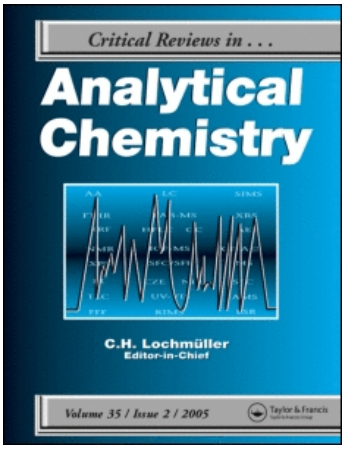

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The Place of Capillary Electrochromatography Among Separation Techniques—A Review

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The Place of Capillary Electrochromatography Among Separation Techniques—A Review

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Replacing pressure-driven flow with electroosmotic flow in chromatography with packed capillary columns results in a new and powerful analytical technique, combining the advantages of high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE): capillary electrochromatography (CEC). In essence, it involves packing CE capillaries with HPLC stationary phases and applying a voltage across the packed capillary, which generates an electroosmotic flow (EOF) that transports solutes along the capillary toward the detector; on the way, both differential partitioning and electrophoretic migration of the solutes occur, resulting in their separation. This dual separation mechanism can afford unique selectivities. Also, the plug-flow profile of EOF reduces flow-related band broadening, so that separation efficiencies of several hundred thousand plates per meter are often obtained (i.e., one or two orders of magnitude greater than those of present conventional chromatographic systems). There is no back pressure when EOF occurs, so small particle sizes (1–3 μm) and/or long columns can be used. Generally, carrier electrolytes containing high levels (40–80%) of organic solvents such as methanol or acetonitrile are employed, making it useful for water-insoluble compounds, which can be difficult to analyse by CE. CEC can be faster than HPLC, and gradient-elution CEC is being developed as an alternative to gradient-elution liquid chromatography of polymers.

Keywords capillary electrochromatography, electroosmotic flow, microseparation techniques

INTRODUCTION

The Basis of Electrochromatography

In recent decades a number of new separation techniques employing high voltages and narrow bore capillaries have emerged (1). They include capillary electrophoresis (CE) or capillary zone electrophoresis (CZE), capillary gel electrophoresis (CGE), micellar electrokinetic capillary chromatography (MECC) and capillary electrochromatography (CEC). The first two can be viewed as high-tech instrumental analogues of the gel-slab electrophoresis. MECC makes use of a form of chromatographic partition to achieve the separation of the components. However, the capillary electrotechnique which most closely resembles modern high-performance liquid chromatography (HPLC) is CEC. The essential difference between HPLC and CEC is that in conventional liquid chromatography the flow of eluent is generated by the application of a pressure gradient, which is usually provided by means of a high-pressure mechanical pump; in CEC, the mobile phase is forced to migrate through the chromatographic medium by the application of an electric field. This phenomenon is commonly referred to as electroosmosis or electroosmotic flow (EOF) (2, 3). CEC thus aims to combine the strengths of two powerful analytical techniques, CE and micro-HPLC (Figure 1).

Depending on the particular application, the CEC stationary phase (e.g., unmodified or coated silica gel) is packed into a capillary (packed column CEC), attached to the capillary wall (open tube CEC) or added to the mobile phase as a "pseudo" stationary phase (4, 5). Like CE, CEC offers low solvent and sample consumption together with short analysis times. It also offers much greater separation efficiencies than HPLC. Finally, and most importantly, the combination of chromatographic and electrophoretic separation mechanisms means that completely new separations are possible (e.g., for charged analytes).

EOF differs from pressure-driven laminar flow in three important aspects (6):

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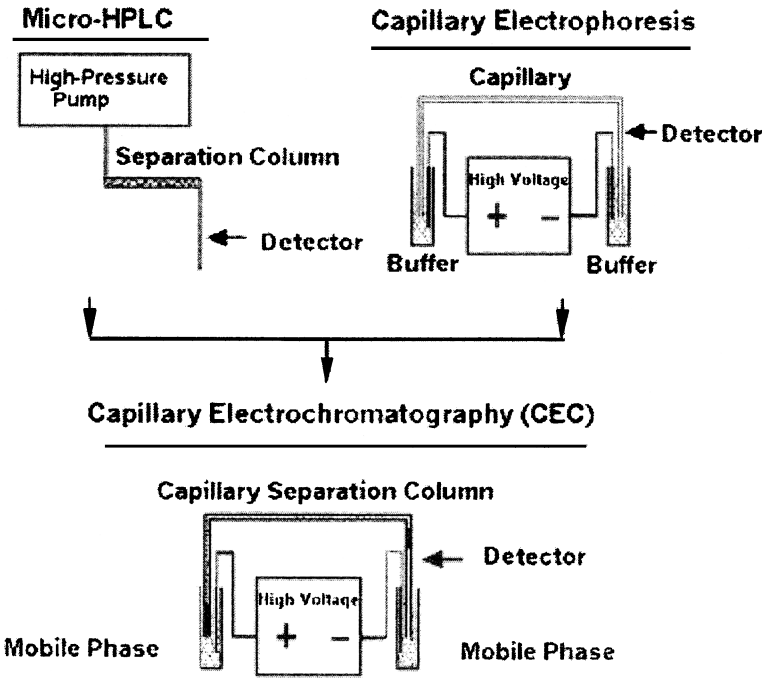

FIG. 1. CEC combines the strengths of CE and micro-HPLC.

1. it has a plug flow profile, that is, at distances $> \sim 1$ nm flow velocity does not vary with distance from the channel wall as it does in hydraulic flow (Figure 2);
2. in channels between 0.1 μm and approximately 150 μm, the linear EOF velocity is independent of the channel width; and
3. no column back pressure is generated.

At surfaces that present charged moieties, the surface charge is compensated by ions from the bulk solution in contact with the surface. When an electric potential is applied parallel to the surface, the ions are electrophoretically dragged toward the oppositely charged electrode. This motion creates shear forces in the bulk liquid and the bulk solution is dragged along the column. An important consequence of this driving mechanism is that it involves no pressure drop, which makes it feasible to use particles <1 μm in diameter as column packing (7). Furthermore, the plug-flow profile means that separation efficiency is much less affected by column packing inhomogeneities than with a pressure-induced parabolic flow

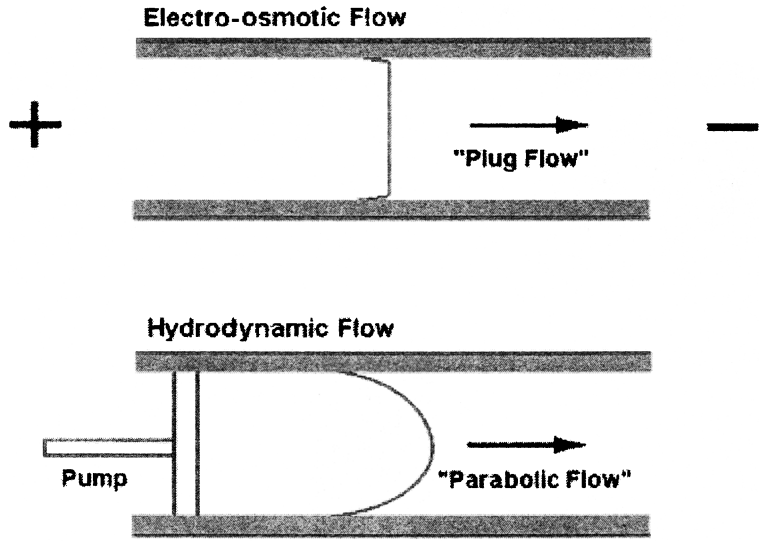

FIG. 2. Flow profile in CEC and micro-HPLC.

profile. With particles with diameters down to 1.5 μm, it has already been demonstrated that peak dispersion in CEC is less than in pressure-driven liquid chromatography (LC) by a factor $2 \sim 3$ (8).

The Place of CEC Among Separation Techniques

As a method characterized by the use of a particular class of stationary phases and a particular mechanism for driving the flow of the mobile phase, CEC occupies a place in the following two-dimensional array of separation techniques (Figure 3).

Of the other voltage-driven techniques in this scheme, capillary electrophoresis is suitable for separating charged components or compounds that are not charged in solution but interact with charged species such as ionic surfactants (9); while electro-driven size-exclusion chromatography (ED-SEC) can be faster and less susceptible to nonideally packed columns than conventional pressure-driven size-exclusion chromatography (SEC), although selectivity (and therefore separation) may be compromised by flow through pores. As we shall see below CEC, an alternative to HPLC, comprises variants such as gradient-elution CEC by the development of pressurized flow CEC (PEC) in which flow is driven by both pressure and voltage (10).

The Purpose of This Review

The aim of this review of CEC is to describe the kinds of separation problems for which it has been found to be suitable, to present guidelines for method optimization, to survey innovations that recently have been developed or currently are being researched, to point to twilight zones in our understanding of CEC, and to suggest areas in which research efforts might be most effective in realizing the full potential of this technique.

METHOD DEVELOPMENT

Instrumentation

Although with careful degassing it is possible to perform CEC on conventional CZE instruments, this usually restricts the operating voltage to <20 kV at ambient temperatures. N. W. Smith and Evans (11) described the use of a modified CZE instrument that allowed electrochromatography to be performed with no bubble formation using up to 90% of organic solvent at 30 kV and 40°C. Bubble formation was originally believed to be due to Joule heating, but subsequent research has suggested that bubbles are in fact formed at the frit adjacent to the detection window as a result of the change in electroosmotic flow on passage from the packed to the unpacked region of the capillary.

Packed Capillaries

Capillaries are usually packed with 3–5 μm C_{18} or C_8 stationary phases held in place with two retaining frits, although some other phases have been studied (12–14). The detection window is immediately adjacent to one of these frits so as to minimize postcolumn band broadening.

Basically, in constructing a column a retaining frit is made from silica, and a slurry of the stationary phase is then pumped into the capillary at high pressure. Once packed, a second

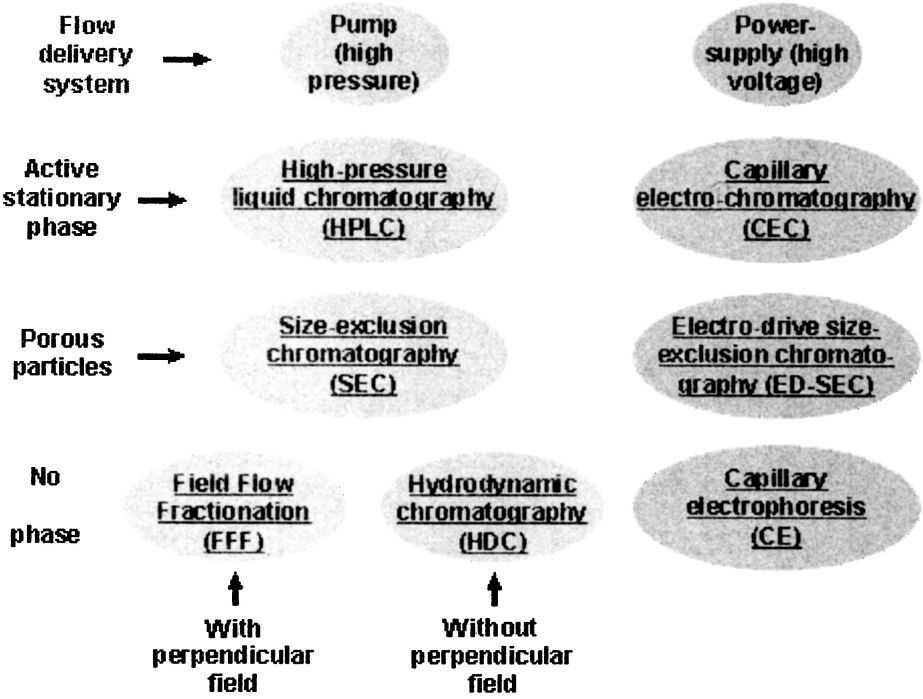

FIG. 3. Separation techniques.

retaining frit is burnt in place and excess packing material is removed by reversing the direction of flow. A detection window is then formed, and the capillary is ready for use. Capillaries of this kind are now available from several commercial manufacturers with a range of dimensions and stationary phases.

Operating Guidelines

Capillaries usually will be supplied by the manufacturer with a test certificate and will have been shipped in the mobile phase used to test their performance. It is highly recommended that the performance of a new capillary be checked against that certified by the manufacturer, especially retention time and efficiency data. Good capillaries packed with 3 μm materials should generate at least 150,000 plates per meter and anything less should be considered as underperforming.

Many problems that can arise in CEC may be avoided by adhering to the following guidelines.

1. Always filter samples prior to injection so that undissolved material does not block the inlet frit, thereby causing a breakdown in current and, consequently, flow.
2. Samples should be dissolved in the mobile phase or a weaker solvent.
3. Conditioning of columns depends very much on the mobile phase. Low pH buffers will take longer to equilibrate since the electroosmotic flow will be lower.
4. When possible, try to use organic buffers since these produce much lower currents and so allow the use of higher concentrations.
5. If working at low pH, use a mixed-mode phase which will provide a much better EOF than conventional octadecylsilica (ODS)-type phases.
6. Because capillaries are fragile, capillaries should be stored in their cell/cassette. It is removal of the capillary from the cell that is most likely to lead to breakage.
7. In CEC the overall buffer concentrations used are low, with the result that operating currents rarely exceed 10 μA. Where the buffer concentration or type leads to high currents, Joule heating can be prevented by capillary cooling systems.
8. Current breakdowns are usually due to bubble formation in the capillary. This normally can be rectified either by pressurizing one side of the capillary while applying a moderate voltage (e.g., 10–15 kV), or by attaching the capillary to an HPLC pump and passing either water or mobile phase through until the bubbles have been swept out (this normally takes less than 10 min). In the latter case, care should be taken to connect the capillary to the pump at the outlet end and not the end containing the inlet frit, which is easily broken. If water is the solvent used to sweep the capillary, there is no need to reintroduce the mobile phase before continuing electrochromatography, since the mobile phase will be electrophoresed into the capillary on application of a voltage even though the capillary contains water.
9. Pressurizing the capillary at both ends during each run helps suppress bubble formation.

Several studies have compared CEC with other microseparation techniques (gas chromatography (GC), LC, and CE) from a developmental viewpoint. To ensure the reliability and reproducibility of CEC for a given phase-bonded packed column, the parameters to be optimized are buffer pH, ionic strength, organic solvent content, temperature, and voltage, while other strategies are required to enhance sensitivity (15–18).

NONAQUEOUS ELUENTS AND GRADIENT ELUTION

Nonaqueous CEC

Jorgenson and Lukacs (19) were the first to employ nonaqueous mobile phases in CEC. Using 100% acetonitrile, a capillary packed with 10 μm Partisil ODS-2, a voltage of 30 kV, and a fluorescence detector at 58 cm from the inlet, they were able to resolve 9-methylanthracene from perylene with an efficiency of 31,000 theoretical plates for the 9-methylanthracene peak and 23,000 for the perylene peak.

Sepaniak (20) found that large polycyclic aromatic hydrocarbons (PAHs) and fullerenes could be separated by using acetonitrile-based CEC with efficiencies of 160,000 plates per meter, and that large amounts of less polar modifiers, such as methylene chloride and tetrahydrofuran, produced predictable decreases in k' values for these nonpolar test compounds. However, large volumes of modifiers ($\sim$50%) reduced flow rate fourfold, with a consequent increase in retention time despite the reduction in k' values. This suggests that these nonaqueous mixtures have a significantly lower zeta potential than 100% acetonitrile. Addition of organic salts to the mobile phase also gave rise to a large reduction in EOF.

Dorsey (21) reported electrophoretic velocities and mobilities in nonaqueous systems that were similar to or better than those measured in totally aqueous media. For example, with 100% acetonitrile electroosmotic flow was three times faster than with a 100% aqueous buffer of pH 10.9. Using 100% acetonitrile, Dorsey (22) was able to separate highly water-insoluble dyes on a 3 μm Hypersil ODS phase after the addition of triethylamine (TEA) as a competing base that significantly improved peak shape. In contrast to open tube electrophoresis, in a packed system (CEC) EOF increases steadily with increasing acetonitrile concentration (23–26).

Pressurized Flow CEC (PEC) and Gradient Elution

To achieve gradient elution, several workers have driven the mobile phase with a pump as well as electroosmotically (27–29). Behnke and Bayer (28), for example, coupled a microbore gradient HPLC system to a modular capillary electrophoresis system. A six-port rotary valve was used for injection, and the eluent from the micropump was passed through a stainless steel T-piece in order to split the flow. As a precaution, this splitter was earthed to prevent any voltage leaking

back and causing damage to these equipment. According to these authors, pressurization increased the stability and reproducibility of the separations and also prevented bubble formation. Among the samples separated with high efficiency were mixtures of oligonucleotides.

Taylor et al. (29, 30) separated drugs from equine biofluids using gradient elution CEC with electrospray ionization (ESI) mass spectrometric detection. The samples analysed included a mixture of seven thiazide diuretics, equine plasma samples spiked with hydrocortisone, and biofluids containing corticosteroids. The mobile phase consisted of 5 mM ammonium acetate in an acetonitrile/water gradient. For prolonged capillary life, sample clean-up was essential: a two-stage solid-phase extraction was used to purify equine urine, followed by dialysis to deproteinate equine plasma. It was possible to perform approximately 200 injections of urine extracts and still maintain sufficient efficiency, even though there was a 40% increase in peak width at half height.

Zare and coworkers (31) described a gradient CEC system in which the gradient was formed by controlling the electroosmotic flow from two separate solvent reservoirs by the use of two high-voltage power supplies. These two solvents flowed into the separation capillary via a low-dead-volume tee, and the required gradient was formed using a computer program to control the voltages of the two power supplies. Samples were introduced by removing the packed capillary from the tee piece, placing its inlet into a sample vial, and applying a small voltage. It was possible to resolve 16 PAHs using a gradient from 55% acetonitrile to 80% acetonitrile in aqueous buffer and a 26 cm capillary packed with 3 μm porous ODS stationary phase.

COMPATIBILITY WITH MASS SPECTROMETRY

The first article on capillary electrophoresis using on-line mass spectrometry (MS) as the detection system was published by R. D. Smith and coworkers in 1987 (32), while off-line CE-MS was developed in the early 1990s (33). Several reviews on this topic have appeared in the mid-1990s (34–36). Special attention should be paid to an article by R. D. Smith (37), because the microdialysis membrane tubing junction he described appears to alleviate many problems encountered with on-line MS. CE-MS is still not widely accepted for routine use because of the limitation on the volumes that can be analyzed without compromising separation efficiency, which results in poor concentration detection limits (38). Moreover, MECC is incompatible with MS. Off-line MS may be attractive to laboratories that do not wish to tie up an ESI mass spectrometer by coupling it to a CE unit. At the present time the off-line mode is the only commercial option for matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). In the case of HPLC, the advantages of HPLC miniaturization when coupling to electrospray mass spectrometers have been recognized for many years. For concentration detection, changing from a 4.6 mm i.d. to a 0.1 mm i.d. column, for example, theoretically should improve sensitivity significantly, although in practice improvement is limited by technical difficulties. Electrically driven separation methods offer solutions to many of these problems.

Verheij et al. (39) and Hugener et al. (40) have independently reported the use of PEC with mass spectrometric detection. Gordon et al. have reported CEC-MS coupling with both continuous flow fast atom bombardment (41) and ESI-MS (42), but noted a loss of resolution as a result of postdetection window dispersion in the length of unpacked capillary necessary for coupling the commercial CE instrument to the mass spectrometer. Lane et al. (43) showed that when fully packed capillaries were interfaced to the mass spectrometer, chromatographic efficiency could be upheld. CEC-ESI-MS separations of steroid mixtures and diastereomeric antibiotics were performed on 95 cm capillaries with excellent efficiencies and MS data, although analyses required up to 70 min due to the weak field strength across the long capillary. These separations were performed using nonvolatile buffers of high pH in order to generate sufficient electroosmotic flow on the C18 stationary phase that was used; these conditions were not optimal for CEC-MS, but were dictated largely by instrumental constraints in coupling the CE instrument to the mass spectrometer. Lane et al. regretted the lack of CEC-specific stationary phases promoting high EOF even with the low pH volatile buffers that are commonly used in MS. In order to be able to use shorter capillaries (43 cm), Lane's group later built a prototype integrated CEC-MS injection-separation interface with which they were able to separate the same steroid test mixture as before in 11 min instead of 70 (44).

ADVANTAGES AND DRAWBACKS

Advantages

The most interesting advantages of CEC are its high efficiency, high resolution, high selectivity, and high speed. Because of the plug-like profile of EOF, the column efficiency in CEC is much higher than that afforded by pressure-induced flow at the same linear velocity in the same column. This high efficiency means dramatic improvement in resolution. Furthermore, because the flow in CEC is independent of the spacing between the particles in the column, longer columns (up to 100 cm) containing very fine particles (down to 0.5 μm) can be used without giving rise to the increase in back pressure normally encountered in micro-HPLC. Consequently, efficiencies up to 200,000 theoretical plates per column (up to 700,000 plates per meter) are achievable (efficiencies of 20,000 plates per column are typically obtained in micro-HPLC) (45). Larger plate counts mean higher peak capacity (i.e., more peaks for given separation time and column length), which enables separation of more complex mixtures thanks to narrower, more symmetrical peaks and improved signal-to-noise (46–48). The amazingly high efficiency of CEC, achieved without loss of the high selectivity and versatility of micro-HPLC, provide the

analyst with an extremely powerful technique that can be used to tackle many challenging analytical problems. For example, although identification of the 14 nitroaromatic and nitramine explosives and their degradation products is very important in forensic and environmental applications, complete separation of these structurally similar compounds by HPLC usually requires gradient elution for 20 min; CEC can perform this task without a gradient. For a given particle size, there is of course a tradeoff between efficiency (longer columns) and speed (shorter columns). With particles of, say 1.5 μm, "ultra-fast" CEC separations can be performed with relatively high efficiencies on columns $<$10 cm in length (49).

CEC is also economic and environmentally friendly. Because of the small internal diameter of the capillary columns used in CEC, both solvent flow ($\sim$100 nL/min) and sample size ($\sim$nL) are reduced by a factor of about 10,000 compared to conventional HPLC.

Drawbacks

The conditions required for generation of high EOF (pH $>$7 so as to ionize silanols on conventional reversed phase (RP) packings) are not ideal for separation of charged molecules. Acidic analytes ionize and tend to migrate toward the anode (i.e., against the EOF) and to maintain their neutrality therefore require ion suppression (use of low pH electrolytes), which reduces EOF and increases retention times. Basic analytes interact with the ionized silanols on the capillary surface and/or packing, leading to peak tailing. Since many analytes contain basic nitrogen groups, this is the greatest obstacle to the development of CEC. Although excellent performance across the pH range 4 to 9 has been achieved with a stationary phase produced by PhaseSeparations, Ltd. (Deeside, UK), in which SO_3H groups are attached to 3 μm porous silica via a propyl linker, overall reliability and reproducibility with this phase are poor, rendering it unsuitable for routine use. It is to be hoped that, once a better understanding of the focusing effect has been achieved, it will be possible to design stationary phases allowing highly efficient CEC analysis of acidic, neutral, and basic compounds (50–54).

OT-CEC Versus Packed-Bed CEC

In a review published a few years ago (55), Wu et al. pointed out that open tube (OT) capillaries (OTCs) can have certain advantages over packed beds. OTCs with inner diameters of around 10 μm can have a smaller plate height and larger height-equivalent theoretical plate (HETP) than packed columns of the same internal diameter due to lack of the band-broadening associated with the presence of packing particles and end-of-column frits. Also the small diameter of OTCs results in high concentration sensitivity and allows the use of high field strength without significant Joule heating, and OTCs can often separate more rapidly than packed columns in which intraparticle diffusion limits separation speed. However, OTCs present serious difficulties with regard to sample injection and detection. The injection volume of OTCs is in the low nL or even pL range, and their very small inner diameters make optical detection difficult so that a detection method such as ESI-TOF-MS may be necessary.

EXISTING APPLICATIONS

Eventually the range of possible applications of CEC is likely to be similar to those of CE and HPLC (56), including impurity analysis, chiral separations, assays of major components of samples, and trace determinations. In recent years most attention has been paid to the separation of neutral drugs (57, 58) and PAHs (59), and to chiral separations (60–62). It is likely that this range of solutes will quickly be extended to include basic and acidic species such as vitamins, agrochemicals, and fine chemicals. It ultimately should be possible to find the optimum conditions for a variety of compounds and to produce generic routine CEC methods to cover a range of quantitative applications in the food, environmental, and pharmaceutical arenas (63–65).

For the reasons discussed in the previous section, the most common applications of CEC are separations of neutral analytes. For example, CEC has been very successfully applied to the separation of parabens and PAHs (59), and PAH-deoxyribonucleoside adducts have also been investigated (66). Uncharged compounds are not separated by conventional CE and, as we have seen, CEC is potentially more efficient and faster than HPLC.

For basic analytes, ion exchange may be used to overcome the difficulties mentioned in the Advantages and Drawbacks section (67). Smith and Evans separated various tricyclic antidepressants and low-molecular-weight neutral and basic pharmaceutical compounds using a strong cation exchange phase (68). Zhang et al. (69) separated erythromycin derivatives using monodisperse porous polymethacrylate microspheres that had been functionalized with quaternary ammonium/octadecyl groups to ensure positive charge over a wide pH range (the functionalized beads were packed into a fused-silica capillary.

For acidic analytes, ion suppression must be used. A quantitative CEC assay for paracetamol (acetaminophen) and aspirin (acetylsalicylic acid) tablets has been developed by Altria et al. (70). Tsuda and coworkers reported the simultaneous separation of anions and cations using anion exchange resins, PEC (to minimize bubble formation and push the anions past the detector), and indirect UV detection (71); cations separated due to their different electrophoretic mobilities through the packed bed, and anions due to both mobility differences and an ion exchange mechanism.

The application of CEC to the analysis of steroids has been investigated in detail by Wang et al. (72), who developed a method for the separation of norgestimate and its degradation products. Efficiencies for the main component and related impurities were in the range of 100,000 plates per meter. Correlation

coefficients of 0.998 or better were obtained for each of the components, and the relative standard deviations for the retention times and peak areas were less than 2%. Quantitation of impurities at relative concentrations of 0.1% was achieved in half the time required by the existing HPLC method.

As we saw in the Pressurized Flow CEC (PEC) and Gradient Elution subsection, Taylor and Teale have used gradient CEC with ESI-MS detection to separate thiazide diuretics, corticosteroids, and benzodiazepines (29, 30). Paterson and coworkers have also investigated the quantification of drugs in plasma (73).

OT-CEC using etched capillaries with a C18 lining were prepared and used by Pesek and Matyska for the separation of tetracyclines (74). The results obtained were better than those achieved using polymeric HPLC columns, and comparable to those obtained on diol columns. The detection limits (5–10 μg/mL) were similar to those of HPLC and CE methods.

Sandra et al. have reported that triglycerides can be analyzed faster and with better resolution using CEC than by micro-LC (75, 76). When applied to vegetable and fish oils, margarines, and pharmaceutical formulations, their CEC methods with photodiode array detection had retention times with *RSDs* less than 2% for hydrodynamic injections and less than 0.5% for electrokinetic injections.

At the U.S. Drug Enforcement Administration laboratories, CEC has been used to analyze complex mixtures of cannabinoids (77).

Fujimoto et al. reported the separation of dansyl amino acids on columns filled with linear and cross-linked polyacrylamides (78, 79). EOF was achieved by the inclusion of moieties such as 2-methyl-1-propanesulfonic acid in the polymer network to create a charged column. Phenylthiohydantoin amino acids have been separated by isocratic and gradient elution on monolithic ODS columns using acetonitrile/phosphate buffer (80–83).

The small reagent volumes required for CEC are an advantage for enantiomeric separations because chiral phases and additives are expensive. Suitable chiral selectors are those typically used in HPLC and CE, such as proteins, cellulose-based and Pirkle-type stationary phases, molecularly imprinted polymers, and cyclodextrins. Efficiencies and resolutions are better than with HPLC, but the detection sensitivity problem found in CE persists in CEC. Selectivity is generally good, but limits of detection are too high for practical application in the pharmaceutical industry (84–93).

CEC appears to offer promise for biopolymer separations due to its sharp, symmetrical peaks and the possibility of further enhancing resolution by the use of counteracting EOF and electrophoretic force (EPF). The judicious use of applied voltage, pH, organic content, and stationary phase should make it possible to generate larger differences in mobilities between similar peptides, proteins, nucleic acids, and (possibly) carbohydrates than with HPLC or high-performance capillary electrophoresis (HPCE) (94–96).

Palm and Novotny have described a monolithic capillary bed formed by copolymerization of polyacrylamide and poly(ethylene glycol) with acrylic or vinyl sulfonic acid additives to produce the desired EOF (97). The presence of free double bonds on the capillary wall permitted the covalent attachment of the polymer gel. Various ratios of linear and cross-linked monomers were used to generate different gel matrices with free sulfonic acid groups and were used to separate peptides and carbohydrates. In order to visualize carbohydrates, they were derivatized by reductive amination with 2-aminobenzamide and detected by laser-induced fluorescence (LIF) using a 325 nm He-Cd laser source (98).

With peptide mixtures, continuous gradient elution is almost essential; since small changes in mobile phase composition can result in large changes in peptide retention times, it is difficult to separate a complex peptide mixture, such as a large protein digest, using isocratic elution or even stepped-gradient programs. Accordingly, one of the few reported separations of oligonucleotides by CEC used a pressurized gradient-elution apparatus (99). The column had a 5 μm C_{18} reversed-phase silica gel packing, and voltage gradients up to 400 V/cm were used.

The affinity strategy known as molecular imprinting has recently attracted great interest (100–103). Although most studies have aimed at small-molecular-mass analytes such as drugs, hormones, and peptides, some have concerned biopolymers such as immunoglobulins. Affinities that have been used include antigen-antibody, hapten-antibody, lectin-sugar, drug-protein, and enzyme-substrate interactions. Ultraviolet, laser-induced fluorescence and mass spectrometer detectors have been employed. The critical issue is background electrolyte selection.

FUTURE PROSPECTS

What decides whether a new analytical technique will become widely accepted is whether or not it can be advantageously applied to real-world samples. It must be able to analyze, qualitatively and quantitatively, samples that are already analyzed by existing methods, and it must do so better, faster, cheaper, with better resolution, greater automation, or less sample preparation, and with equal or better accuracy and precision. Although much of the work done on CEC has dealt with pharmaceuticals, agrochemicals, or other low-molecular-weight analytes (sometimes in crude biofluid matrices), it is not clear that such samples are being routinely analyzed by CEC or any variations thereof (46, 104–106). CEC should show greatest analytical advantages for components where the smallness of differences in size, molecular weight, charge, solubility, and/or hydrophobicity make it difficult to separate them by conventional HPLC or CE. Future applications may include mixtures of peptides, proteins, amino acids, aminoglycosides, or polar or charged drug metabolites. Very complex samples, or analytes that are very difficult to resolve in a given sample matrix, well amenable may be to CEC or CEC-MS, which may be able to achieve beyond the reach of CEC or MS alone. CEC thus basically may remain a research tool, providing unusual resolving capabilities

in nonroutine applications. It nevertheless is reasonable to expect the development and marketing of CEC-specific stationary phases allowing control over EOF and selectivity. For example, the use of submicron particles for CEC packing would lead to high efficiencies and should not cause any decrease in EOF velocity. Also, in view of the low consumption of solvents and packing materials in CEC, it is realistic to consider further study of the use of chiral stationary phases, chiral additives, ion-pair reagents, and normal-phase packing, and, for given applications, optimization of analytical parameters such as temperature, buffer concentration and pH, mobile phase proportions, column diameter and length, and voltage. It ultimately should be possible to find optimal conditions for a variety of compounds and to develop generic routine CEC methods to cover a range of quantitative applications.

Finally an appealing goal would be the combination of all capillary separation techniques into a single instrumental platform, permitting the analyst to switch from HPCE to capillary liquid chromatography (CLC) to CEC to PEC at will and with little downtime (46, 106–108). This type of instrument would allow flow to be driven by pressure alone (by an HPLC pump), voltage alone (for HPCE and CEC), or both (for PEC). The analyst not only would be able to optimize the set of operational parameters for a given separation technique, but also to determine the optimal separation technique.

CONCLUSIONS

There are literally thousands of HPLC applications that could be transferred to CEC. However, although CEC usually affords better resolution than HPLC, commercial CEC capillaries are more expensive than current HPLC columns, are generally less tolerant of dirty samples such as biofluids, and may require more sample preparation and clean-up of crude samples. CEC also has less sample capacity than advanced HPLC apparatus, and therefore higher limits of detection (all capillary techniques are ruled out if preparative or semipreparative separations are required). Also, the better resolution and plate counts of CEC may not matter in applications in which there are only a few analyte peaks of interest, as in most pharmaceutical analyses; in such cases HPLC is simpler, more reproducible, and probably less expensive. More fundamentally, it remains difficult to find an optimal compromise between the often contradictory requirements of reasonable EOF and absence of silanol-analyte interactions (which lead to band-broadening). PEC does not solve the problem, but only forces the analytes to elute more quickly. Thus, CEC may remain restricted to research applications.

On the other hand, the ability of CEC to provide short overall analysis times even in isocratic mode (because of its increased peak capacity and narrower peaks) may mean that it will find many applications for in-process sample analysis in which turnaround time is critical. Also, CEC has great advantages over HPLC for chiral separations, including not only low consumption but also improved resolution, higher efficiency, improved selectivity, and accurate enantiomeric excess determination (109, 110), although commercial chiral CEC capillaries will need to be more widely available, more rugged, and less expensive than at present.

In short, although CEC has flourished in the past few years, its widespread acceptance for routine applications would require prior solution of several problems.

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