Review

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Stationary phases for capillary electrophoresis and capillary electrochromatography

An overview of the most recent developments in column technology employed in capillary electrophoresis (CE) and capillary electrochromatography (CEC), mainly for the separation of small molecules and ions, is presented. Particular emphasis is laid on permanent coating. The wall modification methods in CE include covalent modification, adsorbed coatings and polymeric coatings, while those in CEC include packed columns, open-tubular columns and fritless columns. A short discussion on the characterization and selectivity of the bonded phases is also given.

Keywords: Stationary phases / Wall modification / Review

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Abbreviations: AFM, atomic force microscopy; DRIFT, diffuse reflectance infrared Fourier transform; ODS, octadecylsilica; PMP, 1-phenyl-3-methyl-5-pyrazolone; SEM, scanning electron microscopy

1 Introduction

Capillary electrophoresis (CE) is now acknowledged as a fast, powerful, efficient, cost-effective and high-resolution separation technique. It has been mainly employed in the field of analyzing biological macromolecules such as proteins, peptides and nucleic acids. Recently, however, CE has been increasingly applied to low molecular mass molecules and is being recognized as a powerful method for the separation and simultaneous determination of small molecules and ions [1–11].

Capillary electrochromatography (CEC), a separation technique that potentially combines the separation efficiency of CE with the selectivity and sample capacity of liquid chromatography (LC) has recently generated enormous interest. The hybrid method was originally proposed by Pretorius *et al.* [12] in 1974. However, CEC did not attract much attention until it was demonstrated by Jorgenson and Lukacs [13] using a packed capillary in 1981, and when Knox and Grant [14–17] developed the theory in the late 1980s and early 1990s. This interest is predominantly generated as a result of the mobile phase transport mechanism through the chromatographic stationary phase in CEC, which is electrodriven instead of pressure-driven and thus theoretically offers a number of important advantages which are often realized through increased efficiency values and improved resolution. It is now recognized, however, that a number of significant advances have to be made before CEC can fulfill its potential and ensure a continued interest [18].

The selectivity of CE and CEC is based on three main principles, namely chemical parameters, instrumental parameters, and the capillary column itself. In the present review, we provide a survey of various aspects of column

technology both in CE and CEC with special emphasis given to small molecules and ions. Considerable attention is paid to developments in the last five years.

2 Capillary electrophoresis

The increase in selectivity is an important goal in all analytical techniques. The manipulation of the electroosmotic flow (EOF) in order to optimize the efficiency N , the speed of analyses, and the resolution R is one of the aims of procedures of chemical surface modification. Capillaries can be internally coated to alter selectivity, reverse EOF or to reduce EOF. These coatings can be either permanent (chemically bonded) or temporary (use of electrolyte additives). The latter methods include dynamic coatings. To rapidly separate anions by CZE, cationic surfactants are often added to reverse the EOF. In choosing a surfactant, the detection mode and detection wavelength are important variables. The additives added may change the buffer properties and are likely to result in poorer detection limits [19]. For example, benzyltrimethylammonium bromide is UV active and would interfere with the analyte signal with indirect UV detection. Also, the surfactant counterion can interfere in the separation. It has been shown that the system peak induced by the bromide present in tetradecyltrimethylammonium bromide interferes with thiosulfate determination, whereas the same surfactant in the hydroxide form does not interfere [20].

Precipitation of surfactants with certain background electrolytes (BGEs) at given pH values has been noted [21]. The work by Corr and Anacleto [22] showed that bonding a capillary with strong anion-exchange sites is advantageous when mass spectrometry (MS) is used for the detection of inorganic anions, as current EOF modifiers increase background noise and mass spectral complexity. In other words, this approach of permanent coating was very important for CE-MS work to prevent the large molecules of polymeric modifiers from entering interfaces and ion sources. Improvements in column technology have therefore been one of the main trends in the development of CE over the past few years.

For such an approach, the tubing material should have a defined surface with regard to the concentration of silanols. The presence or absence of contaminants of the silica may change the EOF and the adsorptivity of different types of charged or uncharged small and large polar molecules. Therefore, many applications require a preliminary step of etching or rinsing with strongly acidic or basic aqueous solutions to which the coverage of the surface by silanol groups is changed or made uniform in order to achieve reproducible conditions of the EOF in different capillaries.

2.1 Permanent coating

Bonded and packed capillaries are being used increasingly in CZE, because permanent coatings are not usually destroyed by rinsing with any of the aggressive rinsing solutions that might be applied for regeneration of the capillary surfaces. Any effect on selectivity will depend on the nature of the bonded phase. Treated capillaries can offer a wider accessible pH range, high selectivity and the need for EOF reversal. Various types of coating layers can be generated either by silanization of the SiOH as anchor groups for the fixation of polar, neutral or ionic small and larger oligomers or by multipoint adsorption of neutral and charged polymers that can be cross-linked. Ionic interaction of cationic polymers or surfactant molecules with the negatively charged surface may also be involved for fixation [23]. At present, different permanently coated CE capillaries with reproducible properties have become commercially available, with either hydrophobic (alkylpolysiloxane) or hydrophilic (poly(ethylene glycol) and poly(vinyl alcohol)) coatings and also charged coatings that are fixed by covalent bonding or by strong adsorption and cross-linking of polymeric layers. Several modification techniques in the literature will be described in the following sections.

2.1.1 Covalent modification

Permanent coatings are usually achieved by derivatization of the capillary wall silanols followed by a covalent binding of a material. From literature dealing with the preparation of chromatographic stationary phases, both halo and alkoxy silanes may be coupled to surface silanols through one or more siloxane (Si-O-Si) bonds as seen in the following reaction [24]:

(i) with monofunctional silane

(ii) with polyfunctional silane

where Y is a halo or alkoxy group and X is either a glycid-oxo [25] or amino group [26]. Neither glycidoxo- nor amine-derivatized capillaries are used directly for electrophoresis. In the literature, many glycidoxo-derivatized capillaries have been either converted to the glyceryl derivative or substituted with another hydrophilic substance according to the reaction

where R may be -H, -CH₂CHOHCH₂OH (glycerol), or -(OCH₂CH₂)_nOH (polyoxyethylene).

Liu and co-workers [27] have used 3-glycidyloxypropyltrimethoxysilane to produce capillaries containing 24-membered macrocyclic polyamines, [24]ane-N₆, for the separation of inorganic and organic anions, geometric isomers, polycarboxylates and polyphosphates. With the same strategy, capillaries containing 28-membered macrocyclic polyamines, [28]ane-N₆O₂, have also been prepared for the separation of metalocyanide, inorganic and organic anions, as well as metal speciation [28–30]. The column preparation is shown in Fig. 1. 3-Glycidyloxypropyltrimethoxysilane was also used by Lee and co-workers [31] to produce diol-bonded capillaries at room temperature for CZE. A variety of standard reference compounds and authentic biological samples including ribonucleotides, peptides and proteins were used to test the columns. It was found that a greatly suppressed EOF was measured over a pH range of 3–10.

Several other simple silanes, such as octadecylsilane [32] have also been examined. Maltose and pentafluorobenzoic acid were coupled to γ -aminopropylsilane derivatization capillaries [33]. The limitation of the simple silane coatings is the limited hydrolytic stability above pH 8. Anchor groups, *e.g.*, silanol groups, may also be used to bond molecules which contain functional groups for various types of chemical bonding.

2.1.2 Adsorbed coatings

Adsorbed coatings can be formed either through electrostatic interactions or hydrogen bonding. When the surface of silica is derivatized with an alkyl silane, it is possible to adsorb substances through hydrophobic interactions. Questions that must be considered are whether the chemical and physical properties are compatible with the separation process. Although low-molecular-weight additives can be used as an adsorbed coating, adsorbed coatings are generally oligomers or polymers. Usually high concentration and high molecular weight produce thicker

layers. Thicker coatings probably reduce both EOF and shield the surface more effectively than thin coatings [34, 35].

Neutral and hydrophilic methylcellulose was one of the first adsorbed polymers used in CE. Adsorbed methylcellulose coatings have been used effectively in both CZE and isoelectric focusing [35]. In addition, various molecular sizes of polyvinyl alcohol, and polyvinyl pyrrolidone have been also used to create adsorbed, hydrophilic coatings [36]. Thin-film coatings of cellulose acetate on the surface of fused-silica capillaries were tested for the separation of proteins by CZE. But the coating is destroyed above pH 7.5 and, therefore, cannot be recommended for CZE at alkaline pH or for isoelectric focusing [37].

When polyamine is added to the running buffers and the solution is forced through a capillary, the adsorbed polyamine causes the surface of the column to acquire a positive charge and the EOF is reversed. A polyethyleneimine (PEI) coating on the inner surface of fused-silica capillaries for CE separation of proteins and peptides is reported by Bedia Erim *et al.* [38]. Four polycationic polymers, including polyethyleneimine 1,5-dimethyl-1,5-diazaundecamethylene polymethobromide (Polybrene), poly(methoxyethoxyethyl)ethyleneimine and poly(diallyldimethylammonium chloride) were examined by Cordova *et al.* [39] as noncovalent coatings for capillaries in CE separation of proteins to limit the adsorption of proteins on the walls of fused-silica capillaries.

Cationic polyelectrolytes employed for EOF reversal also include polyarginine [40], chitosan [41], poly(diallyldimethylammonium chloride) [42], and PEI [43]. However, it has been documented that the electroosmotic mobility deteriorates during use. Huber and co-workers [44] reported CE separation of peptides and proteins in fused-silica capillaries coated with derivatized polystyrene nanoparticles. Recently, a successive multiple ionic-polymer layer coating was provided by Katayama *et al.* [45]. An anionic polymer was tightly fixed to the capillary wall by the successive multiple ionic polymer layer (SMIL) coating, in which a cationic polymer was sandwiched between the anionic polymer and the uncoated fused silica capillary by noncovalent bonding. The coating procedures are shown in Fig. 2.

The coated capillary showed a long lifetime for the CE separation of proteins and mixtures of cationic, anionic and neutral amino acids. With this coating, the separation of cresol isomers was also achieved by micellar electrokinetic chromatography. Katayama *et al.* [46] further modified the SMIL coating and developed a stable cation-modified capillary in order to extend its application to the basic analytes. Triple layers of the ionic polymers were

Figure 1. Procedure for covalent surface modification of the fused-silica capillary column. (a) Synthesis of 4, 8, 12, 18, 22, 26-hexaaza-1,15-dioxacyclooctaeicosane ([28]ane- N_6O_2). (b) Surface modification of the fused-silica capillary column. Reprinted from [28], with permission.

formed by attaching Polybrene as a third layer to which an anionic polymer such as dextran sulfate, alginic acid, or hyaluronic acid, was worked as an adhesion material for Polybrene. Graul and Schlenoff [47] also showed that multilayer-coated columns exhibit many desirable features in addition to ease of construction and reproducible control of EOF. The multilayer coatings consisted of 6.5 layer pairs, where the first 3.5 layer pairs were deposited with no salt and the last 3 with 0.5 M NaCl. A layer pair means a layer of cationic polymer, (poly(diallyldimethyl-

ammonium chloride)) plus a layer of anionic polymer, (poly(styrene sulfonate)).

2.1.3 Polymeric coatings

The polymer coating procedure can be applied to surfaces on which chemical bonding cannot be achieved, when performed by cross-linking of suitable presynthesized oligomers containing in variable concentrations those functional groups which are important for the chro-

Figure 2. Successive multiple ionic polymer layer coating procedure. (a) Activation of the silanol groups. (b) First layer coating. (c) Second layer coating. Reprinted from [45], with permission.

matographic process [23]. This is achieved by cross-linking of prepolymers or by polymerization of monomers *in situ* on the nonporous or porous surface. Polymers containing functional groups which may undergo additional chemical bonding to the surface are also known and may be suitable to form polymeric stationary phase coatings which are more stable than those which are cross-linked only [48].

Some polymers can be chemically bound to the inner wall of the capillary to give a permanent positive charge on the surface. Such a surface modification leads to the anodic direction of the EOF without the use of modifiers. Burt and co-workers [49] have separated inorganic anions by CZE with capillaries coated by an azetidinium ion-based reactive polyamide resin using a buffer also containing triethanolamine. The chemistry of the reactive resin is shown in Fig. 3. Polyamines adsorbed to a silica surface may also be cross-linked into a permanent coating [43]. This coating behaves as an anion exchanger.

Silica-containing surfaces, such as the inner wall of a quartz or fused-silica capillary used in CE, are activated by a suitable linking agent which combines with the silanol

Figure 3. The chemistry of the reactive polyamide resin. Reprinted from [49], with permission.

groups on the surface to form Si-O-Si linkages leaving vinyl or acryl groups on the linking agent to form a polymeric coating over the surface. Thermal polymerization of the *N*-carboxyanhydride of glutamic acid-5-methyl ester was used to coat microparticulate silica and the surface of capillaries with poly(methylglutamate) [50].

Lee and co-workers [51] presented a simple method for polymer-coating of fused-silica capillary columns for electrophoresis. In the static coating technique used in GC, the coating solution contains a polymer, a surface derivatization reagent and a cross-linking reagent dissolved in a suitable low-boiling solvent. After coating, the column is subjected to heat treatment to immobilize the polymer film. Nakatani *et al.* [52] prepared a fused-silica capillary deactivated by coating with linear polyacrylamide through Si-C linkages and showed that it was more stable even under alkaline conditions and markedly reduced EOF than linear polyacrylamide through siloxane linkages. In transferring coating procedures from silica liquid chromatographic phases to capillary surfaces, it was possible to prepare stable polymeric coatings. Bonded cation exchangers prepared in this way exhibited a pH-independent flow property, while using bonded anion exchangers, reversal of EOF is possible [53].

Among the bonded material in the literature, polyacrylamide is one of the most popular and successful for achieving good protein separation. However, at high pH the amido bonds in polyacrylamide can hydrolyze, leading to degradation of the attached polymer and a loss of column performance [54]. CE separations of high efficiency and resolution were obtained using poly(acryloylaminoethoxyethanol)-coated capillaries. The polymer was covalently attached to a silica surface previously modified with a 3-methacryloyloxypropyl functionality. The lifetime of the new type coating used at pH 8.5 was more than twice that of conventional polyacrylamide [55]. The high

stability can be attributed to *N*-substituted ethoxy or ethanol acrylamide and carbon silicon bond which both provide resistance to hydrolysis. The same coated capillary was also used for DNA fragment analysis [56]. Chiari *et al.* [57] reported that poly(*N*-(acrylaminoethoxy)ethyl- β -D-glucopyranose was demonstrated to be even more stable.

Epoxy resins, combined with a cross-linker, were coated on organosilane-modified fused-silica capillaries. After cross-linking, a tough three-dimensional network was attached to the capillary surface *via* Si-O-Si-C bonds. The most effective coating consisted of epoxy resins cross-linked with diaminophenylmethane, which was stable over the pH range 2–12 and suppressed protein adsorption effectively [48]. Poly(methacryloyloxypropylhydrosilane) was developed and used as both a deactivating layer and an intermediate layer for stable coating of an uncharged polymer on fused silica capillaries in CE separation of proteins [58]. Van Tassel *et al.* [59] presented a method of controlling protein adsorption during CE which involves the placement of a thermally treated monolayer of adsorbed fibrinogen on the internal surface of the fused-silica capillary. The protein-coated capillaries exhibit an electroosmotic mobility that was 30% smaller than those of bare capillaries and stable over several measurements. The protein-coated capillaries will probably be useful in bio-separations. Poly(2-aminoethylmethacrylate hydrochloride) was chemically bound onto the fused-silica capillary inner wall for the CE separation of basic proteins and drugs [60]. Organic polymer capillaries, polybutyleneterephthalate, ethylene/vinylacetate, polymethylmethacrylate and nylon were tested by Bayer and Engelhard [61] for their suitability as column material in CE for the separation of protein/peptide. Shao *et al.* [62] reported the preparation of columns including cationic, neutral and anionic polymeric-coated columns for the separation of biomolecules. It was found that separations of multivalent biomolecules were difficult using charged-surface columns because of either strong adsorption or high electrophoretic mobilities.

3 Capillary electrochromatography

The high efficiency of CE can be combined with the high selectivity of micro-HPLC and the end result is a hybrid technique known as capillary electrochromatography (CEC). This technique utilizes columns that are similar to those used in micro-HPLC but the mobile phase is driven by an electric potential as in CE. CEC is considered to be complementary to other electroseparation techniques such as capillary electrophoresis, capillary gel electrophoresis and capillary micellar electrochromatography [17]. CEC has been applied for separating a wide range of compounds with high-resolution power as summarized in

some reviews [53, 63–70]. In 1991, Knox and Grant [16] reported a tentative preparation of reversed-phase columns by chemical modification of drawn packed capillaries. This pioneering work has encouraged scientists to explore the possibility of preparing high-quality, laboratory-made columns of variously modified stationary phases.

Applications performed by CEC in all its modes, namely packed-column CEC, open-tubular CEC and pressure-assisted CEC, were reviewed recently by Colon *et al.* [66], Cikalo *et al.* [68], as well as by Dermaux and Sandra [72]. Recently, a review by Pursch and Sander [73] summarized the variety of stationary phases that have been employed for CEC separations. About 70% of CEC research utilizes C18 stationary phases designed for HPLC, but an increasing number of new materials, *e.g.*, ion-exchange phases, sol-gel approaches, organic polymer continuous beds, are under development for use in CEC. Novel aspects of these different materials including the ability to promote EOF, phase selectivity and activity for basic solutes are also discussed. Since the stationary phase is the heart of this technique, improvements in column technology will be of prime importance for the continued growth and development of CEC.

3.1 Packed capillaries

3.1.1 Basic developments

In packed columns, a stationary phase is chemically bonded to a support particle that is then packed into a fused-silica tube of dimensions similar to those used in CE. Most of the reported work relating to CEC separations has been focused on the use of reversed phases [73–80]. However, materials which are suitable for HPLC may not in all cases be suitable for CEC [66]. In liquid chromatography the remaining silanol functions are not favorable for separating basic compounds due to undesired ionic and/or hydrophobic interactions, hence they are usually endcapped by trimethylsilylation. However, such end capping is undesirable in CEC, since the decrease of the density of the silanol group causes reduction of the velocity of EOF due to the decrease of negativity on the particle surface. Some manufacturers (Waters, Hewlett-Packard, Supelco and others) are now providing chromatographic material that has been specifically designed for CEC. Several groups have reported separations on ion-exchange column packing material [80–93], on size-exclusion stationary phases [94], and on chiral stationary phases [95–97]. Djordjevic *et al.* [90] prepared mixed bed stationary phases by blending bare silica and reversed phase. They also studied the retention behavior of neutral compounds in CEC.

Mixed packing CEC with the stationary phase comprising a mixture of strong cation exchanger (SCX) and octadecylsilane (ODS) phase was developed by Zhang *et al.*

[91]. With the existence of a sulfonic acid group on the surface of SCX, not only could the EOF remain high at low pH, but also the hydrophilicity of the stationary phase was increased greatly, leading to broad adaptable ranges of both pH and organic modifier concentration in the mobile phase. At the same time, with the coexistence of C₁₈ on the surface of ODS, both the retention and the resolution of samples were improved. The columns were used for the analysis of strong polar solutes as well as for the high-speed separation of acidic, basic and neutral compounds in a single run. Recently, Ye *et al.* [92] reported the preparation of a column packed with an SCX packing material and dynamically modified with cetyltrimethylammonium bromide (CTAB), which was added to the mobile phase. CTAB was adsorbed onto the surface of the SCX packing material, and the resulting hydrophobic layer on this packing was used as the stationary phase for the separation of neutral solutes. A mixture containing the acidic, basic, and neutral compounds was also separated in this mode with a low-pH mobile phase; however, peak tailing for basic compounds was observed.

Hoffman and Dovletoglou [98] reported the transition metal-mediated separation of isomeric pneumocandins by CEC using capillaries packed or coated with ODS par-

ticles (C18) or with glycerol bound to silica through a carbon chain linker. They found that the separation achieved with the glycerol-coated capillary was much better than the separation achieved with the C18 coated or C18 packed capillaries. Suzuki *et al.* [99] prepared chemically bonded silica gels by pumping an ethanolic solution of a silylating reagent, such as octadecyltrimethoxysilane, 3-aminopropyltrimethoxysilane and dimethyloctadecyltrimethoxysilylpropylammonium chloride into a heated capillary packed with bare silica particles. The silylation reactions were completed in a short time and columns so prepared showed high column efficiency and high reproducibility. Examples are shown for the separation of 1-phenyl-3-methyl-5-pyrazolone (PMP) derivatives of aldopentoses on a 3-aminopropylated silica column (Fig. 4) and benzoate homologues as well as PMP derivatives of the component monosaccharides of glycoproteins on an octadecylammonium column (Fig. 5). The advantage of in-column derivatization is simultaneous introduction of ionic groups to both packing materials and capillary inner wall. In this paper, the authors fixed the bed of modified silica gel particles to the capillary inner wall by a cross-linking technique to solve the drawback of frit making. The separation of dimethylphthalate and thiourea was rather good as compared to that on a column with frits.

Figure 4. Comparison of the separation of 1-phenyl-3-methyl-5-pyrazolone (PMP) derivatives of aldopentose isomer on various columns. Columns prepared (a) by in-column 3-aminopropylation of Nucleosil silica gel; (b) by packing a commercial sample of amino silica, Develosil NH₂, in an APTMS-treated capillary; (c) by packing Develosil-NH₂ in an uncoated capillary. Eluent, (25 mM HEPES-NaOH, pH 6.0)-acetonitrile (2:1 v/v); sample concentration, 50 nmol in 100 μ L of eluent; injection, -2 kV for 3 s (from the cathodic end); applied voltage, -20 kV; detection, UV absorption at 245 nm. Peaks: Ara, D-arabinose; Xyl, D-xylose; Rib, D-ribose; Lyx, D-lyxose, all as PMP derivatives. Reprinted from [99], with permission.

Figure 5. Comparison of the separation of alkylbenzoate homologues on (a) an octadecylammonium column and (b) an ODS column. The columns were prepared by in-column derivatization of DaisoGel silica (30 nm, 3 μ m) with dimethyloctadecyltrimethoxysilylpropylammonium chloride. Reprinted from [99], with permission.

Silica particles coated with a mixture of sulfonic acid groups or amino groups and alkyl chain moieties were also reported [85, 100]. The alkyl chains with sulfonic acid groups at low pH will give cathodal EOF, while those with amino groups at low pH will give anodal EOF. Huang *et al.* [100] used a mixed-mode packing and voltage tuning for peptide mixture separation in pressurized CEC (PCEC) with an ion trap storage/reflection time-of-flight mass spectrometer detector. PCEC is a novel analytical method in which both pressure and electric field are applied to a packed capillary to achieve separation of analytes [101]. PCEC combines various aspects of CEC and LC. In PCEC, an EOF caused by the voltage is superimposed on a pressure-induced hydrodynamic flow. Therefore, the separation efficiency is intermediate between that of pure CEC and LC. The major advantages over pure CEC include the stability of the mobile phase flow due to bubble suppression, the increased speed of separation, and the enhanced selectivity for charged particles. The macrocyclic antibiotics vancomycin and teicoplanin, widely used as chiral selectors in HPLC, have also been employed in packed CEC [102–104].

3.1.2 Packing methods

Several protocols have been reported for the fabrication of packed capillary columns for CEC. Colon *et al.* [105] reported that one can still consider column fabrication in CEC as an art. As in HPLC, variables affecting column packing include quality of packing material, slurry composition, packing procedure, velocity at which particles

arrive to the accumulating bed, and the characteristics of the tube to be packed. A reliable and reproducible column performance depends on the column fabrication. The fabrication of a typical packed column for CEC is schematically presented in Fig. 6 [105].

Columns that have been packed poorly can lead to low efficiency, poor resolution and asymmetric peak shapes. Packing CEC columns is really a skill that requires experience. Five different packing methods are discussed by Colon and co-workers [105] to deliver packing material into the capillary column: slurry pressure packing, packing with supercritical CO₂, electrokinetic packing, using centripetal forces, and packing by gravity. Entrapment of particulate material by sintering and sol-gel technology is also mentioned. The efficiency of columns packed by the different procedures mentioned above varies considerably. Colon *et al.* [105] reported that electrokinetic packing is the simplest and easiest method to implement and use. In general, the preference of which method to use seems to depend on the familiarity of a particular procedure in a given laboratory.

The performance of bed-retention frits in CEC is likely the most important experimental parameter influencing bubble formation. A variety of approaches to frit preparation have been reported [67, 73, 106, 107]. However, good quality frits can be difficult to achieve, especially when small particles (*i.e.* < 3 μ m particle size) are used. Recently, Cassidy and co-workers [106] reported the preparation of porous silica frits *via* spot-heating of a sili-

Figure 6. Representation of the steps involved in the column fabrication processes. (A) Tapping empty column into a container with wet silica material. (B) The silica material in place ready to fabricate a temporary frit. (C) Formation of the temporary frit with a heating element. (D) Flushing out the excess of silica material in the column after temporary frit is formed. (E) Packed capillary pressurized with water to form the retaining frits with a heating element. (F) A fabricated column with frits and detection window in place. Reprinted from [105], with permission.

cate solution, and the effects of several experimental parameters on their performance. Zare and co-workers [107] reported that macroporous polymer frits can be fabricated in fused-silica capillaries by the UV photopolymerization of a solution of glycidyl methacrylate and trimethylolpropane trimethacrylate. This *in situ* preparation is a simple, rapid, and reproducible process.

3.2 Open-tubular capillaries

There are some fundamental problems in using packed columns. The first is the difficult fabrication of the frits used to hold the packing materials in place. Another drawback is the tendency to form bubbles around the packing material or at the frit during the electrochromatographic run. These would result in an unstable baseline, nonreproducible migration times or the interruption of current in the system. Therefore, several alternatives have been developed such as the solvent can be thoroughly degassed and pressure applied to one or both buffer vials [108, 109], with low concentrations of electrolytes and/or a high proportion of organic solvent in the buffer [16, 110], with zwitterionic species buffer [111] or the addition of surfactants to the buffer [112]. An alternative approach to packed columns has been developed in order to overcome these problems. The simplest solution would be to bond the appropriate moiety to the wall of the capillary and utilize solute/bonded phase interactions in a manner similar to open-tubular GC [113–117]. However, the first approach to open-tubular CEC was reported by Tsuda *et al.* [118]. The column used was fabricated by drawing

large bore soda-lime tubes, treating the inner surface with NaOH, and attaching the stationary phase (*i.e.*, ODS) at the inner surface through silane chemistry. A series of hydrocarbons was separated in this way using acetonitrile/water as the mobile phase and applying 13 kV as the separation voltage.

Mayer and Schurig [119, 120] reported the use of a cyclodextrin-coated capillary to resolve the enantiomers of binaphthyl derivatives and 1-phenylethanol using a phosphate-borate buffer at pH 7.0. Recently, Schurig and co-workers [121] have also performed an efficient enantiomer separation by pressure-assisted, micropacked CEC which was carried out using a permethyl- β -cyclodextrin-modified silica. Liu and co-workers [122] demonstrated that a bonded phase capillary column containing hydroxamate functional groups could be used for the separation of transition metal ions. Macrocyclic polyamine-bonded phases have also been prepared and evaluated for their use in the electrophoretic separation of organic and inorganic anions and metal ion speciation [27–30]. A highly selective property can be attributed to anion complexation, anion exchange and reversal of the EOF provided by the wall-bonded functional groups. Supercomplex formation resulting from the second-sphere interaction between metallocyanide and polyamine was also indicated (Fig. 7) [28].

The effect of macrocycles on the separation of aromatic acids is shown by the electropherograms in Fig. 8 [29]. In the work of CEC separation of metal ion species with on-line detection by inductively coupled plasma MS, Liu and

Figure 7. (A) Migration time of metalocyanides as a function of pH. Column, covalent surface modification with polyamine, [28]ane- N_6O_2 fused-silica capillary (50 cm $\times$ 75 μm ID); background electrolyte, 10 mM phosphate buffer; sample concentration, $[\text{Fe(CN)}_6^{3-}]$: 10 μM ; $[\text{Fe(CN)}_6^{4-}]$: 2 μM ; sample injection, electrokinetic (–10 kV, 10 s); detection, direct UV at 220 nm. (B) Separation of metalocyanides. Conditions as in (A), except electrolyte pH of 10.4 and applied voltage –20 kV. Reprinted from [28], with permission.

co-workers [30] found that the matrix effect of the established system with the macrocyclic polyamine bonded phase was smaller than that with bare fused silica (Fig. 9). However, one significant drawback of this method is its low loading capacity since only the inner wall is available as a site for coating or bonding a stationary phase; very low EOF can be another problem, especially for neutral solutes, if the capillary wall is coated or derivatized.

The use of liquid crystals as a separation material in HPLC is rapidly gaining interest. Recently, two liquid crystal compounds, cholesterol-10-undecenoate and 4-cyano-4'-pentoxybiphenyl, attached to the inner wall of a fused-silica capillary, were reported by Pesek and co-workers [123] as a separation medium evaluated by electrochromatographic experiments using mixtures of protein, pyrimidine/purine bases and a nucleoside, benzodiazepines, the synthetic and metabolic compounds of serotonin, and other small basic molecules. Pesek and Matyska [124] gave a thorough review involving the etching of the inner wall of the capillary in order to increase the surface area by a factor estimated to be up to 1000 followed by chemical modification to provide the desired selectivity. The chemical modification process used for attaching organic moieties is the silanization/hydrosilation method through a stable silicon-carbon bond [125].

3.3 Fritless columns

A new generation of columns that holds great potential for CEC consists of a continuous bed inside the column. Continuous beds were first prepared by Hjertén *et al.*

[126] in 1989 for use in LC, later a similar procedure was applied by Svec *et al.* [127]. Now a number of reports have been published describing capillaries prepared by *in situ* polymerization of organic monomers [128–138]. This eliminates the need of frits in the column, and all problems associated with their preparation. Schmid *et al.* [139] reported the preparation of on-column frits in packed fused-silica capillaries by sol-gel technology. Lee and co-workers [140] proposed a method for preparing monolithic capillary columns. A fused-silica capillary packed with porous ODS particles using CO_2 slurry was partially filled with a siliceous sol formed by hydrolysis and polycondensation of tetramethoxysilane and ethyltrimethoxysilane. After gelling and aging of the siliceous sol at room temperature, the column was dried with supercritical CO_2 . The scanning electron micrograph of a cross-section of the capillary column revealed that the ODS particles were bonded to each other and to the column inner wall by the sol-gel, forming a monolith. The monolithic column was evaluated for CEC, using small aromatic compounds and polycyclic aromatic hydrocarbons with Tris buffer in an aqueous acetonitrile mobile phase.

Recently Lee, and co-workers [141] also reported the preparation of continuous-bed columns of 200 μm inner diameter containing sol-gel bonded small-pore and large-pore ODS particles. It is expected to have high commercial potential or usage as a CEC packing, although it was employed only for capillary liquid chromatography. Tang and Lee [142] also prepared the sol-gel bonded packing materials in continuous-bed columns for CEC. The proce-

Figure 8. Electrophoretic separation of aromatic acids. Conditions: column, fused-silica capillary (50 cm $\times$ 75 μ m ID) with covalent surface modification using macrocyclic polyamine, [28]ane- N_6O_2 , sample injection, 3 mL syringe for 2 s; background electrolyte, acetate (20 mM, pH 4.5); applied voltage, -20 kV; detection, at 220 nm; sample concentration, 10 μ M for each, except 3-hydroxy-2-naphthoic acid which was 5 μ M. Peaks: 1, pyromellitic acid; 2, trimellitic acid; 3, 2,6-pyridine-dicarboxylic acid; 4, *o*-phthalic acid; 5, *m*-phthalic acid; 6, *p*-phthalic acid; 7, salicylic acid; 8, mandelic acid; 9, *p*-sulfanilic acid; 10, benzoic acid; 11, 3-hydroxy-2-naphthoic acid; 12, gallic acid; 13, *p*-anisic acid; 14, *p*-hydroxybenzoic acid. Reprinted from [29], with permission.

cedure for preparing sol-gel bonded continuous-bed columns is depicted in Fig. 10. They found that columns containing sol-gel bonded large-pore packing materials gave significantly higher efficiency but lower retention than those with small-pore packing materials. Sol-gel bonded particles with mixed-mode stationary phases generated stable EOF over a wide pH range and can be used for fast separations at low pH; however, they demonstrated strong interaction with basic compounds.

Monolithic materials have quickly become a well-established stationary phase format in the field of CEC. Both the simplicity of their *in situ* preparation method and the large variety of readily available chemistries make the monolithic separation media an attractive alternative to capillary columns packed with particular materials.

Recently, a review focus on monolithic capillary columns prepared from synthetic polymers was presented by Svec *et al.* [143, 144]. Various approaches employed for the preparation of the monoliths are detailed and the material properties of the resulting monolithic capillary columns are shown. Their chromatographic performance is demonstrated by numerous separations of different analyte mixtures in varying modes.

Another continuous polymeric network using molecular imprinting for the preparation of chiral sorbents was shown recently. Molecular recognition-based systems have received much attention in various fields because of their high selectivity for target molecules. Molecular imprinting has been recognized as a promising technique for the development of such systems, where the molecule to be recognized is added to a reaction mixture of cross-linker(s), solvent(s), and functional monomer(s) that possess functional group(s) capable of interacting with the target molecule. Binding sites in the resultant polymers involve functional groups originating from the added functional monomer(s), which can be constructed according to the shape and chemical properties of the target molecules. After removal of the target molecules, these molecularly imprinted complementary binding sites exhibit high selectivity and affinity for the template molecules. The resolution obtained from CEC seems to be better than the results of HPLC [145].

Recent developments in CE have been reviewed by Schweitz *et al.* [146], Takeuchi and Haginaka [147] as well as by Remcho and Tan [148]. Schweitz *et al.* [149] used an *in situ* photo-initiated polymerization process to prepare a molecular imprinting polymer for the separation of the enantiomers of ropivacaine as well as structural analogs mepivacaine and bupivacaine. A simple and quick method for the *in situ* preparation of monolithic molecularly imprinted flow-through polymers was also described by Schweitz *et al.* [133]. Enantiomeric resolution of dansyl amino acids with simultaneous noncovalent molecular imprinting of two chemically distinct functional monomers, vinylpyridine and methacrylic acid, was demonstrated in ethylene glycol dimethacrylate-based copolymers [150].

4 Characterization

4.1 Capillary electrophoresis

The characterization of capillaries before and after modification is extremely difficult. In GC and LC, numerous standard tests and test mixtures are available for characterizing stationary phases. Furthermore, especially in LC, there are many independent physicochemical methods

Figure 9. Matrix effect for the determination of arsenic species. Conditions: capillary column, 160 cm $\times$ 100 μ m ID, (A) and (B) [28]ane- N_6O_2 bonded phase; (C) bare fused silica; sample injection, electrokinetic method (-20 kV, 20 s) with the nebulizer gas flow at 1 L min^{-1} ; separation voltage, -20 kV; background electrolyte, phosphate buffer (20 mM, pH 6.2); sample concentration: (A) and (C) arsenate (1 $\mu\text{g mL}^{-1}$) in chloride ion matrix (1000 $\mu\text{g mL}^{-1}$), (B) chloride ion only (1000 $\mu\text{g mL}^{-1}$). Peak identification: 1, HAsO_4^{2-} ; 2, Cl^{-} . Reprinted from [30], with permission.

- Column packing

- Sol filling

- Gelling and aging at 25 °C

- Supercritical CO₂ Drying

Figure 10. Procedure for preparing sol-gel bonded continuous-bed columns. Reprinted from [142], with permission.

such as elemental analysis, FTIR or NMR to obtain definitive information on surface modification. However, the total length of the CE capillary columns is conventionally less than 1 m. The corresponding inner surface area is less than 10^{-2} cm^2 . Therefore, the change in the EOF caused by the coating is generally viewed as the major characteristic property. Moreover, since most coatings were developed to suppress protein-wall interactions, it is not surprising that the separation of protein standard mixtures serves as an index of the effectiveness of the surface modifications achieved.

Engelhardt and Cuna-Walter [151] used plate numbers achieved in capillary electrophoretic protein separations for characterization of capillary coatings. But plate numbers for proteins are not a good measure for comparing the efficiency of surface coatings in CE, and should be used only when all measurements are made under identical conditions with the same instrument. Kaupp *et al.* [152] reported that it was possible to characterize the inner surfaces of CE capillaries by scanning electron microscopy (SEM). However, it is impossible to investigate coated capillaries using SEM. The use of atomic force microscopy (AFM) is advantageous: grooves were found that were down to 500 nm deep using this technique [153]. However, AFM can provide topographical but not chemical information about the surface. Pesek *et al.*

[154] utilized a more sensitive mercury-cadmium-telluride detector to apply diffuse reflectance infrared Fourier transform (DRIFT) measurements to the etched chemically modified capillaries.

4.2 Capillary electrochromatography

Recently, a review article by Pesek and Matyska [124] described that an etched inner surface of the fused-silica tube can be characterized spectroscopically by DRIFT and EOF measurements. Porous silica-bonded phases can be characterized by a variety of techniques such as DRIFT, solid-state cross-polarization magic angle spinning (CP-MAS) NMR, photoelectron spectroscopy (ESCA) and optical methods such as UV-visible or fluorescence spectroscopy. SEM has been used for the characterization of particle-loaded monolithic sol-gel columns by Dulay *et al.* [155] and the fabrication of silicate-entrapped columns by Chirica and Remcho [156]. Recently, Ratnayake *et al.* [157] reported the preparation of particle-loaded monolithic sol-gel columns and characterized the basic construction, electrical properties and specific permeability of these columns. Pullen *et al.* [158] reported the preparation of octadecyl-derivatized fused silica and characterized the surface property by AFM.

5 Column performance

5.1 Capillary electrophoresis [23]

The efficiency of CE separations can be described by plate heights H or the number of theoretical plates N as follows:

$$N = \frac{1}{H} = \frac{(\mu_{eo} + \mu_{ep})Vl}{2DL} \quad (1)$$

where μ_{eo} and μ_{ep} are the electroosmotic mobility and electrophoretic mobility, respectively, V is the applied voltage, l is the capillary length to the detector, D is the diffusion coefficient, and L is the total capillary length. The resolution R can be described by the equation

$$R = \frac{1}{4} \frac{\Delta\mu}{\mu_{eo} \pm \mu_{ep}} \sqrt{N} \quad (2)$$

where $\Delta\mu$ is the mobility difference. These equations indicate what influence changes in the EOF, that occur with surface modifications for the suppression of analyte-wall interactions, may also have on the efficiency and resolution of an electromigration separation with a separation factor α :

$$\alpha = \frac{(\mu_{ep})_2}{(\mu_{ep})_1} \quad (3)$$

5.2 Capillary electrochromatography [159]

The van Deemter equation in CE is $H = B/u$, while that in CEC is not the same. Because CEC involves the use of a stationary phase, the principles of band broadening are similar to those occurring in conventional liquid chromatography. Therefore, the plate height can be expressed using a modified van Deemter plate height equation:

$$H = Ad_p(ud_p/D_m)^{1/3} + B/u + Cud_p^2/D_m \quad (4)$$

where A , B and C are constants, u is the linear velocity, D_p is the particle diameter, and D_m is the diffusion coefficient. The A -term in the plate height equation relates to the diffusion arising from different flow paths that solute molecules can take through the packed bed. This so-called "eddy diffusion" is more significant in HPLC where the mean flow rate will vary from channel to channel, but in CEC, because the velocities between the channels will be identical, the contribution to band broadening would be expected to be significantly lower than in conventional chromatography for a given particle size of packing. It is clear from the plate height equation that a reduction in the particle diameter will lead to more densely packed and uniform columns and in turn a lower A -term. The B -term is the contribution to the plate height resulting from longitudinal diffusion and arises from the tendency of the band to diffuse away from the band center as it moves down a column and is proportional to the time that the sample spends in the column and also to its diffusion coefficient in the mobile phase. The longer a solute spends in the column, the greater the extent of diffusion and therefore the B -term only becomes significant at low flow rates. The C -term reflects band broadening due to slow equilibration between the mobile and stationary phases and is increased as the mobile phase velocity increases, because less time is available for equilibration. The contribution to band broadening from the C -term also can be reduced by the use of small diameter packing materials. When 1 μm particles are applied to optimum linear velocities, the column efficiency in CEC approaches that of CZE. Only axial molecular diffusion is a contributing factor to band broadening and plate height is given by the equation:

$$H = \frac{2D_m}{u} \quad (5)$$

6 Conclusions

6.1 Capillary electrophoresis

One of the most important choices in chromatographic method developments is column selection. The capillary column in CE is in the words of Whatley: "The capillary is a reagent" [160]. Surface coatings in CE should (i) be able

to modify or suppress the EOF, (ii) be stable in the presence of aqueous buffer solutions, and (iii) suppress strong or even irreversible adsorption of analyte molecules, e.g., proteins [161].

6.2 Capillary electrochromatography

Most of the commercial stationary phases employed today in CEC originate from HPLC. The synthesis of stationary phases designed for CEC has just started, and the potential of stationary phases in CEC from reversed phase to mixed mode and to ion-exchange packings has not yet been fully exploited. Some manufactures (e.g., Waters, Agilent, Metachem, Microsolve and others) are now offering C₁₈ phases that are optimized for CEC in the sense that the materials are not endcapped and/or may have a lower surface coverage of alkyl ligands [73]. If CEC shows a distinctly different selectivity pattern when compared with HPLC and CE, and the stability of CEC columns is drastically improved, CEC might become a powerful method in automated miniaturized separation systems. With increasing maturity of CEC column technology, a greater variety of CEC columns can be expected. The separation mechanism of the packed column might be based on adsorption, partition, ion exchange, chelation (including cation complexation and anion complexation), ligand-exchange, host-guest inclusion, macrocyclic effect, etc., besides the true mobility of the analyte.

For CEC work, there are a number of practical considerations that must be taken into account over and above those for conventional CE [162]. The first is that detection will be more effective if a small length of the tubing remains free of packing material, which then functions as the detection zone. However, in the case of fluorescence detection which is emission spectrum, the packing material may occupy the full length of the capillary [163]. To date, particles as small as 0.2 μm have been investigated for their potential use in CEC [164]. El Rassi and co-workers [165] reported that the CEC separation of the neutral glycosphingolipids required a nonporous octadecyl sulfonated silica (ODSS) stationary phase and a mobile phase of high eluent strength while the CEC separation of the gangliosides was readily achieved on a porous ODSS stationary phase with a mobile phase of moderate eluent strength. Perhaps the development of open-tubular columns and fritless columns, such as the monolithic and entrapped structures, will advance the technique in the CEC area.

Finally, I would like to mention that the accurate determination of trace elements in complex matrices will continue to be one of the most challenging areas in analytical chemistry. While most analytical techniques are readily

applicable to simple matrices, many may fail when applied to samples that represent complex matrices. This is often the case for quantification of trace and ultratrace elements in sea water, estuarine water, brines, biological fluids, and acid-decomposed biological, botanical and geological materials [166]. Fritz [10] has reported in his review [10] that a high concentration of a sample matrix ion can be a problem in both ion chromatography (IC) and CE techniques, but tend to be much more serious in CE. In CE, a high concentration of a sample ion may form a broad zone on the capillary column that will overlap with those of much lower concentrations of other sample ions. To solve the problem, the preparation of a highly selective column would be promising in CE and CEC for the analysis of complex matrices samples.

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7 References

- [1] Pacakova, V., Coufal, P., Stulik, K., *J. Chromatogr. A* 1999, 834, 257–275.
- [2] Jandik, P., Bonn, B., *Capillary Electrophoresis of Small Molecules and Ions*, VCH, New York 1993.
- [3] Macka, M., Haddad, P. R., *Electrophoresis* 1997, 18, 2482–2501.
- [4] Jandik, P., Jones, W. R., *J. Chromatogr.* 1991, 546, 431–443.
- [5] Jandik, P., Jones, W. R., Weston, A., Brown, P. R., *LC-GC Mag. Sep. Sci.* 1991, 9, 634.
- [6] Jones, W. R., Jandik, P., *J. Chromatogr.* 1991, 546, 445–458.
- [7] Romano, J., Jandik, P., Jones, W. R., Jackson, P. E., *J. Chromatogr.* 1991, 546, 411–421.
- [8] Wildman, B. J., Jackson, P. E., Jones, W. R., Alden, P. G., *J. Chromatogr.* 1991, 546, 459–466.
- [9] Crompton, T. R., *Determination of Anions-A Guide for the Analytical Chemist*, Springer, Berlin 1996.
- [10] Fritz, J. S. in: Camilleri, P. (Ed.), *Capillary Electrophoresis: Theory and Practice*, CRC Press, Boca Raton, FL 1998, Chapter 6.
- [11] Haddad, P. R., Doble, P., Macka, M., *J. Chromatogr. A* 1999, 856, 145–177.
- [12] Pretorius, V., Hopkins, B. J., Schieke, J. D., *J. Chromatogr.* 1974, 99, 23–30.
- [13] Jorgenson, J. W., Lukacs, K. D., *J. Chromatogr.* 1981, 218, 209–216.
- [14] Knox, J. H., Grant, I. H., *Chromatographia* 1987, 24, 135–143.
- [15] Knox, J. H., *Chromatographia* 1988, 26, 329–337.
- [16] Knox, J. H., Grant, I. H., *Chromatographia* 1991, 32, 317–328.
- [17] Knox, J. H., *J. Chromatogr. A* 1994, 680, 3–13.
- [18] Wikstrom, H., Svensson, L. A., Torstensson, A., Owens, P. K., *J. Chromatogr. A* 2000, 869, 395–409.
- [19] Kaniansky, D., Masar, M., Marak, J., Bodor, R., *J. Chromatogr. A* 1999, 834, 133–178.

- [20] Kelly, R. G., Brossia, C. S., Cooper, K. R., Krol, J., *J. Chromatogr. A* 1996, 739, 191–198.
- [21] Harakuwe, A. H., Haddad, P. R., *J. Chromatogr. A* 1999, 834, 213–232.
- [22] Corr, J. J., Anacleto, J. F., *Anal. Chem.* 1996, 68, 2155–2163.
- [23] Schomburg, G., in: Khaledi, M. G. (Ed.), *High-Performance Capillary Electrophoresis-Theory, Techniques and Applications*, Wiley, New York 1998.
- [24] Kirkland, J. J., Glajch, J. L., Farlee, R. D., *Anal. Chem.* 1989, 61, 2–11.
- [25] Jorgenson, J. W., *Trends Anal. Chem.* 1984, 3, 51–54.
- [26] Herren, B. J., Shafer, S. G., Alstine, S. V., Harris, J. M., Snyder, R. S., *J. Colloid Interface Sci.* 1987, 115, 46–55.
- [27] Hsu, J. C., Chen, W. H., Liu, C. Y., *Analyst* 1997, 122, 1393–1398.
- [28] Liu, C. Y., Chen, W. H., *J. Chromatogr. A* 1998, 815, 251–263.
- [29] Chen, W. H., Liu, C. Y., *J. Chromatogr. A* 1999, 848, 401–406.
- [30] Chen, W. H., Lin, S. Y., Liu, C. Y., *Anal. Chim. Acta* 2000, 410, 25–35.
- [31] Shao, X., Shen, Y., O'Neill, K., Lee, M. L., *J. Chromatogr. A* 1999, 830, 415–422.
- [32] Towns, J. K., Regnier, F. E., *Anal. Chem.* 1991, 63, 1126–1132.
- [33] Swedberg, S. A., *Anal. Biochem.* 1990, 185, 51–56.
- [34] Regnier, F. E., Wu, D., in: Guzman, N. A. (Ed.), *Capillary Electrophoresis Technology*, Marcel Dekker, New York 1993, Chapter 8.
- [35] Hjertén, S., *J. Chromatogr.* 1985, 347, 191–198.
- [36] Gilges, M., Kleemiss, M. H., Schomburg, G., *Anal. Chem.* 1994, 66, 2038–2046.
- [37] Busch, M. H. A., Kraak, J. C., Poppe, H., *J. Chromatogr. A* 1995, 695, 287–296.
- [38] Bedia Erim, F., Cifuentes, A., Poppe, H., Kraak, J. C., *J. Chromatogr. A* 1995, 708, 356–361.
- [39] Cordova, E., Gao, J., Whitesides, G. M., *Anal. Chem.* 1997, 69, 1370–1379.
- [40] Chiu, R. W., Jimenez, J. C., Monnig, C. A., *Anal. Chim. Acta* 1995, 307, 193–201.
- [41] Sun, P., Landman, A., Hartwick, R. A., *J. Microcol. Sep.* 1994, 6, 403–407.
- [42] Liu, Q., Lin, F., Hartwick, R. A., *J. Chromatogr. Sci.* 1997, 36, 126–130.
- [43] Towns, J. K., Regnier, F. E., *J. Chromatogr.* 1990, 516, 69–78.
- [44] Kleindienst, G., Huber, C. G., Gjerde, D. T., Yengoyan, L., Bonn, G. K., *Electrophoresis* 1998, 19, 262–269.
- [45] Katayama, H., Ishihama, Y., Asakawa, N., *Anal. Chem.* 1998, 70, 2254–2260.
- [46] Katayama, H., Ishihama, Y., Asakawa, N., *Anal. Chem.* 1998, 70, 5272–5277.
- [47] Graul, T. W., Schlenoff, J. B., *Anal. Chem.* 1999, 71, 4007–4013.
- [48] Liu, Y., Fu, R., Gu, J., *J. Chromatogr. A* 1996, 723, 157–169.
- [49] Burt, H., Lewis, D. M., Tapley, K. N., *J. Chromatogr. A* 1996, 739, 367–371.
- [50] Bentrop, D., Kohr, J., Engelhardt, H., *Chromatographia* 1991, 32, 171–178.
- [51] Malik, A., Zhao, Z., Lee, M. L., *J. Microcol. Sep.* 1993, 5, 119–125.
- [52] Nakatani, M., Shibukawa, A., Nakagawa, T., *J. Chromatogr. A* 1994, 672, 213–218.
- [53] Kohr, J., Engelhardt, H., *J. Microcol. Sep.* 1991, 3, 491–495.
- [54] Pesek, J. P., Matyska, M. T., *Electrophoresis* 1997, 18, 2228–2238.
- [55] Chiari, M., Nesi, M., Sandoval, J. E., Pesek, J. J., *J. Chromatogr. A* 1995, 717, 1–13.
- [56] Talmadge, K. W., Tan, A. K., Zhu, M., *J. Chromatogr. A* 1997, 781, 335–345.
- [57] Chiari, M., Dell'Orto, N., Gelain, A., *Anal. Chem.* 1996, 68, 2731–2736.
- [58] Fridstroem, A., Lundell, N., Nyholm, L., Markides, K. E., *J. Microcol. Sep.* 1997, 9, 73–80.
- [59] Van Tassel, P. R., Miras, D., Hagege, A., Leory, M., Voegel, J. C., Schaaf, P., *J. Colloid Interface Sci.* 1996, 183, 269–273.
- [60] Liu, Q., Lin, F., Hartwick, R. C., *J. Liq. Chromatogr. Relat. Technol.* 1997, 20, 707–718.
- [61] Bayer, H., Engelhardt, H., *J. Microcol. Sep.* 1996, 8, 479–484.
- [62] Shao, X., Shen, Y., Lee, M. L., O'Neill, K., *J. Chromatogr. A* 1998, 809, 173–179.
- [63] Stahlberg, J., *J. Chromatogr. A* 2000, 892, 291–302.
- [64] Crego, A. L., Gonzalez, A., Marina, M. L., *Crit. Rev. Anal. Chem.* 1996, 26, 261–304.
- [65] Rathore, A. S., Horváth, C., *J. Chromatogr. A* 1997, 781, 185–195.
- [66] Colon, L. A., Reynolds, K. J., Alicea-Maldonado, R., Fermier, A. M., *Electrophoresis* 1997, 18, 2162–2174.
- [67] Altria, K. D., Smith, N. W., Turnbull, C. H., *Chromatographia* 1997, 46, 664–674.
- [68] Cikalo, M. G., Bartle, K. D., Robson, M. M., Myers, P., Euerby, M. R., *Analyst* 1998, 123, 87R–102R.
- [69] Fujimoto, C., *Trends Anal. Chem.* 1999, 18, 291–301.
- [70] Wistuba, D., Schurig, V., *J. Chromatogr. A* 2000, 875, 255–276.
- [71] Steiner, F., Scherer, B., *J. Chromatogr. A* 2000, 887, 55–83.
- [72] Dermaux, A., Sandra, P., *Electrophoresis* 1999, 20, 3027–3065.
- [73] Pursch, M., Sander, L. C., *J. Chromatogr. A* 2000, 887, 313–326.
- [74] Dittmann, M. M., Wienand, K., Bek, F., Rozing, G. P., *LC-GC Int.* 1995, 13, 800.
- [75] Yan, C., Dadoo, R., Zhao, H., Zare, R. N., Rakestraw, D. J., *Anal. Chem.* 1995, 67, 2026–2029.
- [76] Vouros, P., Ding, J., *Anal. Chem.* 1997, 69, 379–384.
- [77] Gillott, N. C., Euerby, M. R., Johnson, C. M., Barrett, D. A., Shaw, P. N., *Anal. Commun.* 1998, 35, 217–220.

- [78] Zhang, Y., Shi, W., Zhang, L., Zou, H., *J. Chromatogr. A* 1998, 802, 59–71.
- [79] Seifar, R. M., Kraak, J. C., Kok, W. Th., Poppe, H., *J. Chromatogr. A* 1998, 808, 71–77.
- [80] Lurie, I. S., Meyers, R. P., Conner, T. S., *Anal. Chem.* 1998, 70, 3255–3260.
- [81] Smith, N. W., Evans, M. B., *Chromatographia* 1995, 41, 197–203.
- [82] Li, D., Knobel, H. H., Remcho, V. T., *J. Chromatogr. B* 1997, 695, 169–174.
- [83] Kitagawa, S., Tsuda, T., *Anal. Sci.* 1998, 14, 571–575.
- [84] Cikalo, M. G., Bartle, K. D., Myers, P., *Anal. Chem.* 1999, 71, 1820–1825.
- [85] Euerby, M. R., Gilligan, D., Johnson, C. M., Roulin, S. C. P., Myers, P., Bartle, K. D., *J. Microcol. Sep.* 1997, 9, 373–387.
- [86] Zhang, M. Q., El Rassi, Z., *Electrophoresis* 1998, 19, 2068–2072.
- [87] Moffatt, F., Cooper, P. A., Jessop, K. M., *Anal. Chem.* 1999, 71, 1119–1124.
- [88] Smith, N., Evans, M. B., *J. Chromatogr. A* 1999, 832, 41–54.
- [89] Zhang, M. Q., El Rassi, Z., *Electrophoresis* 1999, 20, 31–36.
- [90] Djordjevic, N. M., Fitzpatrick, F., Houdiere, F., Lerch, G., *J. High Resol. Chromatogr.* 1999, 22, 599–603.
- [91] Zhang, L., Zhang, Y., Shi, W., Zou, H., *J. High Resol. Chromatogr.* 1999, 22, 666–670.
- [92] Ye, Mingliang, Zou, H., Liu, Z., Ni, J., Zhang, Y., *Anal. Chem.* 2000, 72, 616–621.
- [93] Spikmans, V., Lane, S. J., Smith, N. W., *Chromatographia* 2000, 51, 18–24.
- [94] Venema, E., Kraak, J. C., Poppe, H., Tijssen, R., *Chromatographia* 1998, 48, 347–354.
- [95] Li, S., Lloyd, D. K., *J. Chromatogr. A* 1994, 666, 321–335.
- [96] Lloyd, D. K., Li, S., Ryan, P., *J. Chromatogr. A* 1995, 694, 285–296.
- [97] Lelievre, F., Yan, C., Zare, R. N., Gareil, P., *J. Chromatogr. A* 1996, 723, 145–156.
- [98] Hoffman, Jr., M. J., Dovletoglou, A., *J. High Resol. Chromatogr.* 1999, 22, 309–314.
- [99] Suzuki, S., Kuwahara, Y., Makiura, K., Honda, S., *J. Chromatogr. A* 2000, 873, 247–256.
- [100] Huang, P., Jin, X., Chen, Y., Srinivasan, J. R., Lubman, D. M., *Anal. Chem.* 1999, 71, 1786–1791.
- [101] Wu, J. T., Huang, P. Q., Li, M. X., Lubman, D. M., *Anal. Chem.* 1997, 69, 2908–2913.
- [102] Dermaux, A., Lynen, F., Sandra, P., *J. High Resol. Chromatogr.* 1998, 21, 575–576.
- [103] Wikstrom, H., Svensson, L. A., Torstensson, A., Owens, P. K., *J. Chromatogr. A* 2000, 869, 395–409.
- [104] Carter-Finch, A. S., Smith, N. W., *J. Chromatogr. A* 1999, 848, 375–385.
- [105] Colon, L. A., Maloney, T. D., Fermier, A. M., *J. Chromatogr. A* 2000, 887, 43–54.
- [106] Chen, Y., Gerhardt, G., Cassidy, R., *Anal. Chem.* 2000, 72, 610–615.
- [107] Chen, J. R., Dulay, M. T., Zare, R. N., *Anal. Chem.* 2000, 72, 1224–1227.
- [108] Choudhary, G., Horváth, C., *J. Chromatogr. A* 1997, 781, 161–183.
- [109] Deng, Y., Zhang, J., Tsuda, T., Yu, P. H., Boulton, A. A., Cassidy, R. M., *Anal. Chem.* 1998, 70, 4586–4593.
- [110] Van den Bosch, S. E., Heemstra, S., Kraak, J. C., Poppe, H., *J. Chromatogr. A* 1996, 755, 165–177.
- [111] Boughtflower, R. J., Underwood, T., Paterson, C. J., *Chromatographia* 1995, 40, 329–335.
- [112] Seifar, R. M., Kok, W. Th., Kraak, J. C., Poppe, H., *Chromatographia* 1997, 46, 131–136.
- [113] Schurig, V., Schmalzing, D., Muhleck, U., Jung, M., Schleimer, M., Mussche, P., Duvekot, C., Buyten, J. C., *J. High Resol. Chromatogr.* 1990, 13, 713–717.
- [114] Schurig, V., Schmalzing, D., Schleimer, M., *Angew. Chem. Int. Ed. Engl.* 1991, 30, 987–989.
- [115] Chyueh, S. D., Liu, C. Y., *J. Chin. Chem. Soc.* 1996, 43, 17–20.
- [116] Liu, C. Y., Hu, C. C., Yang, C. L., *J. Chromatogr. A* 1997, 773, 199–208.
- [117] Liu, C. Y., Chen, J. L., Shiue, C. C., Liu, K. T., *J. Chromatogr. A* 1999, 862, 65–83.
- [118] Tsuda, T., Nomura, K., Nakagawa, G., *J. Chromatogr.* 1982, 248, 241–247.
- [119] Mayer, S., Schurig, V., *J. High Resol. Chromatogr.* 1992, 15, 129–131.
- [120] Mayer, S., Schurig, V., *J. Liq. Chromatogr.* 1993, 16, 915–931.
- [121] Wistuba, D., Czesla, H., Roeder, M., Schurig, V., *J. Chromatogr. A* 1998, 815, 183–188.
- [122] Chen, G. J., Lee, N. M., Hu, C. C., Liu, C. Y., *J. Chromatogr. A* 1995, 699, 343–351.
- [123] Matyska, M. T., Pesek, J. J., Katrekar, A., *Anal. Chem.* 1999, 71, 5508–5514.
- [124] Pesek, J. J., Matyska, M. T., *J. Chromatogr. A* 2000, 887, 31–41.
- [125] Pesek, J. J., Matyska, M. T., Sandoval, J. E., Williamsen, E. J., *J. Liq. Chromatogr. Rel. Technol.* 1996, 19, 2843–2865.
- [126] Hjertén, S., Liao, J. L., Zhang, R., *J. Chromatogr.* 1989, 473, 273–275.
- [127] Svec, F., Fréchet, J. M. J., *Anal. Chem.* 1992, 64, 820–822.
- [128] Fujimoto, C., *Anal. Chem.* 1995, 67, 2050–2053.
- [129] Fujimoto, C., Fujise, Y., Matsuzawa, E., *Anal. Chem.* 1996, 68, 2753–2757.
- [130] Liao, J. L., Chen, N., Ericson, C., Hjertén, S., *Anal. Chem.* 1996, 68, 3468–3472.
- [131] Petro, M., Svec, F., Gitsov, I., Fréchet, J. M. J., *Anal. Chem.* 1996, 68, 315–321.
- [132] Fields, S. M., *Anal. Chem.* 1996, 68, 2709–2712.
- [133] Schweitz, L., Andersson, L. I., Nilsson, S., *Anal. Chem.* 1997, 69, 1179–1183.
- [134] Fujimoto, C., Kino, J., Sawada, H., *J. Chromatogr. A* 1995, 716, 107–113.
- [135] Peters, E. C., Petro, M., Svec, F., Fréchet, J. M. J., *Anal. Chem.* 1997, 69, 3646–3649.

- [136] Palm, A., Novotny, M. V., *Anal. Chem.* 1997, 69, 4499–4507.
- [137] Ericson, C., Hjertén, S., *Anal. Chem.* 1999, 71, 1621–1627.
- [138] Peters, E. C., Petro, M., Svec, F., Fréchet, J. M. J., *Anal. Chem.* 1998, 70, 2288–2295.
- [139] Schmid, M., Bauml, F., Kohne, A. P., Welsch, T., *J. High Resol. Chromatogr.* 1999, 22, 438–442.
- [140] Tang, Q., Xin, B., Lee, M. L., *J. Chromatogr. A* 1999, 837, 35–50.
- [141] Tang, Q., Wu, N., Lee, M. L., *J. Microcol. Sep.* 2000, 12, 6–12.
- [142] Tang, Q., Lee, M. L., *J. High Resol. Chromatogr.* 2000, 23, 73–80.
- [143] Svec, F., Peters, E. C., Sykora, D., Yu, C., Fréchet, J. M. J., *J. High Resol. Chromatogr.* 2000, 23, 3–18.
- [144] Svec, F., Peters, E. C., Sykora, D., Fréchet, J. M. J., *J. Chromatogr. A* 2000, 887, 3–29.
- [145] Lin, J. M., Nakagama, T., Uchiyama, K., Hobo, T., *J. Liq. Chromatogr. Relat. Technol.* 1997, 20, 1489–1506.
- [146] Schweitz, L., Andersson, L. I., Nilsson, S., *J. Chromatogr. A* 1998, 817, 5–13.
- [147] Takeuchi, T., Haginaka, J., *J. Chromatogr. B* 1999, 728, 1–20.
- [148] Remcho, V. T., Tan, Z. J., *Anal. Chem.* 1999, 71, 248A–255A.
- [149] Schweitz, L., Andersson, L. I., Nilsson, S., *J. Chromatogr. A* 1997, 792, 401–409.
- [150] Lin, J. M., Uchiyama, K., Hobo, T., *Chromatographia* 1998, 47, 625–629.
- [151] Engelhardt, H., Cunat-Walter, M. A., *J. Chromatogr. A* 1995, 717, 15–23.
- [152] Kaupp, S., Steffen, R., Watzig, H., *J. Chromatogr. A* 1996, 744, 93–101.
- [153] Kaupp, S., Watzig, H., *J. Chromatogr. A* 1997, 781, 55–65.
- [154] Pesek, J. J., Matyska, M. T., Menezes, S., *J. Chromatogr. A* 1999, 853, 151–158.
- [155] Dulay, M. T., Kulkarni, R. P., Zare, R. N., *Anal. Chem.* 1998, 70, 5103–5107.
- [156] Chirica, G., Remcho, V. T., *Electrophoresis* 1999, 20, 50–56.
- [157] Ratnayake, C. K., Oh, C. S., Henry, M. P., *J. Chromatogr. A* 2000, 887, 277–285.
- [158] Pullen, P. E., Pesek, J. J., Matyska, M. T., Frommer, J., *Anal. Chem.* 2000, 72, 2751–2757.
- [159] Smith, N., *Capillary Electrochromatography*, Beckman Coulter, Palo Alto, CA USA 1999.
- [160] Whatley, H., *LC.GC* 1999, 12, 762–766.
- [161] Schomburg, G., *Trends Anal. Chem.* 1991, 10, 163–169.
- [162] Grant, I. H., in: Altria, K. D. (Ed.), *Capillary Electrophoresis Guidebook*, Human Press, Totowa, NJ 1996.
- [163] Banholczer, A., Pyell, U., *J. Microcol. Sep.* 1998, 10, 321–328.
- [164] Adam, T., Ludtke, S., Unger, K. K., *Chromatographia* 1999, 49, S49–S55.
- [165] Zhang, M., Ostrander, G. K., El Rassi, Z., *J. Chromatogr. A* 2000, 887, 287–297.
- [166] Siriraks, A., Kingston, H. M., *Anal. Chem.* 1990, 62, 1185–1193.