Review

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Recent applications in capillary electrochromatography

The most recent and important applications in capillary electrochromatography (CEC) are summarized, covering literature published since May 2001. A selection of new developments in stationary phases for CEC is highlighted, and enantiomeric separations and chiral stationary phases are discussed. Also, CEC applications of biological molecules, pharmaceuticals, and applications in the field of industrial and environmental analysis are summarized. For this review three modes of CEC were taken into account, *i.e.*, packed-column CEC, CEC using monolith technology, and open-tubular CEC.

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Abbreviations: AMPS, 2-acrylamido-2-methylpropane-sulfonic acid; **COMOSS**, collocated monolith support structures; **ODS**, octadecyl silane; **OT**, open-tubular; **p-CEC**, pressure-assisted CEC; **PDMS**, poly(dimethylsiloxane); **SEEC**, size-exclusion electrochromatography

1 Introduction

Capillary electrochromatography (CEC) is a miniaturized separation technique that combines aspects of both interactive chromatography and capillary electrophoresis. Analytes may be separated according to differences in partitioning ratio between the stationary and the mobile phase, and/or to differences in electrophoretic mobility. High separation efficiencies can be obtained in CEC in comparison to high-performance liquid chromatography (HPLC). This is mainly due to the characteristics of the electroendoosmotic flow (EOF). First, because of the absence of backpressure in CEC, smaller particles can be employed as stationary phase than in the pressure-driven HPLC. Moreover, the EOF is more homogeneous and less influenced by packing differences than a pressure-driven flow. Also, when a porous material is used as stationary phase, a flow through the pores of the particles may further improve the CEC separation efficiencies.

There are several modes to perform CEC. Packed-column CEC is still the most often used technique. Typically, 50–100 μm ID columns are employed packed with HPLC-type stationary phase particles. However, much research is devoted to the development of monolith technology for

the preparation of CEC columns. An advantage of using a monolithic stationary phase is that its properties in terms of active sites for interaction and charged moieties for generation of the EOF can be tailored. Also for the implementation of CEC on chips, the possibility of *in situ* preparation of the monolithic stationary phase is very promising. Another mode of CEC is open-tubular electrochromatography (OT-CEC). In OT-CEC, the stationary phase is attached to the wall of the capillary. This may increase the separation efficiency compared with a packed column because the eddy diffusion contribution is eliminated. In OT-CEC column diameters smaller than 25 μm are used. Compared to packed-column CEC, the loadability of OT-CEC is very low, and lower retention factors are obtained.

Although currently still much research is directed on the further development of CEC as a separation method, the technique is already mature enough for real-life applications. This review focuses on applications in the biochemical, pharmaceutical, environmental, and industrial fields. The tables in the Addendum summarize the compounds that are analyzed with CEC and mention the stationary and mobile phase used in these applications. The applications that are summarized have been published in the literature since the appearance of a similar review in this journal in 2001 [1].

2 New developments in stationary phases for CEC

Several new stationary phases have been developed for CEC. Since this review mainly describes applications of CEC, only a selection of these new developments is highlighted in this chapter.

2.1 Monolithic columns

Several methods are used for the preparation of columns packed with silica stationary phase particles [2, 3]. The preparation of these columns requires considerable expertise because of the many practical difficulties encountered. Creating robust frits and stable column packing are well-known problems. Therefore, much attention has been focused on the *in situ* preparation of different types of monolithic columns. Monolith technology can be used in order to create separation media that fulfill the requirements needed for specific applications. Great progression has been made with the preparation of monolithic columns allowing at the same time the ability to control the surface chemistries for interaction and generation of the EOF, and control over the porous properties.

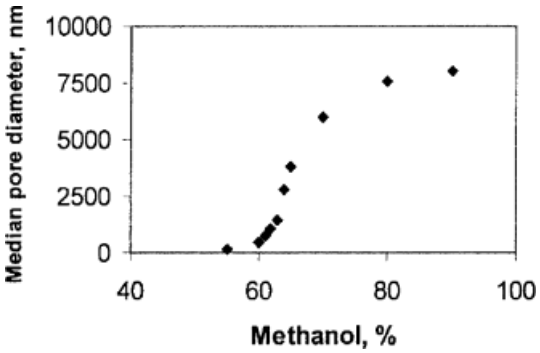

Figure 1. Effect of the composition of methanol-ethyl acetate mixture used as the porogenic solvent on the median pore size of poly(glycidyl methacrylate-*co*-ethylene dimethacrylate)monoliths. Reprinted from [4], with permission.

The preparation of methacrylate ester-based monolithic stationary phases has been extensively investigated by Svec and co-workers [4, 5]. The porous properties could be influenced by the composition of the porogenic solvents. An example is given in Fig. 1, where the effect of a change in composition of the porogenic mixture on pore diameter is depicted for a poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) monolith. With increasing methanol content an increase in pore diameter was observed. This is mainly caused by the increase in polarity of the reaction mixture, giving a lower polymer solubility and a faster phase separation.

In order to change the hydrophobicity of the monolith, to be used for reversed-phase separations, different functional monovinyl methacrylate monomers were used in the polymerization, with ethylene dimethacrylate as a cross-linker [4]. A strong cation-exchange stationary phase could also be obtained after alkylation of 2-dimethylaminoethyl methacrylate used as functional monomer [5]. The ability to tailor the functionality of the monolithic columns for different separation mechanisms (reversed phase, normal phase, ion exchange), allows the use of these columns for a wide variety of applications. The addition of different ionizable monomers provided negatively or positively charged functionalities that allow the possibility to influence the direction of the EOF [4].

Instead of a copolymerization reaction where the monolith is prepared in a single step, photografting can be used in order to modify the functionality of the surface of the monolith [6, 7]. This approach is especially valuable to create patterning and therefore differences in the properties of the monolith at preselected places [7]. Figure 2 shows a macroporous monolithic stationary phase that has been selectively functionalized in different areas by

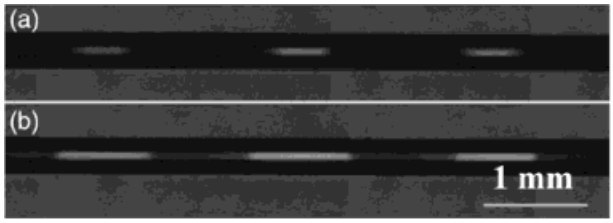

Figure 2. Fluorescence microscopy image of a porous (butyl methacrylate-*co*-ethylene dimethacrylate) monolith photographted with poly(4,4-dimethyl-2-vinylazlactone) through a mask for (a) 1 and (b) 3 min and subsequently reacted with Rhodamine 6C. Reprinted from [7], with permission.

photografting with vinylazlactone through a mask for 1 and 3 min, respectively. Next, the grafted chains were labeled with Rhodamine 6G in order to obtain fluorescent areas. The length of the fluorescent areas after 1 min of irradiation time was slightly less than the expected length of 1 mm, probably due to light scattering at the edges of the mask features. After 3 min of irradiation time the areas had a length of 1 mm. An extra advantage in column development is that photografting is less time-consuming than the single-step approach, because it is not necessary to optimize the polymerization mixture and process for every new surface chemistry.

Photografting might also be useful in proteome analysis. In order to generate an EOF, ionized monomers are used. Covering the surface with another layer may prevent coulombic interaction of (charged) biomolecules with the charged surface. Rohr *et al.* [6] demonstrated the potential of this photografting concept. Alternating layers of 2-acrylamido-2-methylpropanesulfonic acid (AMPS) and butylacrylate were photografted on a spin-coated cyclic olefin copolymer surface. The sulfur-to-carbon ratio of the surface (see Fig. 3) clearly indicates the availability of ionized *versus* hydrophobic surface groups, depending on the final grafting.

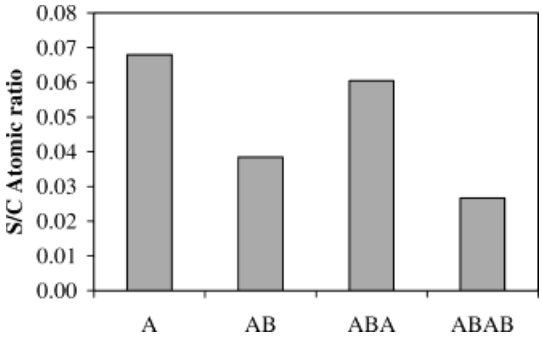

Figure 3. Sulfur to carbon atomic ratio for subsequently photografted layers using (A) AMPS (B) and butylacrylate. Reprinted from [6], with permission.

As was stated before, much research effort has been devoted to the preparation of different types of monolithic stationary phases. Other recent reviews summarize the current status in monolith research [8, 9].

2.3 Stationary phases for OT-CEC

In OT-CEC a distinction has to be made between two types of adsorbed stationary phases. A physically adsorbed stationary phase is strongly attached to the surface of the capillary wall. The columns are prepared by pretreatment of the capillary wall in order to activate the silanol groups, followed by a single flushing with a solution of the adsorbing agent. The second type is a so-called dynamically adsorbed stationary phase. Here, the stationary phase is only weakly attached to the surface of the capillary. After conditioning of the capillary wall with the adsorbing agent, eluent that contains the stationary phase is used continuously in order to obtain equilibrium between the adsorbing agent on the wall and in the eluent.

Compared to packed-column CEC the low phase ratio is a drawback of OT-CEC. In order to enhance the loadability of OT-CEC columns the wall can be etched before the stationary phase is applied. Liu *et al.* [10] investigated the change in phase-ratio when using a capillary etched with HF/NH_4F. The etched capillary was reported to have a surface area approximately 1000 times higher than an unetched capillary. However, when using advidin as a physically adsorbed stationary phase, the phase ratio was found to be higher by a factor of 1.6 only. As a possible explanation it was proposed that the density of silanol groups on the surface had been decreased by side reactions during the etching procedure. Still, the resolution between racemic biomolecules was found to be improved slightly when using the etched capillary.

Sol-gel chemistry for OT-CEC columns has been used in order to obtain fine-tuned stationary phase selectivity through adjusting the composition of the sol solution [11]. Constatin and Freitag [12] developed several new silica-based stationary phases for OT-CEC columns using a sol-gel process. These hydrophobic, hydrophilic, and charged stationary phases allowed the separation for a wide variety of analytes. Also, Zhao *et al.* [13] used sol-gel technology for the preparation of a C_{18} ester-bonded stationary phase. Excellent separations of basic pentapeptides were obtained with efficiencies of up to 480 000 plates per meter.

Kapnissi *et al.* [14] and Kamande *et al.* [15] prepared a polyelectrolyte multilayer coating consisting of a physically adsorbed cationic polymer of a quaternary ammo-

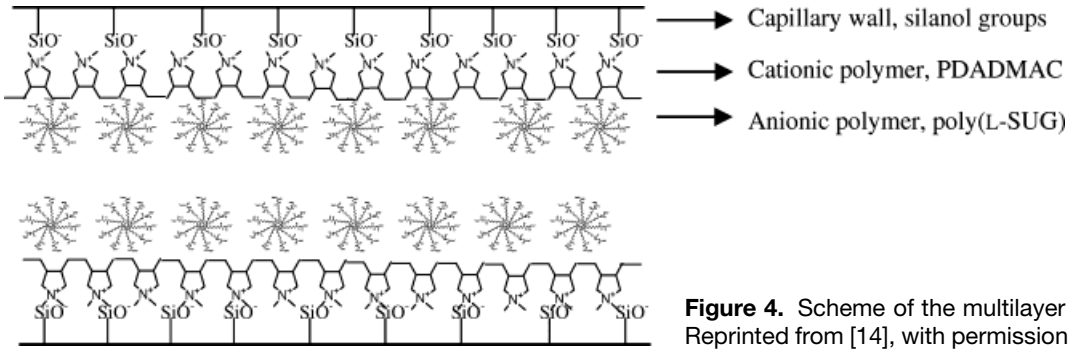

Figure 4. Scheme of the multilayer coating for OT-CEC. Reprinted from [14], with permission.

nium salt (poly-diallyldimethylammonium chloride) and a dynamically adsorbed anionic polymeric surfactant (poly-sodium undecylenic sulfate), as depicted in Fig. 4.

Using this coating, a good selectivity for different phenolic compounds was achieved with OT-CEC. The run-to-run and batch-to-batch repeatability of the EOF velocity was found to be satisfactory, with an RSD of less then 1.5%. The coating was found to be stable for at least 100 subsequent runs. Several other OT-CEC coatings have been developed, especially for enantiomeric separations. These stationary phases will be described in the next section.

3 Enantiomeric separations

3.1 Chiral stationary phases

Especially in biochemistry and in the pharmaceutical industry it is of major importance that a distinction can be made between enantiomers of a substance. This is because enantiomers might have dissimilar interactions and consequently have a totally different biological activity. Enantiomeric separations are based on differences in affinity for chiral selectors. Therefore, an extensive effort is made into the development of new chiral stationary phases.

Several groups have developed CEC columns using the macrocyclic antibiotic vancomycin as a chiral selector. Different support materials have been studied to make vancomycin columns widely applicable. Acrylamide continuous beds were prepared from methacryloxypropyl modified fused-silica capillaries by Kornyšova *et al.* [16]. The continuous-bed chiral stationary phase was subsequently prepared by immobilizing the vancomycin by reductive amination. An extremely high EOF was observed with both nonaqueous polar organic and aqueous solvents. Enantiomeric separations of racemic thalidomide, warfarin, coumachlor, and felodipine were obtained. Fanali *et al.* [17, 18] applied a vancomycin-derivatized silica stationary phase for the separation of basic compounds such as antidepressants, bronchodilators, and antihypertensives. Different eluent compositions were tested in order to optimize the enantiomeric separations. The analysis of clinical samples of patients under depression therapy showed a stereoselective metabolism for venlafaxine [17].

Another type of macrocyclic antibiotic used as chiral selector is teicoplanin glycone. Schmid *et al.* [19] used immobilized teicoplanin glycone on 3.5 μm silica and investigated the separation of glycopeptides in the polar-organic and reversed-phase mode. In the polar-organic mode, using methanol with acetonitrile as eluent, very broad peaks were obtained with only partial or no resolution between the enantiomers. In the reversed-phase mode baseline resolution was obtained under all conditions investigated. An example of a chiral separation of Gly-Asn, Gly-Nva, and Gly-Phe is shown in Fig. 5. Sym-

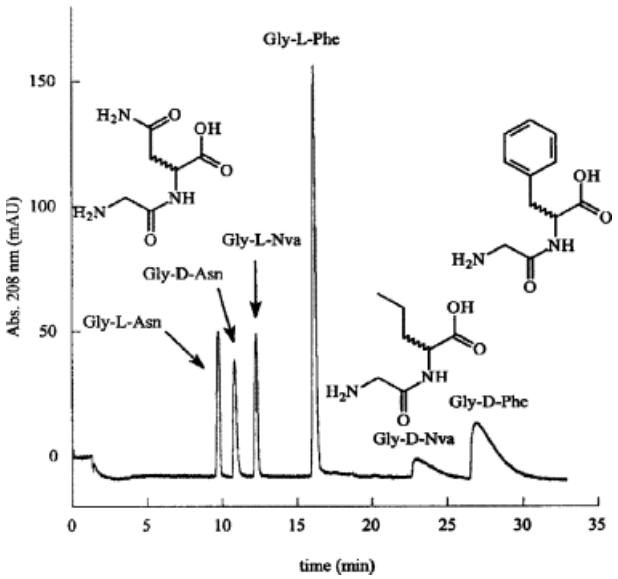

Figure 5. Chiral separation of Gly-Asn, Gly-Nva, and Gly-Phe with RP-CEC. Reprinted from [19], with permission.

metric peaks were obtained except for Gly-D-Nva and Gly-D-Phe. The retention times observed are relatively high for CEC. Using the same stationary phase, this group also investigated the enantiomeric separation of amino acids and β-blockers [20]. High enantioselectivity for amino acids was observed but limited chiral recognition for drugs was obtained. Fanali *et al.* [21, 22] prepared a teicoplanin chiral stationary phase, by reacting a glycopeptide antibiotic, MDL 63,246 (a member of the teicoplanin family), with 5 μm diol silica particles and mixed this with 5 μm amino silica particles. They investigated the effect of a change in eluent composition on the separation of hydroxy acids. At optimum conditions, an enantiomeric separation of racemic mandelic acid could be obtained within 72 s. In comparing this CEC method to CE they found several advantages: a lower amount of chiral selector was used, and detection problems were avoided because the antibiotic was not present in the eluent.

Different groups have performed CEC separations of enantiomers using cellulose or amylose phenyl carbamate stationary phases. Chen *et al.* [23] investigated a cellulose TRIS-phenylcarbamate-bonded chiral stationary phase for the enantiomeric separation of *trans*-stilbene oxide, the enantiomeric separations of warfarin, praziquantel, bendroflumethiazide and benzoin under nonaqueous and aqueous conditions. Using the same chiral selector Kawamura *et al.* [24] showed racemic separations of indapamide, benzoin and PTH-methionine. Plate numbers up to 220 000 per meter were obtained. Wakita *et al.* [25] developed an OT-CEC method with Tris(3,5-dimethylphenylcarbamate) covalently bound to cellulose as chiral selector. Separations of racemic etozolin, *trans*-stilbene oxide, laudanosine, and piprozolin were obtained. Girod *et al.* [26] used cellulose- and amylose-bonded stationary phases, and investigated the influence of the pore size of the silica on the separation characteristics. A higher efficiency was obtained when the intraparticle perfusion transport was increased using stationary phases with a pore size from 60 to 200 Å. Chankvetadze and co-workers [27, 28] also used amylose-bonded stationary phases for the separation of several different enantiomers. They observed a decrease in efficiency and EOF generation with an increase of the amount of amylose bonded to the silica stationary phase.

Enantiomeric separation of racemic dansyl-amino acids was performed on a cyclodextrin-modified stationary phase using nonaqueous CEC by Wistuba *et al.* [29]. For this, β- or γ- cyclodextrin was attached to silica *via* a carbamate link. The authors investigated the experimental conditions and optimized the enantioselectivity and speed of the analysis. They found that adding some water to the nonaqueous mobile phase enhanced the resolution of partially resolved enantiomers.

Wang *et al.* [30] studied sol-gel coated β-cyclodextrin columns for the separation of isomeric aminophenols, dihydroxybenzenes, and nitrophenols. This OT-CEC method showed improved separations in comparison to sol-gel matrix capillary chromatography. Complete baseline resolution was obtained for the OT-CEC separation of the positional isomers, but not in the sol-gel matrix modified capillary column.

The on-line coupling of CEC with coordination ion spray-mass spectrometry for the separation of enantiomers was studied by von Brocke *et al.* [31]. Enantiomers of barbiturates and chlorinated alkyl phenoxypropanoates were separated using a permethylated β-cyclodextrin stationary phase. An increase in sensitivity in MS detection was attained through the addition of different central complexing ions to the sheath flow, such as silver(I), cobalt (II), copper(II), and lithium(I).

Crown ether capped β-cyclodextrin (β-CD) bonded silica was prepared for CEC by Gong and Lee [32]. The recognition sites, the crown ether and β-CD, allowed enantiomeric separations for a wide range of components, *e.g.*, indapamide, nadolol, pindolol, and promethazine. After adding metal ions to the buffer the bonded phase became positively charged, providing extra electrostatic interactions with some polar neutral solutes. Gong and Lee [33] also prepared two novel types of substituted cyclam-capped β-CD bonded silica particles. The structures are depicted in Fig. 6. The chiral selector consisted of three recognition sites; β-CD, cyclam, and the side arm of cyclam. Again, by adding metal ions to the buffer enhanced selectivity was obtained. The cyclam-capped β-CD-bonded stationary phases showed better enantioselectivity than the crown-ether capped analogues.

Zeng *et al.* [34] used *p-tert*-butylcalix[6]-1,4-crown-4 and *p-tert*-butylcalix[6]arene as host molecule for the coating of OT-CEC columns with γ-glycidoxypropyl-trimethoxysilane as bridging agent. Isomeric dihydroxybenzenes, pyridines, picolines, and dihydroxybenzenes were successfully separated. Koide and Ueno [35] investigated the separation of enantiomeric primary amino acids with acrylamide monolithic stationary phase bonded with a chiral crown ether. High efficiencies of more than 100 000 plates per meter were obtained for some compounds. The columns were stable for at least 13 months.

Different sol-gel silica columns for the separation of enantiomers have been prepared. Kang *et al.* [36] described the preparation of a silica monolithic column using a sol-gel process. The monolith was coated with

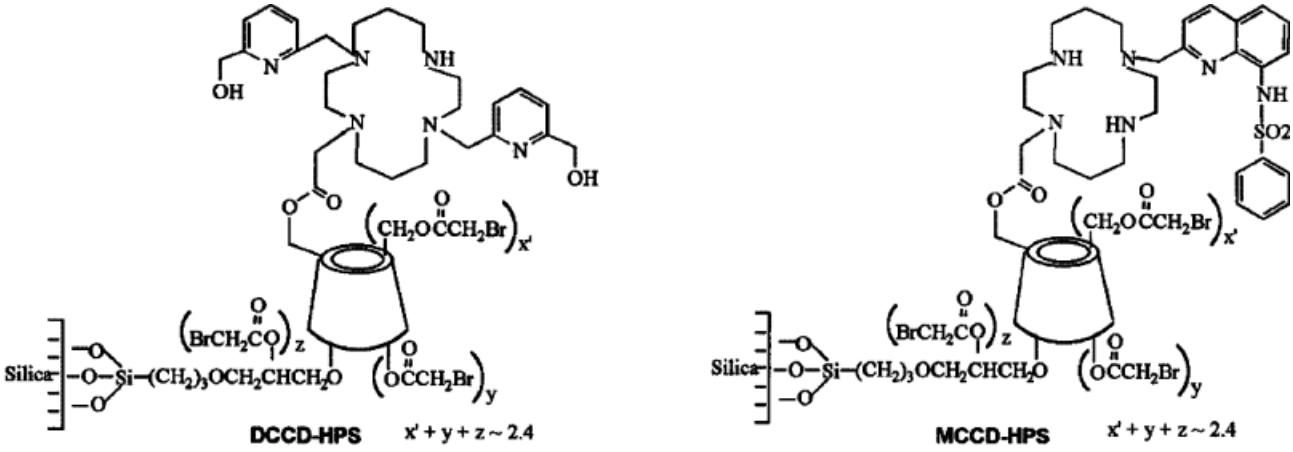

Figure 6. Structures of crown ethers bonded to silica particles. Reprinted from [33], with permission.

Chirasil-β-Dex by heat treatment to give a nonextractable coating. Separations of racemic mephobarbital, hexobarbital, benzoin, and carprofen were obtained. Sakai-Kato *et al.* [37] prepared a bovine serum albumin-encapsulated sol-gel column for the separation of DL-Trp. They observed that the electroosmotic mobility varied under different gelation conditions using different starting materials. The separation of dansyl amino acids and hydroxyacids with a L-prolinamide modified silica sol-gel column was shown by Chen and Hobo [38]. Chen *et al.* [39] used Cu(II) complexes with different L-amino acid amides as chiral selectors using chemically modified acrylamide sol-gel columns, and investigated the separation mechanisms. They observed that the separation behavior depended on the differences in chemical structures of both the chiral selectors and the selectands.

For the separation of chiral bases different cation exchange stationary phases have been developed. Tobler *et al.* [40] and Zarbl *et al.* [41] used a cation-exchange-type chiral stationary phases based on 3,5-dichlorobenzoyl amino acid and amino phosphonic acid derivatives with silica as chromatographic support. Enantiomeric separations of a wide variety of chiral bases and β-blockers were achieved under nonaqueous mobile phase conditions. Also, the influence of several experimental parameters such as mobile phase composition, acid-base ratio, and the counter-ion concentration was investigated [40]. They also showed that in the separation of chiral bases, the ion-exchange retention mechanism dominated over electrophoretic migration [41]. Silica-supported strong sulfonic acid-based cation exchange material proved to be widely applicable as well as useful in a wider range of experimental conditions. Schmid *et al.* [42] investigated L-4-hydroxyproline as chiral selector on acrylamide continuous beds for the separation of hydroxy acids. The resolution of the hydroxy acids was found to depend on the substitution pattern of the aromatic moiety of the analytes.

Rehder and McGown investigated DNA oligonucleotides that form intramolecular G-quartet structures as stationary phase for OT-CEC for the separation of the isomeric dipeptides Trp-Arg and Arg-Trp [43] and lactoglobulin [44]. Fluorescence, circular dichroism, and UV detection were used to indicate interactions between the dipeptides and the biplanar G-quartet structure. Their results suggest that the most favorable aptamer conformation for resolution of the dipeptides is one in which the G-quartet is partially destabilized. For the separation of bovine β-lactoglobulin variants Tris and phosphate buffer were investigated. Tris buffer provided greater resolution and better compatibility with MS detection than phosphate buffer. However, a disadvantage was found in the strong interaction with Tris that could lead to protein denaturation [44].

An on-line sample concentration method for the OT-CEC analysis of negatively charged dansyl-threonine enantiomers was studied by Liu *et al.* [45]. The sample solution prepared in water was introduced with electrokinetic injection after the hydrodynamically introduction of a water plug into the capillary inlet. This approach resulted in a 1000-fold increase in sensitivity.

Several excellent reviews about CEC enantiomeric separations that describe the fundamental aspects and applications to pharmaceutical and biomedical analysis have been published in literature recently [46–49]. Also, the CEC separation of enantiomers on molecular imprinted polymer (MIP) monolithic columns has been reported [50, 51]. The recent status and applications of MIP columns will be discussed in another review in this journal.

3.2 Chiral additives

Ye *et al.* [52] investigated a dynamically modified strong anion-exchange stationary phase for the chiral separations of several enantiomers. After the addition of sulfated β-cyclodextrin to the mobile phase tryptophan, praziquantel, atropine, metoprolol, and verapamil enantiomers were successfully separated. The influence of the ionic strength, additive concentration and methanol content on the separation was studied. It was observed that the methanol content played an important role in improving the peak shape and column efficiency. This group also used affinity CEC to study the competitive binding of enantiomers to a pre-adsorped protein [53]. After the addition of a competitive additive D- or L-tryptophan to the mobile phase, it was observed that bovine serum albumin has a primary site to which L-tryptophan binds strongly, but not D-tryptophan. Both D- or L-tryptophan shared a weak binding site. Using this approach benzoin enantiomers could be resolved. Machtejevas and Maruška [54] used acetyl salicylic acid and L-tryptophan as additives to interact with the active sites of immobilized human serum albumin during the protein allylation and polymerization step. When L-tryptophan was used, a higher enantioselectivity was observed than when using acetyl salicylic acid.

4 Applications in biochemical analysis

4.1 Amino acids and amines

Domoic acid is a naturally occurring amino acid found in some marine algae, which may accumulate in fish and shellfish and therefore may enter the human food chain. Reversed-phase CEC with photodiode array detection of domoic acid in biological samples was performed by Martins and co-workers [55]. They showed that CEC is a promising technique for the analysis of domoic acid. However, according to the authors further research has to focus on the increase in sensitivity in order to monitor domoic acid in contaminated samples using CEC.

Oguri *et al.* [56] separated and detected biogenic amines in extracts obtained form two types of aged fish, using reversed-phase CEC with on-column derivatization and UV detection. The pH and concentration of the buffer, and the concentration of derivatization agent *o*-phthalaldehyde/2-mercaptoethanol were optimized in order to obtain maximum response. A highly sensitive CEC laser-induced fluorescence (LIF) method for the separation of dansylated dialkylamine tags in encoded combinatorial libraries was presented by Liu *et al.* [57]. With a combination of LIF detection with a bubble-cell detection window the limit of detection (LOD) was found to be 58 amol at a signal-to-noise ratio of 2, which was much better than a LOD of 58 fmol as found in HPLC.

Yu *et al.* [4] investigated the separation of a mixture of amines using butyl methacrylate monolithic stationary phases with AMPS and 2-methacryloyloxy-ethyl-trimethylammonium chloride as negatively and positively charged monomers, respectively, for the generation of the EOF. In both cases baseline resolution between the amines was achieved. Enlund *et al.* [58] explored the feasibility to use acrylamide monolithic columns for the separation of hydrophobic amines. The hydrophobicity and charge density of the stationary phase were systematically varied, which resulted in an optimal performance of 200 000 N/m at a molar ratio of 1:80 for the sulfonate and isopropyl groups. A complex migration/retention mechanism was observed in which adsorption, electrophoretic migration, and ion-exchange played a role.

In order to eliminate adsorption effects of amino acids with the capillary silica wall and to obtain separation selectivity, Charvátová and co-workers [59, 60] used (metallo)porphyrin derivates as wall modifier for the separation of underivatized amino acids with OT-CEC. The separation was found to be based on π-π interaction, electrostatic repulsion, and axial ligation between the metal atom and the analytes. With OT-CEC slightly better results in resolution and lower LODs for Thr, Gly, and Val were obtained in comparison with CZE. Pesek *et al.* [61] studied the feasibility of different types of stationary phases for OT-CEC for the separation of heterocyclic aromatic amines. Anionic and zwitterionic fluorosurfactants, physically adsorbed polymers, and covalently bound C_{18}, cholesteryl 10-undecanoate and diol layers were used as stationary phases. The separation of an amine mixture depended strongly on the experimental conditions. The best resolution was obtained using a capillary coated with a polymer containing a positively charged functional group.

4.2 Peptides

Unger and co-workers [62] studied the retention behavior of hormonal cyclic and linear peptides using *n*-alkyl silica reversed-phase particles and mixed mode stationary phases containing cation-exchange and *n*-alkyl groups. The separation mechanism for charged peptides depended on the pH and composition of the eluent, and on the type of stationary phase. Figure 7 shows the change in retention coefficient (K_{CEC}) as a function of the volume fraction of acetonitrile (ψ). The electrophoretic migration of the charged peptide is dominant when the K_{CEC} values are negative, whereas solid-surface interaction domi-

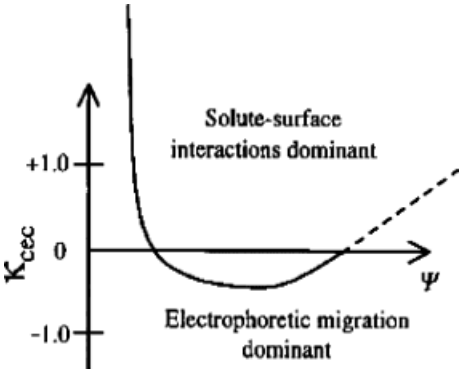

Figure 7. CEC retention coefficient as function of the volume fraction of acetonitrile for positive charged peptides. Reprinted from [62], with permission.

nates when the K_{CEC} values become positive. Increasing the molarity of the buffer and adjusting the pH could suppress the electrostatic interaction between silanol groups of the *n*-alkyl stationary phase and charged peptides. Using the mixed-mode stationary phase, interaction was suppressed at low pH values and high ionic strength. Using reversed-phase stationary phase, this group showed that also the temperature-dependent viscosity might have a major effect on the resolution of a peptide separation [63]. Therefore, eluent properties can be tailored in order to favor either electrophoretic mobility or chromatographic retention.

Zhang *et al.* [64] investigated the use of silica-based tentacular weak cation-exchange particles for the separation of basic peptides. The retention mechanism of peptides was studied by examining the effect of salt concentration on the migration behavior. The separation of peptides by gradient pressure-assisted CEC (p-CEC) was performed by Zhang *et al.* [65]. Effects of the applied voltage, supplementary pressure, and ion-pairing agents on the resolution were investigated. The experiments demonstrated that p-CEC with gradient elution capability is more powerful in separating similar peptides than isocratic elution. Also, Ye *et al.* [66] used p-CEC for the separation of peptides. A mathematical model was developed that described the quantitative relationship between retention factors of the charged peptides and the applied voltage and pressure.

CEC separation of peptides with an ion-trap mass spectrometric detector was performed by Gaspari *et al.* [67]. The sensitivity and scanning speed of both full mass spectrometry and tandem mass spectrometry detection was evaluated. Also, a comparison between sheath flow and sheathless interfacing was made. Despite the 20–40 fold loss in sensitivity obtained using the sheath flow interface, this interface showed to be superior in terms of ruggedness and allowed the use of a higher electric field to achieve faster analysis time.

A neutral hydrophobic lauryl methacrylate column was developed by Wu and co-workers [68]. They observed a poor EOF generation on such columns. However, some peptide isomers that could not be separated by CZE were separated with CEC. In order to improve the generation of the EOF this group developed a mixed-mode methacrylate monolithic column that combines the properties of a reversed-phase stationary phase with that of a strong cation-exchange [69]. An efficiency of over 280 000 N/m for the unretained component was obtained. Throckmorton *et al.* [70] and Shediac *et al.* [71] reported the use of acrylate-based porous monoliths for the separation of peptides and amino acids on chips. The main advantage of using chips compared with packed-column CEC was the significant improvement in analysis time. A separation of six bioactive peptides was obtained within 45 s instead of 9 min as needed in packed-column CEC with the same stationary phase. An example chromatogram of a on-chip separation is shown in Fig. 8, in which naphthalene-2,3-dicarboxaldehyde labeled peptides were separated on a lauryl acrylate monolith with

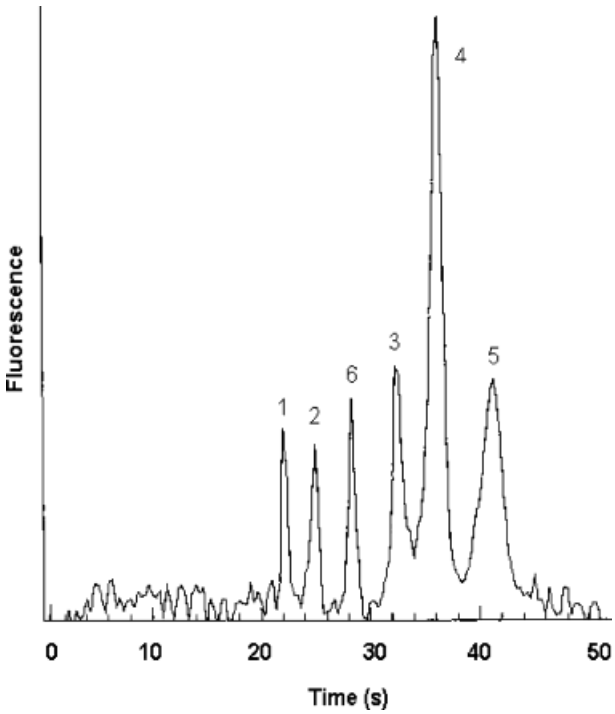

Figure 8. RP-CEC chromatogram of naphthalene-2,3-dicarboxaldehyde labeled peptides in a microchip packed with a lauryl acrylate monolithic stationary phase. Peaks: (1) papain inhibitor, (2) proctolin, (3) opoid peptide (α-casein fragment 90–95), (4) Ile-angiotensin III, (5) angiotensin III, (6) GGG. Reprinted from [70], with permission.

LIF detection. On-chip separations could be performed at higher electrical field strength than packed-column CEC. Another advantage was the extremely high efficiency of up to 600 000 *N*/m obtained.

Charvátová *et al.* [72] used porphyrin derivates as wall modifier for the OT-CEC separation of aromatic peptides. The main separation mechanism was based on the ionic properties of the separated analytes. Pesek *et al.* [61] investigated the feasibility of OT-CEC for the separation of peptides. The stationary phases used for OT-CEC were already described in Section 4.1. With OT-CEC it was possible to separate six peptides that could not be separated by CE or HPLC. Matyska *et al.* [73, 74] investigated the experimental conditions for the separation of synthetic peptides using etched chemically modified OT-CEC columns. The coating was prepared through a silanization procedure followed by a hydrosilation to attach an organic moiety. They found that the combination of partitioning between mobile and stationary phase and electrophoretic migration could be advantageous for the analysis of charged biomolecules.

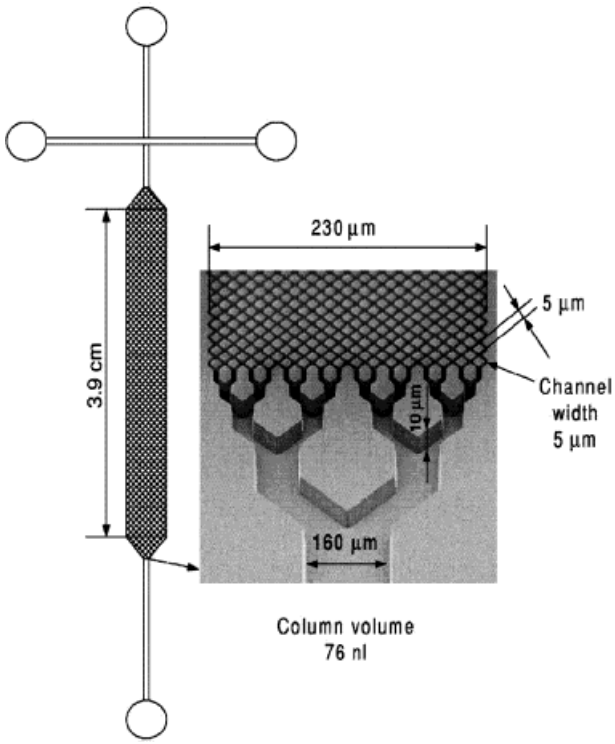

Figure 9. Scheme of a COMOSS column with its typical dimensions. Reprinted from [77], with permission.

4.3 Proteins

Zhang *et al.* [75] synthesized and functionalized polymeric anionic microspheres of glycidyl methacrylate-divinylbenzene in order to separate basic peptides and cytochrome *c* variants with CEC. They also used these columns for µ-HPLC separations with a salt gradient. The efficiency observed using gradient HPLC was comparable with the efficiency obtained with CEC under isocratic conditions.

Protein identification is often achieved through peptide cleavage; proteins are digested before a separation is performed. Slentz *et al.* [76, 77] showed on-chip separations of a tryptic digest of fluorescein isothiocyanate-labeled bovine serum albumin (FITC-BSA) using modified collocated monolith support structures (COMOSS). An example of such a support structure with its typical dimensions is shown in Fig. 9. The structure acts as an ensemble of dividers that create a bundle of homogeneous interconnected open-tubular capillaries. In this way, a unique homogeneity in packing structure is obtained. Another advantage is that there is no need for retaining frits.

Slentz *et al.* [77] investigated the possibility to use poly (dimethylsiloxane) (PDMS) for the fabrication of COMOSS columns. To generate an EOF, the surface was grafted with 2-acrylamido-2-methylpropane sulfonic acid. In order to increase the hydrophobicity of the PDMS columns, alkyl silanes with C_8-C_{18} groups, poly(styrenesulfonic acid), polyacrylic acid and poly(vinylsulfonic acid) were grafted onto the COMOSS surface. On a C_{18}-AMPS modified column, efficiencies up to 620 000 *N*/m were observed.

Great efforts have been directed on the development of micrototal analysis systems (µTAS) for the analysis of proteins. Slentz *et al.* [78] reported the analysis of proteins using a three-dimensional electrochromatography system in a chip. They integrated an electroosmotically driven trypsin digestion step, copper(II)-immobilized metal affinity chromatography [Cu(II)-IMAC] selection for histidine-containing fragments, and reversed-phase CEC separation of the selected peptides on a modified COMOSS column with fluorescence detection. Figure 10 shows chromatograms of on-chip reversed-phase separations of FITC-BSA (A) before and (B) after on-chip trypsin digestion, and (C) after digestion and Cu(II)-IMAC selection using the µ-TAS design. Slentz *et al.* observed some differences between off-chip and on-chip digestions, probably due to incomplete proteolysis using the on-chip design. Still, this design gives the first three-dimensional chromatography on a chip that combines digestion, affinity selection, and reversed-phase chromatography.

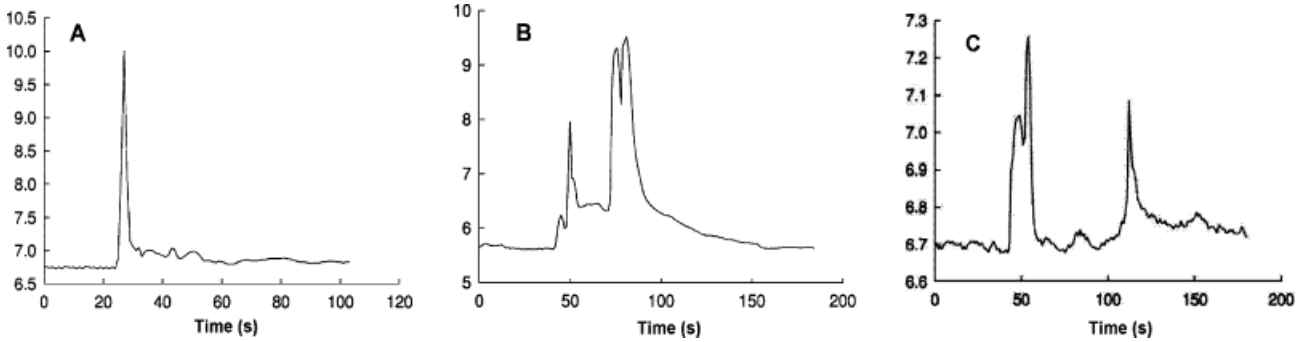

Figure 10. Reversed-phase separations of (A) before trypsin digestion, (B) after, (C) after digestion and affinity chromatography. Reprinted from [78], with permission.

4.4 Nucleosides and nucleotides

Mesplet *et al.* [80, 81] developed a CEC method for the analysis of nucleoside HIV reverse transcriptase inhibitors (NRTIs) using a β-CD-bonded silica stationary phase. In order to decrease analysis time, CEC was carried out on a packed capillary with 10 cm of effective length. Under optimal experimental conditions a baseline separation of six nucleosides was achieved within 7 min. An OT-CEC method to analyze nucleoside mono-, di- and triphosphates was described by Charvátová *et al.* [82]. Sapphyrin (see Fig. 11) was used as wall modifier, which resulted in an increase in electroosmotic flow and a combined partitioning and electrophoretic separation mechanism. The coating was easily applied and stable for at least 20 subsequent runs.

Galloway *et al.* [83, 84] described a separation of double-stranded DNA molecules on a chip using contact conductivity detection. In order to obtain selectivity, octadecylsilane was covalently attached to the glass surface.

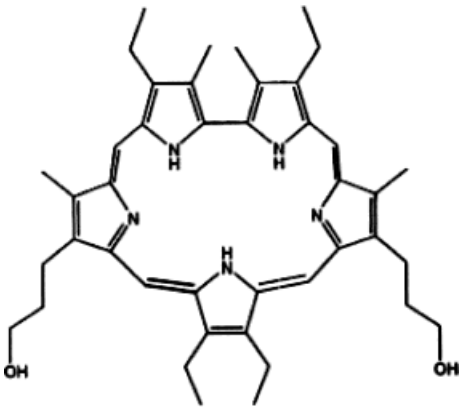

Figure 11. Structure of the sapphyrin molecule. Reprinted from [82], with permission.

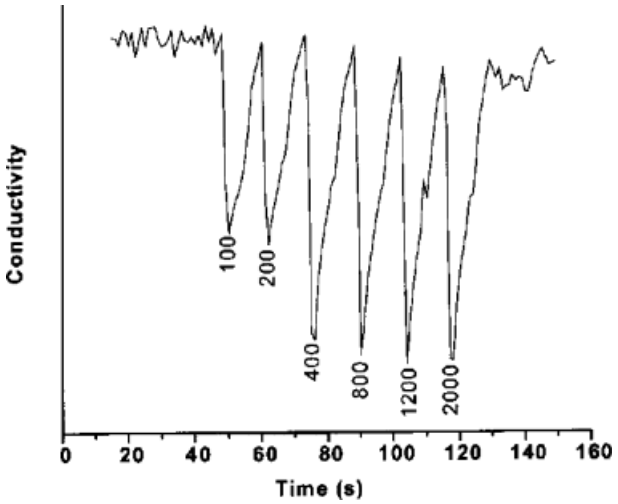

Figure 12. CEC separation of a double-stranded DNA ladder. Reprinted from [83], with permission.

Figure 12 depicts the separation of a low-molecular-weight ladder consisting of 100, 200, 400, 800, 1200, and 2000 bp fragments. The individual DNA fragments were well resolved. Negative peaks were obtained due to the lower conductivity of the oligonucleotide zones compared to the background electrolyte.

4.5 Carbohydrates

It is often difficult to obtain a satisfactory resolution between the many possible carbohydrate compounds differing in size, monomer composition, and (branching) structure. Therefore, CEC with its combined separation mechanism may be a powerful tool for the analysis of carbohydrates. Zhang *et al.* [85] described a method to determine cellular carbohydrate digests in peanut fungal

pathogens and baker's yeast. Because of the absence of chromophores, precolumn derivatization with 6-aminoquinoline *via* reductive amination of chitin and glucan was needed for UV detection. CEC was shown to be very suitable for rapid and accurate qualitative and quantitative analysis.

Size-exclusion electrochromatography (SEEC) was used by Stol *et al.* [86] to obtain molecular mass distributions of celluloses in order to study aging of paper. In SEEC, macromolecules are separated on differences between their hydrodynamic volumes as in size-exclusion chromatography. However, in SEEC, the eluent flow is generated by the EOF, giving higher separation efficiency. Also the typical column dimension used in SEC (4.6 mm ID) is miniaturized and 75–150 μm ID columns are used in SEEC. Derivatization of cellulose with phenylisocyanate (PIC) was needed for UV detection. Solvent selection was required because of the need to dissolve the biopolymers, generate an EOF and maintain UV sensitivity. The electroosmotic mobility using acetone containing 1 mM tetrabutylammonium tetrafluoroborate (TBAFTFB) was found to be sufficient. Narrow PIC-derivatized polysaccharide standards were used for mass calibration of the SEEC system. Mass calibration curves were constructed for columns packed with 10 and 30 nm pore particles. The upper mass limit of 500 kDa found with the 30 nm pore particles was found to provide an adequate mass range for the cellulose samples. A typical electropherogram of a fibrous cellulose sample is shown in Fig. 13. Three peaks were obtained, a broad peak in the front is the cellulose derivate, and the second and third peaks are due to MeOH-PIC and pyridine, respectively. The molecular mass distribution could be calculated from the chromatograms and calibration curve. The molecular mass distribution was found to correlate with the age of the paper.

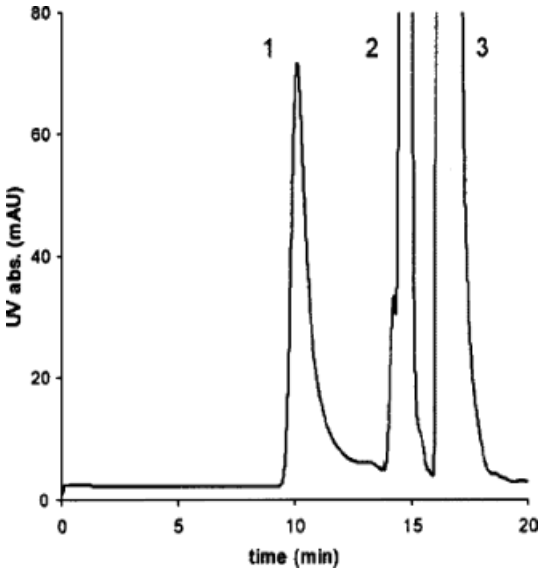

Figure 13. SEEC separation of PIC-derivatized fibrous cellulose. Reprinted from [86], with permission.

Que *et al.* [87] investigated novel types of polar acrylamide monolithic CEC columns for the separation of complex mixtures of saccharides, using mass spectrometry-compatible mobile phases. Using an ion-trap mass spectrometer, low-femtomole LODs were obtained for mono- and disaccharides. This group also investigated the analysis of glycan mixtures released from glycoproteins using CEC coupled to Fourier transform ion cyclotron resonance-mass spectrometry [88]. Two-dimensional contours plots showed a very high resolving power. The analysis of carbohydrates as 1-phenyl-3-methyl-5-pyrazolone derivatives by CEC/microchip electrophoresis has been reviewed by Honda *et al.* [89].

5 Pharmaceutical applications

5.1 Steroids

Jiskra *et al.* [90] developed a CEC method for the separation of hormonal steroids. Hypersil C_8 MOS stationary phase performed best together with ACN as organic modifier and Tris buffer. The method was tested for ruggedness with respect to selectivity and repeatability. For quantification purposes the detection limits were investigated and found to be in the range of 2–4 μg/mL.

For immunoaffinity screening of cardiac glycosides Mayer *et al.* [91] used a CEC method. Varies types of CEC stationary phases were compared for the separation of cardiac glycosides and other steroids. At an eluent composition required for optimal separation the EOF was increased with a factor of 2 using C_4 reversed stationary phase in comparison with C_{18} or C_8 materials. The addition of antibody-carrying particles to the sample allowed screening for high affinity antigens, reflecting the cross-reactivity of eleven steroids. In Fig. 14 the chromatograms (A) before and after addition of (B) 4 mg and (C) 8 mg bioaffinity beads to 2 mL of sample are shown. The peak areas of cardiac glycosides (peaks 1, 2, 4, 10), their impurities (8, 9) and proscillaridin (7) decreased strongly, whereas the peak area of estriol (3) decreased only slightly after adding the immunoaffinity beads.

Chankvetadze *et al.* [92] compared CEC with capillary LC for the determination of the enantiomeric purity of levonorgestrel, using the same cellulose-based stationary phase. Although with CEC higher separation efficiencies were obtained, low levels of enantiomeric impurity were

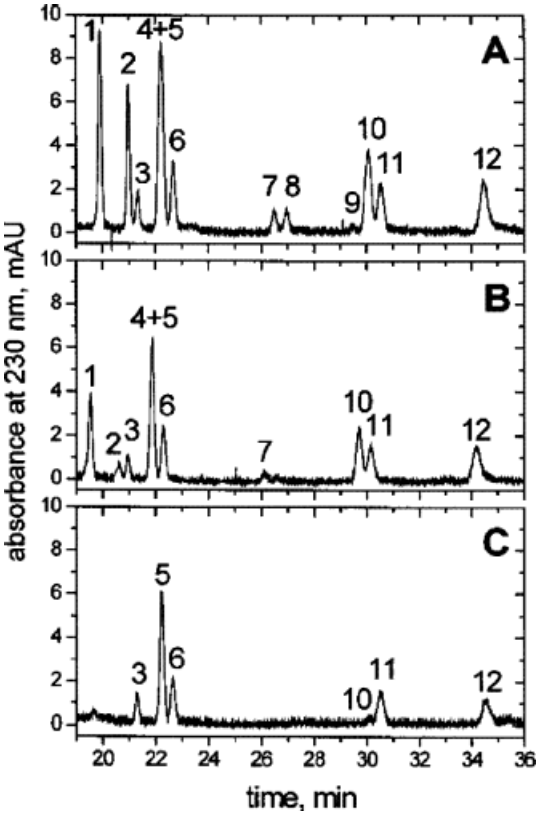

Figure 14. Immonoaffinity screening in a mixture of 13 compounds. Chromatograms of the same mixture are shown (A) before addition of immunoaffinity beads and after addition of (B) 4 and (C) 8 mg, respectively. Peaks; (1) digoxigenin; (2) digoxigenin-bis-digitoxoside; (3) estriol; (4), (5) β-acetyldigoxin and hydrocortisone; (6) prednisone; (7) proscillaridin A; (8), (9) impurities of, respectively, digoxigenin-bis-digitoxoside and β-acetyldigoxin; (10) digitoxigenin; (11) prednisolone acetate; (12) testosterone. Reprinted from [91], with permission.

not more easily detected than with capillary LC. The major reason for this was the higher baseline noise in CEC compared with the LC experiments.

5.2 Acidic drugs

Hydrophilic macroporous weak and strong-anion-exchange stationary phases were prepared by Lämmerhofer *et al.* [5] for the separation of various organic anions. Therefore, a bulk polymer was prepared from methacrylate and ethylene dimethacrylate (EDMA) in the presence of a porogenic solvent using thermal and UV-initiation. Afterwards, the tertiary amino functionalities were alkylated in order to create strong cation-exchangers. A mixture of profens was separated within 13 min with a column efficiency of up to 231 000 *N*/m. Also benzoic acids and weakly acidic, neutral and basic compounds such as phenols, xanthines, and aromatic amines were separated in normal-phase electrochromatographic mode.

An OT-CEC method to separate nonsteroidal anti-inflammatroy drugs (NSAIDs) was described by Pai and Liu [93]. Using a wall modifier that contained histidine functional groups allowed a good separation of seven NSAIDs. The effect of pH, concentration of the buffer and eluent composition on efficiency were investigated. Several ways for the analytes to interact with the bonded phase were found. Electrophoretic mobility, anion exchange, and partitioning were found to play an important role. De Rossi and Desiderio [94] described the reversed-phase CEC analysis of NSAIDs. The use of zwitterionic 2-morpholinethanesulfonic acid or acetate mobile phases strongly modulated the migration order and separation efficiencies. They demonstrated the applicability of this CEC method for the analysis of anti-inflammatory drugs in drops and tablets.

The affinity of acidic drugs for liposomes attached to a capillary was investigated by Manetto *et al.* [95]. The OT-CEC capillaries were prepared by flushing with a liposome suspension followed by a drying step and a wash step with NaOH in order to remove the excess of loosely bound liposomes. They expressed the drug-liposome binding as a free binding enthalpy change relative to acetylsalicylic acid as a standard. Good agreement was found between CEC and CZE experiments.

5.3 Basic drugs

The analysis of basic drugs in HPLC and CEC is often problematic and peak tailing may be observed. This is caused by combined hydrophobic and ion-exchange interaction with the stationary phase. In order to prevent ion-exchange interaction with the silanol groups the pH should be high enough to obtain neutral molecules. However, a high pH is not recommended for silica-based stationary phases. Therefore, current research is focused on using additives in the mobile phase and on the development of new stationary phases.

Jiskra *et al.* [96] described a method for the separation of central nervous system drugs after derivatization to transform a zwitterionic drug into a basic form, using Hypersil MOS C_8 and Hypersil phenyl stationary phases. Basic additives were added to the mobile phase for shielding. They observed a day-to-day repeatability of 5.2% for the retention factors.

CEC with LIF detection was used by Lurie *et al.* for the analysis of heroin [97]. Columns packed with 1.5 μm nonporous octadecyl silica (ODS) particles were comparable

in efficiency with sulfonic acid C_{12} monolithic columns for compounds eluting between 7–10 min. The efficiency of the monolithic columns for fast eluting compounds was found to be much lower than with packed columns. When multistep gradient CEC was compared with gradient HPLC, approximately 30% more peaks were detected with CEC. This can be explained by differences in capacity, selectivity, and detection sensitivity.

Enlund and co-workers [98] used basic drug substances in order to study peak compression effects on a strong-cation-exchange stationary phase. Dissolving the analytes in a stronger solvent (higher acetonitrile concentration) than the mobile phase composition resulted in sharp peaks. Peak compression was also observed when injecting an acetonitrile plug right before or after the sample injection. Their explanation was that a narrow zone is formed due to the difference in migration speed in the sample zone and in the mobile phase. Analytes are strongly retained after passing through a boundary in the sample zone, which results in a continuous sample stacking. Pesek *et al.* [99] studied the separation of the components of two basic antibiotics (ampicillin and gentamycin) by OT-CEC. The best results were obtained with etched capillaries coated with C_{18} or cholesterol layers. More information about the CEC analysis of basic compounds in pharmaceuticals can be found in a review published by Enlund and co-workers. [100].

5.4 Vitamins and food components

Much attention has been given to the separation of vitamin E. Several isomers occur of Vitamin E with different antioxidative properties, some of which are depicted in Fig. 15.

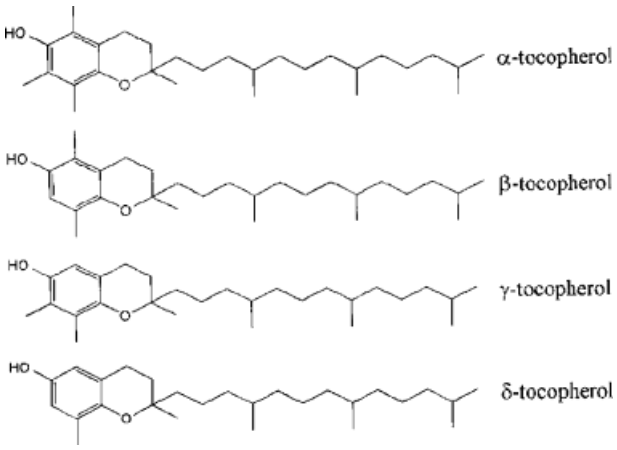

Figure 15. Structures of tocopherol isomers.

Fanali *et al.* [101] reported a CEC method for the separation of α-, γ-, and δ-tocopherols within 5 min using an 8.4 cm effective length reversed-phase column. This method was applied for the qualitative analysis of vitamin E in human serum extracts. Henry *et al.* [102] investigated several RP-stationary phases for the separation of tocopherol α-, β-, γ-, and δ-isomers. Using a C_{30} monolithic stationary phase, the effect of the eluent composition was systematically studied. A baseline separation between β- and γ-isomers could be obtained after the addition of 1% water to the mobile phase (Fig. 16).

CEC of tocopherols and tocotrienols was reported by Abidi *et al.* [103, 104]. Different stationary phases were investigated for their suitability to separate tocopherols and tocotrienols [103]. The elution patterns obtained with CEC were similar to those observed in reversed-phase HPLC. Octylsilica appeared to give the most satisfactory

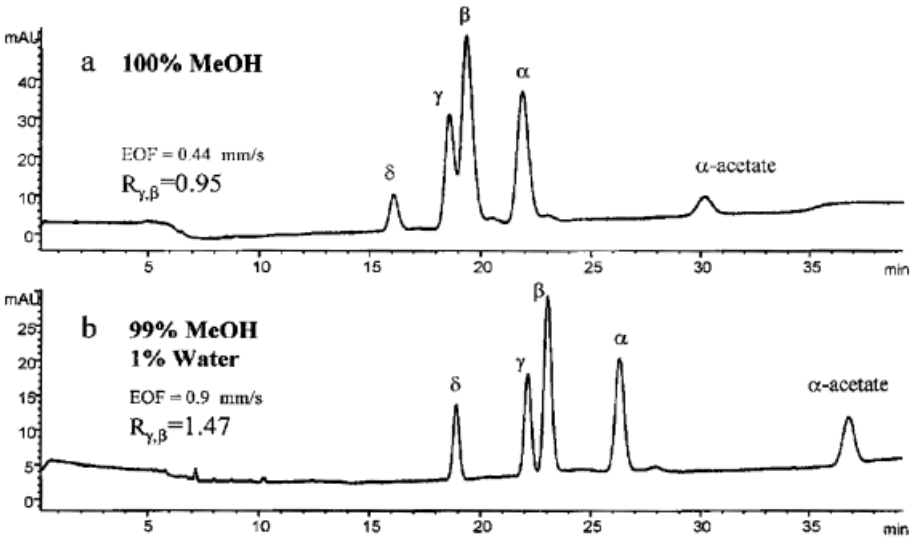

Figure 16. Effect of the addition of 1% water to the methanol mobile phase on the separation between β-, γ-tocopherol isomers using a C_{30} monolithic stationary phase. Reprinted from [102], with permission.

separation and peak characteristics. The authors also reported pentafluorophenyl silica (PFPS), triacontyl silica (TCS), and ODS stationary phases for the separation of unsaponifiable lipids [104]. Complete CEC separation was obtained using the PFPS stationary phase.

Temperature-programmed nonaqueous CEC separations of retinyl esters (vitamin A) on continuous bed columns of C_{30} material were investigated by Roed *et al.* [105]. Temperature programming was used in order to increase resolution of early eluting compounds and to decrease the total analysis time. Lipid extracts of bearded seal liver were analyzed using this method. Starkey *et al.* [106] analyzed isoflavone phytoestrogens in soy-based food using acrylate monolithic columns. Quantification of trace isoflavones in biological samples (*e.g.*, human serum) was performed after on-line preconcentration. A CEC mass spectrometric method for the analysis of withanolides in plant extracts was developed by Cherkaoui [107]. Mass spectrometric detection in the selected ion mode allowed a sensitive and reliable determination of withanolides in Iochroma gesnerioides plant extract. Lignans, biologically active compounds from certain Asian medicinal plants, were determined by Kvasnickova *et al.* using CEC [108]. The polyphenol compounds could be separated using a polymer-based monolithic stationary phase and methanol-water mixtures as mobile phase. The results obtained in the analysis of plant extracts were in good quantitative agreement with HPLC data.

5.5 Miscellaneous

Quaqlia *et al.* [109] used CEC to determine losartan and hydrochlorothiazide in tablets. The method gave accurate and precise results. Enlund *et al.* [110] investigated cation-exchange materials with different pore sizes for the analysis of tricyclic antidepressants. The use of wide-pore material, with the intention to generate pore flow, did not give satisfactory results because retention factors were too low. Cahours *et al.* [111] compared CEC with microemulsion electrokinetic chromatography (MEEKC) for the separation of flunitrazepam and its main metabolites. For the CEC experiments a column packed with 3 μm Hypersil C18 was used. They found that MEEKC gave better separations. However, in contrast to MEEKC, with CEC a mobile phase could be used that was compatible with MS detection.

6 Industrial and environmental applications

6.1 Inorganic anions and cations

Breadmore *et al.* [112, 113] investigated the implementation of sol-gel stationary phases for CEC on a microchip. This group prepared silica monolithic stationary phases from alkyl silane, tetramethyl orthosilicate, by introducing polyethylene oxide (PEO) [114]. With varying molecular weight of the added PEO the size of the macro- and mesopores could be changed. After dynamic coating with a cationic polymer, the monoliths were tested in the ion-exchange mode for the separation of inorganic anions. Differences in efficiency of columns prepared with PEO of different molecular weight were observed, which was attributed to the differences in gel structure. This group also investigated the feasibility of using a sol-gel silica monolith with a polyelectrolyte multiplayer (PEM) coating [113]. They observed two ways to influence the capacity of the column. First, by changing the exposed polyelectrolyte from polydiallyldimethylammonium chloride to dextran sulfate the direction of the EOF could be changed and the chromatographic capacity could be varied. Another way to influence the capacity was to change the thickness of the PEM coating. The retention of inorganic anions increased with the number of PEM layers.

Hilder *et al.* [114] used anion exchange CEC with indirect UV and direct contactless conductivity detection. They investigated the suitability of different eluents for simultaneous indirect UV and direct conductivity detection. Salicylate and *p*-toluenesulfonate were found to be competing anions with sufficient eluotropic strength to induce changes in separation selectivity. However, salicylate gave baseline instability and was found to be unsuitable. Baseline resolution of eight anions was achieved within 2.5 min. Direct conductivity detection was found to be superior to indirect UV detection with regard to both baseline stability and detection sensitivity.

The on-column ion-exchange preconcentration of inorganic anions was investigated by Breadmore *et al.* [115, 116]. For this, anions were retained on a preconcentration zone of cationic latex particles, while the separation was achieved in a zone comprised of cationic modified fused silica. Elution of the adsorbed analytes was achieved using an eluotropic gradient formed by a transient isotachophoretic boundary.

6.2 Synthetic polymers

SEEC has been used for the determination of the molar-mass distributions of synthetic polymers [117, 118]. Ding *et al.* [117] used 5 μm Nucleosil stationary phase with 300 Å pores to construct a mass calibration curve with polystyrene standards. Polystyrene and polycarbonate samples were analyzed and the most-abundant molecular mass, the number-averaged molecular mass, the mass-averaged molecular mass, and the polydispersity were calculated from the chromatograms. Also, the feasi-

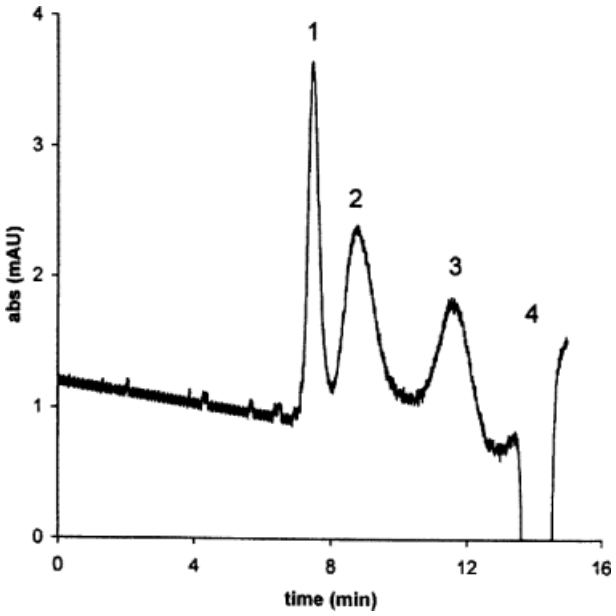

Figure 17. Separation of three different PMMA standards by SEEC. Peaks; (1) PMMA 67 000; (2) PMMA 15 100; (3) PMMA 2400; (4) acetone. Reprinted from [117], with permission.

bility of using hexafluoro-isopropanol (HFIP) as mobile phase on a sulfonated acid-modified Nucleosil stationary phase was shown. Polymethylmethacrylate (PMMA), and other polymers that are difficult to dissolve, such as polyethylenetheraphtelate and polycaprolactam, could be handled in this way. An example of a SEEC separation of PMMA standards using 0.1 mM TBATFB in hexafluoro-isopropanol (HFIP) as mobile phase is given in Fig. 17. The peaks were well resolved and could be detected at 220 nm. The precision and accuracy of the SEEC methods were comparable with those of traditional size-exclusion chromatography.

Mistry *et al.* [118] investigated sulfonated polystyrene/divinylbenzene resins with different exchange capacities and pore sizes for the separation of (neutral) synthetic polymers. A significant increase in electroosmotic mobility was obtained by adding $\leq 3\%$ water to the THF used as the mobile phase. The generation of the EOF was studied with total reflectance Fourier transform infrared spectroscopy. They found that the EOF is generated due to the existence of a ternary eluent mixture: free THF, free water, and a THF-water complex. The SEEC analysis of polyurethane and polystyrene was found to correlate well with conventional liquid chromatography techniques.

6.3 (Poly)aromatics

The separation of aromatic compounds by Zhang *et al.* [119, 120] was performed under different experimental conditions in order to check the prediction of the migration behavior of neutral solutes in gradient elution mode. A difference between the calculated and experimental values of below 3.3% was obtained. Wu *et al.* [121] showed the feasibility of polysiloxane containing metal complexes, $Co(TACN)_2^{3+}$ (TACN = 1,4,7,-triazacyclononane) as coating for the OT-CEC separation of alkylbenzenes and basic compounds. Through cross-linking and covalent bonding, the positively charged polymers, depicted in Fig. 18, were bound to silica supports. The

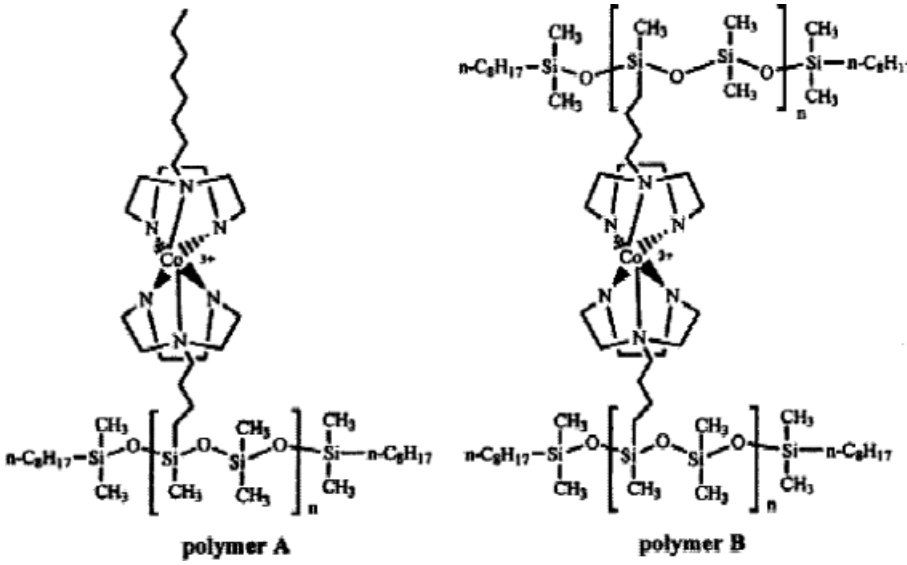

Figure 18. Structures of the metal complex containing coatings. Reprinted from [121], with permission.

separation of alkylbenzenes was achieved within 7 min and the system had a higher resolving power than conventional ODS packings.

Kapnissi *et al.* [14] and Kamande *et al.* [15] investigated a polyelectrolyte multilayer as a coating for OT-CEC columns, as described in Section 1.3. For both phenolic and benzodiapines mixtures a good selectivity between the compounds was obtained. The OT-CEC method was shown to be superior over MEKC and CZE in the separation of benzodiazepines. Scherer and Steiner [122] developed a hydrophobic strong-anion-exchange stationary phase (SAX/C_{18} mixed mode). The synthesis of the stationary phase was based on polymer encapsulation of porous silica. A fast and constant EOF could be generated from pH 3 to 9. The stationary phases showed a good compatibility for the separation of neutral, acidic and weakly basic analytes.

6.4 Pesticides, insecticides, and herbicides

Wall *et al.* [125] investigated the electrochromatographic retention behavior of underivatized and derivatized phenol pesticide metabolites. With various eluent compositions, the underivatized phenols behaved as neutral solutes and a reversed-phase separation was obtained. A combination of partitioning and electrophoretic migration, needed to separate monochlorophenol isomers, could not be achieved. This is because ionization is obtained at pH values higher than 8.5 and silica-based stationary phases are not stable at these pH values. However, after derivatization with a fluorescent tag, carbazole-9-*N*-acetic acid, the selectivity required for the separation of the isomers was obtained.

Tegeler and El Rassi [126] presented an on-column enrichment method for CEC of carbamate insecticides. Various experimental parameters were investigated in order to find optimal conditions for preconcentration. The effectiveness was found to depend on the composition of the injection solvent, mobile phase composition and the applied voltage during injection. At optimum conditions a 500-fold increase in sensitivity was achieved for carbofuran. Tegeler and El Rassi [127] also described another way for trace enrichment. A segmented capillary column containing of two types of stationary phase was used for on-column enrichment of carbamate insecticides and pyrethroid insecticides. The first short segment (2.5 cm) meant for preconcentration contained Zorbax C_8. The second segment (22.5 cm), used for the separation, consisted of ODS reversed-phase material. Under optimal conditions a 282- and 620-fold sensitivity enhancement was obtained for carbaryl and methiocarb, respectively. Using a z-cell UV detection window instead of on-column UV detection increased the sensitivity 752- and 1100-fold, respectively.

Tegeler and El Rassi [128] introduced a novel concept for the separation of pyrethroid insecticides. Surfactant-mediated capillary electrochromatography (SM-CEC) is based on using packed-column CEC and including a charged surfactant in the mobile phase. Sodium di-2-ethylhexyl sulfosuccinate was used as surfactant, acting as a moving pseudostationary phase. In Fig. 19 it is shown that with SM-CEC geometric isomers and diastereomers of pyrethriod insecticides were better resolved than using simple packed-column CEC.

Bedair and El Rassi [129] introduced a sulfonated stearyl acrylate monolithic stationary phase for the separation of neutral and charged analytes. The monolith was prepared by the copolymerization of pentaerythritol diacrylate monostearate (providing a hydrophobic surface) with AMPS (to support the EOF) in the presence of a ternary porogenic mixture. In CEC mode using a 8.5 cm column, a separation of 17 charged and neutral pesticides was achieved within 135 s. More research about the electrochromatographic separation of pesticides and metabolites has been reviewed last year in this journal [130].

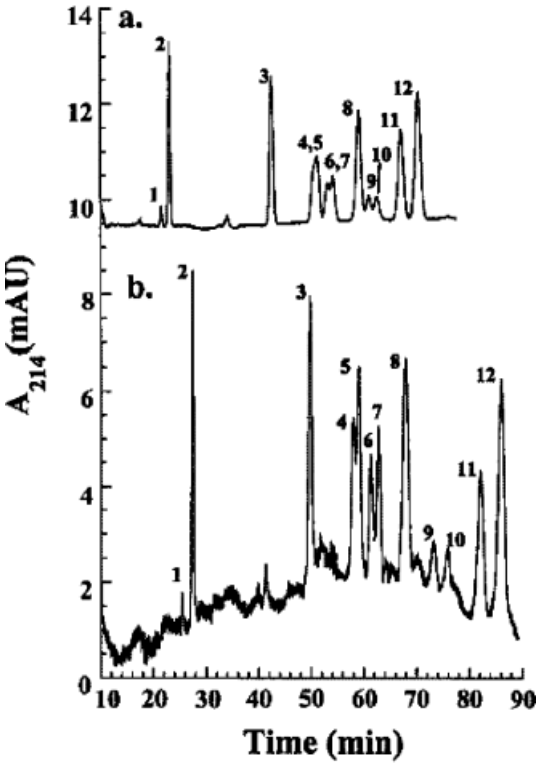

Figure 19. Electropherograms of a mixture of pyrethroids. (a) Measured with SM-CEC, (b) measured using packed-column CEC. Reprinted from [128], with permission.

6.5 Miscellaneous

Liu *et al.* [131] investigated 4-dimethylamino-6-(4-methoxy-1-naphthyl)-1,3,5-triazine-2-hydrazine (DMNTH) as derivatization agent for the reversed-phase separation of carbonyl compounds. A comparison with CEC and micellar electrokinetic chromatography (MEKC) was made, as depicted in Fig. 20. The carbonyl derivatives as well as the derivatization reagent were separated. The separation selectivities of CEC and MEKC were found to be different, especially for the polar compounds.

De Rossi and Desiderio [132] demonstrated the applicability of fast CEC for the analysis of parabens and 4-hydroxybenzoic acid in drugs and cosmetics. Under optimized conditions, separation efficiencies of 129 000–140 000 *N*/m were obtained for parabens and analyte quantitation limits were in the range of 1–3 μg/mL. A

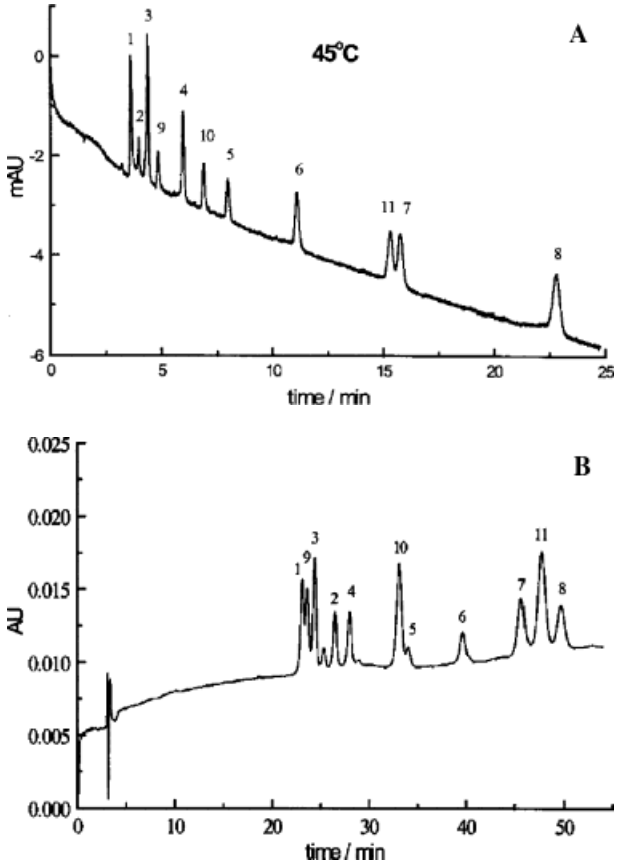

Figure 20. Comparison of (A) a CEC separation, and (B) a MEKC separation of (1) DMNTH, (2) formaldehyde DMNT hydrazone, (3) acetaldehyde DMNT hydrazone, (4) propaldehyde DMNT hydrazone, (5) butyraldehyde DMNT hydrazone, (6) valeraldehyde DMNT hydrazone, (7) hexaldehyde DMNT hydrazone, (8) heptalhyde DMNT hydrazone, (9) acetone DMNT hydrazone, (10) crotonaldehyde DMNT hydrazone, (11) *p*-tolualdehyde DMNT hydrazone. Reprinted from [131], with permission.

method for the determination of phenols in soil by supercritical fluid extraction – CEC was developed by Fung and Long [133]. The use of supercritical CO_2 with methanol as organic modifier was found to be sufficient for the extraction of alkylphenols from soil. Under optimized experimental conditions, baseline resolution between ten phenols was achieved using RP-CEC. A review about recent advances in CEC of pollutants has appeared in this journal [134].

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8 Addendum

Table 1. Applications in enantiomeric separation

Compounds	Mode	Stationary phase	Mobile phase	Ref.
Chiral stationary phases				
Thalidomide, warfarin, coumachlor, felodipine	CEC	Vancomycin immobilized acrylamide continuous bed	ACN:MeOH:TEA:HOAc 20:80:0.1:0.1 v/v% ACN:thiethylamine acetate (pH 5–6.5) 15:85 v/v%	[16]
Antidepressants (venlafaxine, *O*-desmethyl-venlafaxine)	CEC	5 μm vancomycin derivatized Lichrospher silica	ACN:ammonium acetetate buffer 100 mM (pH 6) 90:10 v/v% with varying ACN content	[17]
Antidepressants (mianserin, venlafaxine, *O*-desmethyl, venlafaxine)	CEC	5 μm vancomycin derivatized Lichrospher silica	ACN:MeOH: ammonium acetate buffer 13 mM with varying ACN:MeOH ratio	[18]
Bronchodilators (cienbuterol, salbutamol)				
Antihypertensives (alprenolol, oxprenolol, propranolol)				
Glycyl-dipeptides	CEC	3.5 μm Teicoplanin aglycone immobilized silica gel	MeOH:ACN varying compositions ACN:EtOH: 0.2% trietylamine acetate buffer (pH 4.1), varying compositions	[19]
Amino acids, β-blockers	CEC	3.5 μm Teicoplanin aglycone immobilized silica	MeOH: 0.2% TEAA (pH 4.1) 40:60 v/v% MeOH:ACN:HOAc:TEA varying compositions	[20]
Hydroxy acids (mandelic acid, *m*-, *p*-hydroxymandelic acid, 3-hydroxy-4-methoxymandelic acid, 4-chloromandelic acid, 2-phenyllactic acid)	CEC	5 μm (Hepta-Tyr) Lichrospher diol silica mixed mode with 5 μm amino silica	ACN:ammonium actate buffer (pH 6), varying ACN content ACN:MeOH:ammonium acetate buffer (pH 6), varying ACN and MeOH content	[21]
Hydroxy acids (mandelic acid, *m*-, *p*-hydroxymandelic acid, 3-hydroxy-4-methoxymandelic acid, 2-phenyllactic acid, mandelic methyl ethyl esters)	CEC	5 μm (Hepta-Tyr) Lichrospher diol silica mixed mode with 5 μm amino silica	ACN:ammonium acetate buffer (pH 6), varying ACN content ACN:MeOH:ammonium actate buffer (pH 6), varying ACN and MeOH content	[22]
Trans-stilbene oxide, warfarin, praziquantel, bendroflumethiazide, benzoin	CEC	5 μm Cellulose Tris-phenylcarbamate bonded silica	MeOH:hexane; 2-propanol-THF/5 mM acetic triethylamine buffer (pH 6.5), different compositions ACN:phosphate buffer 2 mM (pH 6.9) 60:40 v/v%	[23]
Indapamide, benzoin, PTH-Mer, phenylbutanol, pindolol	CEC	3 μm OD cellulose Tris-phenylcarbamate bonded silica	ACN: phosphate buffer 10 mM (pH 7.2), varying ACN content	[24]
Tozolin, *trans*-stilbene oxide, laudanosine, piprozolin	OT-CEC	Cellulose (3,5-dichlorophenylcarbamate), covalently bonded	Hexane:2-propanol 95:5 v/v% MeOH	[25]
Ambucetamide, aminoglutethimide, etozolin, nifurtimox, norgestrel, omeprazole, thalidomide analog, Tröger's base	CEC	5 μm Amylose Tris(3,5-dimethylphenyl carbamate bonded silica	ACN:ammonium acetate buffer, 5 mM 60:40 v/v%, varying compositions	[27]
Ambucetamide, benzyl-2-(benzylsulfinyl) benzoate, etozolin, *C*-methylthalidomide, metomidate, miconazole, nomifensine, norgestrel, omeprazole, piprozolin, thalidomide	CEC	5 μm amylose Tris-(3,5-dimethylphenyl carbamate bonded silica	MeOH: ammonium acetate buffer 15 mM	[28]

Table 1. Continued

Compounds	Mode	Stationary phase	Mobile phase	Ref.
Dansyl amino acids	CEC	β- and γ-CD carbamate bonded silica	5–10 mM MES in MeOH (pH 3–6); 5 mM phosphate buffer (pH 6); 20 mM MES in MeOH (50:50 v/v%; 9 mM MES in MeOH:H_2O (pH 6), 90:10 and 80:20 v/v%	[29]
Aminophenols, dihydroxybenzenes, nitrophenols	OT-CEC/ CEC	Sol-gel coated β-CD columns, β-cd matrix columns	EtOH:phosphate buffer, 20 mM (pH 5.3–8), 20:80 v/v%	[30]
Barbiturates, chlorinated alkyl phenoxypropanoates	CEC	Permethylated β-CD modified 5 μm Nucleosil	MeOH: ammoniumacetate buffer, 0.5 mM (pH 6.6) 60:40 and 70:30 v/v%	[31]
Indapamide, nadolol, pindolol, promethazine	CEC	Crown ether capped β-CD bonded silica	ACN:Tris 10 mM (pH 8), varying ACN content, ACN:phosphate buffer, 5 mM (pH 8.8), varying ACN content	[32]
Propranolol, tolperisone, α-methyl-benzylamine, ketoprofen, phynylalanine, mandelic acid benzyl ester, e.o.	CEC	Cyclam-capped β-CD bonded silica particles	MeOH:Tris 10 mM (pH 8.6); ACN:Tris, 10 mM (pH 8.6); ACN:Tris, 10 mM (pH 8.6), containing 2 mM $Ni(ClO_4)_2$	[33]
Toluidines, pyridines, picolines, dihydroxybenzene	OT-CEC	*p-tert*-Butylcalix[6]-1,4-crown-4 and *p-tert*-butylcalix[6]arene	20 mM phosphate buffer (pH 5–9), MeOH:phosphate buffer, 20 mM (pH 6–8), 20:80 v/v%	[34]
Primary amino compounds	CEC	(+)-Tetraallyl 18-crown-6 carboxylate, (+)-18 crown-6 tetracarboxylic acid 2-allyl ester, both covalently bound to an acrylamide monolith	200 mM triethanolamine: 300 mM boric acid (pH 6); ACN:aq boric acid, 300 mM, 50:50 v/v% 200 mM triethanolamine: 300 mM boric acid (pH 6): ACN 80:20 v/v%	[35]
Mephobarbital, hexobarbital, benzoin, carprofen	CEC	Silica monolithic coated with Chirasil-β-Dex	MeOH:MES (pH 6), varying MeOH content; MeOH:MES-Tris (pH 6), 40:60 v/v%	[36]
Tryptophan	CEC	Bovine serum albumin-encapsulated sol-gel column	20 mM phosphate (pH 7)	[37]
Dansyl amino acids, hydroxy acids	CEC	L-Prolinamide modified silica sol-gel colum	ACN:0.25 mM $Cu(Ac)_2$/50 mM NH_4Ac at different ratios (pH 5.5)	[38]
Dansyl amino acids	CEC	Acrylamide sol-gel column modified with L-ProA, L-PheA, L-AlaA	ACN:$Cu(Ac)_2$- NH_4Ac at different ratios (pH 5.5)	[39]
β-Blockers, amino alcohols, anesthetics, antimalarial agents, phenothiazines, antihistaminics	CEC	*N*-(4-allyloxy-3,5-dichlorobenzoyl -1-amino-3-methylbutane phosphonic acid modified 3.5 μm silica Kromasil	ACN:MeOH 80:20 v/v% containing: 10 mM malonic acid and 5 mM TEA; 100 mM triethanolamine and 10 mM acetic acid; 50 mM 2-aminobutanol and 4–12 mM formic acid, with varying eluent composition	[40]
Basic compounds	CEC	*N*-(4-Allyloxy3,5-dichlorobenzoyl) -2-amino-3,3-dimethylbutane-sulfonic acid , phosphonic acid, and carboxylic acid modified 3.5 μm silica Kromasil	ACN:MeOH 80:20 v/v% with formic acid 50 mM, and 2-amino-1-butanol 25 mM	[41]
Hydroxy acids	CEC	L-Hypro as chiral selector on acrylamide continuous beds	20 mM phosphate buffer, 0.1 mM Cu(II) (pH 4.5–7)	[42]
Trp-Arg, Arg-Trp	OT-CEC	G-quartet-forming DNA 5'-GGTTGGTGTGGT TGG-3'; 5'-GGGGTTGGGGTGTGGGG TTGGGG-3'	Tris 25 mM (pH 7.2), 2 mM KCl; NH_4HCO_3 50 mM (pH 7.2) 2 mM KCl	[43]
β-Lactoglobulin A and B	OT-CEC	G-quartet-forming DNA 5'-GGTTGGTGTGGTTGG-3', 5'-GGGGTTGGGGTGTGGGG TTGGGG-3'	Tris 25 mM (pH 7.3); phosphate buffer 10 mM (pH 7.3)	[44]

Table 1. Continued

Compounds	Mode	Stationary phase	Mobile phase	Ref.
Dansyl threonine, ketoprofen	CEC	Avidin physically adsorped to capillary	10 mM phosphate buffer (pH 5.95); MeOH:phosphate buffer 10 mM (pH 5.95), 15:85 v/v%	[45]
Chiral additives				
Tryptophan, praziquantel, atropine, metoprolol, verapamil	CEC	5 μm strong-anion-exchange stationary phase	MeOH: HAC-TEA 20–30 mM (pH 4) and 2 mg/mL sulfated β-CD with varying eluent compositions	[52]
Benzoin, warfarin, tryptophan	CEC	5 μm strong-anion-exchange stationary phase	ACN:phosphate buffer 5 mM (pH 6.5) 5:95 v/v% with 0–250 μM tryptophan	[53]
Kynurenine	CEC	Human serum immobilized acrylamide continuous bed	Phosphate buffer, 20 mM (pH 7.2)	[54]

Abbreviations: TEA, triethylamine; TEAA, triethylamine acetate

Table 2. Applications in biochemical analysis

Compounds	Mode	Stationary phase	Mobile phase	Ref.
Amino acids and amines				
Domoic acid	CEC	3 μm C_{18} Unimicro Technologies	ACN:Tris 5 mM (pH 8), 80:20 v/v%; ACN:phosphate buffer 5 mM (pH 2.5), varying ACN content	[55]
Biogenic amines (histamine, Ser, Tyr, putrescine, cadaverine)	CEC	5 μm C_{18} UG 120	ACN:borate buffer, 10 mM (pH 10), 70:30 v/v/%	[56]
Dansylated dialkylamines:	CEC	3 μm C_{18} Hypersil	ACN:Tris 50 mM (pH 8) 90:10 v/v%	[57]
ethanolamine, butylamine, hexylamine	CEC	Methacrylate monolith	ACN:phosphate buffer 5 mM (pH 6), 80:20 v/v%	[4]
Hydrophobic amines	CEC	Acrylamide monoliths	ACN:phosphate buffer 12 mM (pH 2.75), 70:30 v/v%	[58]
Amino acids	OT-CEC	Rh(III)TPP(m-Oph)$_4$OAC	50 mM Na-100 phosphate buffer 100 mM (pH 2.25); 50 or 100 mM Tris-phosphate buffer 100 mM (pH 2.25); 100 mM phosphate buffer (pH 2.5–10.0)	[59]
Amino acids, diglycine, triglycine	OT-CEC	Rh(III)TPP(m-Oph)$_4$OAC	0.1 M phosphoric acid, 0.05 M Tris (pH 2.1); 0.05 M disodium tetraborate buffer (pH 9)	[60]
Heterocyclic aromatic amines; amino(di)methylimidazoquinolines, aminodimethylimidazoquinoxaline, aminopyridoindole, aminomethylphenylimidazopyridine	OT-CEC	Fluorosurfactants dynamically modified DMA-SO^{3-}; DMA-$N+(CH_3)_3$ physically adsorbed C_{18}; cholesteryl 10-undecanoate and diol chemically modified	30 mM phosphate-19 mM Tris (pH 2.06); 30 mM citric acid-25 mM β-alanine (pH 3.16); 30 mM acetic acid-37.5 mM GABA (pH 4.4); 30 mM MOPS-20.7 mM imidazole (pH 6.72)	[61]
Peptides				
Carbetocin, B-peptide, A-peptide, oxytocin, desmopressin, terlipressin, triptorelin, angiotensin ii	CEC	3 μm C_8, C_{18} Hypersil C_{18}/SCX Spherisorb	ACN:phosphate buffer (pH 2–5); ACN:ammonium acetate buffer (pH 4–5.2); ACN:Tris (pH 8)	[62]

Table 2. Continued

Compounds	Mode	Stationary phase	Mobile phase	Ref.
Peptides	CEC	3 μm C_{18} Hypersil	ACN:acetate buffer (pH 3.8–5.2), varying ACN content	[63]
Basic peptides: Angiotensin I and II, [Sar^1,Ala^8] angiotensin II, [Phe^7]bradykinin	CEC	5 μm 180 Å Glycolic cation-exchange silica particles	20–70 mM NaCl in phosphate buffer 20 mM (pH 4.5)	[64]
Oligopeptides	p-CEC	3 μm C_{18} Unimicro	ACN:TFA gradient	[65]
Charged peptides	p-CEC	5 μm 300 Å PolyHydroxethyl A	ACN:TEAP 10 mM (H 3)	[66]
VYV, [Met^5]-enkephalin, [Leu^5]-enkephalin, desmopressin, carbetocin, oxytocin, peptide A	CEC	3 μm C_{18} Hypersil	ACN:acetate buffer 3 mM (pH 6.7), 50:50 v/v%	[67]
Di- and tripeptides	CEC	Lauryl methacrylate monolith	Phosphate buffer 40 mM (pH 2.1)	[68]
Dipeptides: pyridine, quinoline, nitroaniline, dinitroaniline, dichlornitroaniline benzyl alcohol, benzylpropanol, anisole, ethyl benzene, trimethyl benzene, tetramethylbenzene, butylbenzene	CEC	Mixed-mode 2-(sulfooxy)ethyl methacrylate monolith	ACN:phosphate buffer 8–64 mM (pH 3), varying ACN content	[69]
Papain inhibitor, proctolin, opoid peptide, Ile-angioensin III, angiotensin III, GGG, Arg, Ser, Gly, Phe, Trp	CEC/Chip	Acrylate monolith	ACN:borate buffer 25 mM (pH 8.2), 30:70 v/v% ACN:phosphate buffer 20 mM, 10:90 v/v%	[70]
Bioactive peptides: Leu-enkephalin-Lys, Met-enkephalin-Arg-Phe, α-casein, Met-enkephalin-Lys, β-lipotropin, thymopentin, splenopentin, kyotorphin, PTH amino acids	CEC/Chip	Acrylate monolith	ACN:phosphate buffer 25 mM (pH 7.3), varying ACN content	[71]
Tryp, Ser, Trap peptides	OT-CEC	*n*-Octadecyl modified, cholesterol modified	60 mM phosphoric acid buffer -38 mM Tris (pH 2.14)	[73]
Synthetic peptides	OT-CEC	Fluorosurfactants dynamically modified DMA-SO^{3-}; DMA-$N+(CH_3)^3$ physically adsorbed C_{18}; cholesteryl 10-undecanoate and diol, chemically modified		[61]
Aromatic tripeptides	OT-CEC	$(H_2TTP(m\text{-}PPh)_4$ (Rh(III)TPP(m-Oph)$_4$	Phosphate buffer 100 mM (pH 2.5–10); Tris 50 mM-phosphoric acid (pH 2.25, 8)	[72]
Proteins				
Basic proteins (myoglobin, ribonuclease, α-chymotrypsinogen, cytochrome *c*) cytochrome *c* variants (bovine, horse, rabbit)	CEC	3–5 μm Glycidyl methacrylate-divinylbenzene beads	50–200 mM NaCl in 20 mM phosphate buffer (pH 6–8)	[75]
Bovine serum albumin	CEC/Chip	AMPS-modified COMOSS columns; poly(styrenesulfonate)-modified PDMS COMOSS ODS-modified COMOSS	1 mM Tris (pH 8.5) 1 mM Tris (pH 8.5) and 2.5% 1-propanol; 1 mM carbonate buffer (pH 8.7); 1 mM carbonate buffer (pH 8.7) and 0.2% SDS	[76]
Bovine serum albumin	CEC/Chip	COMOSS columns modified with: AMPS; C_{18}-AMPS; PS-SA; VSA; PAA; StMA-AMPS; C_8; C_{18}	1 mM carbonate buffer (pH 9)	[77]
Bovine serum albumin	CEC/Chip	Trypsin-modified silica (5 μm) Cu(II)-IMAC of 5 μm silca, AMPS-modified RP-COMOSS (5 × 5 μm)	1 mM phosphate buffer (pH 7)	[78]

Table 2. Continued

Compounds	Mode	Stationary phase	Mobile phase	Ref.
Lysozyme tryptic digest	CEC	Methacrylate monolith containing Startburst dendrimers	ACN:phosphate buffer 40 mM (pH 2)	[79]
Nucleosides and nucleotides				
HIV reverse transcriptase inhibitors: zidovudine, lamivudine, didanosine, stavudine, hivid	CEC	5 μm β-CD-bonded silica 3 μm phenyl silica Hypersil	ACN:acetic acid/ammonia buffer 20 mM (pH 5), 80:20 v/v%	[80]
HIV reverse transcriptase inhibitors: zidovudine, lamivudine, didanosine, stavudine, hivid	CEC	5 μm β-CD-bonded silica 3 μm phenyl silica Hypersil	ACN:acetic acid/ammonia buffer 20 mM (pH 5) 80:20 v/v%	[81]
Nucleoside mono-, di-, triphosphates	OT-CEC	Sapphyrin-modified column	25 mM boarate-acetate buffer (pH 7); 25 mM borate-phosphate buffer (pH 7)	[82]
Double-stranded nucleotides	CEC/Chip	ODS-modified chip	ACN:TEAA 50 mM (pH 7.4), 25:75 v/v%	[83]
Double-stranded nucleotides	CEC/Chip	ODS-modified chip	ACN:TEAA 50 mM (pH 7.4), 25:75 v/v%	[84]
Carbohydrates				
Cellular carbohydrates in peanut fungal pathogens and baker's yeast	CEC	5 μm, 120 Å Nucleosil silica, in-house converted into C_{18}	ACN:phosphate buffer 3 mM (pH 5), 60:40 v/v%	[85]
Celluloses 2–500 kDa	SEEC	5 μm 100 Å Nucleosil silica 5 μm 300 Å Nucleosil silica	Acetone, containing 0.1 mM TBATAFB	[86]
Maltooligosacchardides, deoxyribose, fucose, xylose, *N*-acetylglucosamine, fructose, glucose, sucrose, maltose, fucitol, ribitol, xylitol, *N*-acetyl-gliucosaminitol, glucitol, mannitol	CEC	Acrylamide monoliths	ACN:water: ammonium formate buffer 240 mM (pH 3), varying eluent composition	[87]
Oligosaccharides: Dextran ladder *O*-glycans	CEC	Acrylamide monoliths	ACN:ammonium acetate buffer - 2.4 mM (pH 3) and 0.2 mM Na acetate 50:50 v/v%	[88]

Abbreviations: DMA, *N*,*N*'-dimethylacrylamide; GABA, γ-amino-*n*-butyric acid; TEAP, triethylamine phosphate; PS-SA, poly(styrenesulfonic acid); VSA, poly(vinylsulfonic acid); PAA, polyacrylamide; StMA-AMPS, stearyl methacrylate 2-acryl-amido-2-methylpropanesulfonic acid; TBATAFB, tetrabutylammonium tetrafluoroborate

Table 3. Pharmaceutical applications

Compounds	Mode	Stationary phase	Mobile phase	Ref.
Steroids				
Steroid hormones	CEC	Hypersil C_8 MOS Unimicro C_{18}	ACN, MeOH, THF: Tris 5 mM (pH 8), varying compositions	[90]
Cardiac glycosides and other steroids	CEC	3 μm ODS Hypersil 3 μm, 300 Å Grom-sil Octyl-4 FE (C_8) 3 μm, 300 Å Grom-sil butyl-2 FE (C_4) 5 μm, 300 Å Grom-sil butyl Si NP-1 (C_4)	ACN:phosphate buffer 2 mM (pH 7.1), 40:60 v/v% ACN:acetate buffer 25 mM (pH 5), 65:35 v/v%	[91]
Acidic drugs				
Profens	CEC	Methacrylate monolith	ACN:MeOH 80:20 v/v% containing 0.4 M acetic acid and 4 mM TEA	[5]

Table 3. Continued

Compounds	Mode	Stationary phase	Mobile phase	Ref.
Nonsteroidal anti-inflammatory drugs (flurbiprofen, ketoprofen, naproxen, indoprofen, suprofen, ibuprofen, fenprofen)	OT-CEC	Hisitidine-modified wall	MeOH, EtOH, propanol:acetate buffer 10–25 mM (pH 2–5), varying compositions	[93]
Nonsteroidal anti-inflammatory drugs	CEC	5 μm LiChrospher 100 C_{18}	ACN:acetate buffer 5 mM (pH 6), 80:20 v/v%; ACN:MES, 5 mM (pH 2.5–5), varying ACN content; ACN:MeOH: MES 5 mM (pH 5), 37.5:37.5:25 v/v%; MeOH: MES 5 mM (pH 5), 80:20 v/v	[94]
Acetylsalicylic acid, ketoprofen, phenytoin, propranolol, salicylic acid, warfarin	OT-CEC	Liposome wall modifier		[95]
Basic drugs				
Central nervous system drugs	CEC	3 μm Hypersil C_8 MOS 3 μm Hypersil Phenyl	ACN: acetate buffer (pH 7), 70:30 v/v% with ammonia, ethylenediamine, 1,3-diaminopropane as additives	[96]
Acidic and neutral impurities in heroin	CEC	1.5 μm nonporous ODS II particles sulfonic C_{12} monolith	ACN:Tris 5 mM (pH 9), varying ACN content	[97]
Metoprolol, iprenolol, formoterol, salbutamol, lidocaine, ropivacaine, nortriptyline, amitriptyline, 1-phenyl, 1,2-ethanediol	CEC	3 μm, 100 Å bare GromSil particles Activated, silylated, and oxidized	ACN:phosphate buffer (pH 2.8), varying ACN content	[98]
Vitamins and food components				
α-, δ-, γ-Tocopherol (vitamin E)	CEC	5 μm RP_{18} LiChrospher	MeOH – ACN: 0.01% v/v acetate buffer, varying ACN contents	[101]
α-, β-, δ-, γ-Tocopherol α-acetate	CEC	3 μm Hypersil ODS 3 μm Polymeric C_{30} Bischoff (140 Å) 3 μm Monomeric C_{30} Nomura	MeOH Acetone MeOH:acetone 50:50 v/v% MeOH:acetone:water 45:45:10 v/v%, MeOH:water varying MeOH content	[102]
β-, γ-, δ-, ζ_2-, ε- Tocopherol, tocotrienols	CEC	3 μm C_8 silica Hypersil 3 μm C_{18} silica Hypersil 3 μm phenyl silica Hypersil	ACN, MeOH, ACN/MeOH Tris, phosphate buffers, with varying compositions	[103]
Tocopherols, tocotrienol, sterols (lanosterol, ergosterol, stigmasterol, sitosterol)	CEC	3 μm pentafluorophenyl silica 5 μm triacontylsilica 3 μm Hypersil ODS	'MeOH:Tris 25 mM (pH 8) ACN:Tris 25 mM (pH 8) MeCN in MeOH 2.5 mM Li acetate DMF in MeOH 2.5 mM Li acetate, with varying compositions	[104]
Trans-retinyl esters	CEC	Sol-gel C_{30} material using 5 μm Nucleosil 4000 Å	DMF, ACN, MeOH different eluent compositions containing 2.5 mM lithium acetate	[105]
Isoflavones (daidzin, genistin, daidzein, genistein, apigenin, glycitin, glycitein, acetyl daidzin, acetyl henistin, malonyl genistin	CEC	Acrylate monolith	ACN:MeOH ammonium formate buffer 2.4 mM (pH 2.7) with varying ACN composition	[106]
Withanolides (withaferin, iochromolide, withacnistin)	CEC	3 μm Hypersil C_{18}	ACN 10 mM HCOOH/NH4OH (pH 3–8) with varying ACN composition	[107]
Micelleneous				
Losartan, hydrochlorothiazide	CEC	5 μm RP-18 LiChrosorb	ACN:H_2O 50 mM NH_4acetate buffer (pH 7) 75:15:10 v/v%	[108]
Tricyclic antidepressants	CEC	5 μm, 80–800 Å silica	ACN phosphate buffer 0.125 M (pH 2.8–7.5) with varying ACN composition	[110]

Table 4. Industrial and environmental applications

Compounds	Mode	Stationary phase	Mobile phase	Ref.
Inorganic anions and cations				
Synthetic peptides	OT-CEC	C_{18} and cholesterol-modified capillary	MeOH:buffer, various pH	[112]
Inorganic anions: Br^-, I^-, NO_2^-, NO_3^-	CEC	Silica monolithic sol-gel columns	30 mM Tris buffer (pH 8.05) with 0.025% PDDAC	[113]
Inorganic anions: Cl^-, IO_3^-, BrO_3^-, NO_2^-, Br^-, NO_3^-, I^-,F^-, SO_4^{2-},PO_4^{3-}	CEC	Dionex AS9-HC	HCl 10 mM (pH 8.05); H_2SO_4 5 mM (pH 8.05); $HClO_4$ 5 mM (pH 8.05); Sorbic acid 10 mM: arginine 20 mM (pH 8.9); Na_2CrO_4 5 mM: histidine 25 mM (pH 7.7); *p*-toluenesulfonic acid 2.5–50 mM:Tris 5–100 mM (pH 8.05)	[114]
Inorganic anions	OT-CEC	Coating of 75 nm latex particles	10 mM $HClO_4$ or naphthalenedisulfonic acid in 20 mM Tris buffer	[115]
Inorganic anions	OT-CEC	Coating of 75 nm latex particles	10 mM naphthalenedisulfonic acid in 20 mM Tris buffer	[116]
Synthetic polymers				
Polystyrene, polymethylmethacrylate, polycarbonate, polycaprolactam, poly(ethylene terephthalate)	SEEC	5 μm 300 Å Nucleosil silica 10 μm 100 Å Nucleosil silica	DMF: LiCl 0.1 mM HFIP: TBATFB 1.0 mM	[117]
Polystyrene, polyurethane, polycarbonate	SEEC	Sulfonated polystyrene/divenylbenzene	THF:H_2O varying compositions	[118]
Polyaromatics				
Aromatics	CEC	3 μm ODS, 5 μm silica gel	MeOH:Tris 4 mM, 80:20 v/v%; ACN:Tris 4 mM, 80:20 v/v%; ACN: Tris 4 mM 90:10 v/v%	[119]
Aromatics	CEC	3 μm ODS, 5 μm silica gel	MeOH:Tris 4 mM (pH 8.7), 60:40 (v/v)%, varying ACN compositions	[120]
Novel polysiloxane with metal complex	CEC	3 μm 80 Å and 3.5 μm 100 Å bare silica	ACN in 10 mM Tris-HCl, 80:20 v/v%	[121]
Benzodiapines	OT-CEC	Polyelectrolyte multiplayer-coated capillary	0.5 w/v% PDADMAC, 0.5 w/v% poly(L-SUG) in 0.2 M NaCl	[14]
Benzodiapines phenols	OT-CEC	Polyelectrolyte multiplayer-coated capillary	0.5 w/v% PDADMAC, 1 w/v% poly(L-SUG) in 0.2 M NaCl	[15]
Toluene, ethylbenzene, *p*-ethylaniline,acetyl salicylic acid, salicylic acid, dihydroxybenzoic acid, 2-hydroxyhippuric acid	CEC	SAX/C_{18} mixed mode monolith	ACN: phosphate buffer 5–10 mM (pH variable), 70:30 v/v% ACN: Tris 20 mM (pH 7), 70:30 v/v%	[122]
Benzylamine hydrochloride, diphenylhydramine chloride, nortriptyline hydrochloride, salbutamolsulfate, nicotine, procainamide hydrochloride	CEC	3 μm Hypersil CEC; basic C_{18}, Hypersil ODS, Hypersil BDSA C_{18}, Hypersil BDS C_{18}, Hypersil C_{18}, HyPURITY C_{18}	ACN:buffer 60:40 v/v% (phosphate, Tris and MES buffers)	[123]
Phenones, benzaldehydes	CEC	5 μm C_{18} Hypersil	ACN:acetate buffer 2 mM (pH 3.7–4.7), varying ACN content ACN:phosphate buffer (pH 7.7), 30:70 v/v%	[124]
Pesticides, insecticides and herbicides				
Aromatic phenols	CE CEC	5 μm, 120 Å Nucleosil Silica in-house converted into ODS	ACN:phosphate buffer 5 mM (pH 6), varying ACN content	[125]
Carbamate insecticides	CEC	5 μm, 120 Å Nucleosil silica in-house converted into ODS ODS in lab	ACN:(THF):NaH_2PSO_4 10 mM (pH 6), varying compositions	[126]

Table 4. Continued

Compounds	Mode	Stationary phase	Mobile phase	Ref.
Carbamate and pyrethroid insecticides: carbofuran carbaryl methiocarb fenpropanthrin sanmarton permethrin	CEC	5 μm, 120 Å Nucleosil silica in-house converted into ODS 5 μm 100 Å Nucleosil ODS 5 μm 120 Å ODS (J.T. Baker) 5 μm C_8 zorbax	ACN:THF:acetate buffer 5–14 mM (pH 6), varying compositions	[127]
Pyrethroid insecticides	SM-CEC	5 μm, 120 Å Nucleosil in-house converted into ODS	ACN:acetate buffer 20 mM (pH 6.0), 75:25 v/v% ACN:Tris (pH 8): DOSS, varying compositions	[128]
Pesticides, alkyl phenols, polyaromatic hydrocarbons, phenones	CEC	Diacrylate monostearate monolith	ACN:phosphate buffer (pH 6), 60:40 v/v% ACN:ammonium acetate buffer (pH 4.5)	[129]
Miscellaneous				
Carbonyls	CEC	5 μm Spherisorb ODS I 3 μm C_{18} Hypersil	MeOH: Tris 2 mM (pH 8.8), with varying compositions ACN: Tris 2 mM with varying compositions and different pH	[131]
4-hydroxbenzoic acid, methylparaben, ethylparaben, propylparaben, butylparaben, benzylparaben	CEC	5 μm C_{18} particles	ACN: formate buffer 5 mM (pH 3), varying composition; ACN: acetate buffer 5 mM (pH 5), 80:20 v/v% ACN: MES 5 mM (pH 6), 80: 20 v/v%	[132]
Phenols	SFE-CEC	3 μm ODS (Unimicro)	ACN:Tris 4 mM (pH 7) 65:35 v/v%	[133]

Abbreviations: PDDAC, poly(diallyldimethylammonium chloride); SAX, strong-anion-exchange; poly(L-SUG), poly(sodium *N*-undecylenyl-L-glycinate); SM-CEC, surfactant-mediated CEC; DOSS, dioctyl sulfosuccinate