1 / VOL. 2, NO. 2
THE CHEMICAL EDUCATOR

ISSN 1430-4171
http://journals.springer-ny.com/chedr
10.1007/s00897970120a

In the Classroom

Capillary Electrokinetic Chromatography (CEC): An Introduction to a High-Efficiency Microanalytical Technique

VINCENT T. REMCHO
Department of Chemistry
West Virginia University
Morgantown, WV 26506-6045
remcho@wvu.edu

Several capillary electroseparation methods (CES methods) have captured the interest of analytical chemists over the last few years.

Capillary electrokinetic chromatography (CEC, also called "capillary electrochromatography") is a relatively new separation technique based on a combination of liquid chromatographic and electrophoretic separation methods. CEC offers both the *efficiency* of capillary electrophoresis (CE) and the *selectivity and sample capacity* of packed capillary high performance liquid chromatography (HPLC). These advantages are provided in part because of the favorable flow characteristics of electroosmosis, a method of pumping a liquid by applying a high potential axially to a thin, fluid-filled tube. The speed at which analytes move through the separation conduit under the influence of electroosmosis is quite uniform, regardless of the position of the analyte with respect to adjacent surfaces (within certain limits). This results in very little spreading of zones of analyte; narrow, compact bands of analytes are therefore maintained, which results in high efficiency. Because the

ISSN 1430-4171
http://journals.springer-ny.com/chedr
S 1430-4171 (97) 02120-1

capillaries used in CEC are packed with HPLC packing materials, the wealth of selectivities available in HPLC are also available in CEC. The high surface area of these packing materials enables CEC capillaries to accommodate relatively large amounts of sample, making detection a simpler task than it is in capillary electrophoresis (CE). This paper will briefly visit the theory and practice of CEC, and will provide examples of how CEC can be applied in sample analysis.

Introduction

Several capillary electroseparation methods (CES methods) have captured the interest of analytical chemists over the last few years. These techniques use 25-to-200-μm inner diameter (i.d.) capillaries, 0.5 to 1.5 m long, across which high voltages are applied. The methods include capillary zone electrophoresis (CZE) [1], micellar electrokinetic capillary chromatography (MEKC) [2], capillary gel electrophoresis (CGE) [3], and capillary electrokinetic chromatography (CEC) [4]. All of these techniques offer high efficiency (>100,000 theoretical plates/m).

Of all these CES methods, capillary electrokinetic chromatography (CEC) is the least developed. In the most common form of CEC, silica-based reversed-phase particles (2—10-μm diameter) are packed into fused silica capillaries with inner diameters of 25–100 μm. In commonly used buffer systems, the silica particles have a negative surface charge (due to ionized silanol groups), which is compensated by counterions in the eluent solution. By applying a potential across the capillary, a surface-originated flow is generated, the electroosmotic flow (EOF). The flow profile of EOF in electrochromatography is essentially flat as compared to the parabolic flow profile of pressure driven HPLC. This flat flow profile is the primary contributing factor to the high efficiencies observed in CEC. The highest reported efficiencies to date were obtained by Smith and Evans [5] who obtained 387,000 plates per meter for a retained solute on a 50-μm-i.d. capillary packed with 3 μm particles of Spherisorb ODS-1, and > 300,000 plates per meter for a retained solute on a 50-μm-i.d. capillary packed with 1.8-μm particles of 1 Zorbax SBC8.

The Theory of CEC: How Does it Work?

Electroosmosis in a Packed Capillary

In CEC there are two forces which act on a molecule to cause it to migrate. The two

ISSN 1430-4171
http://journals.springer-ny.com/chedr
S 1430-4171 (97) 02120-1

processes are electrophoresis and electroosmosis, and they will be addressed separately. These processes act in concert with partitioning between the mobile and stationary phases, which results in chromatographic separation.

In the case of electrophoresis, analytes are moved within the separation conduit by virtue of their charge in solution. (Nonionized species are not moved through the tube by an electrophoretic process.) Ionized solutes are drawn toward the pole of opposite charge through the bulk solution at velocities which are dictated by the charge and hydrodynamic volume of the solute. The fact that this solute is ionized in solution results in the formation of an electrochemical double layer around the solute, the first layer of which is tightly associated with the solute and which is termed the Stern layer. The second or outer layer is called the Gouy layer, and is more loosely associated with the solute, such that species in the Gouy layer can freely diffuse into the bulk solution and exchange with ions residing there. When this electrochemical double-layer system is placed in an electric field, a shear force is exerted between the Gouy and Stern layers as the ions in the system are attracted to their opposite pole. Viscous drag on large solutes results in lower velocities, while increases in net charge result in higher velocities. This process of movement describes electrophoresis. The electrophoretic velocity, μ_{ep}, of a material is related to its zeta potential, ζ, and the field strength, E, by

$$\mu_{ep} = (0.67)\frac{\varepsilon_0 \varepsilon_r \zeta E}{\eta} \tag{1}$$

where η is the solution viscosity, ε_o is the vacuum permittivity, and ε_r is the dielectric constant of the separation medium.

An interesting and critical feature of CEC is that neutral species in solution, as well as charged species, are capable of being moved through the capillary electrokinetically. Remember that no geometric constraint is placed on the formation of an electrochemical double layer. This means that an electrochemical double layer exists at the interface of the wall of a capillary tube and the solution filling the tube and at the interface between particles in the packed bed and the solution filling the tube. When a potential field is applied along the length of the tube, ions in the Gouy layer associated with the wall of the tube and the particles filling it will migrate towards their opposite pole, as described earlier. These surfaces, which carry a net negative charge by virtue of the silanol moieties, have cationic species associated with them, as illustrated in

ISSN 1430-4171
http://journals.springer-ny.com/chedr
S 1430-4171 (97) 02120-1

Figure 1. These cationic species are drawn towards the cathode, and (because of the enormous friction between species in solution) will drag the surrounding bulk solution along with them. This phenomenon is termed *electroosmosis*, and results in the transport of neutral species in the direction of the cathode in fused silica capillaries when a potential field is applied. Unlike the flow profile in pressure-driven flow, which is parabolic, the flow profile in electroosmotic flow is flat, as illustrated in Figure 2.

The velocity of electroosmosis, v_{eo}, at any radial point a can be predicted from the following equation:

$$v_{eo} = -\frac{\varepsilon\zeta E}{4\pi\eta}\left(1 - \frac{I_0(\kappa_d a)}{I_0(\kappa_d r)}\right) \tag{2}$$

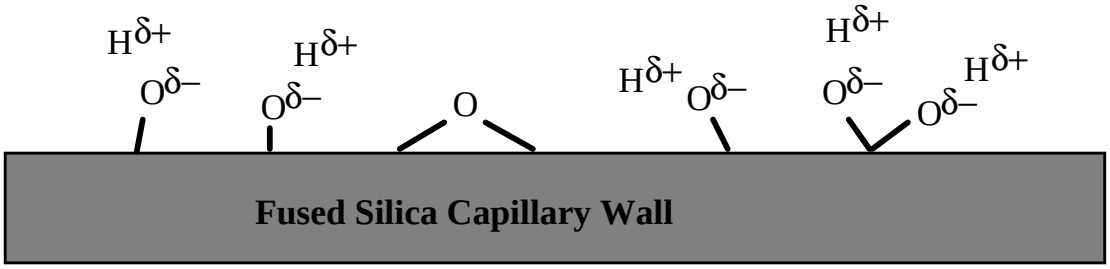
where ε is the dielectric constant of the mobile phase, ζ is the zeta potential across the solution-surface interface, κ_d is the reciprocal of the thickness of the double layer, a is the distance from the center of the capillary, r is the radius of the capillary, and I_o is a zero-order Bessel function of the first kind.

The double layer thickness is typically quite small (on the order of 1 nm), and so equation 2 reduces to the following form:

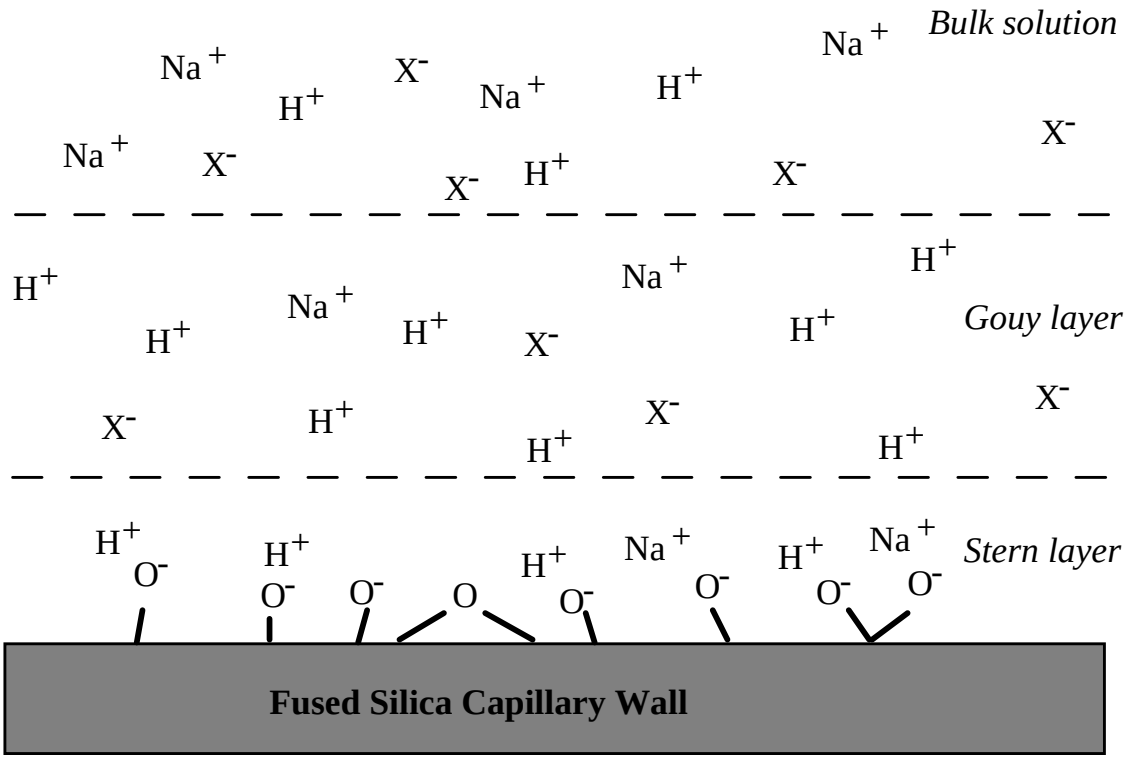
$$\langle v_{eo} \rangle = -\frac{\varepsilon\zeta E}{4\pi\eta} \tag{3}$$

Here, the brackets $\langle\ \rangle$ indicate and average over the cross section of the capillary. In CEC, the capillary is tightly packed with HPLC sorbent particles, typically 5 microns in diameter. Like electroosmosis in open tubes, a zeta potential is measurable at the interface of the glasslike packing material and the filling solution. This means that the motive force for flow in the packed-tube system comes from the packing material as well as from the walls of the container in which the sorbent is packed. Furthermore, the interstitial spaces between the particles of packing material are sufficiently large that there will be no impedance of electroosmosis. As a result, the flow velocity in the interstitial spaces between particles will be essentially equal throughout the cross-sectional area of the column, and equation 3 can be used to predict the mean

ISSN 1430-4171
http://journals.springer-ny.com/chedr
S 1430-4171 (97) 02120-1



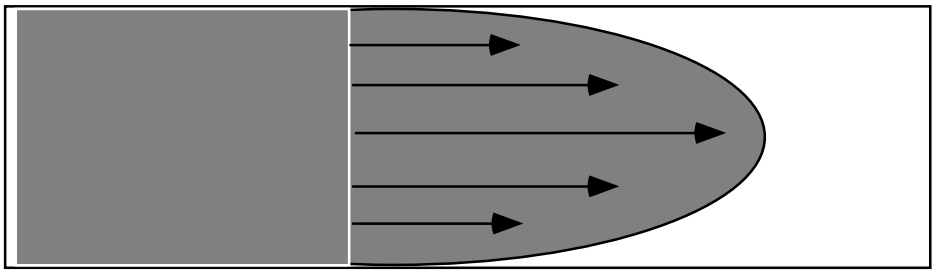
A representation of the surface of fused silica tubing.



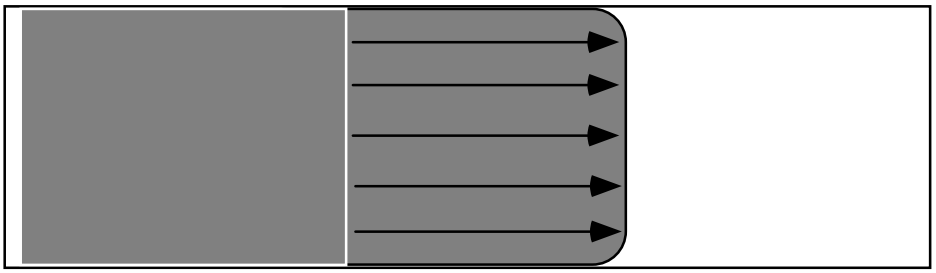
Hydrated silica surface illustrating the electrochemical double layer.

FIGURE 1. A REPRESENTATION OF THE SURFACE OF A FUSED SILICA CAPILLARY WHEN FILLED WITH AN AQUEOUS SOLUTION.

ISSN 1430-4171
http://journals.springer-ny.com/chedr
S 1430-4171 (97) 02120-1



Laminar Flow arising from a Pressure-driven system.



Bulk Flow arising from an Electroosmotically-driven system.

FIGURE 2. A COMPARISON OF LAMINAR & PLUG FLOW VELOCITY PROFILES IN OPEN TUBES (SIDE VIEW).

electroosmotic flow velocity. The flow profile is illustrated in Figure 3, and is responsible for the high efficiencies typical of CE and CEC.

The Source of High Resolving Power in CEC.

CEC is becoming the focus of much research attention because of the need for a technique that can accommodate greater sample loads than CE (for improved limits of detection) and that works with organic mobile phases. Although packed capillary liquid chromatography (capillary LC) has the necessary qualities, efficiency is limited by the laminar flow profile. CEC represents a marriage of CE and capillary LC, yielding high efficiency, good selectivity, high mass loading, and good detectivity. Among the first descriptions of electroosmotically-driven HPLC in a capillary format is that contributed by Knox [6] in 1988. Knox coined the term capillary electrokinetic chromatography (CEC).

ISSN 1430-4171
http://journals.springer-ny.com/chedr
S 1430-4171 (97) 02120-1

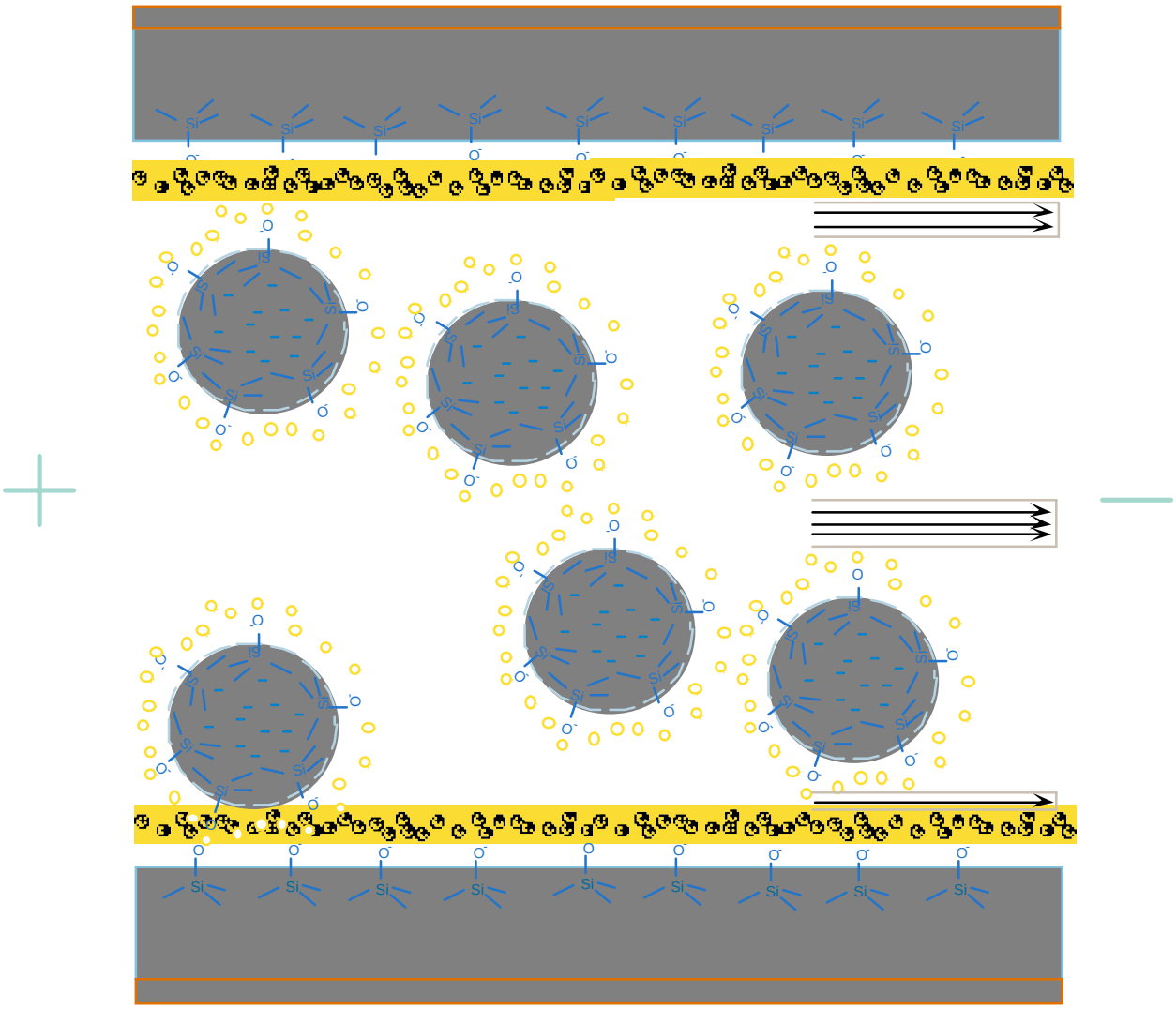


FIGURE 3. A SCHEMATIC SHOWING THE ELECTROOSMOTIC FLOW PROFILE IN A PACKED CAPILLARY COLUMN AS USED IN CEC (SIDE VIEW). THE ARROWS REPRESENT FLOW VELOCITY VECTORS, WHICH ARE VERY UNIFORM IN THE SPACES BETWEEN ADJACENT PARTICLES AND BETWEEN PARTICLES AND THE CAPILLARY WALL.

In order for CEC to be deemed worthy of study, a careful assessment of its potential benefits must first be conducted. Since CEC is a chromatographic technique, chromatographic theory should first be consulted. According to general chromatographic theory, the resolving power, which defines that ability of a technique to separate two components, is described by the master resolution equation:

$$R_s = \frac{1}{4}\left(\frac{k'}{1+k'}\right)\left(\frac{\alpha-1}{\alpha}\right)\sqrt{N} \qquad (4)$$

ISSN 1430-4171
http://journals.springer-ny.com/chedr
S 1430-4171 (97) 02120-1

Here R_s is termed the resolution, which according to equation 4, depends on three primary factors: the capacity factor k' for an analyte; the selectivity α of a column for one analyte relative to another; and the efficiency defined by the number of plates N of the separation system. Each of these three factors is described separately below.

The capacity factor is a thermodynamic parameter, which is directly related to the partition coefficient K of the analyte:

$$K = \beta k' \tag{5}$$

Here β is the phase ratio, which is the volume of mobile phase in the column divided by the volume of stationary phase. The partition coefficient K is defined as the concentration of analyte in the stationary phase divided by the concentration of analyte in the mobile phase at equilibrium [7]. K is therefore a measure of the affinity of an analyte for the stationary phase relative to its affinity for the mobile phase. Since the capillaries and mobile phases used in CEC are essentially identical to those used in capillary LC, the capacity factor for a particular analyte is the same in both techniques. As a result, the capacity factor term of the master resolution equation does not promise better resolution in CEC compared to capillary LC.

The α term in equation 4 conveys the importance of selectivity in the resolution of complex mixtures. Selectivity is defined as the ratio of partition coefficients for the two analytes, and is a measure of one analyte's affinity for the stationary phase relative to that of another analyte. It is selectivity that drives the chromatographic separation. In HPLC, the selectivity for a group of analytes can be tuned by changing the composition of either the mobile phase or the stationary phase. Once again, the similarities of CEC and capillary LC indicate that neither will have the edge in providing more resolving power through changes in selectivity. The two techniques should in fact offer almost identical selectivities for analytes with zero net electrophoretic mobility. In CEC, as in HPLC or capillary LC, selectivity is an important factor in the resolving power, because both the mobile and stationary phases can be changed.

Efficiency is measured as a total number of theoretical plates N. The greater the value of N the better the resolving power. The number of theoretical plates is calculated from chromatographic data using the following equation [8]:

ISSN 1430-4171
http://journals.springer-ny.com/chedr
S 1430-4171 (97) 02120-1

$$N = \left(\frac{t_r}{\sigma}\right)^2 \tag{6}$$

where t_r is the retention time of an analyte and σ is the standard deviation of the eluting peak that contains that analyte. Efficiency is often cast in terms of the plate height H which is related to N by

$$H = \frac{L}{N} \tag{7}$$

where L is the length of the separation conduit. Efficiency is not the strong suit of HPLC where columns generate about 12,000 theoretical plates; however, efficiency is the strong suit of CE, as well as other electrokinetic separations techniques, which generates on the order of 100,000 theoretical plates. As a result, the efficiency term of the master resolution equation is greater in CEC compared to capillary LC; this translates to a higher resolving power.

In summary, the benefits offered by CEC include: (1) high efficiencies due to the bulk flow profile; (2) high selectivities typical of liquid chromatographic sorbents; (3) greater sensitivity compared to CE due to larger sample loads; (4) ease of interfacing to existing CE detectors; and (5) high resolving power compared to either CE or capillary LC.

Practical Aspects of CEC: Building the System

Preparing CEC Columns

Fused silica capillaries with inner diameters of 50 to 100 μm are cut to a length of 50 to 100 cm. Frits are installed in the outlet end of the column by sintering (softening and adhering together) particles of silica gel. This results in a porous ceramic filter that can hold in a particulate packing material, but which readily passes liquids through.

The columns are slurry packed using a procedure similar to that developed for preparing columns of larger inner diameter. A slurry is typically prepared in a ratio of 80:1 (ml:g) slurry liquid to packing material. The slurry liquid may be an aqueous buffer or an organic solvent such as acetonitrile. Silica-based reverse-phase packing material is most often used in producing the packed capillaries. The slurry is ultrasonicated for 10 to 15 min and transferred to a reservoir. The reservoir is

ISSN 1430-4171
http://journals.springer-ny.com/chedr
S 1430-4171 (97) 02120-1

connected to a high-pressure pump, which is operated in the constant pressure mode at 3,000–10,000 psi. While packing takes place, the slurry reservoir may be placed in an ultrasonic bath to minimize settling of the particles. The particles going into the column can be observed periodically with a zoom stereomicroscope throughout the packing process. Once the desired length of the column is filled, the pressure is maintained, and an outlet frit is made using a fiber optic fusion splicer or another source of highly focused heat to sinter a portion of the packing material. Following this, the column is removed from the packing apparatus and flushed with mobile phase. A narrow (0.5 mm) detection window is made immediately adjacent to the outlet frit using the fusion splicer.

CEC Instrument Assembly

The instrumentation used in CEC is essentially the same as that used in CE, and consists of a high voltage power supply (typically a 30 kV, 10 mA supply), an on-column capillary-format UV detector (other detection schemes are also possible), and a safety interlock box. Some systems are equipped with an external pressure device that allows the capillary to be operated at 50 to 150 psi above ambient pressure in order to prevent formation of vapor bubbles, which ruin the separation experiment. Each end of the packed capillary is immersed in a separate reservoir containing an appropriate mobile phase, and the capillary is filled with the mobile phase as well. An electrode is immersed into each of the two reservoirs; one of these serves as earth ground and the other is used to apply a high potential (5–30 kV) along the capillary. The resulting electric field causes movement of mobile phase due to electrosomosis. Finally, a small section of the polyimide coating on the outside of the fused silica capillary is removed as described above so that a UV detector can be placed in line between the reservoirs for in situ detection. A schematic of the instrumentation is shown in Figure 4. This represents a homebuilt instrument, though a number of commercial instruments are also available.

Victor Pretorius [9] once called sample introduction in GC the “Achilles Heel” of the technique. In CE, this characterization applies not only to sample introduction, but equally to detection. Concentration-based limits-of-detection are in the tens of parts-per-million for CE with UV detection. The reasons for this insensitivity are twofold. First, as an open tubular technique, CE provides low sample loading capabilities. In

ISSN 1430-4171
http://journals.springer-ny.com/chedr
S 1430-4171 (97) 02120-1

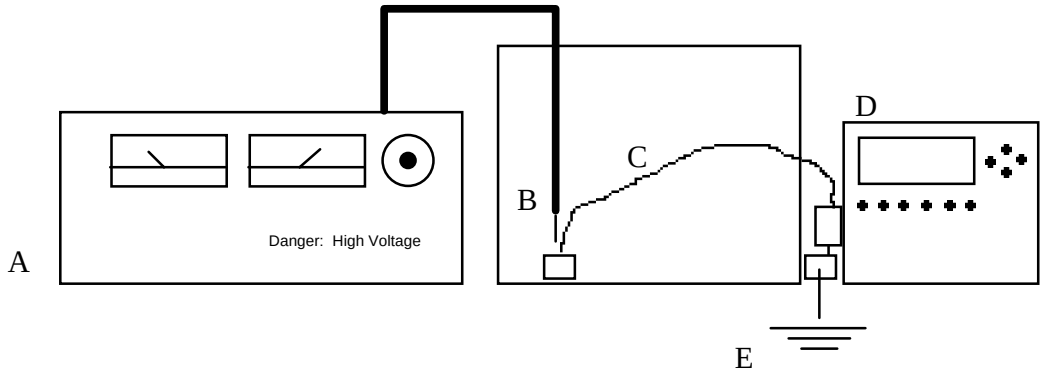


FIGURE 4. A SCHEMATIC OF THE INSTRUMENTATION USED IN CEC**.** A: HIGH VOLTAGE POWER SUPPLY. B: PLATINUM WIRE ELECTRODE. C: CAPILLARY. D: UV DETECTOR. E: EARTH GROUND. OPERATOR IS PROTECTED FROM ELECTRICAL SHOCK BY ENCLOSING THE HIGH VOLTAGE ELECTRODE IN A PLEXIGLAS BOX.

other words, only very small masses (1 to 20 nanoliters) of sample can be injected before the capacity of the system is exceeded and efficiency suffers. For this reason, the amount of sample present for detection is necessarily small. Secondly, when CE is used in conjunction with UV detection, the detection cell is actually a part of the fused silica capillary separation tube from which the polyimide coating has been removed in order to allow for in situ UV detection. As CE is conducted in very small i.d. tubes (again, 20 to 100 microns), the path length for detection is likewise very small. As indicated by Beers' law, the path length is directly proportional to absorbance intensity, meaning that the detector signal achieved in the case of a short path length as in CE will be miniscule. The end result of these two factors is poor sensitivity due to small sample size and geometric constraints. The higher loading capacity of a packed capillary column, as is used in CEC, allows for better detectivity simply because more sample is available to be detected.

Examples of CEC Data

Control of the Electroosmotic Flow Velocity

Columns packed with either 3 μm or 7 μm particles of Nucleosil C_{18} with 100-Å pores were operated under identical conditions, except that the field strength was varied between 150 V/cm and 500 V/cm. The test mixture consisted of acetone (an unretained marker with zero net electrophoretic mobility), methyl paraben, and ethyl paraben. The mobile phase consisted of 20% acetonitrile and 80% 100-mM phosphate buffer pH 6.9. No attempt was made to thermostat the capillary.

ISSN 1430-4171
http://journals.springer-ny.com/chedr
S 1430-4171 (97) 02120-1

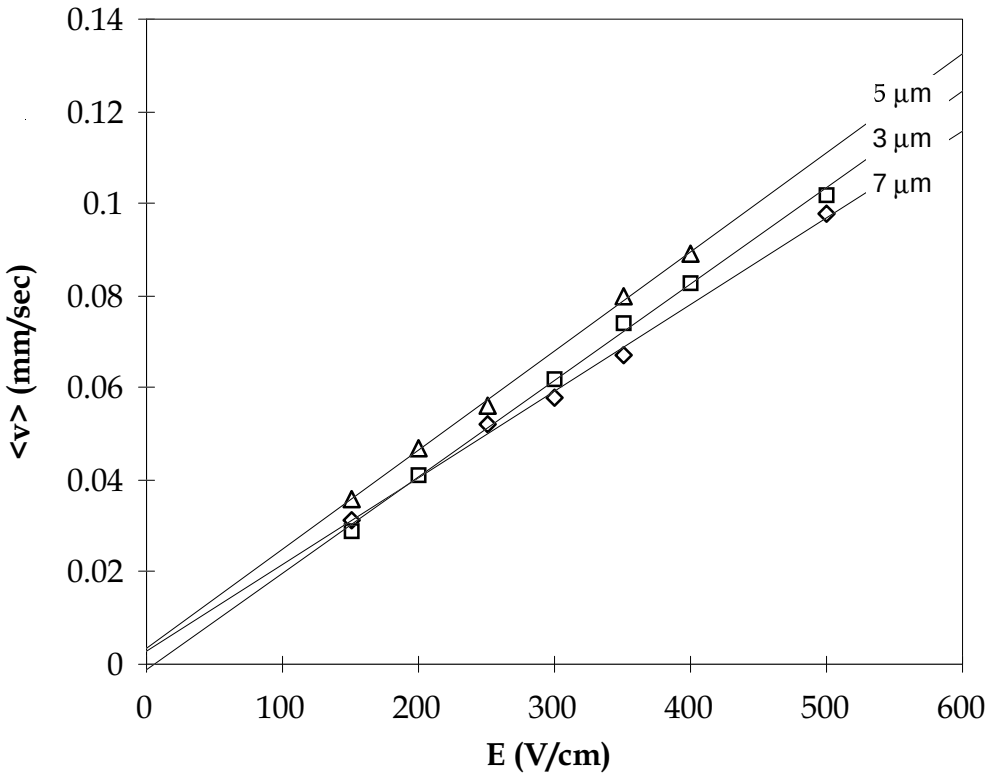


FIGURE 5. LINEAR VELOCITY (<V>) AS A FUNCTION OF FIELD STRENGTH (E) FOR 3, 5 AND 7 μm DIAMETER NUCLEOSIL C18 PARTICLES.

As illustrated in Figure 5, a linear relationship exists between field strength *E* and linear velocity <*v*> over the range of field strengths studied. This is in accordance with equation 3. More importantly, the particle size of the packing material has no effect on the linear velocity for the range of particle diameters studied. This indicates that the interstitial pore space (the space between adjacent particles of packing material) is sufficiently large to promote electroosmosis.

Effect of Particle Size on Efficiency

The columns used in determining the dependence of flow velocity on field strength were also employed in studying the effect of particle diameter on plate height in CEC. As expected, the well established tenets of rate theory hold true for CEC as they do in conventional HPLC: smaller particle sizes yield lower plate heights, as illustrated in Figure 6. Thus, the system is more efficient; that is, it does a better job separating mixtures and is, therefore, able to accommodate more complex samples. The very shallow slope of the mass transfer dominated portion (high flow velocity region) of the

ISSN 1430-4171
http://journals.springer-ny.com/chedr
S 1430-4171 (97) 02120-1

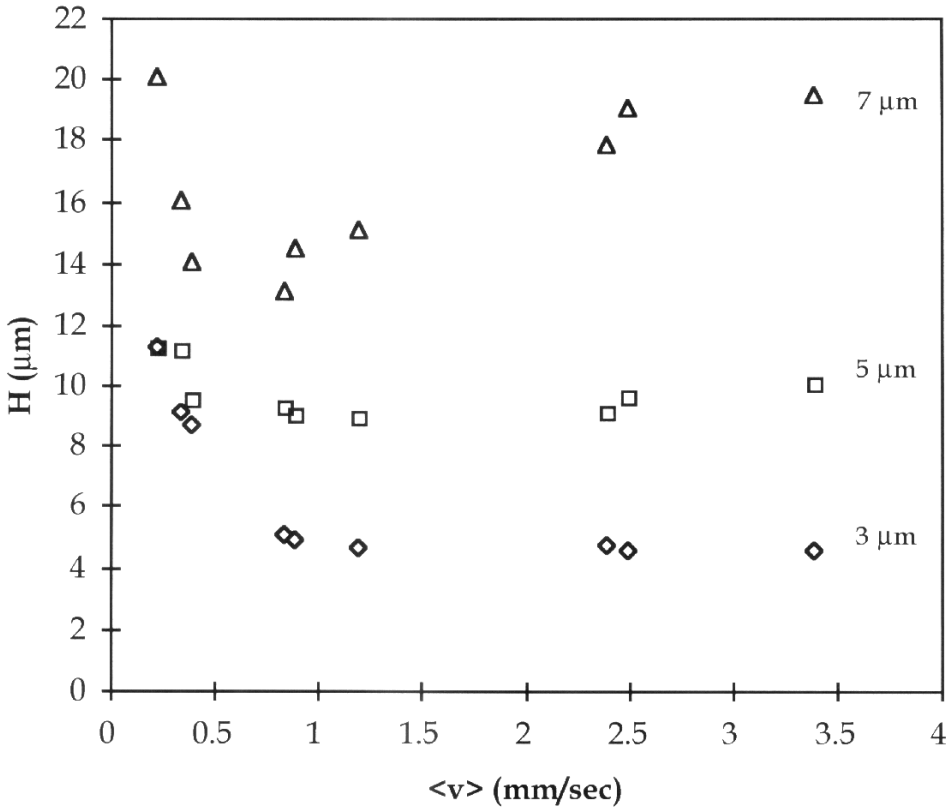


FIGURE 6. PLATE HEIGHT (H) AS A FUNCTION OF LINEAR VELOCITY (<V>) FOR THREE PARTICLE SIZES (3, 5, AND 7 μm) OF NUCLEOSIL C18. IN CEC, AS IN ALL CHROMATOGRAPHIC SEPARATION METHODS, SMALLER PARTICLE SIZES YIELD INCREASED EFFICIENCY.

rate curves is notable in CEC, and means that CEC systems can be operated at high flow velocities in order to yield very high efficiencies while providing for rapid analysis.

Reproducibility

To study the reproducibility of a single CEC packed capillary column, more than twenty consecutive runs were performed on the analysis of acetone using a single capillary. Figure 7 illustrates the run-to-run reproducibility of electroosmotic flow velocity using acetone, which is a neutral marker.

Sample Applications

Figures 8 and 9 illustrate typical chromatograms. The very narrow peaks are a manifestation of the very high efficiency of CEC. The separation of the three parabens (preservatives) in the first example demonstrates the potential use of CEC in the

ISSN 1430-4171
http://journals.springer-ny.com/chedr
S 1430-4171 (97) 02120-1

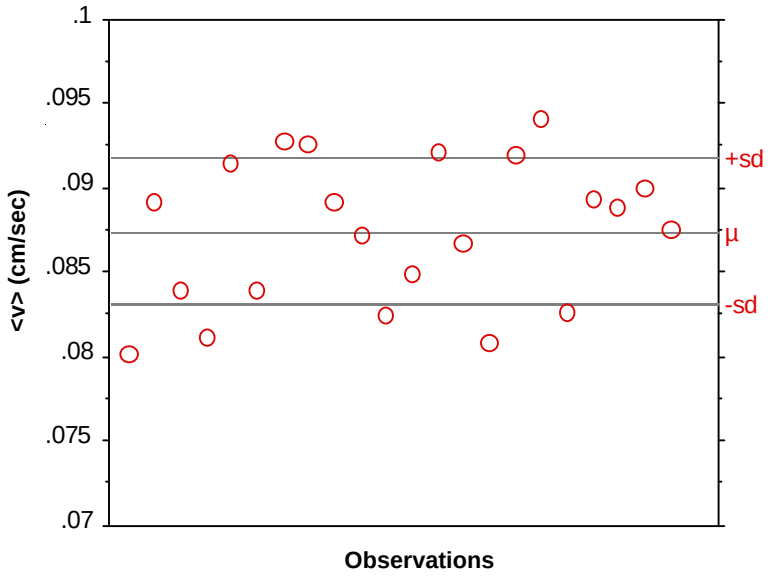


FIGURE 7. QUALITATIVE REPRODUCIBILITY IN CEC, EVALUATED USING AN UNRETAINED TEST SOLUTE (ACETONE) AS A MARKER OF THE ELECTROOSMOTIC FLOW VELOCITY. THE RELATIVE STANDARD DEVIATION (RSD) IN ELUTION TIME IS 4.6%. IN PRESSURE-DRIVEN MICROSCALE HPLC, RSD'S AS LOW AS 1.5% ARE COMMON. THIS POINTS TO THE NEED FOR USE OF INTERNAL STANDARDS IN CEC FOR BOTH QUALITATIVE AND QUANTITATIVE ANALYSIS.

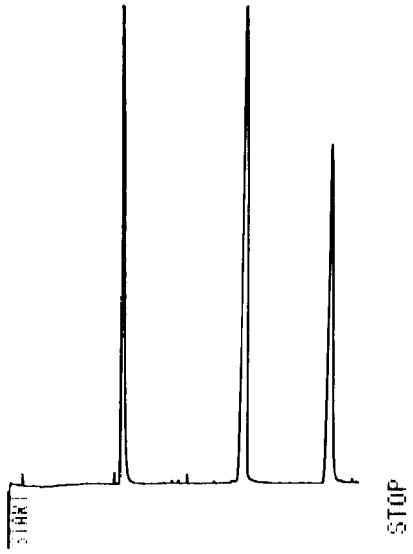


FIGURE 8. REVERSE-PHASE PACKED CEC SEPARATION OF ACETONE (A MARKER OF ELECTROOSMOTIC VELOCITY) AND METHYL- AND ETHYL- PARA-HYDROXY BENZOIC ACID ESTERS (COMMON PRESERVATIVES FOUND IN COSMETICS AND FOODS). CAPILLARY: 100 μm I.D.; TOTAL LENGTH 300 mm; BED AND EFFECTIVE LENGTH 227 mm. PARTICLES: 5 μm DIAMETER LICHROSORB ODS. INJECTION: ELECTROKINETIC, 50 V/cm, 3 SEC. CONDITIONS: 400 V/cm APPLIED POTENTIAL, 20:80 ACETONITRILE:50 mm NA PHOSPHATE pH 6.9. DETECTION: UV ABSORBANCE AT 254 nm.

ISSN 1430-4171
http://journals.springer-ny.com/chedr
S 1430-4171 (97) 02120-1

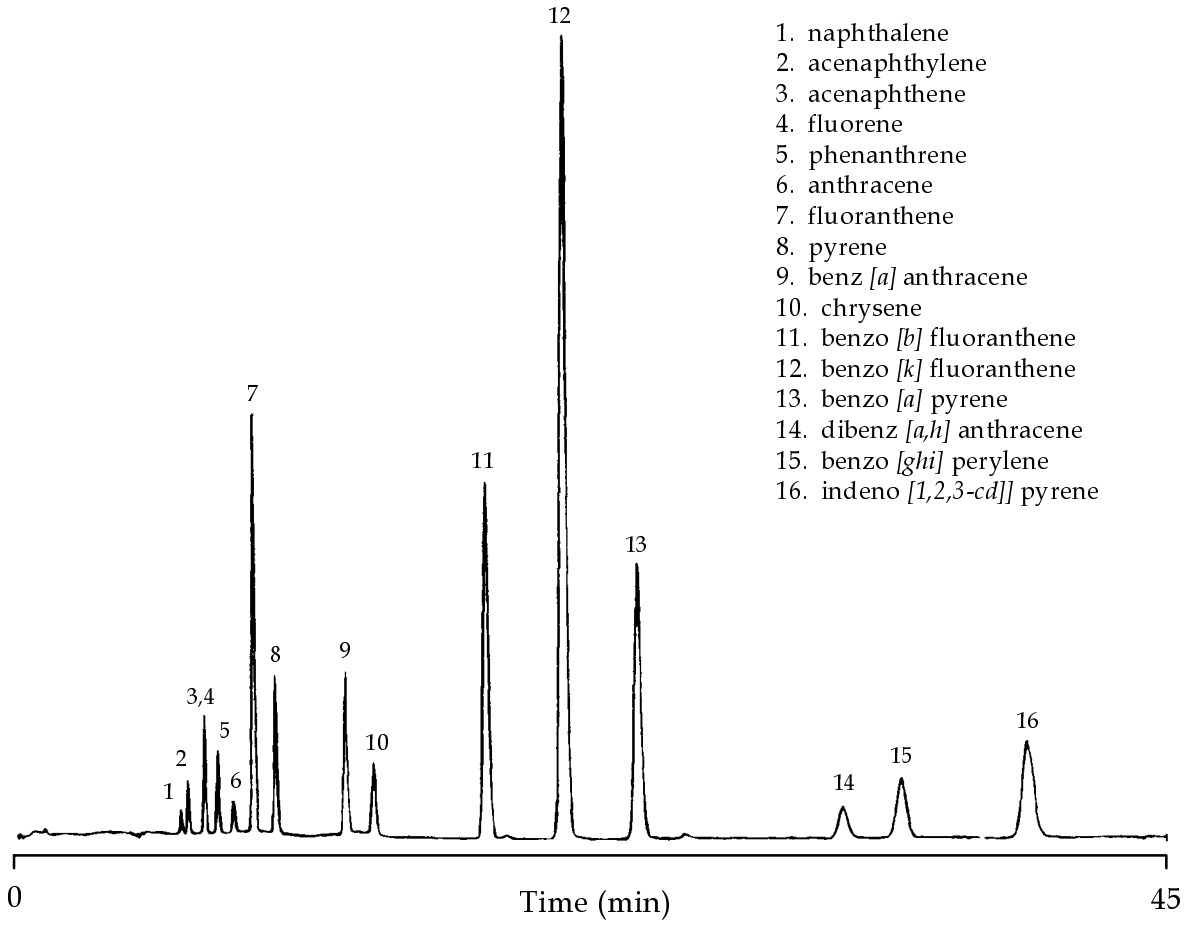


FIGURE 9. REVERSE-PHASE PACKED CEC SEPARATION OF POLYCYCLIC AROMATIC HYDROCARBONS (COMMON ENVIRONMENTAL CONTAMINANTS FROM OLD ELECTRICAL TRANSFORMERS). CAPILLARY: 75 μm I.D.; TOTAL LENGTH 30 cm; BED AND EFFECTIVE LENGTH 20 cm; PARTICLES 3 μm DIAMETER ODS SI. INJECTION: ELECTROKINETIC, 50 V/cm, 7 SEC. CONDITIONS: 60:40 ACETONITRILE:10 mm SODIUM BORATE; E = 600 V/cm. DETECTION: XE-ARC LAMP FLUORESCENCE; EXCITATION WAVELENGTH 260 nm; EMISSION WAVELENGTH 280-600 nm.

cosmetics or food products industries. The second example demonstrates the applicability of CEC to environmental analysis. Many other applications of CEC are possible, both in the reverse-phase mode and in others. References 10–15 provide a few more samples of applications in CEC and related areas.

REFERENCES

1. Jorgenson, J. W.; Lukacs, K. D. *J. Chromatogr.* **1981,** *218*, 209.

2. Terabe, S.; Otsuka, K.; Ando, T. *Anal. Chem.* **1985,** *57*, 834.

ISSN 1430-4171
http://journals.springer-ny.com/chedr
S 1430-4171 (97) 02120-1

3. Guttman, A.; Paulus, A.; Cohen, A. S.; Grinberg, N.; Karger, B.L. *J. Chromatogr.* **1988**, *448*, 41.

4. Knox, J. H.; Grant, I. H. *Chromatographia* **1987**, *24*, 135.

5. Smith, N. W.; Evans, M. B. *Chromatographia* **1994**, *38*, 649.

6. Knox, J. H. *Chromatographia* **1988**, *26*, 329.

7. Braithwaite. A.; Smith, F. *Chromatographic Methods*; Chapman & Hall: London. 1985.

8. Giddings, J. C. *Dynamics of Chromatography*; Marcel Dekker: New York, 1965.

9. Pretorius, V.; Bertsch, W. *J. High Res. Chrom.* **1983**, *8*, 64.

10. Tan, Z. J.; Remcho, V. T. *Analytical Chemistry* **1997**, *69*, 581.

11. Remcho, V. T.; Ballou, N. E.; Ducatte, G. R.; Quang, C. *J. High Resol. Chromatogr.* **1996**, *19*, 183.

12. Dittmann, M. M.; Rozing, G. P. *J. Chromatogr. A* **1996**, *744***,** 63.

13. Smith, N. W.; Evans, M. B. *Chromatographia* **1995**, 414, 197.

14. Li, D.; Remcho, V. T. *J. Chromatogr. B*, **1997**, 695.

15. Robson, M. M.; Roulin, S.; Sheriff, S. M.; Raynor, M. W.; Bartle, K. D.; Clifford, A. A.; Myers, P.; Euerby, M. R.; Johnson, C. M. *Chromatographia*, **1996**, *43*, 313.