Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1044 (2004) 67–81
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Review

# Recent highlights in stationary phase design for open-tubular capillary electrochromatography

Elizabeth Guihen, Jeremy D. Glennon*

*Department of Chemistry, Analytical and Biological Chemistry Research Facility (ABCRF), University College Cork, Cork, Ireland*

**Abstract**

This review examines the most recent innovations made to achieve high performance in open-tubular capillary electrochromatography (OT-CEC) separations, focusing on the ingenious chemical and physical solutions made to increase the surface area and equip the stationary phase with exploitable selectivity. Among the approaches taken are chemically bonded ligands, etching with chemical bonding, sol–gels, molecularly imprinted polymers, porous layers, physically attached or adsorbed phases, and nanoparticle coatings. Particularly noteworthy are modern developments with macrocyclic receptor ligands, nanoparticles and open channel electrochromatography on-chip.


*Keywords:* Reviews; Stationary phases, electrochromatography; Electrochromatography; Molecular imprinting; Chip technology

**Contents**



## 1. Introduction

To meet the ever increasing demand for high performance, rapid separations from the life sciences of genomics, proteomics, metabolomics, and drug discovery/medicine, novel micro and nanoseparation strategies, chemical innovation and miniaturized devices are needed if sufficient chromatographic efficiency and performance are to be produced.

The quest for higher performance chromatographic phases and tailored uniform chemically bonded surfaces for micro and nanoscale chemical analysis has reached a new signif-

* Corresponding author. Fax: +353-21-4274097.
*E-mail address:* j.glennon@ucc.ie (J.D. Glennon).

0021-9673/$ – see front matter © 2004 Published by Elsevier B.V.
doi:10.1016/j.chroma.2004.05.107

icance. Nowhere is this more evident than in the changing face or inner surface of open tubular fused silica capillaries or microchannels on-chip, designed for electrochromatographic separations.

An open tubular chromatographic column is a capillary or channel where the inner surface is coated with a stationary phase, which bestows on it a chromatographic separation mechanism, be it partition, ion exchange, molecular or chiral recognition, when placed in intimate contact with a mobile phase. Open-tubular capillary electrochromatography (OT-CEC) pertains when the mobile phase is electroosmotically driven through the column, as opposed to pressure driven flow in open tubular liquid chromatography. The flat flow profile induced by electroosmosis inherently confers higher efficiency in open tubular capillary electrochromatography than in pressure driven open tubular liquid chromatography where a parabolic flow profile is observed.

However, it is the powerful combination of selectivity control from solute interaction with immobilized stationary phase chemistries with electrophoretic discrimination that draws the continued attention of separation scientists to CEC and OT-CEC. In this review, some of the most recent advances in OT-CEC will be highlighted.

OT capillaries have important advantages over packed capillary columns including ease of preparation (no end frit formation), variety and availability of surface modification and immobilization chemistries, no bubble formation and higher compatibility with smaller internal diameter columns and future miniaturized microfluidic channels on-chip. Small internal diameters in the range 2–25 μm are required in OT-CEC to facilitate efficient solute diffusion to the stationary phase surface. Low sample capacities and low concentration sensitivity result from the short optical path length. Since the first OT-CEC column by Tsuda et al. [1] relying on monolayer silica surface functionalisation with octadecylsilane, innovative approaches have been made to increase the stationary phase area of OT-CEC columns (Table 1). This search for selective intimate contact between stationary phase and solute has continued into the new century. Analytical separations in open tubular capillary electrochromatography have been recently reviewed [19], and the emphasis here is, in the main, on more recent highlights and innovations.

This review examines the most recent innovations made to achieve high performance in OT-CEC separations, focusing on the ingenous chemical and physical solutions made to increase the surface area and equip the stationary phase with exploitable selectivity.

Table 1
Historical development of OT-CEC

| | | | |
|---|---|---|---|
| Tsuda et al. | Octadecylsilane bonded inner surface | 1982 | [1] |
| Bruin et al. | Functionalized porous silica layer | 1990 | [2] |
| Pfeffer and Yeung | Polymer-coated polyvinylsiloxane phase | 1990 | [3] |
| Mayer and Schurig | Chiral separations using permethylated β-CD | 1992 | [4] |
| Armstrong et al. | Chiral cyclodextrin immobilized capillary | 1993 | [5] |
| Jacobson et al. | Octadecyl modified channel on-chip | 1994 | [6] |
| Guo and Colon | Sol–gel porous organic–inorganic film | 1995 | [7] |
| Pesek and Matyska | Etched silica capillary, octadecyl modified | 1996 | [8] |
| Francotte and Jung | Cellulose-coated chiral capillary | 1996 | [9] |
| Tan and Remcho | Bonded linear polymethacrylate thick film | 1997 | [10] |
| Schweitz et al. | MIP for enantiomeric separation | 1997 | [11] |
| Kutter et al. | Octadecyl silica channel on-chip | 1998 | [12] |
| Hofstetter et al. | Chemically bonded BSA capillary | 1998 | [13] |
| Liu et al. | Adsorbed protein, peptide amino acid phase | 1999 | [14] |
| Sawada and Jinno | Linear polymer coated capillary | 1999 | [15] |
| Huang et al. | In situ porous polymerization, dodecyl chains | 1999 | [16] |
| Fujimoto et al. | Etched peek capillary | 1999 | [17] |
| Xu and Regnier | Polyaspartic acid phase | 1999 | [18] |

## 2. Chemically bonded ligand phases

In the design of selective separation phases for OT-CEC, it is not surprising that synthetic ligands, studied previously in LC or CEC separations, should be chosen for study. Thus, to the established functionalities of choice such as octadecylsilica, cyclodextrin and protein-based, have been added more selective molecular recognition and aptamer phases.

### 2.1. Amino acids

The amino acid histidine was chemically immobilized to the inner wall of a capillary for open tubular electrochromatography, chosen for its pH controllable charge and for its potential hydrophobic, hydrogen bonding and ion-exchange interactions with selected solutes. The optimum conditions for the electrochromatographic separation of non-steroidal anti-inflammatory drugs were determined. Electroosmotic flow (EOF) reversal was a distinct feature at a pH below the isoelectric point [20].

### 2.2. Molecular recognition ligands

In the search for higher selectivity in capillary electrophoretic separations, the established use of cyclodextrin derivatives as buffer additives or immobilized ligands, is recognized for its effectiveness in achieving enantioselectivity. The use of macrocyclic ligands or molecular recognition reagents in OT-CEC is gaining momentum, highlighting the exciting future possibilities for host–guest complexation in attaining advanced electrochromatographic selectivity. In particular, considerable interest has been shown recently in N-containing macrocycles and in calixarene derivatives.

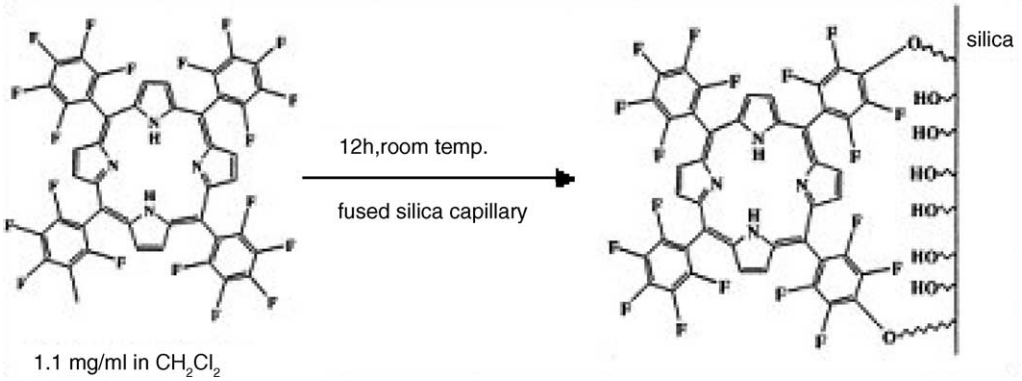


Fig. 1. The structure and the manner of covalent attachment of porphyrin derivative, 5,10,15,20-tetrakis(pentafluorophenyl)porphyrin, $H_2$TPFPP, onto the fused-silica capillary surface [25].

*2.2.1. Polyamines and porphyrin macrocycles*

The selectivity of macrocyclic polyamines in the electrophoretic separation of organic and inorganic anions, has been demonstrated by Chen and Liu [21] at the close of the last century, while Hu et al. [22] investigated macrocyclic dioxopolyamines as buffer additives in capillary zone electrophoresis (CZE). Fused silica capillaries, etched with sodium hydroxide and chemically bonded with a macrocyclic dioxopolyamine using an activated silylating agent, showed improved OT-CEC separations of phenolic isomers and selected neurotransmitters [23,24].

Porphyrin moieties have also received attention as both covalent and dynamic coatings in OT-CEC. For the preparation of the chemically bonded phase, the known affinity of $H_2$TPFPP toward nucleophilic attack was used; the synthetic strategy for coating was based on the generation of anionic silanol groups on the inner capillary surface, which in turn were employed for nucleophilic substitution at the para position of $H_2$TPFPP. Using 5,10,15,20-tetrakis(pentafluorophenyl)porphyrin, Charvatova et al. [25] found that the separation efficiency and resolution of aromatic carboxylic acids were better in coated capillaries than in bare fused silica capillary. Increases in EOF were also observed at pH 5 and 6 with covalently modified capillary but decreasing of EOF was observed at pH 8.5. At pH 5, only the covalent modification can be used as the physically adsorbed porphyrin coating lead to irreproducible results. An immobilization scheme is presented in Fig. 1 below, although the exact nature of the bonding to silica has not been fully characterised.

*2.2.2. Calixarene and calix–crown host phases*

Calixarenes, cyclic oligomers of phenol–formaldehyde condensates, have emerged as attractive molecules for host–guest chemistry, with well-defined cavities composed of aromatic rings linked by methylene units at the *meta*-position. Since the first applications in liquid chromatography [26] and electrophoresis [27], calixarenes, functionalisable at upper and lower rims, have been widely used as stationary phases in chromatographic and capillary electrophoretic separations (Fig. 2).

Water-soluble calixarene derivatives, e.g. sulfonated calixarene and carboxycalixarenes have been employed as buffer additives in CE, and resorcarenes for pseudostationary phases in micellar electrokinetic chromatography (MEKC). The chemical bonding of these macrocyclic receptors to the capillary inner wall as stationary phases for electrochromatography is expected to allow observations of advanced separation selectivity. Thus, recently, calix–crown derivatives, where the phenolic oxygens of the calixarene are linked intra-molecularly via flexible polyoxyethylene chains, have received particular attention. Higher complexation selectivity results for organic and cationic guests in these preorganised calixarene–crown macrocycles [28].

Zeng et al. [29] prepared two host molecules, *p-tert*.-butylcalix[6]-1,4-crown-4 and *p-tert*.-butylcalix[6]arene

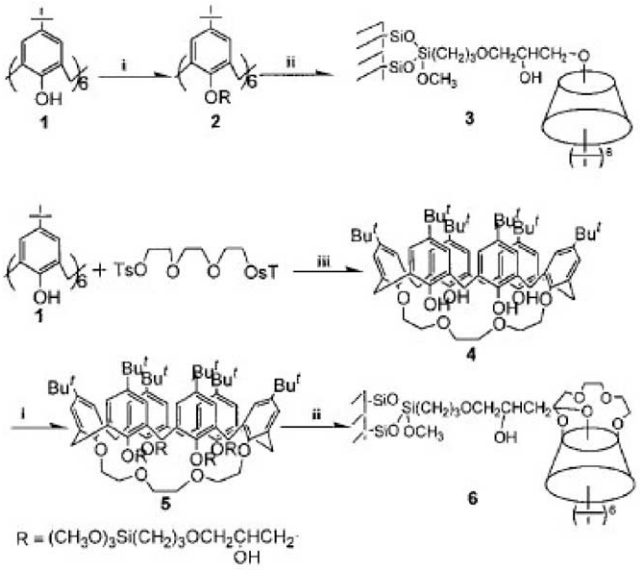


Fig. 2. Scheme of modification of stationary phases onto the capillary wall. Reagents and conditions: (i) $HClO_4$, KH560, toluene, reflux; (ii) 3 h, 120 °C, the etched capillary inner wall and (iii) $K_2CO_3$, toluene, reflux, 24 h. From ref. [29] with permission.

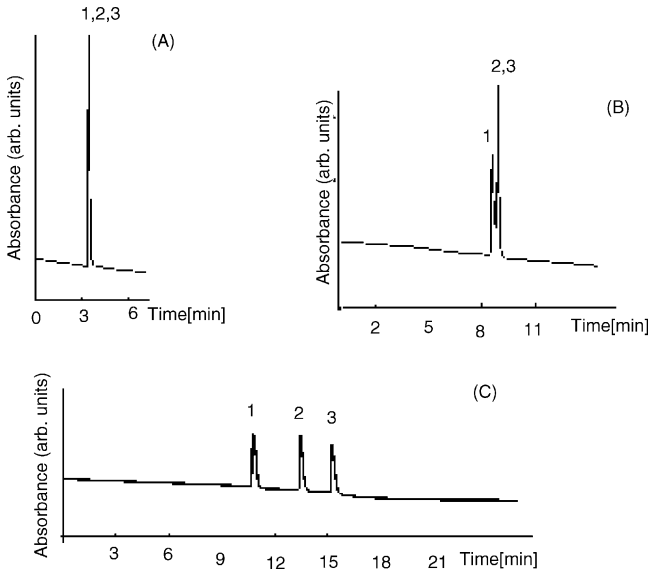


Fig. 3. Separation of toluidine isomers in CZE and in OT-CEC columns. (A) CZE: 49 cm (effective length 37 cm) × 50 μm bare fused-silica capillary; (B) OT-CEC: 49 cm (effective length 37 cm) × 50 μm fused-silica capillary modified with calix[6]arene and (C) OT-CEC: 49 cm (effective length 37 cm) ×50 μm fused-silica capillary modified with calix[6]crown. Mobile phase, 20 mM aqueous sodium phosphate, pH 6.0; voltage, 20 kV; hydrodynamic injection, 10 s; wavelength, 254 nm. Peak identification: 1, *p*-toluidine; 2, *m*-toluidine; 3, *o*-toluidine. From ref. [29] with permission.

for attachment onto the inner surfaces of capillaries for open-tubular electrochromatography with the aid of γ-glycidoxypropyl-trimethoxysilane (GOPS) as coupling agent. Interestingly, the successful bonding was confirmed by infrared (IR) results and greatly decreased EOF. By removing the external coating of the OT-CEC column prior to grinding and by using a higher blending ratio of bonded phase capillary to potassium bromide than conventionally used, IR spectral analysis was used to identify functional groups of the bonded calix–crown ligands. Using isomeric toluidines (Fig. 3), a mixture of pyridine and isomeric picolines, and isomeric dihydroxybenzenes, the selectivity of the calix[6]crown-coated capillary was reported to be enhanced relative to the both the bare capillary and calix[6]arene-bonded capillary.

In addition, Zhang and co-workers [30] prepared and characterized *p-tert*-butylcalix[8]arene bonded capillaries for OT-CEC, also with γ-glycidoxypropyl-trimethoxysilane as a bridge. The bonded calix[8]arene capillary displayed low and steady EOF values over the pH range from 4 to 9 and displayed good separation selectivity for *o*-, *m*- and *p*-benzenediols, α- and β-naphthols, and α- and β-naphthylamines. The observed selectivity was attributed to significant interactions between the analytes and the bonded *p-tert*-butylcalix[8]arene, which contributed to the electrochromatographic separation mechanism.

### 2.2.3. Aptameric stationary phases in OT-CEC

The use of aptamer molecules in analyses requiring molecular recognition shows promise and has recently been

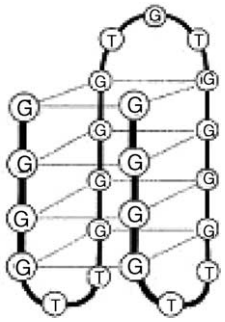

Fig. 4. Structure of the four-plane aptamer. From ref. [33] with permission.

reviewed by Clark and Remcho [31]. As described by these authors, aptamers consist of single-stranded oligonucleotides that are isolated and amplified on the basis of their affinity for a target molecule by "systematic evolution of ligands by exponential enrichment" (SELEX) [32] (Fig. 4). The large number of possible oligonucleotide sequences and their molecular diversity make possible the isolation of aptamers that show affinity for a large variety of molecules, including small molecules, peptide sequences, proteins, and other oligonucleotides. Aptamers, applied in such techniques as flow cytometry, sensors and biosensors, in affinity chromatography, affinity capillary electrophoresis, and capillary electrochromatography, have now been applied in OT-CEC.

Rehder and McGown [33] have investigated the open-tubular capillary electrochromatography of bovine β-lactoglobulin variants A and B using an aptamer stationary phase. In their reported work, DNA aptamers, that form a G-quartet conformation, were covalently attached to a capillary surface via a linker group for open-tubular capillary electrochromatographic separation of bovine β-lactoglobulin variants A and B, which vary by two of their 162 amino acid residues. The oligonucleotides were covalently attached to the inner capillary surface using an organic linker molecule, sulphosuccinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (S-SMCC).

Separation was best achieved using a four-plane, G-quartet aptamer stationary phase with tris(hydroxymethyl) aminomethane (Tris) as the mobile phase buffer. In contrast, separation did not occur using either an oligonucleotide of similar base composition but which does not form a G-quartet structure, or using on a bare capillary under similar experimental conditions. While separation was also achieved using a capillary coated only with the covalent linker molecule, the retention times are almost twice those for the aptamer-coated capillary and the peaks are much broader, leading the authors to suggest that aptamer-based separations may be gentler and less likely to denature the proteins than separations on organic stationary phases.

In work by Clark and Remcho [32], an RNA aptamer consisting of 35 bases, isolated on the basis of its affinity for the flavin moiety of the small biological

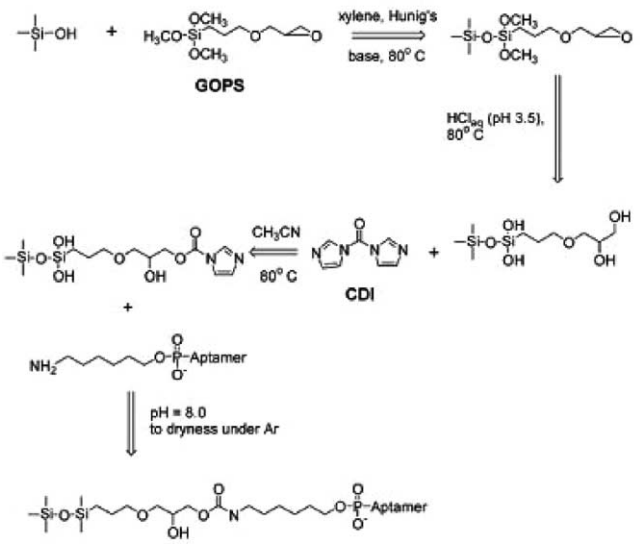


Fig. 5. Scheme resulting in covalent bonding of an RNA aptamer onto the inner walls of a fused-silica capillary. From ref. [32] with permission.

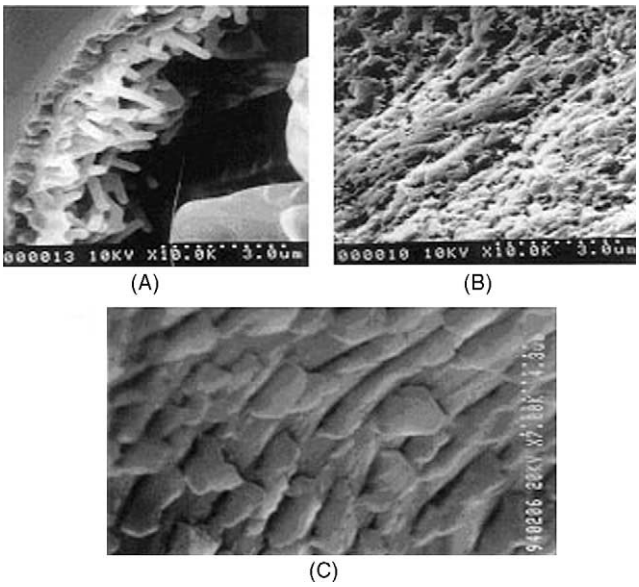


Fig. 6. Scanning electron micrographs (SEM) of etched capillary surfaces. Etching process was carried out for (A) 3 h at 300 °C, (B) 2 h at 300 °C and 2 h at 400 °C and (C) 2 h at 300 °C and 1 h at 400 °C [34].

cofactors flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), was immobilized inside the fused-silica capillaries (Fig. 5) used for OT-CEC. Immobilisation was achieved using surface silylation with glycidoxypropyl-trimethoxysilane, followed by activation with 1,1′-carbonyldiimidazole (CDI).

Using flow counterbalanced open tubular capillary electrochromatography (FC-OT-CEC), the chromatographic value $k'$ for the aptamer stationary phase was experimentally determined for different mobile phase conditions. By using this new method to evaluate capillaries in OT-CEC combining OT-CEC and flow-counterbalanced capillary electrophoresis, $k'$ and $k_{CEC}$ values were determined for these capillaries. In this way, the affinity exhibited by the immobilized RNA aptamer toward its two target molecules was characterized in different $Mg^{2+}$ concentrations.

## 3. Chemically bonded etched capillaries

Dramatic and effective steps to increase the surface area and sample capacity, and to reduce axial solute migration distances, in OT-CEC have been taken by Pesek and co-workers using etching of the inner capillary surface. Using ammonium hydrogen difluoride with careful time control and high temperature treatment, the surface area of the capillary can be increased by as much as 1000-fold, creating radical radial extensions or protrusions from the surface alongside the modification of the surface composition, EOF and adsorptive properties. These extensive surface changes are shown in Fig. 6 for varied treatment regimes.

The resulting etched capillaries can be used as etched only, etched with a physically adsorbed stationary phase and etched along with chemically bonding, the latter being the most popular. An efficient silanization/hydrosilation method of chemical modification is then used to chemically anchor the desired bonded phase through silicon-carbon bonds.

$$\text{Silanisation:}\quad \text{Si–OH} + (\text{EtO})_3\text{SiH} \rightarrow \text{Si–O–Si–H} + n\text{EtOH}$$

$$\text{Hydrosilation:}\quad \text{Si–O–Si–H} + \text{HCH} = \text{CHR} \rightarrow \text{Si–O–Si–CH}_2\text{–CH}_2\text{R}$$

This method is versatile, allowing the production of monomeric bonded phases, above a surface dominated with residual hydride as opposed to silanol groups. Pesek and Matyska [34,35,40] have released numerous papers on chemically bonded etched OT electrochromatographic separations. Organic moieties that have been bonded include octadecyl, $C_8$, diol, chiral selectors, and cholesterol derivatives.

Matyska et al. [36,37] employed various electrochromatographic experiments to characterise the properties of etched, chemically modified surfaces of open tubular capillaries with peptides as solute probes. Voltage, temperature and solvent composition were varied. The performance characteristics of etched capillaries with either *n*-octadecyl or liquid crystal moieties derived from a cholesterol phase bonded to the surface were compared. With regard to the liquid crystal bonded species, significant variations in retention behaviour of peptides were observed in comparison with the *n*-octadecyl modified surfaces on varying the above listed parameters. Pesek and co-workers [38,39] also reported the performance characteristics of two different types of etched chemically (*n*-octadecyl-, cyano-*n*-pentoxybiphenyl and cholesteryl-10-undecenoate) modified capillaries in OT-CEC for the analysis of selected proteins, synthetic peptides, drugs, bases and metabolites.

Pesek et al. [41] in addition, published a comparison of different modes of OT-CEC in bare and etched capillaries for the separation of impurities in two synthetic peptides and of a mixture of heterocyclic aromatic amines. Three types of stationary phase were evaluated in this multi-modal investigation: flourosurfactants (anionic and zwitterionic) adsorbed in the inner wall of the capillary (electrochromatography with dynamically modified stationary phases), physically adsorbed polymers [DMA-$SO_{3-}$ and DMA-$N^{+}(CH_3)_3$] and chemically modified capillaries (octyldecyl, cholesteryl-10-undecanoate and diol). The authors conclude that OT-CEC is a viable alternative to CE and LC.

Liu et al. [42] examined an extended light path and etched capillary for improving sensitivity and separation capability of OT-CEC with a chiral stationary phase of physically adsorbed avidin. They found that the phase ratio improvement with the etched capillary was less than expected from the surface area increase, likely due to reduced surface silanol density and the generation of nitrogen-containing groups during the etching process.

In more recent work from the same group, Pesek et al. [43] investigated two sets of peptides, with structurally similar amino acid sequences, using etched chemically modified *n*-butylphenyl, *n*-octyldecyl and cholesterol capillaries. The results showed that OT-CEC with etched chemically modified capillaries have great resolving power than gradient RP-HPLC and that under optimized conditions, efficient separations, with highly symmetrical peaks, can be achieved (Fig. 7).

## 4. Sol–gel derived phases

The sol–gel process, as recently reviewed by Smith and co-workers [44], involves the preparation of a sol solution followed by the gelation of the sol to form a network in a continuous liquid phase. Common precursors for the synthesis of sol–gels are metal alkoxides, as they react easily with water, such as tetramethoxysilane (TMOS) and tetraethoxysilane (TEOS). The following overall reactions describe the process.

Hydrolysis

$$\equiv \text{Si–OR} + \text{H}_2\text{O} \rightarrow \equiv \text{Si–OH} + \text{ROH} \quad (1)$$

Alcohol condensation

$$\equiv \text{Si–OH} + \text{RO–Si} \equiv \rightarrow \equiv \text{Si–O–Si} \equiv + \text{ROH} \quad (2)$$

Water condensation

$$\equiv \text{Si–OH} + \text{HO–Si} \equiv \rightarrow \equiv \text{Si–O–Si} \equiv + \text{H}_2\text{O} \quad (3)$$

Polycondensation can take place with the linkage of additional silanol groups, to form cyclic oligomers and eventually cast a silicate network. pH, temperature, reagent concentrations, reaction time, and the nature of the catalyst-employed can alter the properties of each sol–gel matrix. By careful control of each of these factors it is possible to control the monolith properties including through-pore and mesopore sizes.

The main challenge in preparing capillary columns is to have ample retentive and mass loadability features. Retention characteristics of sol–gel-based columns can be controlled by varying the ratio of monomeric precursors in the initial sol–gel solution [45]. The advantages of sol–gel processed stationary phases include high stability, high mass loadability, great column efficiency, large surface area which in turn gives higher retentions, and straightforward preparation [46,70,49]. Wang et al. [50] developed a novel OT-CEC column coated with 2,6-dibutyl-β-cyclodextrin (DB-β-CD) using the sol–gel technique. Due to the three-dimensional network of sol–gel and the strong chemical bond between the stationary phase and the capillary surface, unique selectivity in separating isomers was exhibited. The DB-β-CD column showed high efficiencies for the separation of the isomeric nitrophenols, with migration time reproducibility above 2.2% over five analyses and 4.5% from column to column. In the comparison with the sol–gel matrix column, these sol–gel-coated DB-β-CD columns have demonstrated improved separation of isomeric aminophenols, isomeric dihydroxybenzenes, and isomeric nitrophenols.

Hayes and Malik [51] utilised sol–gel chemistry to fabricate OT-CEC columns with surface-bonded octadecylsilane (ODS) stationary phase coating, achieving efficiencies of over 400 000 theoretical plates/m and pH switchable electroosmotic flow. *N*-Octadecyldimethyl[3-(trimethoxysilyl)-propyl] ammonium chloride, as a sol–gel precursor, provided the necessary sol–gel reactivity, ODS chains for chromatography and a positively charged capillary surface for EOF reversal.

Zhao et al. [47] prepared a new type of OT $C_{18}$ ester-bonded electrochromatographic column using sol–gel technology, followed by on-column octadecyl silylation reaction. Glycidoxypropyl-trimethoxysilane, an activated silane agent, was used as the sol–gel precursor to from a thin coating layer on the wall of the silica capillary. The octadecyl groups were introduced into the coating layer by on-column etherification reaction with stearic acid, yielding a high efficiency OT-CEC column (480 000 plates/m).

Constatin and Freitag [52] developed many new silica-based stationary phases for OT-CEC utilising the sol–gel process. The stationary phases, which were hydrophobic, hydrophilic, and charged, allowed for the separation of a broad number of analytes. Recently, Dube and Smith [53] investigated the separation of charged analytes by electrochromatography on porous-layer OT capillaries prepared using the sol–gel method. Acidic diuretic drug compounds were separated at high pH, as were the *N*-alkylanilines in their basic and neutral forms. The limitation of open tubular columns was apparent when separating some basic pharmaceutical drugs. These components displayed severe peak tailing and were unresolved on a 20 μm

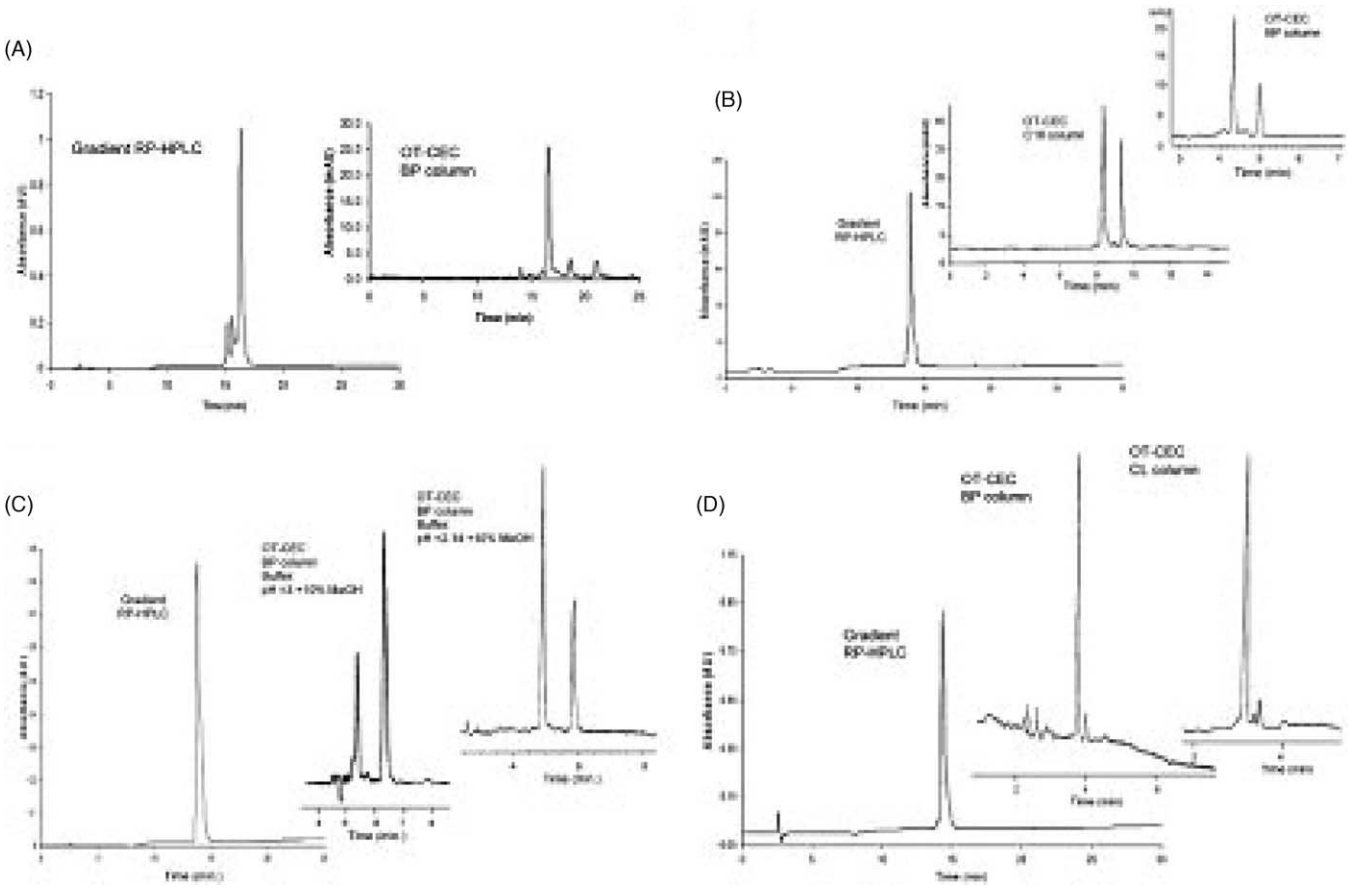


Fig. 7. Comparisons of gradient RP-HPLC analyses of synthetic peptides with electrophoretic determinations using various etched chemically modified capillaries: (A) peptide 6 (HHNSWDHDINR) on butylphenyl capillary at pH 2.14; (B) peptide 9 (QHNHFHR) on $C_{18}$ and butylphenyl capillaries at pH 3.0; (C) peptide 11 (QDQHNHFHR) on butylphenyl capillary using two different pH electrolytes, pH 2.14 and pH 3.0; and (D) peptide 13 (IHQDQHNHFHR) on butylphenyl and cholesterol capillaries at pH 2.14. Solid-phase peptide synthesis, gradient HPLC conditions, OT-CEC conditions, buffers and capillary dimensions given in the text. From ref. [43] with permission.

I.D. porous silica layer open tubular column. Strongly acidic components were undetectable on these columns due to their higher counter electromobilities. Successful separation of neutral aryl alkyl ketones with an efficiency of 101 533 plates $m^{-1}$ for butyrophenone illustrated the improved phase ratio on this type of open tubular column.

Recently, Crosnier de Bellaiste et al. [54] modified the inner surface of a silica capillary using an anhydrous sol–gel method: zirconium propoxide reacted with silanol groups to give, after hydrolysis, a “zirconia-like” surface. The electroosmotic properties of zirconia-modified capillaries were evaluated under different conditions; working parameters were nature and strength of the electrolyte, ionic strength, and pH and also solvent composition. Using different ions such as sodium, potassium, chloride, nitrate or methanoate, a positive electroosmotic flow (cathodic flow) was observed in the pH range 5–11. Below pH 5, EOF reversal (anodic flow) occurred, corresponding to a permanent positively charged inner wall. The value of the electroosmotic flow (including the sign) was easily controlled by addition of multivalent ions to the electrolyte. Some of these modifications of surface charge can be made irreversible. The observed electroosmotic flow has been related to surface characteristics by way of a triple layer model. The large set of working parameters, allows for the optimisation of separations. Flow control was demonstrated for the separation of four antihistaminic compounds (Fig. 8). For further details and recent reviews on sol–gel technology, the following papers are recommended [19,48,55–58].

## 5. Molecularly imprinted polymers

Molecular recognition can be produced by molecular imprinting in a polymeric matrix. As described by Bruggeman et al. [59] the technique involves the creation of a three-dimensional cross-linked polymer network bearing distinct interaction points in the presence of a template molecule. The template or analyte creates a specifically imprinted groove in the polymer, and when removed, leaves a permanent memory of the original template in terms of a complementary shape and chemical functionality.

Due to their high selectivity, molecular imprinted polymers (MIPs) find use in CEC either as thin films

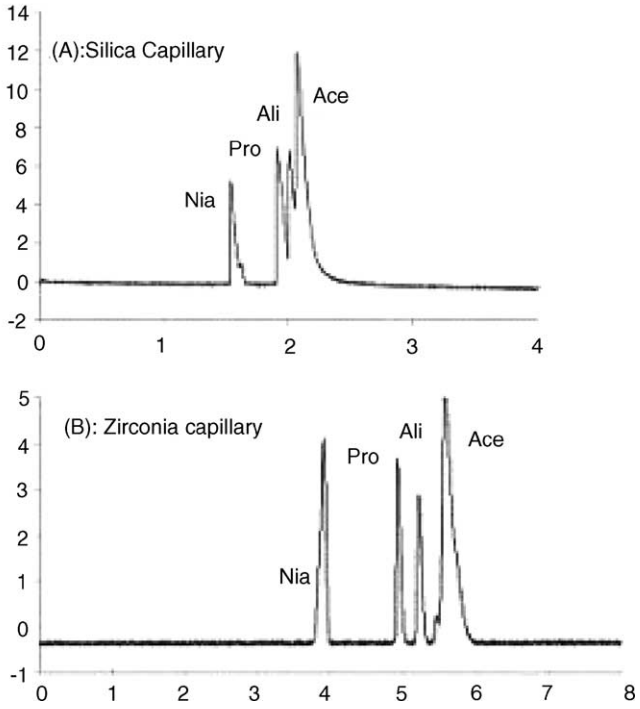


Fig. 8. Separation of four antihistaminic compounds (niaprazine, prometazine, alimeamazine, acepromazine). Capillary: 38 cm (effective length 29 cm) × 50 μm I.D., methanoic acid 60 mM, UV 241 nm. (A) Fused-silica capillary and (B) Zirconia-modified capillary, ref. [54].

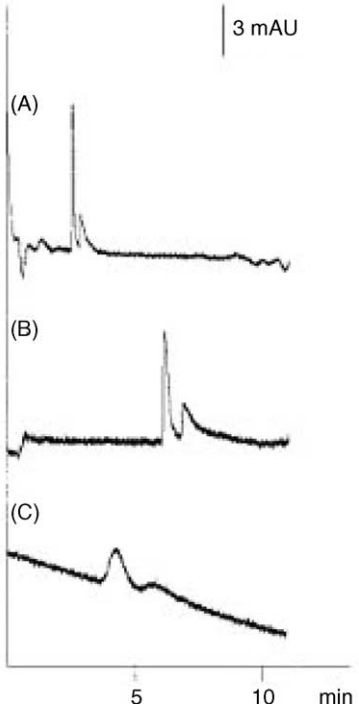


Fig. 9. Electrochromatograms of the enantiomer separation of propranolol using MIP coatings synthesized in different solvents: (A) toluene; (B) dichloromethane; (C) acetonitrile. Elution order: (R)-propranolol followed by (S)-propranolol. Conditions: capillary, 50 μm I.D., 35 cm total length, and 26.5 cm effective length; separation, 15 kV; temperature, 60 °C. From ref. [65] with permission.

coated onto the capillary wall or as packed columns [60]. Electrochromatographic-based separations are thought to improve the efficiency of MIP-based separations and many different formats are being investigated to maximise the performance in capillaries, including monolithic, micro/nanoparticulate, coatings and thin film [61]. Here, the focus is on recent advancements in MIP coatings in an open tubular format [62].

Bruggeman et al. [59] prepared MIP coatings covalently bonded to the inner capillary surface using (methacryloxypropyl)trimethoxysilane derivatised capillaries. Changes in the porogen type and monomer composition resulted in a thin MIP coating on the inner surface of the capillary.

Enantiomeric separations of D- and L-dansyl phenylalanines were achieved by Tan and Remcho [63,64] employing MIPs as stationary phases in OT-CEC. In situ polymerization resulted in thin films of MIPs bonded to the inner walls of 25 μm I.D. fused-silica capillaries. Methacrylic acid and 2-vinyl pyridine were chosen as functional monomers, with either ethylene dimethacrylate or trimethylol propane trimethacrylate as cross-linker.

More recently, Schweitz [65] used a surface-coupled radical initiator to synthesise MIP coatings in fused-silica capillaries. The coatings were prepared using either toluene, dichloromethane, or acetonitrile in the prepolymerization mixtures and were 0.15–2 μm in thickness. The MIP-based OT capillary columns proved capable of separating the enantiomers of propranolol (Fig. 9). An additional novel approach is the use of MIP microparticles in a partial filling application of capillary electrochromatography. Selectivity towards multiple predetermined target solutes in nanoparticle electrochromatograpy has been investigated using mixed singly templated and multiply templated MIP nanoparticle approaches [66,67].

## 6. Porous layers

The main reason for employing porous layer in OT-CEC is to increase the surface area, and in turn the loading capacity of analytes. Evidence in the literature suggests that this area is currently not a main focus in OT-CEC. Huang et al. [16] carried out CEC of basic proteins and peptides with porous-layer open tubular (PLOT) columns which had a functionalized polymeric porous layer grafted to the innerwall of 20 μm I.D. fused silica capillaries. The porous layer was highly cross-linked and prepared by in situ polymerization of vinylbenzyl chloride and divinylbenzene, in the presence of 2-octanol as a porogen inside a pre-treated fused-silica capillary. The chloromethyl functions at the surface of the porous polymeric support layer were reacted with *N*, *N*-dimethyldodecylamine to obtain a positively charged chromatographic surface with fixed

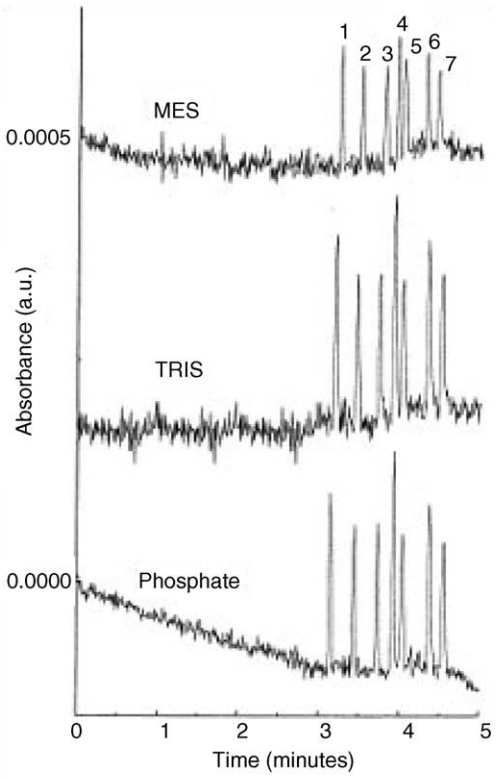


Fig. 10. Separation of a test mixture containing six PAHs by OT-CEC with three different buffers: 2-(*N*-morpholino)ethanesulfonic acid (MES), Tris and phosphate. Experimental conditions: OTC, 41 cm (31 cm to the detector) × 9.60 μm I.D.; mobile phase, MeCN 1 mM buffer pH 7.0 (50:50); hydrodynamic injection, 15 cm for 15 s; applied voltage, 25 kV; detection, UV at 235 nm. The test mixture contained (1) DMF, (2) naphthalene, (3) acenaphthene, (4) phenanthrene, (5) anthracene, (6) fluoranthene and (7) pyrene. Ref. [68].

$C_{12}$ alkyl chains. A mixture of lysozyme, cytochrome *c*, ribonuclease A and α-chymotrypsinogen A was separated isocratically by counter directional CEC with hydro-organic mobile phases containing acetonitrile and phosphate buffer, pH 2.5. The migration behaviour of the four proteins was the result of interplay of chromatographic retention and electrophoretic migration, and was therefore different from that observed in CZE or in reversed-phase chromatography under similar conditions.

Crego et al. [68] investigated the influence of mobile phase composition on electroosmotic flow, solute retention and efficiency using an OT column of 9.60 μm I.D. with a porous silica layer chemically modified with $C_{18}$ as stationary phase. The retention of a group of polycyclic aromatic hydrocarbons (PAHs) (Fig. 10), used as a test mixture, varied significantly on changing the organic modifier content in the hydroorganic mobile phase according to the reversed-phase-like selectivity of the stationary phase. In addition, it was noted that an increase in the percentage of organic modifier resulted in a slight increase in the linear velocity of the EOF. However, when the phosphate buffer concentration was increased over the range 1–50 mM, the electroosmotic mobility reduced dramatically, the retention of the solutes decreased steadily, and the plate height showed a significant increase. The pH rather than the nature of the buffer (phosphate, tris(hydroxymethyl)aminomethane or 2-(morpholino)ethanesulfonic acid) governed the separation behaviour for these organic solutes. Separations of seven PAHs with efficiencies greater than 100 000 plates were obtained within 4 min.

## 7. Physically attached/adsorbed

One problem commonly met in capillary electrophoresis and capillary electrochromatography is unwanted adsorption of analytes onto the capillary wall, a process capable of causing adverse effects such as tailing, loss of efficiency, migration time variation, baseline instability and shortening of capillary lifetime. Adsorption layers, on the other hand, carefully designed and uniformly constructed to change the properties of the inner capillary surface are widely used and continue to be researched. Reversal of EOF direction is readily achievable using a bilayer of the cationic surfactant CTAB. However, in OT-CEC, the objective is to create a stationary phase to introduce a chromatographic mode into the capillary, while avoiding the steps involved in covalent anchoring to the surface. Adsorbed stationary phases can be physically adsorbed or dynamically adsorbed. Physically adsorbed refer to strongly bound stationary phases whereas those dynamically adsorbed possess a weaker interaction and so the modifier is present in the mobile phase. Physically attached layers of a variety of chemistries have been utilised including ligands, cationic surfactants, polymeric surfactants, charged polymers, biopolymers/proteins, chiral and functionalised polymers (chiral, affinity). Liu et al. [69] and Doherty et al. [70] have recently reviewed this area, while Fu et al. [71] reviewed the separation of biomolecules referring to physically adsorbed stationary phases.

### 7.1. Porphyrin derivatives

A continuing highlight is the body of recent work using porphyrin derivatives as capillary surface modifiers. Charvatova et al. [72] investigated two different porphyrin derivatives [$H_2$TPP(m-OPh)$_4$ and Rh(III)TPP(m-OPh)$_4$] with regard to their capability to help resolution of five model aromatic peptides in CE/OT-CEC. In the coating procedure, the capillary was simply filled with the solution of porphyrin derivative in dichloromethane (2 mg/ml) and placed in a vacuum oven where it was dried for 1.5 h at 65 °C. The authors reported that while the main separation mechanism was preferentially based on the ionic properties of the separated analytes, involvement of particularly $H_2$TPP(m-Opt)$_4$–peptide interactions at alkaline pH (8.0) was clearly evident. In combination with Tris–phosphate buffer, a faster separation was observed at pH 2.25 [particularly if Rh(III)TPP(m-OPh)$_4$ was used as capillary coating] yet still allowing a complete

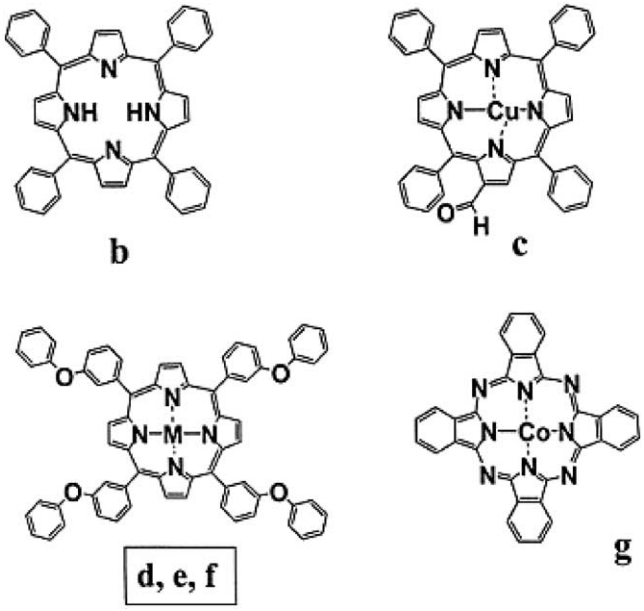


Fig. 11. Structures of (metallo)porphyrin derivatives: (b) 5,10,15, 20-tetraphenylporphyrin, TPP; (c) 3-formyl-5,10,15,20-tetraphenyl-porphyrinate Cu(II), Cu(II)TPP(HCO); (d, e, and f) 5,10,15, 20-tetrakis(phenoxyphenyl)porphyrinate metal (M), (d) M = Rh(III), Rh(III)TPP(*m*-OPh)$_4$, (e) M = Co(III), Co(III)TPP (*m*-OPh)$_4$; (f) M = Ni(II), Ni(II)TPP(*m*-OPh)$_4$; (g) Co(II) phthalocyanine. Ref. [75].

separation of the five model peptides. It is concluded that this coating/buffer combination can be used for a considerable speeding up of long separations of peptides in acidic media with some decrease in the separation power of the system.

In the same year, Charvatova et al. [73] used OT-CEC to study the interactions of synthetic (metallo)porphyrin derivatives (Fig. 11) (immobilized by physical adsorption to the fused-silica capillary wall) with three aromatic amino acids (phenylalanine, tyrosine, tryptophan), three aliphatic amino acids (β-alanine, proline, valine) and two oligopeptides (diglycine, triglycine). Three types of non-covalent interactions, namely axial ligation to the central metal atom, π–π (hydrophobic) stacking and electrostatic repulsion, are believed to take part in the interactions of analyzed amino acids and peptides with porphyrin derivatives, resulting in a better separation of these analytes by OT-CEC than by CZE. Modification of the fused silica capillary was based on the physical adsorption of water- and water-buffer-insoluble porphyrin derivatives dissolved in organic solvent (dichloromethane) and flushed through the capillary. The same research group recently studied physically adsorbed and covalently bonded porphyrin derivative, 5,10,15,20-tetrakis(pentafluorophenyl)porphyrin, $H_2$TPFPP, as a fused-silica capillary wall modifier in OT-CEC. Influence on the EOF velocity and on the selectivity of OT-CEC separations of a set of aromatic carboxylic acids was studied. Coating of the inner fused-silica capillary surface is known to slow down the EOF by shielding the silanol groups of the inner capillary surface. However, in a systematic investigation of oligopyrrolic macrocycles as inner capillary wall modifiers in OT-CEC, it was noted that physically adsorbed or covalently bonded tetrakis(pentafluorophenyl)porhyrin, $H_2$TPFPP, just on the contrary to a set of other porphyrin derivatives, is capable of increasing the EOF of the background electrolyte [25].

Charvatova et al. [74] continued by attempting the separation of 17 “common” underivatized amino acids by OT-CEC in fused-silica capillaries coated with Rh(III)tetrakis(phenoxyphenyl)porphyrinate [Rh(III)TPP(*m*-OPh)$_4$OAc] using sodium phosphate and Tris–phosphate buffers as background electrolytes. The OT-CEC separation of amino acids was compared with that obtained by CZE in bare fused-silica capillaries using the same background electrolytes. Depending on the experimental conditions, at least 15 amino acids could be separated. The best separations were obtained in an Rh(III)TPP(*m*-OPh)$_4$OAc-coated capillary in 50 mM Tris 100 mM phosphate buffer at pH 2.25. The same group employed several (metallo) porphyrins, particularly the porphyrin derivative tetraphenylporphyrin, and complexes of porphyrin derivatives with metal ions ($Zn^{2+}$, $Cu^{2+}$, $Ni^{2+}$, $Co^{2+}$ and $Co^{3+}$) as physically adsorbed stationary phases on the inner fused-silica capillary surface [75]. Four octapeptides, derivatives of the B23–B30 fragment of the B-chain of human insulin with minor changes in their sequences (presence of lysine or ornithine in position B29, presence or absence of phenylacetyl protecting group on the amino group of lysine/ornithine or N-terminal amino group of glycine), were studied as model analytes. Separations were performed both in high (pH 9.0) and in low pH (2.25) background electrolytes, and the changes in the migration/retention behaviour of the model set of peptides were examined with respect to the porphyrin periphery/central metal atom and the charge of the octapeptides modified. Successful separation of these peptides seems to be due to the accessibility of functional groups of the peptides to the interaction with the modifiers tested. In conclusion, modification of the bare fused-silica surface with all (metallo)porphyrin derivatives used resulted in the electroosmotic flow decrease (as compared to the bare fused-silica). In the alkaline BGE, pH 9, the best resolution was obtained when Ni(II)TPP(*m*-OPh)$_4$ was employed as the fused silica capillary inner surface modifier. When Cu(II)TPP(CHO)porphyrin was used partial resolution of octapeptides differing only in Lys/Orn amino acid side chain could be achieved. At least partial resolution of the same pair of octapeptides was obtained in the acid BGE as well, provided that the recognition macrocycle was Co(II)phthalocyanine. The unprotected amino group of Lys/Orn residues of the analysed octapeptides seems to play a vital role in the interaction of the solutes with the (metallo)porphyrin derivatives.

### *7.2. Chiral and affinity polymers*

The separation of enantiomers has been achieved in OT-CEC by chiral selector coating, adsorption and covalent

bonding. The use of polysiloxane-bonded permethyl-β-cyclodextrin capillary coatings has recently been reviewed by Schurig and Mayer [76]. A useful theoretical treatment of OT-CEC is also provided. This open tubular approach to chiral separations is also well described by Laemmerhofer et al. [77] as part of their review on enantiomeric capillary electrochromatography.

Liu et al. [78] showed a new method to prepare stationary phases for OT-CEC based on the adsorption of positively charged compounds on the wall of a fused-silica capillary. The positively charged substances including cationic surfactant such as CTAB and basic chiral selectors including proteins, peptides and amino acids, were physically adsorbed onto the wall under specially selected conditions. The adsorbed stationary phase of CTAB was capable of separating neutral compounds, while others performed chiral separations. Separation efficiency was reflected through theoretical plate numbers of up to 590 000/m. Liu et al. [79] studied chiral separations by OT-CEC with avidin adsorbed on to the capillary wall by physical adsorption. The amount of avidin adsorbed on the wall was estimated to be the order of magnitude of $10^{-12}$ mol for a capillary of 50 cm effective length corresponding to $10^{-8}$ mol/m$^2$ or $10^{-6}$ mol/l. Buffer pH and retention factor of the analyte proved to be key factors in greatly influencing the separation. A total of 16 enantiomeric compounds were separated; however, due to the unfavourable phase ratio, this OT-CEC format is only suitable for enantiomers that interact strongly with the stationary phase. More recently, Liu et al. [80] used physical adsorption for a chiral stationary phase of avidin prepared onto a silica monolith. Enantiomeric separations were performed using CEC, CLC, due to the improved phase ratio and shown to yield a more powerful separation in comparison to OTCEC.

The polymerisation of 3-aminophenylboronic acid (APBA) in aqueous environment has been used for the open tubular modification of CE capillaries by Bossi et al. [81]. Immobilised boronic acids have been previously used for affinity chromatography because of their ability to form reversible covalent complexes with *cis*-diol containing solutes such as sugars and catechols. The APBA polymer is grafted to the capillary surface and being a polymer with boronic acid ligands, aromatic rings and secondary amines groups, it posses a variety of functional groups affecting selectivity. Diastereoisomers (e.g. ascorbic and isoascorbic acid) and proteins (e.g. haemoglobins) were successfully separated on the poly-APBA capillary, by means of a combination of electrophoresis and open tubular electrochromatography. It is included here as an affinity ligand worthy of further attention in OT-CEC, particularly in future affinity separations and chip technologies [81].

### 7.3. Molecular micelles (polymeric surfactants)

Kapnissi et al. [82] investigated fused-silica capillaries coated with thin films of physically adsorbed charged polymers by way of a polyelectrolyte multilayer (PEM) coating procedure. The in situ coating was created by alternating rinses with positively and negatively charged polymers, the negatively charged polymer being a molecular micelle. Poly(diallyldimethylammonium chloride) ((PDADMAC)) was the cationic polymer while poly(sodium-*N*-undecanoyl-L-glycinate) was the anionic surfactant for PEM coating. The performance of the modified capillary was evaluated using seven benzodiazepines and the PEM-coated capillary was stable under extreme pH conditions and very robust (Fig. 12).

Recently, Kamande et al. [83] published investigations of a stable PEM coating for use in OT-CEC. The PEM consisted of a cationic polymer of a quaternary ammonium salt, poly(diallyldimethylammonium chloride) and the anionic surfactant, poly(sodium undecylenic sulphate). The cationic and the anionic polymers were both physically adsorbed to the surface of the fused-silica capillary by a simple coating procedure, involving alternate rinses of the positively and negatively charged polymers. The PEM coating acted as

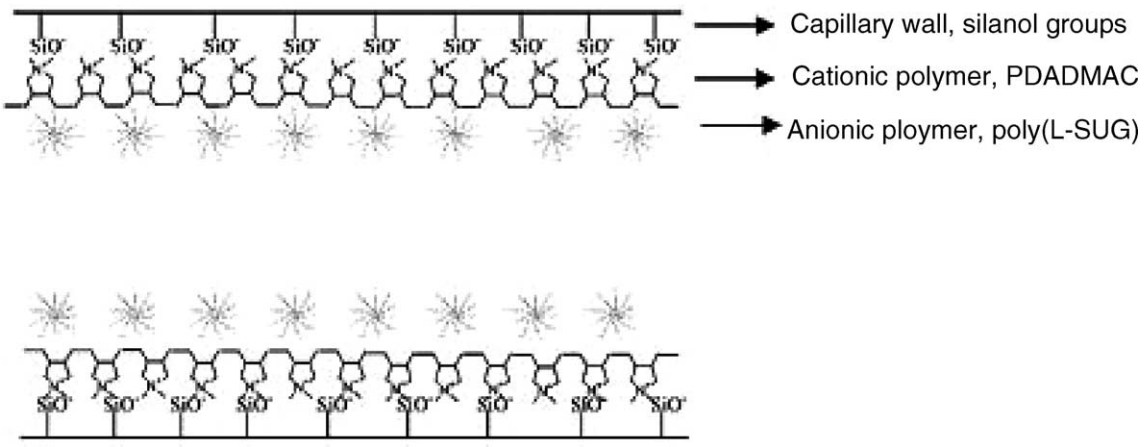


Fig. 12. Schematic diagram of the PEM-coated capillary. From ref. [82] with permission.

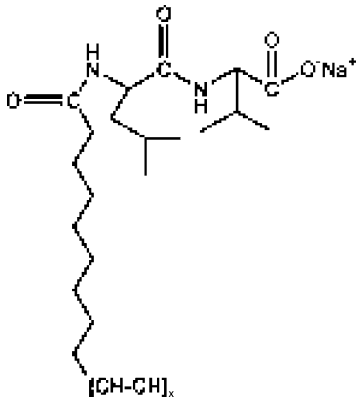

Fig. 13. Structure of poly-L-SULV. From ref. [86] with permission.

a dynamic stationary phase, displayed good selectivity for both phenols and benzodiazepines.

Some of the latest development by Warner and co-workers [84] involved the use of polymeric surfactant poly(sodium undecylenic sulfate) (poly-SUS) as a stationary phase coating in OT-CEC coupled with electrospray ionisation mass spectroscopy (ESI–MS) for the analysis of β-blocker and benzodiazepines. The coating was formed on the inner capillary wall by adsorption of the cationic polymer poly(diallyldimethylammonium chloride), and adsorption of the negatively charged poly-SUS on to the cationic polymer layer via strong physical interaction of the two polymer layers. The method minimizes the introduction of monomeric or polymeric surfactant into the MS system. Results included the separation of four β-blockers and four benzodiazepines.

Dipeptide micelle polymers are a class of polymeric surfactants of which the polysodium undecanoyl-L-leucylvalinate (poly-L-SULV) (Fig. 13) was found to be a generally applicable chiral selector [85]. To overcome the loss of the chiral decriminators during flushing in chiral MEKC, the PEM coating approach using the anionic polymer (poly-L-SULV) was used for OT-CEC [86]. Two-bilayer and three-bilayer coatings were shown to be effective for selected chiral solutes.

## 8. Nanoparticle phases

In order to overcome the limited loading capacity, which is characteristic of OT columns, Breadmore et al. [87] investigated the OT-ion-exchange electrochromatography (IE-CEC) for the separation of inorganic anions, by coating the inner walls of a fused silica capillary with quaternary ammonium anion-exchange latex nanoparticles. Dionex AS5A latex particles were bound electostatically to the inner wall and this caused EOF reversal. Separation of anions was based on anion-exchange interactions, the strength of which could be controlled by the type and concentration of the competing ion used in the background electrolyte. As a result, separation selectivity could be controlled to give an order of elution expected for CE, or IC, or both together. This fritless, simple procedure is a mechanism for controlling the separation selectivity of small inorganic anions. In the same year, this group carried out an experimental study of factors influencing peak shapes in IE-CEC using adsorbed quaternary aminated latex particles as a stationary phase [88]. It was observed that electrophoretic and chromatographic sources both contributed to peak shape in OT-CEC. Though electromigration dispersion is not a major player in band broadening, ion mobility is still significant. Resistance to mass transfer in the mobile phase can be improved by decreasing capillary diameter, which results in a noticeable reduction in peak tailing especially for polarisable anions. Breadmore et al. [89] reviewed the topic of determining inorganic anions by CEC in 2001.

More recently, Breadmore et al. [90] developed a solid-phase extraction method (SPE) on an ion-exchange retention mechanism for in-line preconcentration of inorganic anions prior to separation by CE. Analyte anions were retained on a preconcentration zone comprising an adsorbed layer of cationic latex particles, while separation took place in a zone comprising fused silica modified by adsorption of a cationic polymer. Elution of the adsorbed analytes was achieved by way of eluotropic gradient formed by a transient isotachophoretic boundary between a fluoride electrolyte and a naphthalenedisulfonate electrolyte. Optimisation resulted in separation of sub-ppb levels of inorganic anions. This method was used to determine $NO_3^-$ in Antarctic ice cores.

O'Mahony et al. [91] immobilised dodecanethiol gold nanoparticles on a prederivatised 3-aminopropyltrimethoxysilane (APTMS) or 3-mercaptopropyltrimethoxysilane (MPTMS) fused-silica capillaries. The electroosmotic flow characteristics of Au-APTMS and Au-MPTMS capillary columns were examined by variation of the applied potential and the pH of the run buffer (Fig. 14). OT-CEC separations were achieved for benzophenone and biphenyl solutes on Au-MPTMS and Au-APTMS capillary columns, analogous to reversed phase behaviour in LC. A study of the reproducibility of retention for these solutes on Au-APTMS, Au-MPTMS, and on a loosely coated capillary demonstrated the necessity of a coupling agent to prevent the gold nanoparticles from washing-off. In contrast to benzophenone and biphenyl, organic thiourea solutes (non-fluorinated and fluorinated) injected on these gold nanoparticle phases resulted in poor peak shapes and discriminating ability, possibility due to adsorption of the solutes onto the gold nanoparticles. The work confirmed the use of dodecanethiol gold nanoparticles as a novel phase for OT-CEC, demonstrating reproducible retention and characteristic reversed phase behaviour. Very recently Geng et al. [92] employed capillary-coated layer-by-layer assembly of γ-zirconium phosphate/lysozyme nanocomposite film to achieve OT-CEC chiral separations.

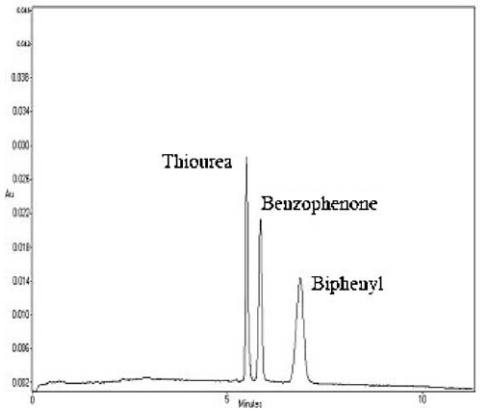


Fig. 14. Electropherogram obtained on dodecanethiol Au-MPTMS-coated capillary, Conditions: capillaries 30 cm × 30 μm I.D.; mobile phase 70% MeOH: 25 mM $Na_2HPO_4$ pH 7.0; voltage 30 kV. Sample: thiourea, benzophenone and biphenyl in methanol–water (70:30). Injection: 3.5 kPa for 4 s. Ref. [91].

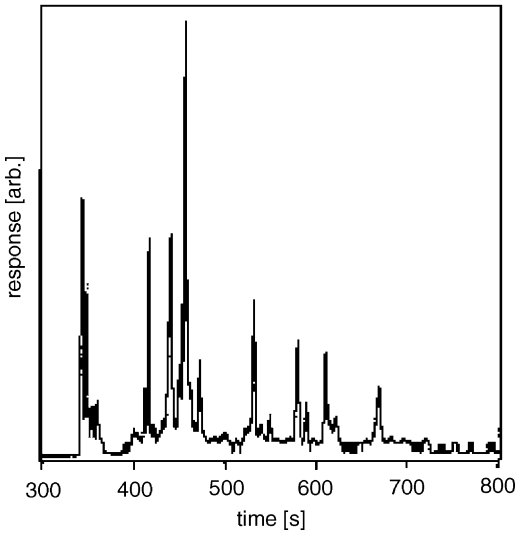


Fig. 15. One-dimensional OCEC separation of TRITC-labelled tryptic peptides of β-casein. The field strength was 220 V/cm in the OCEC channel. The buffer was 10 mM sodium borate with 30% (v/v) acetonitrile. From ref. [93] with permission.

## 9. Microchip open channel CEC

On-chip OT-CEC was demonstrated by Jacobson et al. [6] in 1994 using an ODS modified microchannel fabricated on a glass substrate. More recently, Kutter et al. [12] demonstrated open-channel electrochromatography in conjunction with solvent programming using a microchip set-up. The channel walls were coated with octadecylsilanes at room temperature, yielding chromatographic stationary phases for neutral dye separations. Separations times were as fast as 60 s while efficiency values were comparable to conventional CE set-ups.

The same laboratory showed a two-dimensional separation system on a microfabricated device using open-channel electrochromatography as the first dimension and CE as the second [93] (Figs. 15 and 16). The first dimension was operated isocratically, while the effluent from the first dimension was continually injected into the second dimension every few seconds. The 25 cm separation channel, possessing spiral geometry, was chemically modified with octadecylsilane and coupled to a 1.2 cm straight separation channel for CE. Fluorescently labelled products originating from trypic digests of β-casein were analysed within 13 min. This approach might be suitable for rapid, automated fingerprinting of proteins and protein digests coupled with MS detection.

More recent progress by this group has integrated sample filtration, concentration and subsequent separation on a microchip, coated with ODS stationary phase [94]. On chip sample filtering, solid phase extraction and OT-CEC with solvent programming were demonstrated using the quartz microchip. Four polycyclic aromatic hydrocarbons were separated by open channel electrochromatography in under 50 s using the chip as a multi-task platform.

Constantin et al. [95] demonstrated a fast and easy way to produce self-containing open-tubular μ-CEC columns ($C_8$ moieties for reversed phase applications) by the sol–gel technique. The separation of a mixture of three uncharged analytes (polycyclic aromatic hydrocarbons) is demonstrated. Under optimized conditions, the performance of the chip is reported to be comparable or better than that of capillary-based CEC columns of the same kind.

Burke et al. [96] examined electrophoretically mediated microanalysis (EMMA) in a microfabricated system. This

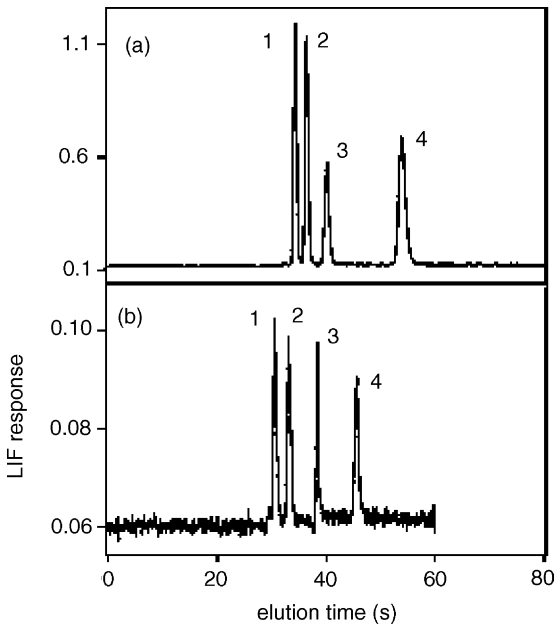


Fig. 16. Separation of four component mixture (1) anthracene, (2) pyrene, (3) 1,2-benzofluorene, and (4) benzo[a]pyrene. Chip 1 and a step gradient were used, and the injection time was 20 s. The concentrations of analytes 1–4 were 2.8, 0.9, 5.8 and 5.0 μM in (a), and 100, 28, 230, and 200 nM in (b), respectively. LIF = laser-induced fluorescence. From ref. [94] with permission.

method of chemical analyses is typically carried out in an open-tubular capillary, using the difference in the electrophoretic mobility between particular reagents. Morishima et al. [97] synthesised a porous photopolymerised sol–gel (PSG) monolith in the separation channel of a borosilicate glass chip via UV irradiation (5 min) of a mixture of 3-methacryloxypropyltrimethoxysilane, an acid catalyst, a porogen, and a photoinitiator. The PSG monolith adhered strongly to the chemically untreated channel walls. Two dyes Coumarin 314 and 510 were successfully separated within baseline resolution in 225 s with fluorescent detection after the PSG section. Very recently, Stachowiak et al. [98] studied UV-initiated grafting of plastic tubes and microfluidic chips with ethylene diacrylate followed by the preparation of porous polymer monoliths. The first step afforded a thin grafted layer of polymer with a multiplicity of pendent double bonds, which allowed for covalent attachment of the monolith to the wall. The procedure eliminated void formation at the monolith-channel interface a common problem associated with monolith shrinkage and lack of compatibility with the material of the device. Irradiation with UV light through a photomask permitted precise patterning specifying both the area subjected to surface modification and the location of the monolith within specific areas of the device.

Microchannel wall coatings for protein separations by capillary and chip based electrophoresis have been reviewed by Doherty et al. [70].

## References


[1] T. Tsuda, K. Nomura, G. Nakagawa, J. Chromatogr. 248 (1982) 241.
[2] G.J.M. Bruin, P.P.H. Tock, J.C. Kraak, H. Poppe, J. Chromatogr. 517 (1990) 557.
[3] W.D. Pfeffer, E.S. Yeung, Anal. Chem. 62 (1990) 2178.
[4] S. Mayer, V.J. Schurig, J. High Resolut. Chromatogr. 15 (1992) 129.
[5] D.W. Armstrong, Y. Tang, T. Ward, M. Nichols, Anal. Chem. 65 (1993) 1114.
[6] S.C. Jacobson, R. Hergenroëder, L.B. Koutny, J.M. Ramsey, Anal. Chem. 66 (1994) 2369.
[7] Y. Guo, L.A. Colon, Anal. Chem. 67 (1995) 2511.
[8] J.J. Pesek, M.T. Matyska, J. Chromatogr. A 736 (1996) 255.
[9] E. Francotte, M. Jung, Chromatographia 42 (1996) 521.
[10] Z.J. Tan, V.T. Remcho, Anal. Chem. 69 (1997) 581.
[11] L. Schweitz, L.I. Andersson, S. Nilsson, Anal. Chem. 69 (1997) 1179.
[12] J.P. Kutter, S.C. Jacobson, N. Matsubara, J.M. Ramsey, Anal. Chem. 70 (1998) 3291.
[13] H. Hofstetter, O. Hofstetter, V. Schurig, J. Microcol. Sep. 10 (1998) 287.
[14] Z. Liu, H. Zou, J. Ni, Y. Zhang, Anal. Chim. Acta 378 (1999) 73.
[15] H. Sawada, K. Jinno, Electrophoresis 20 (1999) 24.
[16] X. Huang, J. Zhang, C. Horvath, J. Chromatogr. A 858 (1999) 91.
[17] C. Fujimoto, M. Sakurai, Y. Muranaka, J. Microcol. Sep. 11 (1999) 693.
[18] W. Xu, F. Regnier, J. Chromatogr. A 853 (1999) 243.
[19] C.P. Kapnissi-Christodoulou, X. Zhu, I.M. Warner, Electrophoresis 24 (2003) 3917.
[20] Y.-F. Pai, C.-Y. Liu, J. Chromatogr. A 982 (2002) 293.
[21] W.H. Chen, C.Y. Liu, J. Chromatogr. A 848 (1999) 401.
[22] S. Hu, E.Q. Fu, P.C.H. Li, J. Chromatogr. A 844 (1999) 439.
[23] N. Guan, Z.R. Zeng, Y.C. Wang, E.Q. Fu, J.K. Cheng, Anal. Chim. Acta 418 (2000) 145.
[24] Y.C. Wang, Z.R. Zeng, C.H. Xie, N. Guang, E.Q. Fu, J.K. Cheng, Chromatographia 54 (2001) 475.
[25] J. Charvatova, V. Kasicka, Z. Deyl, V.J. Kral, J. Chromatogr. A 990 (2003) 111.
[26] J.D. Glennon, E. Horne, K. O'Connor, G.A. Kearney, S.J. Harris, M.A. McKervey, Anal. Proceed. 31 (1994) 33.
[27] D. Shohat, E. Grushka, Anal. Chem. 66 (1994) 747.
[28] J.D. Glennon, in: I.A. Wilson, T.R. Adlard, C.F. Poole, M. Cook (Eds.), Encyclopedia of Separation Science, vol. III, Academic Press, 2000, p. 3400.
[29] Z. Zeng, C. Xie, H. Li, H. Han, Y. Chen, Electrophoresis 23 (2002) 1272.
[30] X. Wu, H. Liu, H. Liu, S. Zhang, P.R. Haddad, Anal. Chim. Acta 478 (2003) 191.
[31] S.L. Clark, V.T. Remcho, Electrophoresis 23 (2002) 1335.
[32] S.L. Clark, V.T. Remcho, Anal. Chem. 75 (2003) 5692.
[33] M.A. Rehder, L.B. McGown, Electrophoresis 22 (2001) 3759.
[34] J.J. Pesek, M.T. Matyska, J. Chromatogr. A 887 (2000) 31.
[35] J.J. Pesek, M.T. Matyska, Am. Lab. May (2002) 22.
[36] M.T. Matyska, J.J. Pesek, R.I. Boysen, M.T.W. Hearn, J. Chromatogr. A 924 (2001) 211.
[37] M.T. Matyska, J.J. Pesek, I. Boysen, T.W. Hearn, Electrophoresis 22 (2001) 2620.
[38] M.T. Matyska, J.J. Pesek, R.I. Boysen, M.T. Hearn, Anal. Chem. 73 (2001) 5116.
[39] M.T. Matyska, J.J. Pesek, A.A. Katrekar, Anal. Chem. 71 (1999) 5508.
[40] J.J. Pesek, M.T. Matyska, H. Tran, J. Sep. Sci. 24 (2001) 1.
[41] J.J. Pesek, M.T. Matyska, S. Sentellas, M.T. Galceran, M. Chiari, G. Pirri, Electrophoresis 23 (2002) 2982.
[42] Z. Liu, K. Otsuka, S. Terabe, J. Chromatogr. A 961 (2002) 285.
[43] J.J. Pesek, M.T. Matyska, G.B. Dawson, Anal. Chem. 76 (2004) 23.
[44] C. L-Quigley, N.D. Marlin, V. Melin, A. Manz, N.W. Smith, Electrophoresis 24 (2003) 917.
[45] Y. Guo, L.A. Colon, Anal. Chem. 67 (1995) 2511.
[46] P. Narang, L.A. Colon, J. Chromatogr. A 773 (1997) 65.
[47] Y. Zhao, R. Zhao, D. Shangguan, G. Liu, Electrophoresis 23 (2002) 2990.
[48] S.A. Rodriguez, L.A. Colon, Anal. Chim. Acta 397 (1999) 207.
[49] Y.C. Wang, Z.R. Zeng, C.H. Xie, N. Guan, E.Q. Fu, J.K. Cheng, Chromatographia 54 (2001) 475.
[50] Y. Wang, Z. Zeng, N. Guan, J. Cheng, Electrophoresis 22 (2001) 2167.
[51] J.D. Hayes, A. Malik, Anal. Chem. 73 (2001) 987.
[52] S. Constantin, R. Freitag, J. Sep. Sci. 25 (2002) 1245.
[53] S. Dube, R.M. Smith, Chromatographia 57 (2003) 485.
[54] M. Crosnier de Bellaiste, O. Mathieu, J. Randon, J.L. Rocca, J. Chromatogr. A 971 (2002) 199.
[55] S. Constantin, R. Freitag, J. Chromatogr. A 887 (2000) 253.
[56] A. Malik, Electrophoresis 23 (2002) 3973.
[57] S. Eeltink, G.P. Rozing, W.T. Kok, Electrophoresis 24 (2003) 3935.
[58] A-M. Siouffi, J. Chromatogr. A 1000 (2003) 801.
[59] O. Bruggeman, R. Freitag, M.J. Whitcombe, E.N. Vulfson, J. Chromatogr. A 781 (1997) 43.
[60] G. Gublitz, M.G. Schmid, Electrophoresis 21 (2000) 4112.
[61] P. Spegel, L. Schweitz, S. Nilsson, Electrophoresis 24 (2003) 3892.
[62] L. Schweitz, P. Spegal, S. Nilsson, Electrophoresis 22 (2001) 4053.
[63] Z.T. Tan, V.T. Remcho, Electrophoresis 19 (1998) 2055.
[64] V.T. Remcho, Z.J. Tan, Anal. Chem. 71 (1999) 248A.
[65] L. Schweitz, Anal. Chem. 74 (2002) 1192.
[66] P. Spegel, L. Schweitz, S. Nilsson, Electrophoresis 22 (2001) 3833.
[67] P. Spegel, L. Schweitz, S. Nilsson, Anal. Chem. 75 (2003) 6608.
[68] A.L. Crego, J. Martinez, M.L. Marina, J. Chromatogr. A 869 (2000) 329.

[69] Z. Liu, R. Wu, H. Zou, Electrophoresis 23 (2002) 3954.
[70] E.A.S. Doherty, R.J. Meagher, M.N. Albarghouthi, A.E. Barron, Electrophoresis 24 (2003) 34.
[71] H. Fu, X. Huang, W. Jin, H. Zou, Curr. Opin. Biotechnol. 14 (2003) 96.
[72] J. Charvatova, V. Kral, Z. Deyl, J. Chromatogr. B 770 (2002) 155.
[73] J. Charvatova, V. Kasicka, V. Kral, Z. Deyl, J. Chromatogr. B 770 (2002) 165.
[74] J. Charvatova, Z. Deyl, V. Kasicka, V. Kral, J. Chromatogr. A 990 (2003) 159.
[75] J. Charvatova, V. Kasicka, T. Barth, Z. Deyl, I. Miksik, V. Kral, J. Chromatogr. A 1009 (2003) 73.
[76] V. Schurig, S. Mayer, J. Biochem. Biophys. Methods 48 (2001) 117.
[77] M. Laemmerhofer, F. Svec, J.M.J. Fréchet, W. Linder, Trends Anal. Chem. 19 (2000) 676.
[78] Z. Liu, H. Zou, M. Ye, J. Ni, Y. Zhang, Electrophoresis 20 (1999) 2891.
[79] Z. Liu, K. Otsuka, S. Terabe, J. Sep. Sci. 24 (2001) 17.
[80] Z. Liu, K. Otsuka, S. Terabe, M. Motokawa, N. Tanaka, Electrophoresis 23 (2002) 2973.
[81] A. Bossi, L. Castelletti, S.A. Piletsky, A.P.F. Turner, P.G. Righetti, J. Chromatogr. A 1023 (2004) 297.
[82] C.P. Kapnissi, C. Akbay, J.B. Schlenoff, I.M. Warner, Anal. Chem. 74 (2002) 2328.
[83] M.W. Kamande, C.P. Kapnissi, X. Zhu, C. Akbay, I.M. Warner, Electrophoresis 24 (2003) 945.
[84] X. Zhu, M.W. Kamande, S. Thiam, C.P. Kapnissi, S.M. Mwongela, I.M. Warner, Electrophoresis 25 (2004) 562.
[85] S.A. Shamsi, B.C. Valle, F. Billiot, I.M. Warner, Anal. Chem. 75 (2003) 379.
[86] C.P. Kapnissi, B.C. Valle, I.M. Warner, Anal. Chem. 75 (2003) 6097.
[87] M.C. Breadmore, M. Macka, N. Avdalovic, P.R. Haddad, Analyst 125 (2000) 1235.
[88] M.C. Breadmore, M. Boyce, M. Macka, N. Avdalovic, P.R. Haddad, J. Chromatogr. A 892 (2000) 303.
[89] M.C. Breadmore, E.F. Hilder, M. Macka, P.R. Haddad, Trends Anal. Chem. 20 (2001).
[90] M.C. Breadmore, A.S. Palmer, M. Curran, M. Macka, N. Avdalovic, P.R. Haddad, Anal. Chem. 74 (2002) 2112.
[91] T. O'Mahony, V.P. Owens, J.P. Murrihy, E. Guihen, J.D. Holmes, J.D. Glennon, J. Chromatogr. A 1004 (2003) 181.
[92] L. Geng, T. Bo, H. Liu, N. Li, F. Liu, K. Li, J. Gu, R. Fu, Chromatographia 59 (2004) 65.
[93] N. Gottschlich, S.C. Jacobson, C.T. Culbertson, J.M. Ramsey, Anal. Chem. 73 (2001) 2669.
[94] B.S. Broyles, S.C. Jacobson, J.M. Ramsey, Anal. Chem. 75 (2003) 2761.
[95] S. Constantin, R. Freitag, D. Solignac, A. Sayah, M.A.M. Gijs, Sens. Actuators B: Chem. 78 (2001) 267.
[96] B.J. Burke, F.E. Regnier, Electrophoresis 22 (2001) 3744.
[97] K. Morishima, B.D. Bennett, M.T. Dulay, J.P. Quirino, R.N. Zare, Electrophoresis 25 (2002) 1226.
[98] T.B. Stachowiak, T. Rohr, E.F. Hilder, D.S. Peterson, M. Yi, F. Svec, J.M.J. Fréchet, Electrophoresis 24 (2003) 3689.