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Capillary electrochromatography with polymeric continuous beds synthesized via free radical polymerization in aqueous media using derivatized cyclodextrins as solubilizing agents

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Abstract

A novel synthetic route to amphiphilic acrylamide-based monolithic stationary phases for capillary electrochromatography (CEC) employing water-soluble cyclodextrins as solubilizing agents was explored. *N*,*N*′-Octamethylenebisacryamide and *N*,*N*′-dodecamethylenebisacryamide were synthesized and their solubilization in aqueous solution with derivatized and underivatized cyclodextrins of different cavity size was studied. Amphiphilic stationary phases were synthesized by free radical copolymerization of the bisacrylamide–cyclodextrin host–guest complexes with hydrophilic monomers and an additional hydrophilic cross-linker in aqueous solution. Complex formation in solution and removal of the complexed cyclodextrin from the polymer during synthesis was studied with ^{1}H-NMR and solid state ^{13}C-NMR spectroscopy and cyclodextrin-modified micellar electrokinetic chromatography. The impact of the incorporated alkylene groups in the acrylamide-based macroporous polymer on retention was studied with neutral solutes by CEC in the normal-phase elution mode and in the reversed-phase elution mode. Batch-to-batch reproducibility of the synthesis procedure and day-to-day repeatability of the separations achieved were investigated. With these capillaries, a sufficiently high electroosmotic flow velocity, a high reproducibility and repeatability of separation parameters and high plate numbers (up to 200 000 m^{-1}) were obtained.

Keywords: Electrochromatography; Monolithic columns; Complexation; Cyclodextrins

1. Introduction

Capillary electrochromatography (CEC) is a rapidly emerging separation technique [1]. According to the IUPAC provisional recommendations [2], CEC is a separation method in which the propulsion of the mobile phase through a capillary filled, packed or coated with a stationary phase, is achieved by electroosmosis. The peculiarities of the electroosmotic flow make it possible to obtain higher plate numbers and higher peak capacities with CEC than with HPLC (in the isocratic mode). Another feature of CEC is the presence of a high electric field strength in the separation chamber, so that electrophoresis and chromatographic principles do both contribute to the separation of solutes.

Several review papers on advances in CEC highlight the advantages of continuous beds (capillaries filled with a monolithic stationary phase) over packed separation capillaries in CEC [3–5]. These advantages include ease of preparation, absence of fragile frits, and low resistance to mass transfer. Several types of organic monoliths (macroporous organic polymers) have been investigated as continuous beds in CEC [6–8]. Organic monoliths are prepared by filling the (pre-treated) capillary with a solution of monomers (and initiator system) dissolved in a porogenic liquid (e.g. mixture of solvents), followed by in situ polymerization. The porosity of the formed macroporous polymer is due to phase separation of the solid polymer from the porogenic liquid during polymerization.

Acrylamide-based continuous beds form one group of organic monoliths. Compressed acrylamide-based continuous beds for HPLC have been developed by Hjertén's group [9–12]. Acrylamide-based continuous beds can be also

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prepared in pre-treated fused-silica capillaries. They have been introduced as stationary phases in CEC by Hjertén [13] and Fujimoto et al. [14]. Some acrylamide monomers are very polar and water-soluble. Polymerization can then be performed with aqueous solutions of the monomers. Recently, Maruška [15] gave an overview on the preparation and properties of continuous beds prepared from water-soluble acrylamide-based monomers.

It is obvious that stationary phases prepared from these hydrophilic monomers will also be relatively hydrophilic. Consequently, retention in reversed-phase chromatography is relatively low. If more hydrophobic monomers are to be incorporated in the organic monolith, mixtures of an aqueous buffer with an organic solvent (e.g. formamide) have been used as porogenic liquid [17] in order to dissolve the hydrophobic monomers during the polymerization procedure. Alternatively, octadecyl groups can be anchored to the polymer via the BF_3 catalyzed reaction of octadecanol with epoxide groups of the polymeric matrix [17] or the reaction of 1,2-epoxyoctadecane with hydroxyl groups of the polymeric matrix [18,19]. Also synthesis of macroporous polymers in aqueous solution with emulsified (Triton-X 100 as surfactant) hydrophobic monomers (stearyl methacrylate or butyl methacrylate) [20], and by suspension polymerization with sonicated stearyl methacrylate [21] have been reported.

The morphological properties (swollen gel or rigid monolith) of the synthesized polymer depend on the composition of the monomer solution, the composition of the porogenic liquid and other reaction parameters. Conditions that allow the synthesis of acrylamide-based monolithic microcolumns for CEC have been investigated [15,22]. Crucial parameters comprise the total monomer concentration in the reaction mixture, the molar fraction of cross-linker relative to the total monomer molar amount, the solubility of the monomers in the porogenic liquid, the volume fraction of an organic solvent in the porogenic liquid, and the polymerization reaction rate. In case of polyacrylamide-based monoliths with hydrophobic sections, phase separation is effected by the presence of a lyotrophic salt (e.g. ammonium sulfate (AS)) in the porogenic liquid via the salting-out effect [23]. The concentration of this salt in the reaction mixture determines the morphology of the produced polymer. The higher the salt concentration is, the lower is the microcolumn backpressure [15,24] and the higher is the roughness of the continuous bed surface [25], indicating larger channels in the polymeric skeleton (and hence higher streaming permeability).

Acrylamide-based monoliths containing hydrophilic and hydrophobic groups can be characterized as amphiphilic [9]. Their amphiphilic nature makes it possible to use them in the normal- [26] and the reversed-phase mode [27]. Taking the elution order of hydrophilic and hydrophobic solutes as an indication of the chromatographic mode, Hoegger and Freitag [16] were able to show that separations with amphiphilic acrylamide-based continuous beds are neither pure normal-phase nor pure reversed-phase chromatographic separations. Keeping in mind that these stationary phases can be characterized as a patchwork of hydrophilic and hydrophobic groups, a mixed-mode selectivity will explain the unique selectivities observed with relatively non-polar mobile phases (methanol–acetonitrile, 1:3, v/v).

In our paper a novel synthetic route to amphiphilic acrylamide-based monolithic stationary phases is explored. Polymerizations of hydrophobic monomers in aqueous media are possible if the monomers are solubilized via host–guest complexation with water-soluble cyclodextrins (CDs) [28]. Cyclodextrins are cyclic oligoamyloses. They are able to enclose hydrophobic molecules to form host–guest complexes, where the hydrophobic guest molecule is encapsulated by the cyclodextrin. The outer hydrophilic surface of the cyclodextrin then interacts with water in order to maintain the water-solubility of the complex. This inclusion complex formation results in significant changes of the solution properties and reactivities of the guest molecule [29]. Ritter and co-workers have demonstrated for several water-insoluble monomers that free radical homopolymerization and copolymerization of monomer–CD complexes (pseudorotaxanes) in aqueous solution is possible. The free radical homopolymerization of *tert*-butyl methacrylate–cyclodextrin host–guest complexes [30], the free radical homopolymerization of cyclohexyl and phenyl methacrylate–cyclodextrin host–guest complexes [31], the free radical homopolymerization of *N*-methacryloyl-1-aminononane–cyclodextrin host–guest complexes [32] and the free radical copolymerization of hydrophobic acrylate–cyclodextrin host–guest complexes [33–35] and methacrylate–cyclodextrin host–guest complexes [36] were reported.

During chain propagation the host [e.g. statistically methylated β-cyclodextrin (Me-β-CD)] may slip off from the monomer. Due to its high water solubility, Me-β-CD remains dissolved in the aqueous phase. Ritter and co-workers report that the resulting hydrophobic polymer will be free of Me-β-CD [30,31,34,35]. In some cases, traces of Me-β-CD remain unextractable in the polymer [32,36,37]. Quantitative evaluation of ^{1}H-NMR spectra of the dissolved polymer reveals that the molar ratio of monomer unit to Me-β-CD is in all cases less than 5%.

In order to verify that host–guest complex formation with cyclodextrins can be also successfully applied in the synthesis of acrylamide-based monolithic stationary phases for CEC, we synthesized a series of *N*,*N*′-alkylenebisacryamides with carbon chain numbers ranging from 8 to 12 and studied the solubilization of these hydrophobic monomers in aqueous solution with derivatized and underivatized cyclodextrins of different cavity size.

Amphiphilic stationary phases were synthesized by free radical copolymerization of the bisacrylamide–cyclodextrin host–guest complexes with hydrophilic monomers and an additional hydrophilic cross-linker in aqueous solution. According to our knowledge synthesis of continuous beds by copolymerization of bisacrylamide–cyclodextrin host–guest

complexes with hydrophilic monomers has not been reported so far. Complex formation and removal of the complexed cyclodextrin from the polymer during synthesis was studied with ^{1}H-NMR and solid state ^{13}C-NMR spectroscopy and cyclodextrin-modified micellar electrokinetic chromatography (CD-MEKC). Impact of the alkylene groups incorporated into the acrylamide-based macroporous polymer on retention was studied with neutral solutes by CEC in the normal-phase elution mode and in the reversed-phase elution mode. Batch-to-batch reproducibility of the synthesis procedure and day-to-day repeatability of the separations achieved were investigated.

2. Experimental

2.1. Chemicals

All chemicals were used without further purification. 3-(Trimethoxysilyl)propyl methacrylate (Bind silane), piperazine diacrylamide (PDA), *N*,*N*-dimethylformamide (DMF), disodium hydrogenphosphate and ammonium sulfate were from Fluka (Buchs, Switzerland). Statistically methylated β-cyclodextrin (mean substitution degree 1.8), *N*-isopropylacrylamide (N-IPA), vinylsulfonic acid [VSA, 25 % (w/v) in aqueous solution], *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED), 4-hydroxy-3-methoxybenzaldehyde, acrylyl chloride, 1,8-diaminooctane, 1,10-diaminodecane, 1,12-diaminododecane, and 4-ethylaniline were from Sigma–Aldrich (Steinheim, Germany). Methacrylamide (MA), ammonium peroxodisulfate (APS), sodium dihydrogenphosphate, acetic acid (100%, analytical-reagent grade), alkylphenones and alkyl benzoates, 4-hydroxybenzaldehyde, 3-ethoxy-4-hydroxybenzaldehyde, and potassium carbonate were from Merck (Darmstadt, Germany), and hexanophenone from Acros Organics (NJ, USA).

2.2. Preparation of mobile phases

With all mobile phases, the specific electric conductivity and the apparent pH (pH*) were controlled. We define here the pH* as the pH that is indicated for a solution containing an organic solvent with a pH-meter calibrated with aqueous standards. Consequently, the pH* was determined with the mobile phase after addition of the organic solvent to the aqueous buffer. The multimeter used (WTW Inolab, Weilheim, Germany) is equipped with a pH electrode (WTW Sentix81) and a conductivity measurement cell. Methanol (MeOH) was HPLC gradient grade (Merck, Darmstadt, Germany). Water was doubly distilled and further purified by a Milli-Q plus ultra pure water system (Millipore, Billerica, MA, USA). Triethylamine (TEA) was from Alfa Aesar (Karlsruhe, Germany).

Mobile phases with constant volume fraction of methanol (30%, v/v) and varied pH* were prepared by addition of MeOH–0.22 mmol L^{-1} aqueous solution of TEA (30:70, v/v) to MeOH–0.31 mmol L^{-1} aqueous solution of acetic acid (30:70, v/v). The desired electric conductivity was obtained by further addition of MeOH–water (30:70) to the prepared buffer solution. Other mobile phases with varying volume fraction of methanol were prepared by addition of a mixture of acetic acid–TEA (1:1, v/v) to the MeOH–water mixtures. The resulting pH* was 5.3 ± 0.3. An electric conductivity of 120 μS cm^{-1} was adjusted by further addition of MeOH–water mixtures.

Mobile phases with pure methanol were obtained by addition of acetic acid–TEA [2.4:1, v/v, pH* 6.4 (separation of phenols) or 1:4.8, v/v, pH* 8.7 (4-ethylaniline as solute)] to methanol. An electric conductivity of 140 or 250 μS cm^{-1} was adjusted by further addition of methanol.

2.3. Synthesis of *N*,*N*'-alkylenebisacryamides

N,*N*′-Octamethylenebisacrylamide (BA8), *N*,*N*′-decamethylenebisacrylamide (BA10) and *N*,*N*′-dodecamethylenebisacrylamide (BA12) were synthesized according to [38]. 0.15 mol acrylyl chloride were dissolved in 150 mL dried toluene, to which 0.2 mol potassium carbonate were added. At 0 °C 0.039 mol of diamine dissolved in 250 mL dried toluene were added. The mixture was stirred for 2.5 h at 0 °C. After completion of the reaction 400 mL distilled water were added. The precipitate was filtrated, rinsed successively with each 100 mL hydrochloric acid (0.1 mol L^{-1}), NaOH solution (0.1 mol L^{-1}), and distilled water and finally dried in vacuo. The crude product was purified by recrystallization in methanol. A white crystalline material (yield about 33%) was obtained. Identification was done by IR, ^{1}H-, and ^{13}C-NMR spectroscopy.

2.4. Synthesis of stationary phases

Fused silica capillaries (100 μm I.D. × 360 μm O.D., Polymicro Technologies, Phoenix, AZ, USA) were pre-treated with 3-(trimethoxysilyl)propyl methacrylate (bind silane) according to Hjertén [39]. First the capillaries were purified by rinsing with acetone, hydrochloric acid (0.1 mol L^{-1}), NaOH solution (0.1 mol L^{-1}), water, and finally acetone. Then the capillary is rinsed 30 min with a solution of bind silane in acetone (30%, v/v) and left overnight filled with this solution at room temperature. After silanization the reaction solution is washed out with acetone and water.

If the polymerization mixture (see Table 1) contains an *N*,*N*′-alkylenebisacryamide, this monomer and statistically methylated β-cyclodextrin are dissolved in 1 mL of methanol. Then the solvent is evaporated in a gentle stream of nitrogen. The residue is dissolved in 1 mL phosphate buffer (50 mmol L^{-1} sodium dihydrogenphosphate, 50 mmol L^{-1} disodium hydrogenphosphate, pH 7). To this solution the other monomers and the lyotrophic salt ammonium sulfate are added according to

Table 1
Composition of polymerization mixtures and dimensions of prepared separation capillaries (I.D. = 100 μm)

Capillary	PDA (mg)	BA8 (mg)	BA12 (mg)	MA (mg)	N-IPA (mg)	VSA (μL)	Me-β-CD (mg)	AS (mg)	L_{eff} (cm)	L_{tot} (cm)
MA1	125	–	–	230	–	20	–	128	17.3	23.5
MA2	78	–	–	71	–	20	–	50	17.3	23.3
MA3	75	4	–	71	–	20	104	50	16.2	22.0
MA4	72	8	–	71	–	20	208	50	16.1	21.9
MA5a; b; c	66	16	–	71	–	20	416	50	15.8; 16.0; 15.8	21.7; 22.1; 21.4
N1	109	16	–	–	133	8	416	24	15.5	21.6
N2	109	–	20	–	133	8	416	16	16.6	22.8
N3	121	–	–	–	133	8	–	24	16.4	22.6

PDA, piperazine diacrylamide; BA8, *N*,*N*′-octamethylenebisacrylamide; BA12, *N*,*N*′-dodecamethylenebisacrylamide; MA, methacrylamide; N-IPA, *N*-isopropylacrylamide; VSA, vinylsulfonic acid; Me-β-CD, statistically methylated β-cyclodextrin; AS, ammonium sulfate; L_{eff}, length of capillary to the detector; L_{tot}, total length of capillary.

Table 1. If the polymerization mixture does not contain an *N*,*N*′-alkylenebisacryamide, the first step is omitted and the monomers are directly dissolved in the phosphate buffer. The free solution radical polymerization is started with a redox initiator. Solutions of TEMED (10%, v/v) and APS (10%, w/v) in distilled water (20–40 μL) were added to the degassed monomer solutions.

Polymerization in capillaries was done with an in-house manufactured capillary-filling apparatus. A similar set-up is given in [18]. First the monomer solutions were degassed with help of a membrane vacuum pump (ca. 15 min) and refilling the space with argon. Then the redox initiator solutions were added under a gentle stream of argon. A few seconds after starting the polymerization the pre-treated capillary is dipped into the reaction mixture. With help of an argon overpressure (ca. 1 bar) the reaction mixture is pressed into the capillary. The filled capillary is taken out, the ends are sealed with silicon grease and the capillary is left overnight at room temperature. After completion of the polymerization the capillary is rinsed with distilled water. The monolith formed is visually inspected with an optical microscope.

Before capillaries filled with a monolith are employed in CEC, they were rinsed with help of an HPLC pump and a flow splitter at 50–100 bar for 3 h with distilled water. During this rinsing procedure the detection window is produced by heating a short section (about 4 mm) of the filled capillary with a red glowing wire. During heating the outer coating of the capillary is removed and the inner polymer is hydrolyzed and removed by the stream of water, so that a short open section is formed. Afterwards the capillary is installed in the CEC apparatus and rinsed for 30 min with mobile phase.

Polymers for subsequent solid phase ^{13}C-NMR studies have been prepared in an analogues fashion. However, no capillary was filled. The produced bulk polymer was crushed and rinsed with distilled water to remove not-immobilized Me-β-CD and dried in a desiccator. The composition of the polymerization mixtures is identical to those given in Table 1 for N1, N2 and N3.

2.5. Chromatographic system

The CEC apparatus consists of a Spellman (Plainview, NY, USA) CZE 1000R high-voltage generator with in-house manufactured electronic steering unit for controlled electrokinetic injection, a Spectra 100 UV–vis detector (Thermo Separation Products, San Jose, CA, USA) with detection cell for in-capillary detection, and a Shimadzu (Kyoto, Japan) LC-10 AD HPLC pump for conditioning of the separation capillary with new mobile phase under pressure. Data treatment and recording was with EZ-Chrom 6.6 (Scientific Software, San Ramon, CA, USA). Sample solutions (200–250 mg L^{-1}) were prepared in methanol. Generally, the injection parameters were set at 6 kV for 3 s. UV detection was at a wavelength of 230 nm. If not otherwise indicated, measurements were repeated four times to control repeatability. DMF was marker of the hold-up time.

Micellar electrokinetic chromatography (MEKC) was done with a P/ACE MDQ system (Beckman, Fullerton, CA, USA) and fused-silica capillaries of 75 μm I.D. (360 μm O.D., Polymicro technologies). Samples were prepared in methanol–water (50:50, v/v).

2.6. NMR studies

^{1}H-NMR and ^{13}C-NMR spectra of solutions were recorded with a Varian INOVA 500 (Varian, Palo Alto, CA, USA).

The ^{13}C solid-state NMR experiments were conducted on a Bruker AVANCE 700 spectrometer (Bruker BioSpin, Rheinstetten, Germany) at Larmor frequencies of 700.13 MHz (^{1}H) and 176.32 MHz (^{13}C). A Bruker double-resonance probehead was used with magic-angle spinning (MAS) capabilities supporting MAS rotors of 4 mm outer diameter. ^{1}H-to-^{13}C cross-polarization (CP) spectra were recorded under 12 kHz MAS, using a contact time of 2 ms and a ramped contact pulse (80–100% amplitude on ^{13}C). While acquiring the ^{13}C signal, TPPM decoupling [40] was applied on ^{1}H using an effective radio-frequency field of ∼100 kHz.

3. Results and discussion

3.1. CEC apparatus

Usually, CEC is performed with upgraded (pressurized vials) instrumentation designed for capillary electrophoresis. This instrumentation has the disadvantage that the separation capillary has to be bent. Bending of monoliths might induce mechanical stress resulting in cracks and other mechanically induced irregularities leading to enhanced instrumental band broadening. In the apparatus used by our group the separation capillary is fixed at the outlet end in a stainless steel t-piece in a similar fashion as previously published [41]. However, in contrast to our previously constructed apparatus the separation capillary is now completely straight installed. The direction of the stream of mobile phase during a chromatographic run is in upwards direction against gravitation. The inlet end is open and dips into the inlet buffer vial. Sample injection can be made very simply at this end. With the presented apparatus, electronically controlled electrokinetic injection was realized.

High voltage is applied between a platinum wire dipping into the inlet vial and the stainless steel t-piece fixed to the outlet end of the separation capillary. It is possible to apply pressure at the outlet end of the separation capillary with help of an HPLC pump and to rinse or condition the capillary with fresh mobile phase without taking it out of the system. During a CEC run the HPLC pump is switched off and no pressure is applied at the end of the packed capillary. For UV detection, the detection volume is an open section of the fused-silica separation capillary.

According to Rebscher and Pyell [42] pressures up to about 1000 bar can be produced by electroosmosis with a capillary of 30 cm × 100 μm I.D., packed with 3 μm octadecyl silica gel. Also with capillaries filled with an organic monolith it can be assumed that the electroosmotic pressure generated is so high that the inversely directed hydrostatic pressure due to the geometry of the apparatus and the hydrostatically induced laminar counterflow can be neglected. The validity of these assumptions was checked with following experiments.

In Fig. 1 the electroosmotic velocity v_{eo} for capillary MA1 with methanol–aqueous buffer (30:70, v/v, pH* 5.1, 140 μS cm^{-1}) as mobile phase (mean value and standard deviation (S.D.) for four runs) is plotted against the electric field strength. If there were a substantial hydrostatically induced laminar counterflow, we would expect a positive *x*-axis intercept and strong deviation from linearity. Indeed, there is a small deviation from linearity at a field strength higher than 600 V cm^{-1}. However, in CEC with hierarchically-structured macroporous monolithic materials [meso(or micro-) porous monolith skeleton], employing mobile phases with low ionic strength a non-linear dependence of v_{eo} on the electric field strength might be attributed to electroosmosis of the second kind, which has been reported by Nischang and Tallarek [43]. At lower field strength than

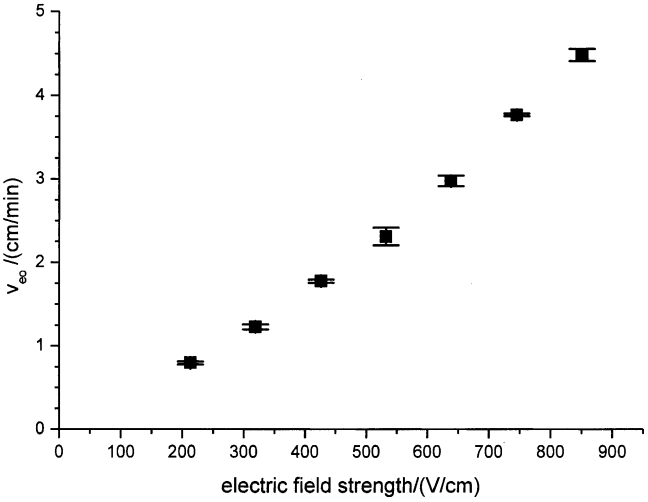

Fig. 1. The electroosmotic velocity v_{eo} determined for capillary MA1 vs. the electric field strength (mean value and S.D. for four consecutive runs). Mobile phase: methanol–aqueous buffer (30:70, v/v) specific electric conductivity χ 140 μS cm^{-1}; capillary dimensions 235 mm (173 mm) × 100 μm; UV detection at 230 nm; injection: 6 kV for 3 s.

600 V cm^{-1} there is a linear dependence of v_{eo} on the field strength. The *x*-axis intercept (taking only the first four measuring points into calculation) is only 52 V cm^{-1}.

The electroosmotic velocity is dependent on the ionic strength of the mobile phase. As we would expect from theory [44], there is a decrease in v_{eo} with higher specific electric conductivity of the mobile phase. At an electric field strength of 638 V cm^{-1} v_{eo} is decreased from 3.41 cm min^{-1} at a specific electric conductivity χ = 30 μS cm^{-1} to 2.59 cm min^{-1} at χ = 590 μS cm^{-1}. In further experiments mobile phases with specific electric conductivities ranging between 70 and 250 μS cm^{-1} were used.

With sulfonic groups (low pK_a) covalently attached to the polymer via copolymerization of vinylsulfonic acid, we would expect that v_{eo} should be independent of the pH of the mobile phase over a wide range. We determined v_{eo} dependent on the pH* in a range from 5.1 to 9.2 at a constant volume fraction of methanol and a constant specific electric conductivity. Only a marginal increase in v_{eo} with higher pH* was observed.

3.2. Host–guest complex formation

One of the most remarkable properties of cyclodextrins is their ability to form inclusion complexes. Complex formation with various compounds has been studied in the past [45]. Interaction forces with guest molecules are generally weak and are mainly based on van der Waals and hydrophobic interactions [46]. Small guest molecules therefore have a large flexibility when incorporated into the cavity. They tend to fill sterically the cavity as complete as possible. Many applications of cyclodextrins are due to the fact that complex formation with cyclodextrins increases dramatically the water solubility of hydrophobic compounds.

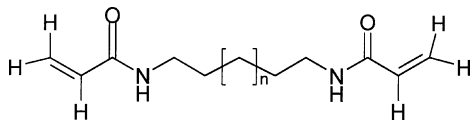

Fig. 2. Structures of the synthesized alkylenebisacrylamides ($n = 4$: *N*,*N*′-octamethylenebisacrylamide; $n = 6$: *N*,*N*′-decamethylenebisacrylamide; $n = 8$: *N*,*N*′-dodecamethylenebisacrylamide).

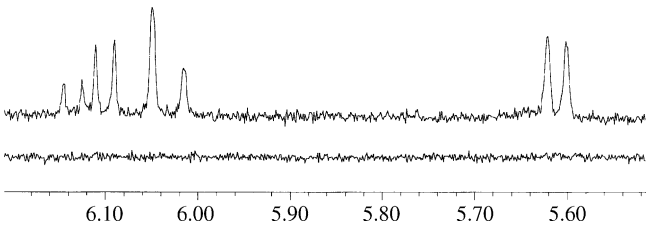

Fig. 3. ^{1}H-NMR spectra (^2H_2O, 500 MHz, 128 scans): upper line: *N*,*N*′-dodecamethylenebisacrylamide with Me-β-CD, bottom line: only *N*,*N*′-dodecamethylenebisacrylamide.

The structures of the alkylenebisacrylamides synthesized in our laboratory are shown in Fig. 2. These compounds have a very low solubility in pure water. First experiments undertaken with an up to 10-fold molar excess of native α- and γ-CD and hydroxypropyl or statistically Me-β-CD were not successful, as we did not succeed in dissolving the hydrophobic alkylenebisacrylamides in aqueous solutions containing a large molar excess of the water-soluble cyclodextrin.

Fischer and Ritter [47] reported the synthesis of five new oxazoline functionalized vinyl monomers. The complexation of these new monomers with 2,6-dimethyl-β-CD was attempted in several solvents including water at various temperatures with poor success. However, the monomers could be quantitatively incorporated into the units of 2,6-dimethyl-β-CD at room temperature by the use of methanol as solvent. It was possible to dissolve the hydrophobic guest compound and the cyclodextrin first in methanol, to precipitate the formed complex by evaporation of the solvent and to redissolve the formed inclusion complex in water. Possibly, direct complex formation in aqueous solution is apparently kinetically hindered or ternary complexes (cyclodextrin, vinyl monomer, and methanol) are formed. For tryptamine forming binary complexes with various cyclodextrins a stabilization of the binary complex in the presence of methanol is reported, probably because methanol is co-included into the cavity, forming a ternary complex [48]. Employing methanol as first solvent we succeeded in dissolving 8 mg BA8 or 10 mg BA12 in 0.5 mL aqueous solution at room temperature using a 5 M excess of Me-β-CD.

Solubilization by complex formation was verified for *N*,*N*′-dodecamethylenebisacrylamide (BA12) by comparing the ^{1}H-NMR spectra for following liquids: Liquid 1 was obtained by stirring 1 mg of BA12 with 1 mL of ^2H_2O at room temperature in a glass vial with a magnetic stirrer bar over night. The suspension was filtrated through a membrane filter (0.45 μm). Liquid 2 has been prepared in the same fashion with the difference, that 16 mg Me-β-CD were added to the suspension. As can be seen in Fig. 3 (both spectra taken under identical instrumental parameters, e.g. scan number), signals due to BA12 are seen only in the spectrum taken for Liquid 2. BA12 is virtually water-insoluble. The improved solubility of BA12 can be attributed to complex formation with Me-β-CD.

Complex formation with water-soluble neutral cyclodextrins has been used by Terabe et al. [49] to separate highly hydrophobic solutes by micellar electrokinetic chromatography. Highly hydrophobic solutes are difficult to separate by MEKC because their micellar medium/aqueous medium distribution coefficients K_{distr} are extremely high. In order to improve their separation, the reduction of the retention factors is needed. Addition of cyclodextrin to the separation buffer results in reduction of observed retention factors k_{ob}, because complex formation with the electroosmotically transported dissolved neutral cyclodextrin and distribution between the electrophoretically migrating micellar phase and the electroosmotically driven aqueous phase are competing processes. With constant K_{distr}, the observed retention factor k_{ob} will be reduced with increasing complex formation constant K_{CD} and with increased concentration of cyclodextrin.

This reduction in k_{ob} with increasing concentration of cyclodextrin was used to demonstrate complex formation with Me-β-CD. The observed retention factor k_{ob} is calculated according to Eq. (1):

$$k_{ob} = \frac{t_{mig} - t_0}{t_0(1 - (t_{mig}/t_{MC}))} \quad (1)$$

where t_{mig} is the migration time of the solute, t_0 the migration time of a polar marker not interacting with the micelles ($K_{distr} \rightarrow 0$), and t_{MC} the migration time of a hydrophobic marker with $K_{distr} \rightarrow \infty$ and $K_{CD} \rightarrow 0$.

The micelle forming surfactant was sodium dodecylsulfate (SDS) at a concentration of 100 mmol L^{-1}. Acetone was used as polar marker of the hold-up time. Quinine hydrochloride served as marker of the migration time of the micelles. Standard solutions were prepared in methanol–water (50:50, v/v). Here the selection of the marker of the migration time of the micelles is the crucial point of the method. It is known from different working groups that quinine hydrochloride has $K_{distr} \rightarrow \infty$ for aqueous solutions of SDS at a concentration above the critical micelle concentration [50,51]. Both hydrophobic interaction of the micelle core with the hydrophobic moiety of quinine hydrochloride and electrostatic interaction of the negatively charged micelle with the positive charge of the protonated base contribute to the high distribution constants K_{distr} observed. It is assumed here that the bulky marker quinine hydrochloride will not interact significantly with the neutral Me-β-CD so that K_{CD} for the marker can be neglected.

In Table 2 the observed retention factors for the three hydrophobic monomers BA8, BA10, and BA12 are given for

Table 2
Observed retention factors for *N*,*N*′-alkylenebisacrylamides dependent on the concentration of Me-β-CD in the separation buffer (100 mmol L^{-1} SDS, 10 mmol L^{-1} $Na_2B_4O_7$, capillary, 600(550) mm × 75 μm, temperature 25 °C, marker of the hold-up time: acetone), for symbols refer to Eq. (1)

	c(Me-β-CD) (mmol L^{-1})			
	0.1	1.0	10	50
BA8	10.12	9.50	5.31	1.29
BA10	63.90	59.07	35.20	2.27
BA12	$t_{mig} = t_{MC}$	$t_{mig} = t_{MC}$	$t_{mig} = t_{MC}$	14.64

concentrations of Me-β-CD between 0.1 and 50 mmol L^{-1}. It is obvious that the observed retention factors are decreased with increasing concentration of Me-β-CD in the separation buffer as it would be expected for solutes forming a complex with the dissolved neutral cyclodextrin. With 50 mmol L^{-1} Me-β-CD in the separation buffer k_{ob} is decreased about one order of magnitude compared to the measurement with 0.1 mmol L^{-1} Me-β-CD in the separation buffer. The results reflect also the increasing hydrophobicity of the synthesized monomers with increased length of the alkyl chain. At constant concentration of Me-β-CD k_{ob} is increased with increased alkyl chain length of the solute.

3.3. Chromatographic properties of formed monoliths in the reversed-phase mode

Complex formation of monomers with cyclodextrins does not only change the solubility in water but also the reaction rate in the polymerization reaction. It has been shown that the reaction rate of the monomer–CD complex can be significantly higher than that of the free dissolved monomer [30]. The reaction rate of the monomer in free radical polymerization will be decreased if the reactive site is blocked by inclusion into the cavity of the cyclodextrin. According to Weickenmeier and Wenz [52] octamethylene segments are covered by β-cyclodextrin. We assume therefore that the reactive groups of the synthesized linear cross-linkers BA8, BA10, and BA12 are not blocked by inclusion into the cavity of the host. Therefore, the two reactive sites of the complexed linear cross-linkers should be accessible for free radical copolymerization.

Assuming here that the reaction rates of the monomers copolymerized are in the same order of magnitude, we expect that the composition of the cross-linked macroporous polymers produced in our work reflects the molar ratios of monomers in the polymerization mixture. In a first experiment the impact of the molar fraction of BA8 in the polymerization mixture on the retention factors for several benzoates under constant chromatographic conditions was determined. Capillaries MA2 to MA5 were synthesized from hydrophilic monomers and the hydrophobic monomer BA8 (see Table 1). Consequently, if Me-β-CD is stripped off from BA8 during polymerization, retention factors for solutes

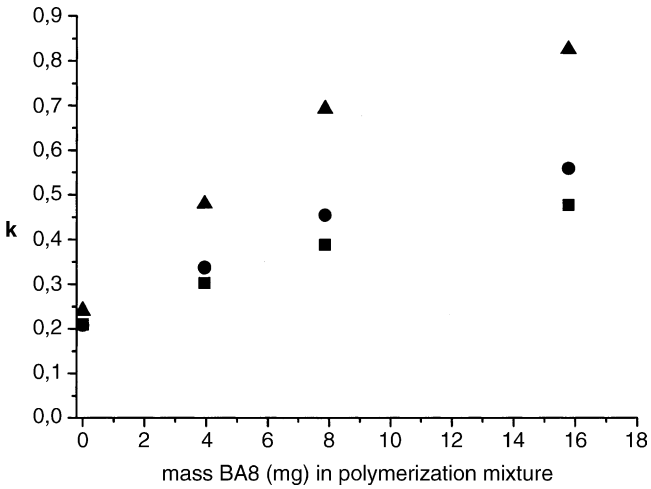

Fig. 4. Retention factor for selected alkyl benzoates vs. the mass of BA8 present in the polymerization mixture, (■) methyl benzoate, (●) ethyl benzoate, (▲) propyl benzoate (capillaries MA2–MA5a, see Table 1). Mobile phase: methanol–aqueous buffer (20:80, v/v), pH* 5.8, χ = 165 μS cm^{-1}. For remaining experimental parameters, refer to Fig. 1.

determined in the reversed-phase mode should be increased with increased molar fraction of BA8 in the polymerization mixture. This expectation is met with the results presented in Fig. 4. For the solute propyl benzoate, with the mobile phase methanol–aqueous buffer (20:80, v/v, pH 5.8, χ = 165 μS cm^{-1}), the retention factor was linearly increased from 0.24 with 0 mg BA8 in the polymerization mixture to 0.83 with 16 mg BA8 in the polymerization mixture.

Due to the high molar fraction of very polar monomers in the polymerization mixture, the retention properties of the monolith produced are unfavorably low in the reversed phase mode. In further polymerization reactions, the monomer MA was therefore replaced by the more hydrophobic monomer N-IPA (see Table 1) [53]. Different monolithic separation capillaries with different molar fractions of BA8 or BA12, respectively, in the polymerization mixture were produced (N1, N2, and N3, see Table 1). Due to the higher hydrophobicity of this monolith, the retention factors determined for alkyl benzoates in the reversed-phase mode are substantially higher. The retention factors are decreased with increasing volume fraction of methanol in the mobile phase as expected for the reversed-phase elution mode (data not shown). However, the addition of BA8 or BA12 to the polymerization mixture has only a marginal impact on the retentive properties of the monolith produced. With a capillary of this type in the reversed-phase elution mode retention is obviously dominated by the non-polar *iso*-propyl groups.

Typical reversed-phase separations of a series of alkyl benzoates and alkyl phenones are shown in Figs. 5 and 6. The decrease in plate numbers with increase in retention factor has been observed in all our experiments with acrylamide-based monoliths [54]. This dependence indicates a considerable resistance to mass transfer in the stationary phase. With solutes of low retention factor high plate numbers up to 200 000 plates m^{-1} are obtained.

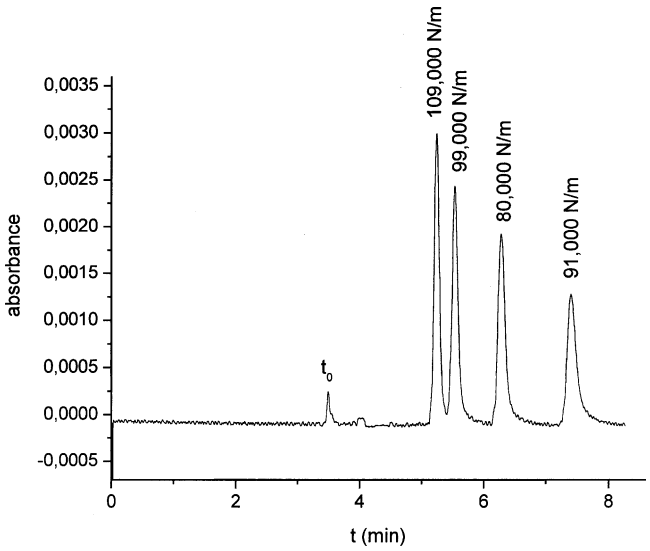

Fig. 5. Separation of alkyl benzoates. Solutes in order of elution: DMF (t_0), methyl benzoate, ethyl benzoate, propyl benzoate, and butyl benzoate. Capillary: N1b. Mobile phase: methanol–aqueous buffer (50:50, v/v), pH* 5.6, $\chi = 120\,\mu S\,cm^{-1}$. Voltage 30 kV. For remaining experimental parameters, refer to Fig. 1.

3.4. Chromatographic properties of formed monoliths in the normal-phase mode

Due to their amphiphilic character acrylamide-based monoliths can be used for normal-phase and reversed-phase separations. With buffered methanol as mobile phase and the capillaries MA2, MA3, and MA5 the impact of the molar fraction of BA8 in the polymerization mixture on retention factors was studied. In Table 3, the retention factors obtained for the polar neutral solutes 4-ethylaniline and resorcinol dependent on the molar fraction of BA8 in the polymerization mixture (see Table 1) are given. Determinations of the electrophoretic mobility (by capillary electrophoresis taking the mobile phase (+5%, v/v, water) as separation buffer) have shown that these solutes are neutral under the conditions of the measurement. The elution order corresponds to that expected for the normal-phase elution mode. As expected for the normal-phase elution mode, there is a decrease in retention factors with increasing content of BA8, which is increasing the volume fraction of hydrophobic segments in the polymer.

Table 3
Retention factor for selected analytes vs. the mass of BA8 present in the polymerization mixture (capillaries MA2, MA3, and MA5a, see Table 1)

m(BA8) (mg)	*k*(resorcinol)	*k*(ethylaniline)
0	0.34	0.04
4	0.32	0.03
16	0.28	0.02

Mobile phase: buffered methanol, pH* 6.4, $\chi = 140\,\mu S\,cm^{-1}$ (employed for resorcinol) and buffered methanol, pH* 8.7, $\chi = 250\,\mu S\,cm^{-1}$ (employed for 4-ethylaniline). For remaining experimental parameters, refer to Fig. 1.

Fig. 7 shows the separation of 3-alkoxy-4-hydroxybenzaldehydes from 4-hydroxybenzaldehyde and resorcinol on MA5a with buffered methanol as mobile phase. The elution order corresponds to the normal-phase elution mode. The run-to-run and day-to-day precision of the electroosmotic flow velocity and of the retention factor for selected solutes was determined with 9–12 repeated injections at 3 days. The results for the retention factors are given in Table 4. For the retention factors R.S.D.s of 0.4–2% have been determined for the run-to-run precision and 2.5–4%

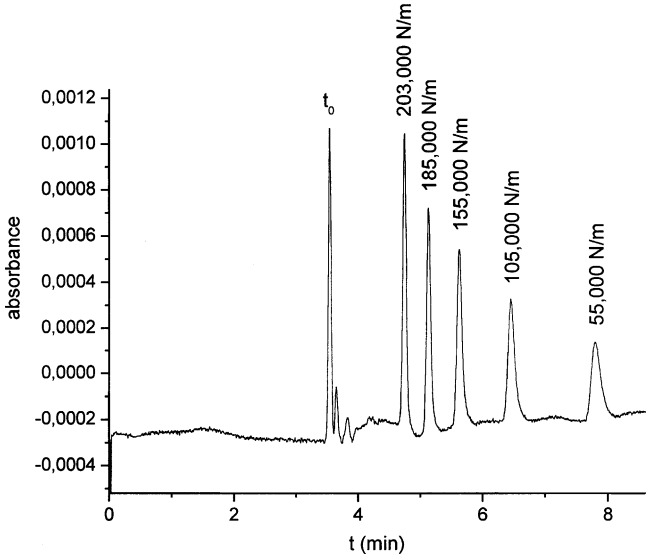

Fig. 6. Separation of alkylphenones. Solutes in order of elution: DMF (t_0), acetophenone, propiophenone, butyrophenone, valerophenone, and hexanophenone. Capillary: N1. Mobile phase: methanol–aqueous buffer (50:50, v/v), pH* 5.6, $\chi = 120\,\mu S\,cm^{-1}$. Voltage 30 kV. For remaining experimental parameters, refer to Fig. 1.

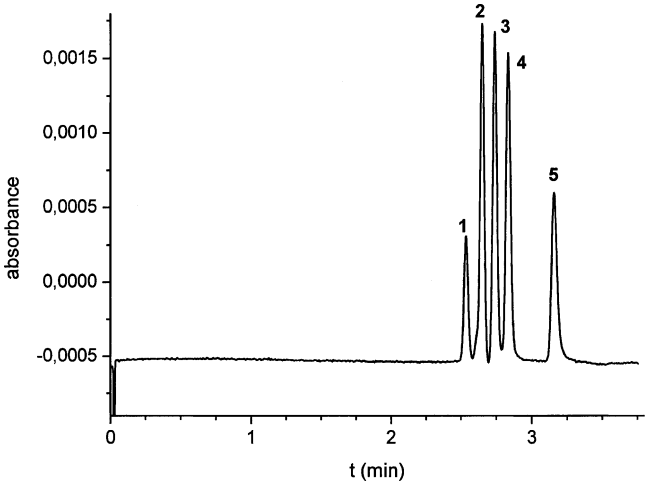

Fig. 7. Separation of polar compounds on capillary MA5a. Solutes: (1) DMF, (2) 3-ethoxy-4-hydroxybenzaldehyde, (3) 3-methoxy-4-hydroxybenzaldehyde, (4) 4-hydroxybenzaldehyde, and (5) resorcinol. Mobile phase: buffered methanol, pH* 6.4, $\chi = 140\,\mu S\,cm^{-1}$. Voltage 30 kV. For remaining experimental parameters, refer to Fig. 1.

Table 4
Day-to-day and run-to-run precision of the retention factors for selected solutes with capillary MA5a

Day	*k*(3-ethoxy-4-hydroxy-benzaldehyde)	*k*(3-methoxy-4-hydroxy-benzaldehyde)	*k*(4-hydroxy-benzaldehyde)	*k*(resorcinol)
1	0.044 (0.9×10^{-3})	0.079 (1.1×10^{-3})	0.116 (1.2×10^{-3})	0.247 (1.1×10^{-3})
2	0.042 (0.6×10^{-3})	0.075 (1.4×10^{-3})	0.113 (0.8×10^{-3})	0.243 (1.2×10^{-3})
3	0.045 (0.7×10^{-3})	0.081 (1.3×10^{-3})	0.120 (1.3×10^{-3})	0.255 (1.6×10^{-3})

n(Day 1) = 12, n(Day 2) = 9, n(Day 3) = 11, mean values and S.D.s in brackets. For experimental parameters, refer to Fig. 7.

for the day-to-day precision. For the electroosmotic velocity R.S.D.s of 0.44–0.46% have been determined for the run-to-run precision and 1.3% for the day-to-day precision.

3.5. Reproducibility and repeatability studies

In further experiments we evaluated the reproducibility of the synthesis of monoliths by preparing three capillaries of type MA5 (see Table 1) at different days (MA5a–c). Fig. 8 shows a typical chromatogram for the separation of phenol from resorcinol with MA5a and buffered methanol as mobile phase. Mean plate numbers for three capillaries and S.D.s for repeated injections according to Fig. 8 are given in Table 5. In Table 6 mean values of the electroosmotic velocity and the retention factors for phenol and resorcinol and S.D.s for repeated injections are given for the three capillaries evaluated. These studies show that under the experimental conditions of this work monolithic capillaries can be synthesized in a very reproducible manner.

Capillary MA5a was used at 4 days for more than 36 chromatographic runs according to Fig. 8. The chromatographic properties remained constant over this whole period. The retention factor for phenol was 0.07 in the first run and 0.06 in the last run. The retention factor for resorcinol

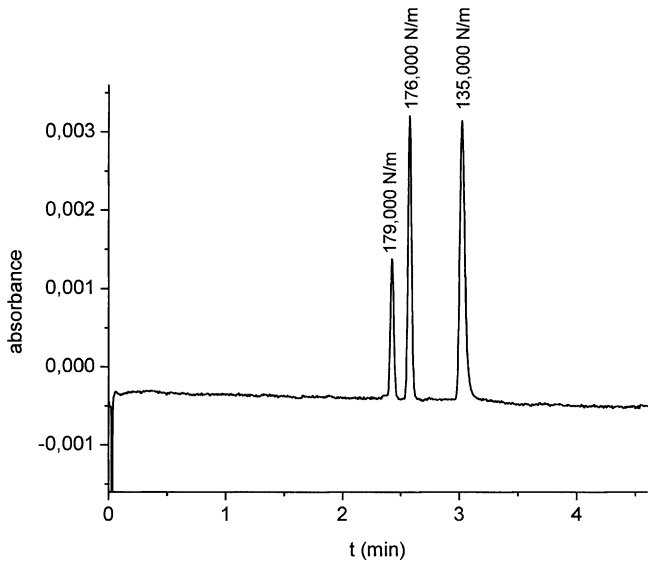

Fig. 8. Separation of phenol and resorcinol on capillary MA5a. Solutes in order of elution: DMF, phenol, resorcinol. Mobile phase: buffered methanol, pH* 6.4, $\chi = 140\,\mu S\,cm^{-1}$. Voltage 30 kV. For remaining experimental parameters, refer to Fig. 1.

Table 5
Batch-to-batch and run-to-run precision of the plate numbers per meter determined for DMF, phenol, and resorcinol for capillaries MA5a-c, mean values and S.D.s in brackets

	N(MA5a) (m^{-1})	N(MA5b) (m^{-1})	N(MA5c) (m^{-1})
DMF	159 000 (5 000)	161 000 (10 000)	n.d.
Phenol	177 000 (2 000)	175 000 (8 000)	159 000 (16 000)
Resorcinol	113 000 (4 000)	128 000 (7 000)	92 000 (14 000)

n(MA5a) = 36, n(MA5b) = 7, n(MA5c) = 7. For experimental parameters, refer to Fig. 8.

was 0.27 in the first run and 0.24 in the last run. The resolution between phenol and resorcinol was 6.1 in the first run and 5.9 in the last run. There was no measurable decrease in efficiency. The electroosmotic velocity decreased from 8.2 cm min^{-1} in the first run to 7.7 cm min^{-1} in the last run. Altogether, this capillary has been in use for more than 160 chromatographic runs without showing any signs of deterioration.

Scanning electron microscopy (SEM) studies of polymer-filled capillaries (Fig. 9a–d, SEM photos of a sector of the polymer cross section in a filled capillary) reveal that the morphology of capillaries synthesized at different days with polymerization mixtures of identical composition is very reproducible. Cross sections of the used capillaries MA5a and MA5b and cross sections of the used capillaries N1 and N2 are compared to each other. In all cases typical macroporous polymers with interconnected microglobules were obtained. The microglobular structure (referring to microglobule size distribution, macroporous channel size distribution and volume ratio microglobules to channels) is very homogeneous over the cross section of a monolithic capillary (I.D. = 100 μm) and over the length of a capillary.

From enlargements of these photos the microglobule diameters were estimated graphically from each 60 randomly selected microglobules. For capillaries of the same type (MA5a and b) an identical mean microglobule diameter of 190 nm with distributions ranging from 100 to 320 nm (MA5a) and from 100 to 370 nm (MA5b) was estimated. For the capillaries synthesized from a different mixture of monomers with a different concentration of lyotrophic salt in the polymerization mixture a different microglobule size distribution was obtained: mean microglobule diameter of 690 nm with a distribution ranging from 300 to 1300 nm (N1) and mean microglobule diameter of 680 nm with a distribution ranging from 200 to 1300 nm (N2).

Table 6
Batch-to-batch and run-to-run precision of the migration time of DMF and the retention factors for phenol and resorcinol for capillaries MA5a–c, mean values and S.D.s in brackets

	MA5a	MA5b	MA5c
t_{mig}(DMF)/min	2.0 (68.8×10^{-3})	2.434 (25.4×10^{-3})	1.938 (8.5×10^{-3})
k(phenol)	0.066 (0.3×10^{-3})	0.062 (0.4×10^{-3})	0.067 (0.8×10^{-3})
k(resorcinol)	0.258 (1.1×10^{-3})	0.247 (1.6×10^{-3})	0.282 (1.0×10^{-3})

n(MA5a) = 36, n(MA5b) = 7, n(MA5c) = 7. For experimental parameters, refer to Fig. 8.

3.6. Cyclodextrin content in polymer

Generally, in free radical polymerizations of acrylamide– or methacrylate–cyclodextrin host–guest complexes in aqueous media the cyclodextrin slips off from the monomer during chain propagation [29–37], although in some cases traces of Me-β-CD remain unextractable in the polymer. However, in case of a complexed cross-linker, as it is the case in this work, also a topological immobilization of the cyclodextrin via rotaxane formation [55] can be considered as possible. A topological immobilization of native and derivatized β-cyclodextrins has been observed by Kornyšova et al. [25,56] during the preparation of acrylamide-based continuous beds from water-soluble monomers in the presence of dissolved native or derivatized β-CD.

In order to determine the content of immobilized Me-β-CD in the continuous beds prepared in our work, $^{1}H - ^{13}C$ CP-MAS-NMR spectra (700/176 MHz, 12 kHz MAS) of the purified bulk polymers synthesized according to N1 and N2 (see Table 1 and Section 2) were compared to the $^{1}H - ^{13}C$ CP-MAS-NMR spectrum of the purified bulk polymer synthesized according to N3 in the presence of the same concentration of Me-β-CD as for the above mentioned preparations. These spectra were compared to that of solid Me-β-CD. An overlay of these spectra is given in Fig. 10.

The spectrum for the polymer synthesized in the presence of Me-β-CD without BA8 or BA12 in the polymerization mixture (line b) reveals that Me-β-CD, which is not topologically immobilized in the polymer via rotaxane formation, will be washed out from the polymer during purification by rinsing procedures. With this polymer under the conditions of this run (43 ± 1 mg sample and 24 576 signal accumulations), signals due to Me-β-CD are below the detection limit (which would be, in this case, at about 0.4–0.5 mg

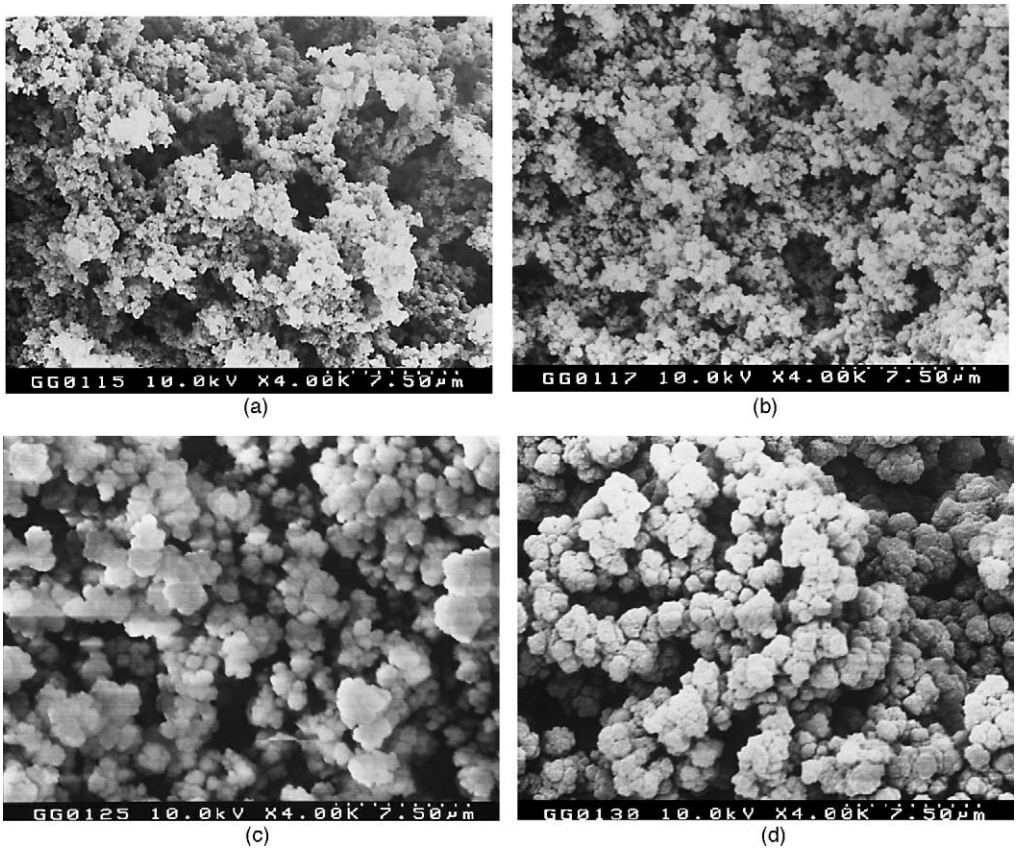

Fig. 9. SEM photographs of the cross-sectional areas of the used capillaries (a) MA5a, (b) MA5b, (c) N1, and (d) N2.

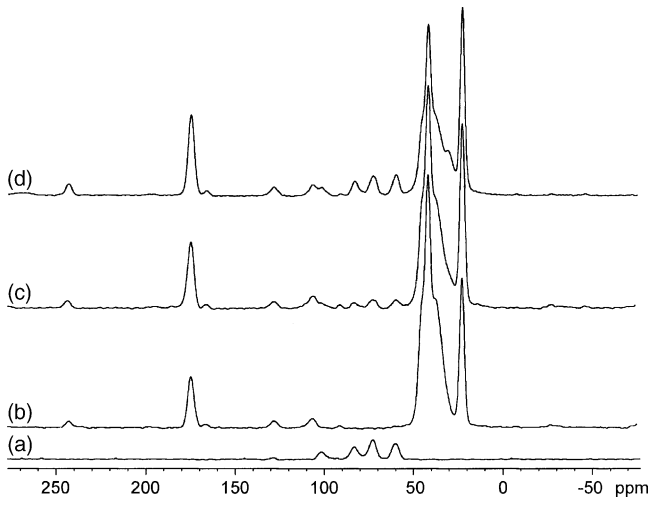

Fig. 10. ^{13}C solid-state NMR spectra for (a) Me-β-CD (128 scans), (b) polymer synthesized according to N3 (24 576 scans), (c) polymer synthesized according to N1 (32 768 scans), and (d) polymer synthesized according to N2 (32 768 scans). Experimental parameters are described in Section 2.

Me-β-CD in the sample). Consequently, the presence of signals (60–82 ppm) due to Me-β-CD (line a) can be regarded as an indication of topologically immobilized Me-β-CD. Indeed with those polymers synthesized by copolymerization of water-soluble monomers with complexed BA8 (line c) or BA12 (line d), the presence of Me-β-CD can be clearly detected under the experimental conditions of this measurement.

From the ratios of the signal integrals for the characteristic signals (comparison of signals at identical chemical shift between spectra) and the number of scans (instrumental parameters kept constant) the mass fraction of Me-β-CD in the polymer can be roughly quantitatively estimated. For the copolymer with complexed BA8 1.3 ± 0.4 mg Me-β-CD and for the copolymer with complexed BA12 5 ± 0.9 mg Me-β-CD were estimated to be contained in the solid sample (43 ± 1 mg). Regarding that the complex formation constant in aqueous solution for the Me-β-CD–BA12 complex will be higher than that for the Me-β-CD–BA8 complex (BA12 is more hydrophobic than BA8), differences in the mass fraction of Me-β-CD in the purified bulk polymer reflect differences in the complex stability constants of the reacting pseudorotaxanes.

Assuming a 1:1 complex formation of Me-β-CD with BA8 or BA12, respectively, and equal reaction rates for all monomers copolymerized, a mass fraction of Me-β-CD of about 24% would be expected for complete conversion of the pseudorotaxanes into rotaxane unities during polymerization. These considerations show that for BA8 the major fraction of the immobilized cross-linker is present in its uncomplexed form in the polymer, which is corroborated by our chromatographic studies with continuous beds of different content of BA8 in the polymerization mixture (see Fig. 4). The NMR studies, however, show also that in case of high formation constant of the formed host–guest complex co-polymerization of complexed hydrophobic bifunctional cross-linkers in aqueous medium with water-soluble monomers is a possible route to polymeric rotaxane structures.

4. Conclusions

Host–guest complex formation of hydrophobic monomers with cyclodextrins can be applied successfully in the synthesis of acrylamide-based monolithic stationary phases for CEC. Complexation with statistically methylated β-cyclodextrin can be used to dissolve monomers, which have a very low solubility in pure water, in aqueous polymerization mixtures containing further water-soluble monomers. Via this reaction route acrylamide-based monolithic capillaries can be prepared with high reproducibility. With these capillaries, a sufficiently high electroosmotic flow velocity, a high repeatability of separation parameters and high plate numbers can be obtained.

Acknowledgements

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References

[1] F. Svec, Adv. Biochem. Eng./Biotechnol. 76 (2002) 1.
[2] http://www.iupac.org/projects/2001/2001-021-1-500.html.
[3] C. Fujimoto, Trends Anal. Chem. 18 (1999) 291.
[4] Q. Tang, M.L. Lee, Trends Anal. Chem. 19 (2000) 648.
[5] J.K. Debowski, J. Liq. Chromatogr. 25 (2002) 1875.
[6] F. Svec, E.C. Peters, D. Sýkora, J.M.J. Fréchet, J. Chromatogr. A 887 (2000) 3.
[7] E.F. Hilder, F. Svec, J.M.J. Fréchet, Electrophoresis 23 (2002) 3934.
[8] C. Legido-Quigley, N.D. Marlin, V. Melin, A. Manz, N.W. Smith, Electrophoresis 24 (2003) 917.
[9] S. Hjertén, J.L. Liao, R. Zhang, J. Chromatogr. 473 (1989) 273.
[10] J.L. Liao, R. Zhang, S. Hjertén, J. Chromatogr. 586 (1991) 21.
[11] S. Hjertén, Y.M. Li, J.L. Liao, J. Mohammad, K. Nakazato, G. Pettersson, Nature 356 (1992) 810.
[12] S. Hjertén, J. Mohammad, K. Nakazato, J. Chromatogr. 646 (1993) 121.
[13] S. Hjertén, Ind. Eng. Chem. Res. 38 (1999) 1205.
[14] C. Fujimoto, Y. Fujise, E. Matsuzawa, Anal. Chem. 68 (1996) 2753.
[15] A. Maruška, in: F. Svec, T.B. Tennikova, Z. Deyl (Eds.), Monolithic Materials—Preparation, Properties and Applications, Elsevier, Amsterdam, 2003, pp. 143–172.

[16] D. Hoegger, R. Freitag, J. Chromatogr. A 914 (2001) 211.
[17] S. Hjertén, K. Nakazato, J. Mohammad, D. Eaker, Chromatographia 37 (1993) 287.
[18] J.L. Liao, Y.M. Li, S. Hjertén, Anal. Biochem. 234 (1996) 27.
[19] C. Ericson, J.L. Liao, K. Nakazato, S. Hjertén, J. Chromatogr. A 767 (1997) 33.
[20] J.L. Liao, N. Chen, C. Ericson, S. Hjertén, Anal. Chem. 68 (1996) 3468.
[21] C. Ericson, S. Hjertén, Anal. Chem. 71 (1999) 1621.
[22] D. Hoegger, R. Freitag, Electrophoresis 24 (2003) 2058.
[23] Y.M. Li, J.L. Liao, K. Nakazato, J. Mohammad, L. Terenius, S. Hjertén, Anal. Biochem. 223 (1994) 153.
[24] D. Hoegger, R. Freitag, J. Chromatogr. A 1004 (2003) 195.
[25] O. Kornyšova, R. Šurna, V. Snitka, U. Pyell, A. Maruška, J. Chromatogr. A 971 (2002) 225.
[26] A. Maruška, C. Ericson, Á. Végvári, S. Hjertén, J. Chromatogr. A 837 (1999) 25.
[27] C. Ericson, J. Holm, T. Ericson, S. Hjertén, Anal. Chem. 72 (2000) 81.
[28] G. Schornick, A. Kistenmacher, H. Ritter, J. Jeromin, O. Noll, M. Born, German Pat., DE 19533269 (1997).
[29] J. Storsberg, H. van Aert, C. vaan Roost, H. Ritter, Macromolecules 36 (2003) 50.
[30] S. Schwarz-Baraæ, H. Ritter, J. Macromol. Sci. 40 (2003) 437.
[31] J. Jeromin, H. Ritter, Macromol. Rapid Commun. 19 (1998) 377.
[32] J. Jeromin, O. Noll, H. Ritter, Macromol. Chem. Phys. 199 (1998) 2641.
[33] S. Bernhardt, P. Glöckner, A. Theis, H. Ritter, Macromolecules 34 (2001) 1647.
[34] P. Glöckner, H. Ritter, Macromol. Rapid Commun. 20 (1999) 602.
[35] S. Bernhardt, P. Glöckner, H. Ritter, Polym. Bull. 46 (2001) 153.
[36] P. Casper, P. Glöckner, H. Ritter, Macromolecules 33 (2000) 4361.
[37] J. Jeromin, H. Ritter, Macromolecules 32 (1999) 5236.
[38] W.A. Skinner, J. Lange, T.E. Shellenberger, W.T. Colwell, J. Med. Chem. 10 (1967) 949.
[39] S. Hjertén, J. Chromatogr. 347 (1985) 191.
[40] A.E. Bennett, C.M. Rienstra, M. Auger, K.V. Lakshmi, R.G. Griffin, J. Chem. Phys. 103 (1995) 6951.
[41] H. Rebscher, U. Pyell, Chromatographia 42 (1996) 171.
[42] H. Rebscher, U. Pyell, unpublished results.
[43] I. Nischang, U. Tallarek, Electrophoresis (2004) in press.
[44] A. Banholczer, U. Pyell, J. Chromatogr. A 869 (2000) 363.
[45] W. Saenger, Angew. Chem. 92 (1980) 343.
[46] L. Liu, Q.X. Guo, J. Inclusion Phenom. Macrocycl. Chem. 42 (2002) 1.
[47] M. Fischer, H. Ritter, Macromol. Rapid Commun. 21 (2000) 142.
[48] R.E. Galian, A.V. Veglia, R.H. de Rossi, Analyst 123 (1998) 1587.
[49] S. Terabe, Y. Miyashita, O. Shibata, E.R. Barnhart, L.R. Alexander, D.G. Patterson, B.L. Karger, K. Hosoya, N. Tanaka, J. Chromatogr. 516 (1990) 23.
[50] P. Tsai, B. Patel, C.S. Lee, Electrophoresis 15 (1994) 1229.
[51] U. Pyell, U. Bütehorn, Chromatographia 40 (1995) 175.
[52] M. Weickenmeier, G. Wenz, Macromol. Rapid Commun. 18 (1997) 1109.
[53] C.M. Zeng, J.L. Liao, K. Nakazato, S. Hjertén, J. Chromatogr. A 753 (1996) 227.
[54] P. Gottschalk, Diploma Thesis, University of Kassel, Kassel, 2001.
[55] A. Harada, Acta Polym. 49 (1998) 3.
[56] O. Kornyšova, E. Machtejevas, V. Kudirkaitė, U. Pyell, A. Maruška, J. Biochem. Biophys. Methods 50 (2002) 217.