the monomer concentration and the cross-linking degree [33–35]. When the cross-linker concentration, but especially when the total monomer concentration was increased for a given combination of monomers, a transition from a gel type consistency to a hard polymer could be observed as the polymer formed. For a given monomer and cross-linker concentration, ammonium sulfate has an additional effect on the pore formation and in general the pore size increases with increasing amounts of salts. This is visualized in Fig. 1. In this figure a polymer where no salt has been added is compared with polymers, where 20 mg/ml, or 120 mg/ml of ammonium sulfate had been added. Concomitantly, porosity measurements revealed that the average pore size increased from 50 nm to 800 nm and finally 1.3 μm under these circumstances. The absolute effects of a given salt concentration are different for each monolith, but once conditions for the synthesis of a rigid, porous structure are found, the average pore size can easily be adjusted to the desired one by carefully optimizing the salt concentration. The addition of increasing amounts of ammonium sulfate hence has similar consequences for an acrylamide-based monolith as the addition of various alcohols to the organic solvents had on the outcome of the polymerization of the more hydro-

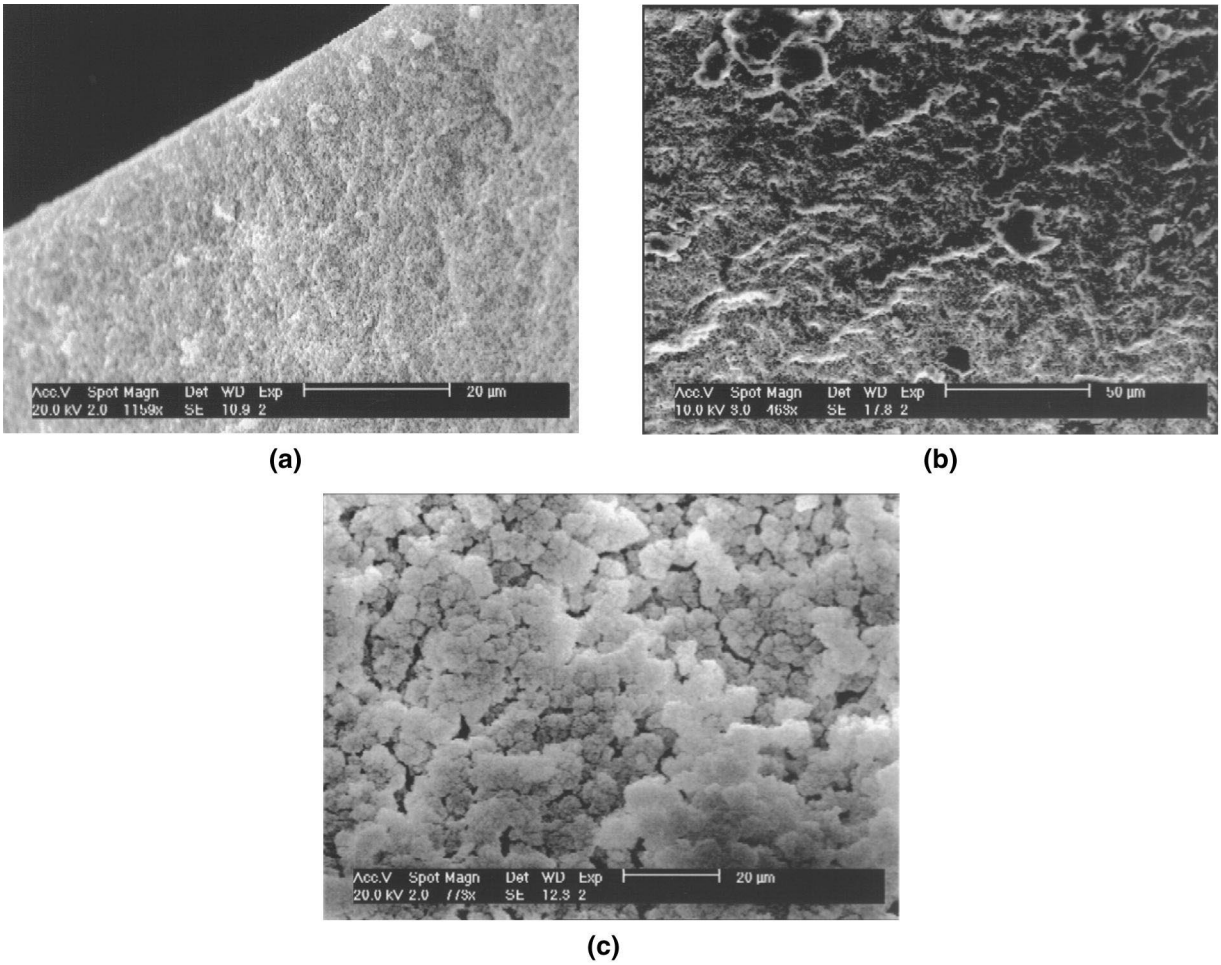


Fig. 1. Effects of ammonium sulfate concentration on the morphology of poly(DMAA–PDA–VSA) monoliths (T=29%, C=52%), prepared in the presence of (a) no, (b) 20 mg/ml, (c) 120 mg/ml ammonium sulfate in 50 m*M* phosphate buffer, pH 7.

phobic methacrylate-based monoliths described by Xie et al. [36].

From these preliminary experiments, monoliths with a high degree of cross-linking (%C=52%) and with a relatively high total monomer concentration (%T=15 or 29%) were chosen as candidates for the development of a robust stationary phase for CEC. PDA was preferred to *N,N′*-methylenebisacrylamide as a cross-linker, because of its better solubility in water. Solubility constraints equally had to be met when monomers with longer alkyl chains were used (C_4 and C_6). In such cases, a mixture of formamide–water (1:1, v/v) could be used successfully as a solvent and still be compatible with the redox initiation of the polymerization reaction used here [15]. However, in such cases the total monomer concentration had to be lowered to T=15% to assure full solubility of the monomers.

The optimized procedure resulted in monolithic columns, which were guaranteed to be stable up to a pressure of at least 170 bar. Good stability of the monolith towards both organic and aqueous solvents was also observed. After the initial flushing, no elution of monomers, e.g., due to decomposition was detected. Once developed, the success rate of the procedure in terms of producing a monolithic column of the expected qualities was close to 100%. The run-to-run reproducibility of the chromatographic performance was good (see Section 3.2) and even the batch-to-batch reproducibility between different columns gave satisfactory results.

3.2. Column performance studies

Four optimized monoliths, see Table 2, which differed mainly in the type of interactive sites were compared for their chromatographic performance. Column I was based on dimethylacrylamide moieties as interactive sites. Column II had a similar chemistry, but was prepared at lower monomer and ammonium sulfate concentration. As a result the average pore size of column II (0.84 μm) was considerably smaller than that of column I (1.3 μm). Column III carried some C_6 groups in addition to the dimethylacrylamide ones and column IV carried exclusively underivatized methacrylamide moieties. By necessity, the values for the mean pore radius were obtained in the dry state. It must be emphasized that this can substantially differ from the wet, even swollen state, under which the monoliths are operated in chromatography. The pore size values given in Table 2 thus represent a semi-quantitative appreciation of the relative differences between the four polymer types in terms of their respective permeabilities and surfaces.

A first experiment was used to demonstrate that the monolith covers the inner wall of the silica capillary well. A column of the general type I, where the vinylsulfonic acid monomer had been omitted, was used for that purpose. As a consequence of this omission, the acrylamide moieties introduced into this monolith carry no charge and therefore are not able to provoke an EOF. When the corresponding monolithic columns were used under conditions, which tended to provoke a strong EOF in naked silica capillaries, almost no EOF was observed for the monolith. A second set of experiments was carried out to determine a suitable procedure for the preparation of the detection window. Long-term column stability seemed to be much better, when the detection window (ca. 0.5 cm) was created by pyrrolyzation of the polymer, while being flushed with water, rather than when a technique was applied, where the capillary was only filled with the monomer mixture to a certain level, leaving the part after the detection window bare. The reasons for this

Table 2
Optimized column compositions

Column	%T	%C	DMAA (mg)	HeA (mg)	MA (mg)	PDA (mg)	$(NH_4)_2SO_4$ (mg)	VSA (mg)	Mean pore size (μm)
I	29	52	136	–	–	150	120	15	1.3
II	15	52	71	–	–	78	50	15	0.84
III	15	52	52	20	–	78	30	15	n.d
IV	15	52	–	–	71	78	50	15	0.84

n.d., Not determined.

kind of behavior are not quite clear yet. Since the pyrrolyzation method for the creation of the detection window resulted in stable, reproducible capillaries, alternative approaches to the creation of the necessary detection widow were not investigated.

Monolithic stationary phases have been used for chromatographic separations in HPLC but also in CEC for some time now. Most of these monoliths contain hydrophobic moieties, and reversed-phase chromatography is usually assumed to take place. Maruska et al., on the other hand, speak of normal-phase chromatography to describe the behavior of their acrylamide-based monoliths in chromatographic separations [37]. At the same time it is generally acknowledged that much of the fluidic and interaction behavior of monolithic stationary phases remains to be elucidated, even when only chromatographic separations are concerned [20]. In CEC, the situation becomes even more complicated by the fact that in addition to the hydrophobic moieties, charged functions have to be present in the stationary phase to generate the EOF.

To investigate the suitability of our acrylamide-based columns for the application in CEC, a sample mixture similar to the one suggested by Maruska et al. [37] was chosen, since we expected our column to have similar characteristics. In addition to the six polar aromatic compounds, however, three neutral polyaromatic hydrocarbons (naphthalene, phenanthrene, pyrene) were also included in the test mixture. These compounds have previously been used repeatedly in the investigations of reversed-phase separations [9,38–40]. The sample compounds should be uncharged under the investigated conditions, a property, which is very important to distinguish chromatographic from electrophoretic behavior in CEC separation. DMF, which gave a sharper signal than, e.g., the commonly used thiourea, was chosen as EOF marker in these experiments.

For column I, a mobile phase consisting of a mixture of acetonitrile–methanol (3:1) gave the best resolution for the nine compounds of the standard sample mixture. An optimized separation is shown in Fig. 2. The elution order observed in our case for an electrochromatographic separation corresponds well to the one observed by Maruska et al. for a purely chromatographic one on a similar column assuming

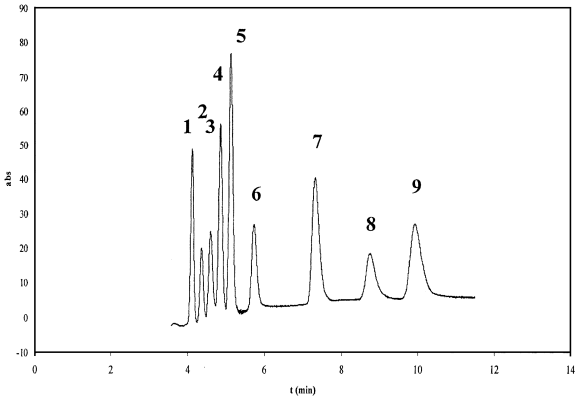


Fig. 2. Electrochromatographic separation of nine aromatic compounds: naphthalene (1), phenanthrene (2), pyrene (3), hydroquinone monomethyl ether (4), phenol (5), 2-naphthol (6), catechol (7), hydroquinone (8), resorcinol (9). UV detection at 200 nm. Mobile phase: methanol–acetonitrile (1:3), injection: 10 kV for 2 s. Voltage: 30 kV, temperature: 25°C.

‘‘normal-phase chromatography’’, independent of the mobile phase composition. When the same mixture was separated on a commercial, particle-based C_{18} CEC column (reversed-phase column), the observed elution order was not just inverted, as it would have been expected, but distinctly different, as the summary of the elution pattern in Fig. 3 shows.

From this it can be deduced that other mechanisms than just differences in hydrophobicity reign the separation. For further underlining of this assumption, monolithic columns with different degrees of hydrophobicity were compared. Column III was of particular interest in this context, since in this column 30% of the DMAA monomers were replaced by hexylacrylate (HeA) (C_6 moieties). According to some evidence from the literature [9,13–15], such acrylamide monoliths, where longish alkyl chains have been introduced, should become more supportive of reversed-phase separations. However, when the separations were compared, the elution order of the sample compounds in column type III appeared to be the same as in column type II, where no such replacement had taken place (Fig. 4). It should be noted that retention and resolution on these columns can not be properly compared, as pore size and specific surface were not adjusted, when the hydrophobicity was changed.

During the studies outlined above and especially

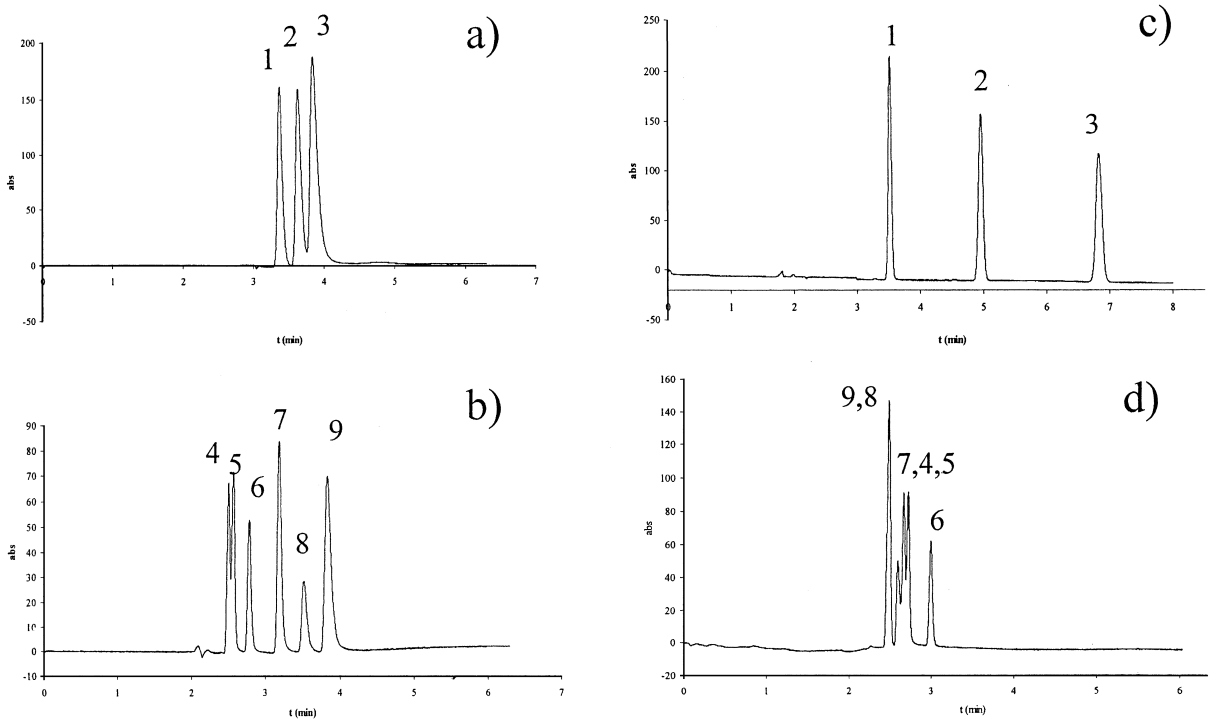


Fig. 3. Comparison of the elution order of aromatic compounds (numbering as in Fig. 2) in separations on a monolithic column type I (a, b) vs. separations on a commercial column packed with ODS C_{18} silica particles (c, d). Mobile phase: methanol–acetonitrile (6:4) (a, b), acetonitrile–5 m*M* phosphate, pH 7 (7:3) (c, d).

during the early mobile phase optimization experiments, columns were used under a variety of different and sometimes quite harsh conditions (pure organic phases to pure aqueous buffers with pH between 2.5 and 9). As expected, chromatographic performance changed with the conditions, and often no separation could be observed. Especially the salt or buffer concentrations of aqueous mobile phases seemed to be crucial. Mobile phases, which contained salts, often resulted in noisy baselines and nonreproducible peaks. However, generally the initial performance was recovered, even after several runs under ''nonfavorable'' conditions, once the former conditions were reestablished. This stability under a variety of chemical conditions augurs well for a systematic investigation of the performance of such columns as a function of their structure and chemistry.

The run-to-run reproducibility of a given capillary column under identical chromatographic conditions, as well as the batch-to-batch reproducibility of columns prepared and used under identical conditions are resumed in Table 3. Standard deviations of less than 1.5% were routinely found for consecutive runs. In order to assure good reproducibility from the first run onward, proper conditioning prior to the introduction of a new mobile phase was found to be extremely important. The optimized procedure consisted of first voltage assisted (7 kV) flushing of the column for 30 min with the mobile phase (11 bar) followed by pure voltage equilibration (25 kV) for another 10 min. Between the runs no conditioning was necessary. For the determination of the batch-to-batch reproducibility, nine monolithic columns (type I) were prepared and compared for the separation of the standard mixture. A relative standard deviation of only 5% was observed for the unretained EOF marker DMF in this case. This high

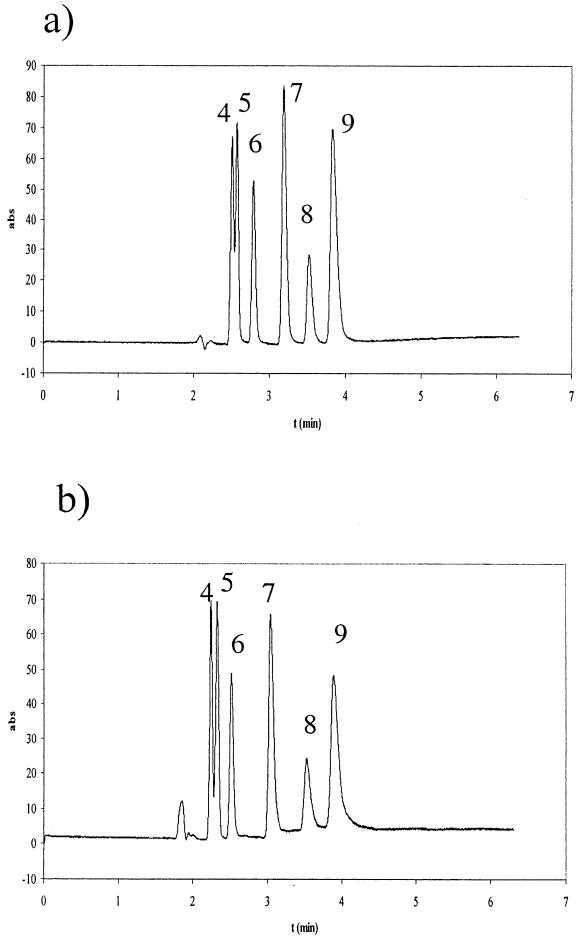


Fig. 4. Electrochromatographic separations of polar aromatic compounds (numbering as in Fig. 2) on a column type II (a), and on a column type III (containing 30% of C_6 moieties) (b). UV detection at 200 nm. Mobile phase: methanol–acetonitrile (6:4), injection: 10 kV for 3 s. Voltage: 30 kV, temperature: 25°C.

reproducibility can be considered as another good portent for the in depth investigations of the chromatographic properties of such monoliths.

The Van Deemter curves for the monolithic stationary phases were determined for DMF as unretained tracer and phenanthrene as a retained substance, Table 4. In Fig. 5, the plate height values are plotted against the linear velocity of the mobile phase for six consecutively obtained individual Van Deemter curves. The plate number N was calculated using the formula $N=5.54(t_R/w_{0.5})^2$, where t_R is the retention time and $w_{0.5}$ is the peak width at the half height of a peak. Accordingly, the plate height H was calculated as $H=L_{eff}/N$, where L_{eff} is the effective column length. The range of linear velocities corresponds to applied field strengths between 180 V/cm and 1110 V/cm (5–30 kV). As expected for a monolithic stationary phase in chromatography, we observed quasi-constant plate heights even at high linear velocities. The absolute values of the plate heights were between 10 and 15 μm at best. This is inferior to values reported for particle-based columns (e.g., Ref. [8]). A possible contribution of the detection widow to the plate height can not be excluded. However, this possibility was not investigated, since comparable capillaries lacking the de-

Table 3
Migration time reproducibilities[a]

Solute	t_R (min)	RSD (%)
(a) Run-to-run for consecutive runs ($n=10$)		
DMF (marker)	2.8	0.94
2-Naphthol	3.6	1.26
Resorcinol	4.8	1.34
(b) Batch-to-batch ($n=9$)		
DMF	4.1	5

[a] Conditions: stationary phase: as column I for (b), as column II for (a); applied voltage: 30 kV for (a), 25 kV for (b); mobile phase: methanol–acetonitrile (6:4) for (a), methanol for (b).

Table 4
Average retention times, plates per meter and plate height optima derived from six consecutive Van Deemter curves measured for the indicated analytes

Column type	Analyte	t_R (min)	N/m	H (μm)
DMAA, I	Phenol	5.9	$3.2\cdot10^4$	31.2
	2-Naphthol	6.8	$6.8\cdot10^3$	39.5
	Naphthalene	3.4	$4.1\cdot10^4$	24.0
	DMF	2.9	$5.8\cdot10^4$	17.4
DMAA, II	2-Naphthol	2.8	$5.2\cdot10^4$	19.1
	DMF	2.4	$1.1\cdot10^5$	9.5
DMAA/HeA, III	2-Naphthol	4.8	$3.8\cdot10^4$	26.5
	Naphthalene	3.2	$1.1\cdot10^5$	8.9
	DMF	2.7	$1.0\cdot10^5$	9.8

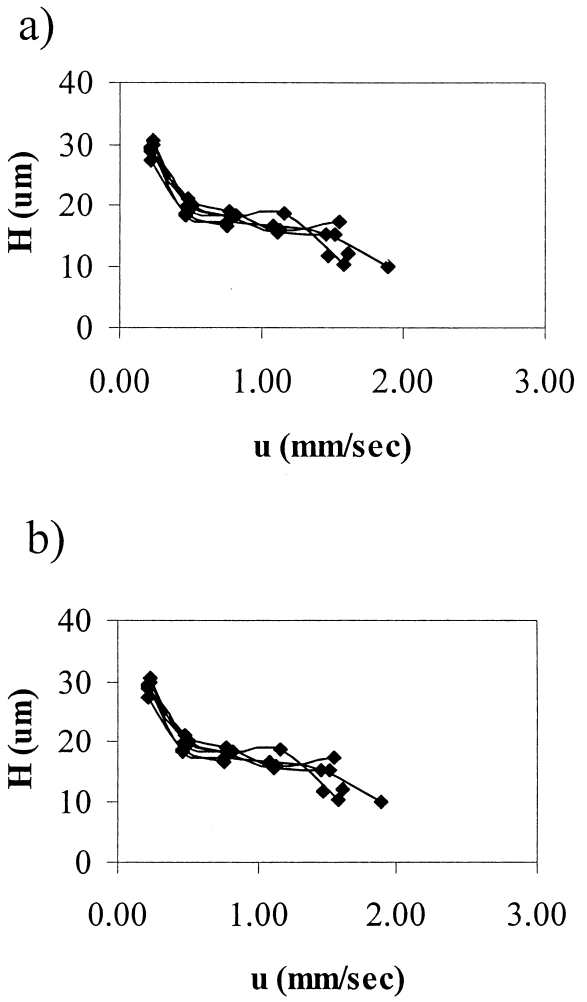


Fig. 5. Six repeated plots (Van Deemter curves) of the plate heights vs. linear velocity of an unretained marker (DMF) (a) and a retained compound (phenanthrene) (b). Stationary phase: column type III, mobile phase: methanol–acetonitrile (6:4). UV detection at 200 nm.

tection window but still compatible to on column detection were not available.

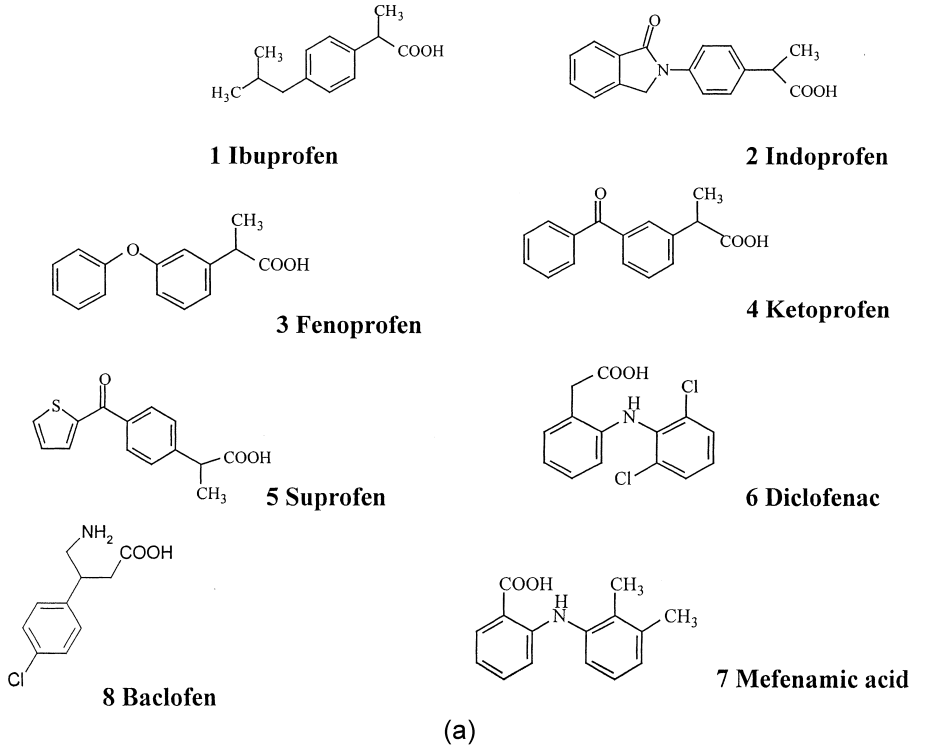
3.3. Analysis of pharmaceuticals

Most previously published separations in CEC used similar compound mixtures as the one used as standard mixture in this study. In order to extend the range of these applications, the separation of a mixture of seven nonsteroidal anti-inflammatory drug molecules was attempted using a monolithic column of type I to develop the method. The chemical structures of these compounds is shown in Fig. 6a. A similar mixture had previously been analyzed by nonaqueous capillary electrophoresis [41]. In that case the apparent pH of the organic mobile phase had been adjusted to 8.5 in order to ensure that the compounds were negatively charged. On the monolithic CEC column, the separation was possible at a pH of 3, where most of the compounds could be expected to be uncharged. A mixed mobile phase composed of methanol–acetonitrile–25 m*M* ammonium acetate in water (6:2:2) was used. The resulting separation is shown in Fig. 6b.

Only six peaks are seen in this electrochromatogram. From spiking experiments is became apparent that the signal of substances 3 and 4 (i.e., fenoprofen and ketoprofen) could not be resolved. The structure of these compounds differs by a single function, where a keto group is replaced by an ether function. In the CE experiment, baclofen had been included in the sample mixture. This component bears a positive charge at low pH and therefore eluted before the EOF marker. If this drug was also introduced in the analyte mixture under CEC conditions, poor peak shapes were observed for this compound (signal not shown). Adsorption of charged compounds, which show charges opposite to the EOF generating monomers, often leads to poor peak shapes in CEC. If the EOF generating charges are of the same sign as those of the charged analytes, little secondary interaction with the stationary phase takes place. Hence the problem can to some extent be ''solved'' by working under conditions where all analytes are either uncharged or bear the same charge as the chromatographic surface [42,43]. However, the simultaneous analysis of samples containing species of opposite charges by CEC remains difficult.

4. Conclusions

CEC may benefit from the use of monolithic rather than particle-based columns. A major aim of the outlined research was the definition of a reliable protocol for the chemical synthesis of such relatively hydrophilic monoliths, but also the definition of an



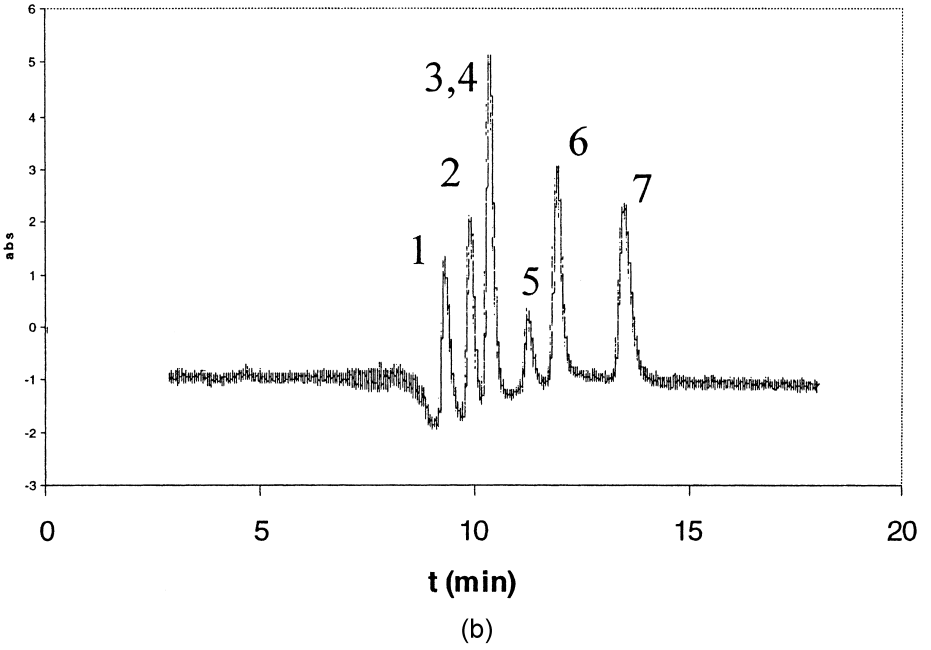


Fig. 6. Electrochromatographic separation (b) of seven nonsteroidal anti-inflammatory drugs (structures in a). Stationary phase: column type I, UV detection at 214 nm. Mobile phase: methanol–acetonitrile–25 m*M* ammonium acetate, pH 3 (6:2:2). Voltage: 30 kV, temperature: 25°C.

algorithm for the straightforward development and optimization of the synthetic procedure, once a given set of components (interactive sites, charged functions) has been chosen. We come to the conclusion that at least in the cases where acrylamide-based stationary phases can be used, the approach outlined here has some advantages. If the polymerization is done in water, respectively, in hydro–organic solutions, the rigidity and also average pore sizes can be adjusted in a simple manner. A major challenge in CEC is the understanding of the interaction mechanism and hence the choice of a suitable stationary phase for a given analyte mixture. The analysis of some drug molecules was intended as a first inclination of which type of molecules may profit from being analyzed on the acrylamide-based monolithic CEC columns.

Acknowledgements

This work was supported by the Swiss National Science Foundation (grant No. 20-58879.99 to R.F.).

References

[1] J.H. Knox, I.H. Grant, Chromatographia 7/8 (1991) 317.
[2] J.H. Knox, I.H. Grant, Chromatographia 24 (1987) 135.
[3] G. Choudhary, Cs. Horváth, J. Chromatogr. A 781 (1997) 161.
[4] E. Wen, R. Asiaie, Cs. Horváth, J. Chromatogr. A 855 (1999) 349.
[5] M.M. Dittmann, K. Wienand, F. Bek, G.P. Rozing, LC–GC 13 (1995) 800.
[6] C. Yu, F. Svec, J.M.J. Fréchet, Electrophoresis 21 (2000) 120.
[7] H. Yamamoto, H. Baumann, F. Erni, J. Chromatogr. 593 (1992) 313.
[8] M.M. Dittmann, G.P. Rozing, J. Chromatogr. A 744 (1996) 63.
[9] C. Fujimoto, Y. Fujise, E. Matsuzawa, Anal. Chem. 68 (1996) 2753.
[10] C. Fujimoto, J. High Resolut. Chromatogr. 23 (2000) 89.
[11] I. Gusev, X. Huang, Cs. Horváth, J. Chromatogr. A 855 (1999) 273.
[12] C. Ericson, J.-L. Liao, K. Nakazato, S. Hjertén, J. Chromatogr. A 767 (1997) 33.
[13] C. Ericson, S. Hjertén, Anal. Chem. 71 (1999) 1621.
[14] J.-L. Liao, N. Chen, C. Ericson, S. Hjertén, Anal. Chem. 68 (1996) 3468.
[15] A. Palm, M.V. Novotny, Anal. Chem. 69 (1997) 4499.
[16] B. Xiong, L. Zhang, Y. Zhang, H. Zou, J. Wang, J. High Resolut. Chromatogr. 23 (2000) 67.
[17] J. Matsui, I.A. Nicholls, T. Takeuchi, Anal. Chim. Acta 365 (1998) 89.
[18] L. Schweitz, L.I. Andersson, S. Nilsson, Anal. Chem. 69 (1997) 1179.
[19] L. Schweitz, L.I. Andersson, S. Nilsson, J. Chromatogr. A 792 (1997) 401.
[20] F. Svec, E.C. Peters, D. Sykora, C. Yu, J.M.J. Fréchet, J. High Resolut. Chromatogr. 23 (2000).
[21] E.C. Peters, M. Petro, F. Svec, J.M.J. Fréchet, Anal. Chem. 69 (1997) 3646.
[22] E.C. Peters, M. Petro, F. Svec, J.M.J. Fréchet, Anal. Chem. 70 (1998) 2288.
[23] T.B. Tennikova, M. Bleha, F. Svec, T.V. Almazova, B.G. Belenkii, J. Chromatogr. 555 (1991) 97.
[24] S. Xie, F. Svec, J.M.J. Fréchet, J. Chromatogr. A 775 (1997) 65.
[25] Q.C. Wang, F. Svec, J.M.J. Fréchet, J. Chromatogr. A 669 (1994) 230.
[26] D. Sykora, F. Svec, J.M.J. Fréchet, J. Chromatogr. A 852 (1999) 297.
[27] C.-M. Zeng, J.-L. Liao, K. Nakazato, S. Hjertén, J. Chromatogr. A 753 (1996) 227.
[28] J.-L. Liao, R. Zhang, S. Hjertén, J. Chromatogr. 586 (1991) 21.
[29] S. Hjertén, J. Mohammad, K. Nakazato, J. Chromatogr. 646 (1993) 121.
[30] P.G. Righetti, M. Chiari, M. Nesi, S. Caglio, J. Chromatogr. 638 (1993) 165.
[31] P.G. Righetti, J. Chromatogr. A 698 (1995) 3.
[32] S. Hjertén, Arch. Biochem. Biophys., Suppl. 1 (1962) 147.
[33] A. Guyot, in: C. Sherington, P. Hodge (Eds.), Syntheses and Separations using Functional Polymers, Wiley, New York, 1988, p. 1.
[34] A. Guyot, M. Bartholin, Prog. Polym. Sci. 8 (1982) 277.
[35] W.L. Sederel, G.J. de Jong, J. Appl. Polym. Sci. 17 (1973) 2835.
[36] S. Xie, F. Svec, J.M.J. Fréchet, J. Polym. Sci. 35 (1997) 1013.
[37] A. Maruska, Ch. Ericson, A. Végvári, S. Hjertén, J. Chromatogr. A 837 (1999) 25.
[38] R. Stol, W.T. Kok, H. Poppe, J. Chromatogr. A 853 (1999) 45.
[39] S. Constantin, R. Freitag, J. Chromatogr. A 887 (2000) 253.
[40] R. Asiaie, X. Huang, D. Farnan, Cs. Horváth, J. Chromatogr. A 806 (1998) 251.
[41] S. Cherkaoui, J.-L. Veuthey, J. Chromatogr. A 874 (2000) 121.
[42] T. Koide, K. Ueno, J. High Resolut. Chromatogr. 23 (2000) 59.
[43] X. Huang, J. Zhang, Cs. Horváth, J. Chromatogr. A 858 (1999) 91.