Nagaoka T, Fujimoto M, Nakao H *et al.* (1994) Electrochemical separation of ionic compounds using a conductive stationary phase coated with polyaniline or polypyrrole film, and ion exchange properties of conductive polymers. *Journal of Electroanalytical Chemistry* 364: 179–189.

Suzuki T, Noble RD and Koval CA (1997) Electrochemistry, stability, and alkene complexation chemistry for copper(I) triflate in aqueous solution. Potential for use in electrochemically modulated complexation-based separation processes. *Inorganic Chemistry* 36: 136–140.

Ting EY and Porter MD (1997) Separations of corticosteroids using electrochemically modulated liquid chromatography: selectivity enhancements at a porous graphitic carbon stationary phase. *Analytical Chemistry* 69: 675–678.

Ting EY and Porter MD (1998) Column design for electrochemically modulated liquid chromatography. *Analytical Chemistry* 70: 94–99.

Electrochromatography

N. Smith, Imperial College of Science, Technology and Medicine, London, UK

Introduction

High performance liquid chromatography (HPLC) is an established technique with a vast number of stationary phases available, but the efficiency is ultimately limited by the size of the particles used to pack the columns. Theory shows that for the highest efficiency the particles should be of a much smaller diameter than those in current use, but a significant move in this direction is constrained by the pressure required from the pumping system used to deliver the mobile phase. Capillary electrochromatography (CEC) uses an electric field rather than hydraulic pressure to drive the mobile phase through the packed bed of stationary phase. Since the resulting flow profile is plug-like rather than parabolic, very high efficiencies can be achieved.

Theory

The ability to drive a liquid through a packed capillary under the influence of an electric field was first described by Pretorius in 1974, but a lack of the necessary hardware at the time meant that his suggestion was not followed up. Pretorius showed that the linear velocity of a liquid (u) under electrical flow conditions was given by the equation:

$$u = \frac{\varepsilon E \zeta}{4\pi\eta} \qquad [1]$$

where E is the applied electrical field in V cm^{-1}; ε is the dielectric constant (dimensionless); ζ is the zeta potential in volts; and η is the viscosity of the liquid in kg m^{-1} s^{-1}.

Pretorius estimated from eqn [1] that, with voltages up to 1500 V cm^{-1}, it should be possible to generate linear velocities of 0.1 to 1 cm s^{-1}, which are similar to those used in modern HPLC. He also noted that eqn [1] is independent of particle diameter, which means that the flow rate could be maintained in a column packed with very fine material. When the various contributions to column plate height are summed and plotted against linear velocity, it can be seen that the overall dispersion in an electrically driven system is approximately half that of a corresponding pressure-driven system. This is almost entirely due to a dramatic decrease in the contribution of the eddy diffusion term in the electrically driven system, as illustrated in **Figure 1.**

The driving force in electrochromatography results from the electrical double layer that exists at any liquid–solid interface. The electrical double layer inside a fused silica capillary filled with an electrolyte is shown in **Figure 2.**

Under alkaline conditions, the surface silanol groups of the fused silica become ionized, leading to a negatively charged surface. This surface will have a layer of positively charged ions in close proximity that is relatively immobile. This surface layer of ions is called the Stern layer. The remainder of the excess charge, constituting the Goüy layer, is solvated and has the characteristics of a typical solvated ion. This layer extends into the bulk of the liquid and is the so-called double layer. The concentration of ions in the double layer is relatively small compared to the total ion concentration and falls off exponentially from the capillary surface, as does the electrical potential, which is proportional to the charge density. The potential at the boundary between the Stern layer and the diffused Goüy double layer is known as the zeta potential, ζ, and ranges from 0 to 100 mV. As the charge density drops off with distance from the surface, so does the zeta potential. The distance from the immobile layer to a point in the bulk liquid at which the potential is 0.37 of that at the interface between the Stern layer and the diffuse layer is defined as the thickness of the double layer, denoted by δ. The

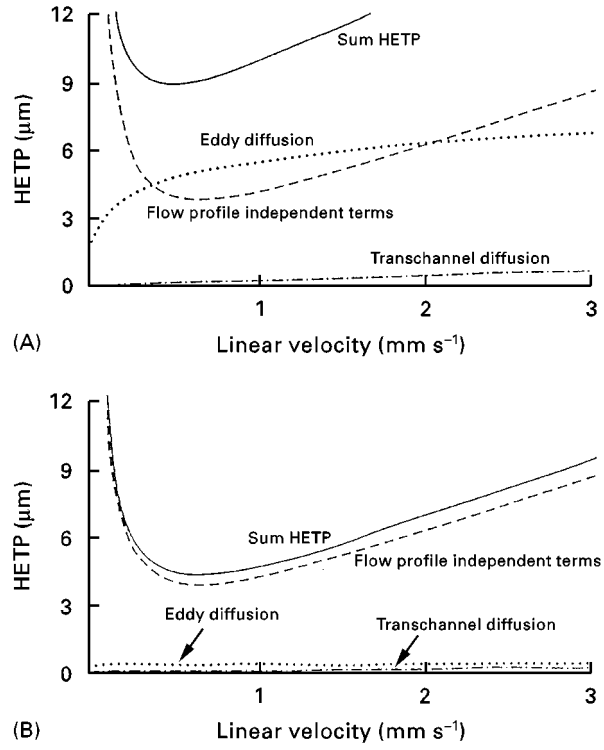

Figure 1 Contributions to plate height in (A) a pressure-driven system (HPLC) and (B) an electrically driven system (electrochromatography). HETP, height equivalent to one theoretical plate. (Reprinted from Dittmann MM and Rozing GP (1996) Capillary electrochromatography – a high efficiency micro-separation technique. *Journal of Chromatography A* 744: 63–74 with kind permission from Elsevier-NL.)

equation describing δ is as follows:

$$\delta = \frac{\varepsilon_r \varepsilon_0 R T^{1/2}}{2cF^2} \qquad [2]$$

where ε_r is the dielectric constant or relative permittivity of the medium; ε_0 is the permittivity of a vacuum (8.85×10^{-12} C^2 N^{-1} m^2); R is the gas constant (8.314 J K^{-1} mol^{-1}); T is the temperature in Kelvin; c is the molar concentration of the electrolyte; and F is the Faraday constant (96 500 C mol^{-1}).

Using the above equation with water ($\varepsilon_r = 80$), the thickness of the electrical double layer for a 1 : 1 electrolyte at a concentration of 0.001 mol L^{-1} in water would be 10 nm, while at a concentration of 0.01 mol L^{-1} it would be 1 nm.

Electroosmotic flow (EOF) in a capillary arises when an electric filed is applied tangentially along the length of the column. This causes the ions in the diffuse (Goüy) layer that are not absorbed into the Stern layer to migrate towards the cathode. Shearing will occur within this region and electroosmosis will result because the core of liquid within this sheath

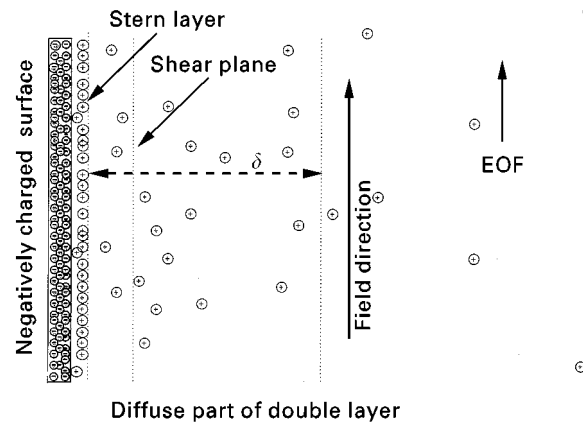

Figure 2 Electrical double layer. Origin of electroosmotic flow (EOF) in a fused silica capillary. (By permission of the author.)

will also be transported to the cathode. Because shearing only occurs within the diffuse layer, the resulting flow profile is plug-like and its velocity is independent of the capillary diameter (d), provided that $d \geq 10\delta$ (d is usually $> 20\delta$).

If d approaches δ, then double layer overlap will occur and the elctroosmotic flow will be considerably reduced, taking on a parabolic profile. In the case of packed capillaries, the capillary diameter is replaced in the equation by the mean channel diameter. Thus for aqueous electrolytes between 0.001 mol L^{-1} and 0.01 mol L^{-1} there would be no double layer overlap as long as $d_p \geq 40\delta$.

If we assume a value of 10 nm for δ in a 0.001 mol L^{-1} aqueous solution, then $d_p = 0.4$ μm and use of these small-diameter particles should give a dramatic increase in column efficiency. Since typical silica-based reversed-phase packing materials also contain silanol groups, these also contribute to the overall EOF, as illustrated in **Figure 3.**

Another important consideration in CEC is the relationship between the linear velocity and concentration of the electrolyte. Since u is directly propor-

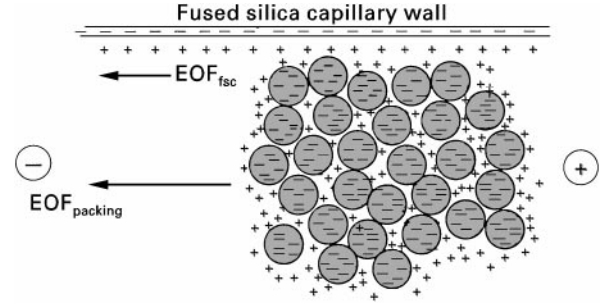

Figure 3 EOF at high pH in a capillary packed with standard HPLC material (e.g. C_{18}, C_8). (By permission of the author.)

tional to the zeta potential, which itself decreases with increasing electrolyte concentration, it is an important variable to consider during method development. The effect of electrolyte concentration on the zeta potential has been measured using 5 μm Hypersil ODS. The results showed that 10^{-4} mol L^{-1} $NaNO_3$ had a zeta potential of $\sim$50 mV, while at 10^{-3} mol L^{-1} the potential was $\sim$45 mV and at 10^{-2} mol L^{-1} it was $\sim$25 mV. When plate height and linear velocity were plotted against NaH_2PO_4 concentration, the reduced plate height was lowest at 10^{-3} mol L^{-1} and the linear velocity altered little over the range 4×10^{-5} mol L^{-1} to 2×10^{-2} mol L^{-1}. The best overall performance, i.e. the lowest values of plate height (H), at high EOF would be achieved at electrolyte concentrations of *c.* 0.002 mol L^{-1}.

Experimental Considerations

If higher concentrations of buffer are used, particularly with high concentrations of organic solvent, then bubble formation can become a problem. This can be overcome by operating the whole capillary under pressure. **Figure 4** is a schematic diagram showing such a pressurized apparatus.

The packing of small-diameter particles into narrow capillaries represents an enormous challenge. The initial stage is the formation of a suitable retaining frit, which must be capable of retaining particles of not more than 1 μm and also capable of withstanding pressures of the order of 10 000 psi (68 000 kPa). Outline details of how to form such frits can be found in the literature. The frits must have a good porosity if the packing process is to be successful; porosity can be tested with a conventional HPLC pump at 6000 psi (41 000 kPa) and a flow of water of 1 mL min^{-1}. For a good frit the pressure should rise to 6000 psi fairly rapidly and then stop due to the pressure cut-out. An ideal frit is one where the pressure decays to zero over a period of 10–20 s. If the pressure decay is much quicker then the frit has probably failed; if the decay is very slow then the frit is not porous enough for efficient column packing. Once a satisfactory frit has been formed, then the capillary can be slurry packed as outlined in **Figure 5.**

Although the packed capillary contains water it is not necessary to condition it with mobile phase since there is a sufficient EOF to allow the mobile phase to be pumped into the system. Once a steady current has been established the column is ready for use.

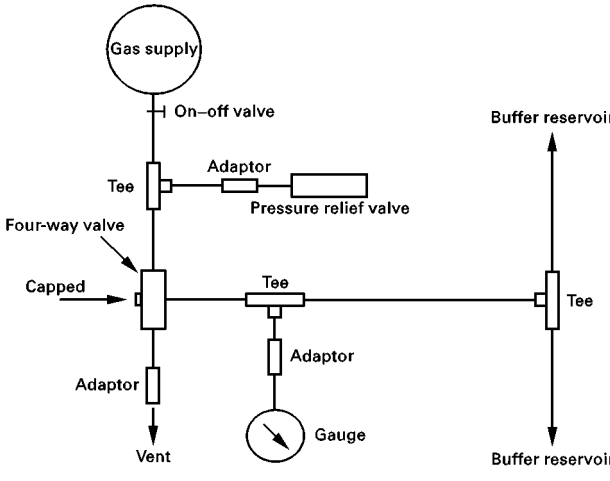

Figure 4 Schematic layout of the pressurization system for electrochromatography. (By permission of the author.)

Applications

Figure 6 shows the separation of the steroid fluticasone propionate from two closely related impurities using micellar electrokinetic chromatography (MEKC) with sodium dodecyl sulfate (SDS) as the micelle while **Figure 7** shows the corresponding HPLC trace using 3 μm Spherisorb ODS-1 stationary phase with gradient elution (impurity 3 was not present in the MEKC experiment).

If the sample is analysed by CEC with the same batch of 3 μm Spherisorb ODS-1, then the chromatogram shown in **Figure 8** is obtained. Apart from a reversal in elution order from the MEKC/HPLC to the CEC the latter is extremely efficient with peak 3 in the CEC trace exhibiting an efficiency of almost 4×10^5 plates m^{-1} with a reduced plate height of 0.9.

If the electrolyte is changed from phosphate to borate and the organic content increased from 75 to 80%, then the chromatogram shown in **Figure 9** is obtained. Although the capillary length has been increased, the analysis time has been reduced significantly. Also, a peak previously undetected has now shown up between peaks 1 and 2.

Increasing the organic content of the electrolyte can drastically reduce run times, but in order to maintain high efficiencies it is desirable to work at high buffer concentration. To meet this criterion it is necessary to work with so-called biological buffers since traditional inorganic buffers are insoluble in high concentrations of organic solvents. **Figure 10** shows the fast, highly efficient analysis of the three impurity peaks from the parent steroid in less than 11 min using a mobile phase containing 0.1 mol L^{-1} Tris buffer (2-amino-2-hydroxymethylpropane-1,3-diol).

Figure 11 shows the chromatogram of a crude sample of a prostaglandin, GR63779X, obtained by using an unadjusted phosphate buffer on a 3 μm Spherisorb ODS-1 phase, while **Figure 12** is the same

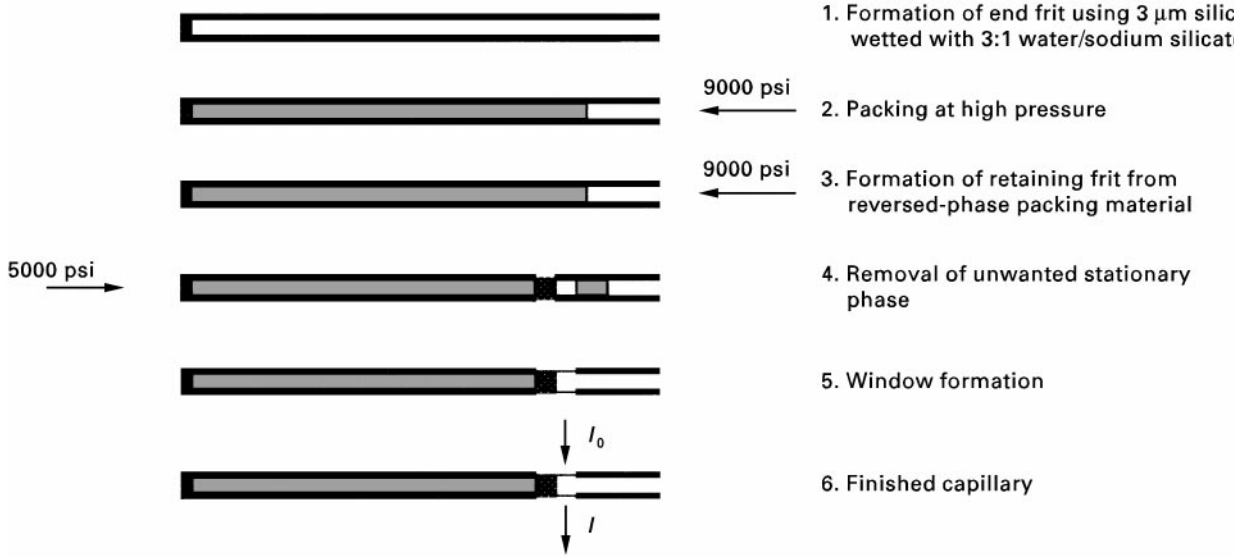

Figure 5 Capillary packing procedure. 5000 psi ≈ 34 000 kPa; 9000 psi ≈ 62 000 kPa.

sample run under identical conditions on a 1.5 μm Zorbax C_8 column.

The expected increase in efficiency on going from 3 μm to 1.5 μm particles has not been realized. The efficiency for the peak eluting at 20 min on the smaller-particle Zorbax column is about 300 000 plates m^{-1} with a reduced plate height of 1.6. This is not as good as the figures for the 3 μm column for two reasons: first, there is the difficulty in packing the smaller particles, and second, Zorbax itself is a difficult material to pack. However, for the two early eluting peaks the 1.5 μm Zorbax column gives much better peak shapes and slightly different selectivity. **Figure 13** shows a highly efficient separation of an angiotensin compound from 13 impurities. Apart from the two late emerging compounds, all the peaks are symmetrical with no apparent evidence of peak tailing. In contrast many of the compounds in this mixture gave tailing peaks when run on an HPLC column containing the same stationary phase. This may be due to the fact that the CEC separation was at high pH while the HPLC separation was at a pH less than 5. Experience has shown that it is possible to run columns under electrodrive conditions at a much higher pH than is possible in HPLC without any deterioration in performance and often with a considerable improvement in separation.

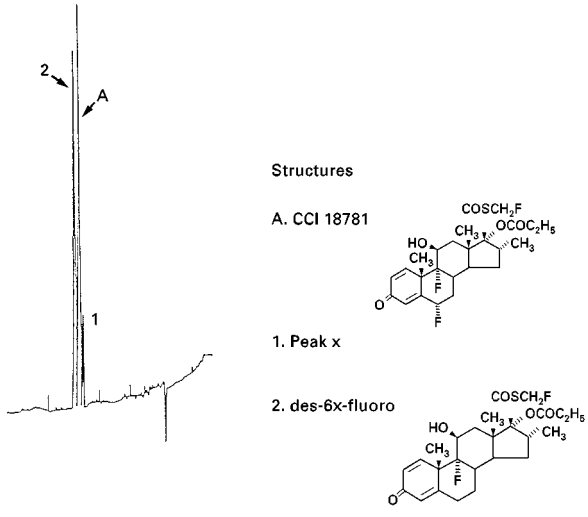

Figure 6 Separation of fluticasone from related compounds by MEKC. Capillary, 72 cm × 75 μm i.d.; detection at 238 nm with a range of 0.02 and rise time of 0.5 s; applied voltage, 30 kV; temperature, 60°C; carrier, 0.01 mol L^{-1} Na_2HPO_4/0.006 mol L^{-1} $Na_2B_4O_7 \cdot 10H_2O$/0.05 mol L^{-1} SDS in 20% methanol; injection, 1.0 s vacuum.

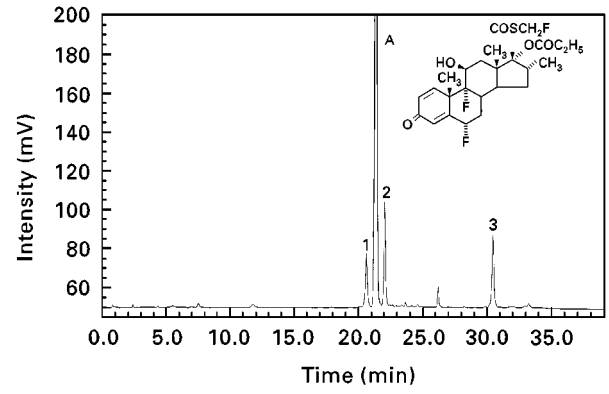

Figure 7 Separation of fluticasone from related compounds by HPLC. Column, 15 cm × 4.6 mm i.d. packed with 3 μm ODS-1; flow rate, 1.0 mL min^{-1}; detection at 238 nm, 0.05 absorbance units full scale (aufs); gradient 40% acetonitrile/H_2O → 70% acetonitrile/H_2O in 20 min. (By permission of the author.)

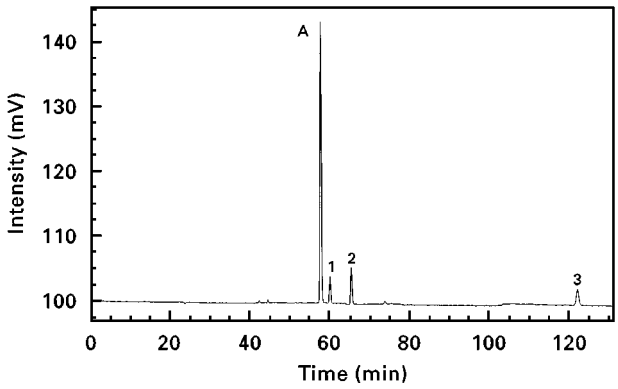

Figure 8 Separation of fluticasone from related compounds by capillary electrochromatography (CEC) with a phosphate buffer. Column, 40 cm × 50 μm i.d. packed with 3 μm ODS-1; detection at 238 nm, 0.05 aufs; voltage, 30 kV; temperature, 30°C; carrier, 75% acetonitrile/H_2O; buffer 2 mmol L^{-1} Na_2HPO_4, pH 8.3; injection, 0.4 min at 20 kV.

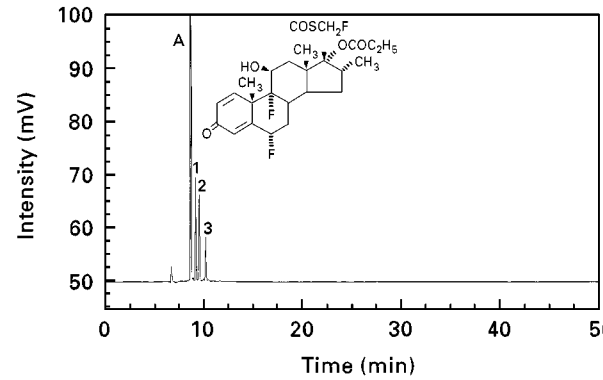

Figure 10 Rapid separation of fluticasone from related impurities by CEC with a Tris buffer. Column, 40 cm × 50 μm i.d. packed with 3 μm ODS-1; detection at 238 nm, 0.05 aufs; voltage, 30 kV; temperature, 30°C; carrier, 90% acetonitrile/10% 0.1 mol L^{-1} Tris; injection, 0.05 min at 10 kV.

Cephalosporins are a class of antibiotic compounds. As well as the chiral centre in the β-lactam ring, the compound shown in **Figure 14** possesses a chiral centre in the ester group on the adjacent six-membered ring, giving rise to a pair of diastereoisomers. In addition the oxime group can be in one of two positions – either the *syn* (*E*) or the *anti* (*Z*) – both of which give rise to isomers. Both pairs of diastereoisomers are readily resolved on the 3 μm Spherisorb ODS-1 column with a length of 40 cm. The use of such a column length for HPLC would be impractical because the pressure requirements would be beyond the range of current instruments. Since there is no pressure drop in CEC, the use of long columns presents no practical problems.

If the extremely high efficiencies obtained with CEC for achiral compounds could be matched with chiral compounds, then there would be less need to achieve high α values in order to separate the isomers. **Figures 15** and **16** show the separation of bendroflumethiazide and hexobarbital on an α-glycoprotein and a Cyclobond stationary phase, respectively. Although large α values are obtained with these columns, the overall efficiencies are extremely poor. This is possibly due to column overload, since these phases have a low sample capacity.

Nearly all CEC separations to date have been carried out on C_{18} or C_8 phases. However, because EOF drops off substantially below pH 6, most studies have been carried out above pH 7 and as a result most CEC has been for neutral molecules. One way of promoting EOF at lower pH values is by the use of columns packed with a strong cation exchanger (SCX); such

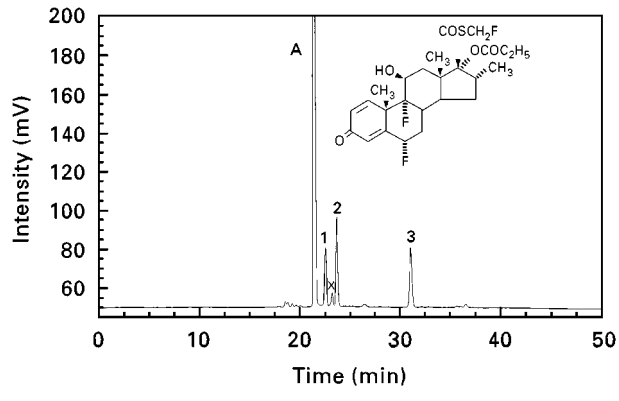

Figure 9 Separation of fluticasone from related compounds by CEC with a borate buffer. Column, 60 cm × 50 μm i.d. PC20 packed with 3 μm ODS-1; detection at 238 nm, 0.05 aufs; voltage, 30 kV; temperature, 30°C; carrier, 80% acetonitrile/20% 5 mmol L^{-1} borate, pH 9; injection, 0.4 min at 30 kV.

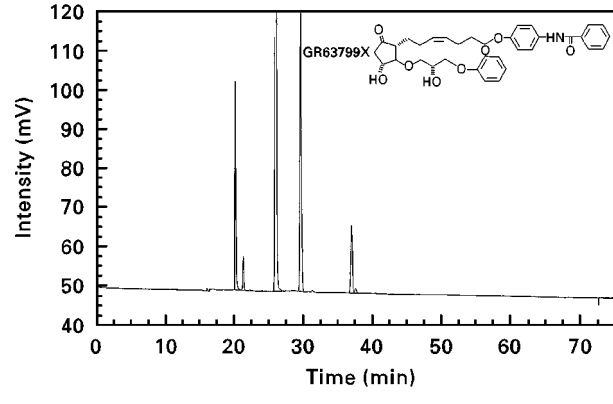

Figure 11 Separation of a prostaglandin from related impurities by CEC. Column, 60 cm × 50 μm i.d. packed with 3 μm ODS-1; detection at 270 nm, 0.05 aufs; voltage, 30 kV; temperature, 35°C; carrier, 70% acetonitrile/30% 0.1 mol L^{-1} Na_2HPO_4; injection, 0.04 min at 30 kV. (By permission of the author.)

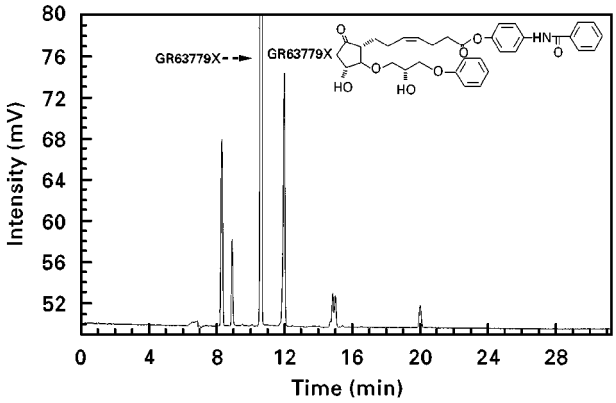

Figure 12 Separation of a prostaglandin from related impurities by CEC with a column packed with 1.5 μm C_8 silica. Column, 40 cm × 50 μm i.d. packed with 1.5 μm Zorbax; detection at 270 nm, 0.05 aufs; voltage, 30 kV; temperature, 30°C; carrier, 70% acetonitrile/30% 0.1 mol L^{-1} Na_2HPO_4; injection, 0.04 min at 20 kV.

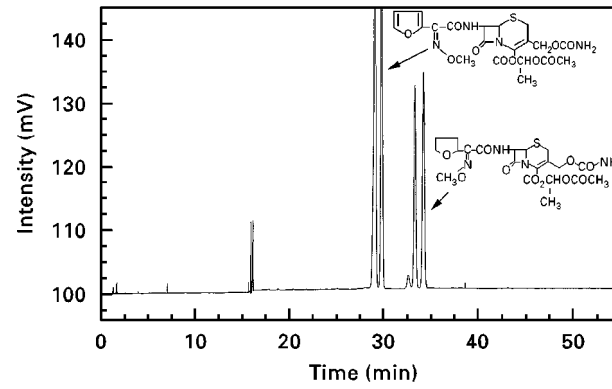

Figure 14 Separation of diastereoisomers by CEC. Column, 40 cm × 50 μm i.d. packed with 3 μm ODS-1; detection at 276 nm, 0.03 aufs; voltage, 30 kV; temperature, 30°C; carrier, 50% acetonitrile/50% 0.01 mol L^{-1} Na_2HPO_4; injection 0.2 min at 20 kV.

a material has been used for the CEC of neutral and charged analytes. The SCX used (manufactured by Phase Separations Ltd, Deeside, UK) had a sulfonic acid group linked to 3 μm silica via a propyl group to give a loading of 0.031 mEq g^{-1}.

Figure 17 shows the structures of a group of tricyclic antidepressants. Because of the highly basic nature of these compounds, with typical pK_a values of around 8, severe tailing is encountered with HPLC and even the new base-deactivated phases made from ultrapure silica still give some tailing.

When a test mixture containing bendroflumethiazide and four of the antidepressants was analysed on a Spherisorb ODS-1 capillary, only the neutral bendroflumethiazide eluted (**Figure 18**). However, CEC analysis at low pH on the 3 μm SCX phase gave unexpectedly high efficiencies. **Figure 19** shows the separation of three tricyclic antidepressants on the cation exchange phase at pH 3.5. It is noticeable in this chromatogram that the neutral compound bendroflumethiazide, despite eluting first, gives a significantly broader peak than the later eluting compounds. This suggests that some form of focusing effect is taking place. Plate numbers on this phase have been measured in excess of 8×10^7, which is many orders of magnitude above that explicable by current theory.

Attempts to use a mixed-mode column with a C_{18} ligand and a $-SO_3H$ functional group attached to the same silica particle were not successful. When the antidepressant test mixture is injected onto the mixed-mode column at both pH 9.8 and pH 5.7, only

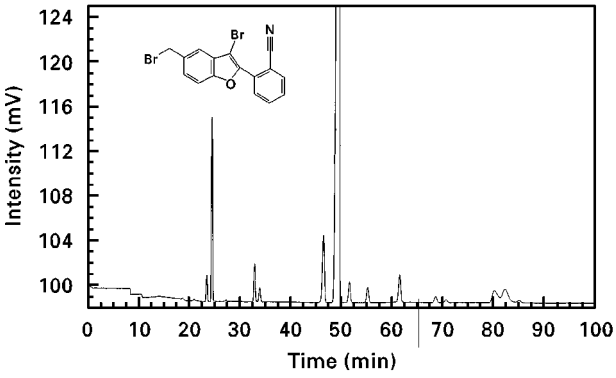

Figure 13 Separation of an angiotensin compound from impurities by CEC at a relatively high pH. Column, 30 cm × 50 μm i.d. packed with 3 μm ODS-1; detection at 220 nm, 0.03 aufs; voltage, 30 kV; temperature, 28°C; carrier, 76% acetonitrile/H_2O in 2 mmol L^{-1} Na_2HPO_4 buffer, pH 7.3; injection, 0.4 min at 20 kV.

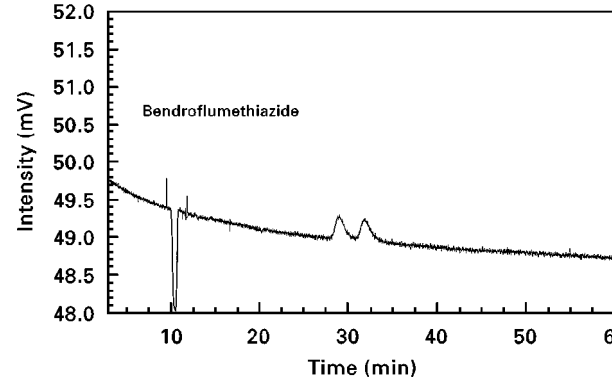

Figure 15 Chiral separation of bendroflumethiazide by CEC with an α-glycoprotein stationary phase. Column, 40 cm × 50 μm i.d.; detection at 225 nm, 0.05 aufs; voltage, 30 kV; temperature, 30°C; carrier, 10% IPA/0.01 mol L^{-1} Na_2HPO_4 buffer, pH 7; injection, 0.4 min at 30 kV. (By permission of the author.)

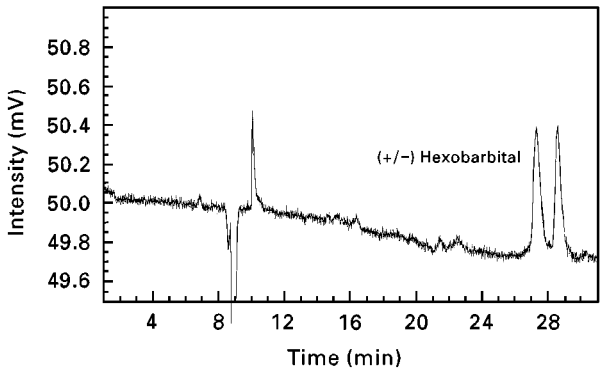

Figure 16 Chiral separation of hexobarbital by CEC with a Cyclobond 1 stationary phase. Detection at 210 nm, 0.05 aufs; carrier, 5% acetonitrile/95% Na_2HPO_4, pH 7.1; injection, 0.3 min at 10 kV. (By permission of the author.)

the neutral bendroflumethiazide is eluted. Similar results were obtained with a mixed-mode phase with a C_6 chain. It is believed that the hydrophobic ligands

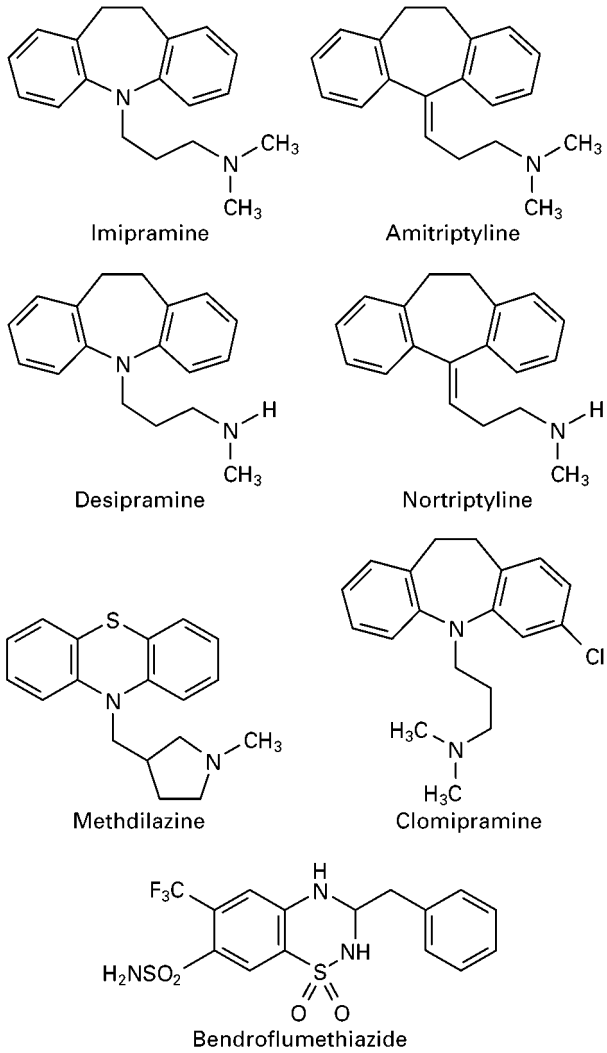

Figure 17 Structures of some tricyclic antidepressants separated by CEC.

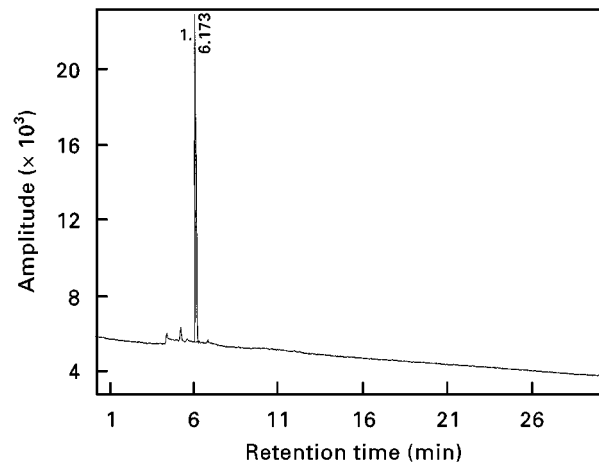

Figure 18 CEC of bendroflumethiazide and four tricyclic antidepressants by CEC on a 3 μm ODS-1 column. Detection at 210 nm, 0.05 aufs root temperature 0.2; voltage, 30 kV; temperature, 30°C; carrier, 70% acetonitrile/30% 0.01 mol L^{-1} Na_2HPO_4, pH 9.8 unadjusted; injection, 0.3 min at 3 kV. Peak 1 represents bendroflumethiazide. Nortriptyline, clomipramine, methdilazine and imipramine were not eluted.

collapse onto the $-SO_3H$ groups and shield them from participation in the focusing process. If a strong cation exchanger is produced, using a different link from the propyl group used for the original material, then the focusing effect returns. **Figure 20** shows the electrochromatogram of methdilazine, clomipramine and imipramine on a column containing a stationary phase where the sulfonic acid group is attached to the silica via a phenyl group. Once again, highly efficient separations are obtained. Methdilazine now elutes before clomipramine, whereas on the propyl $-SO_3H$ phase elution was the other way round.

Nonporous materials are used in HPLC for the analysis of highly hydrophobic compounds that

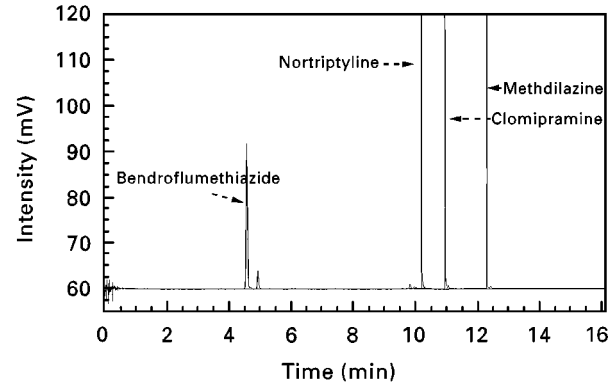

Figure 19 CEC of bendroflumethiazide and three tricyclic antidepressants on a 3 μm Spherisorb SCX column. Detection at 220 nm, 0.045 aufs; voltage, 30 kV; temperature, 30°C; carrier, 70% acetonitrile/30% 0.05 mol L^{-1} Na_2HPO_4, pH 3.5; injection, 0.5 min at 2 kV.

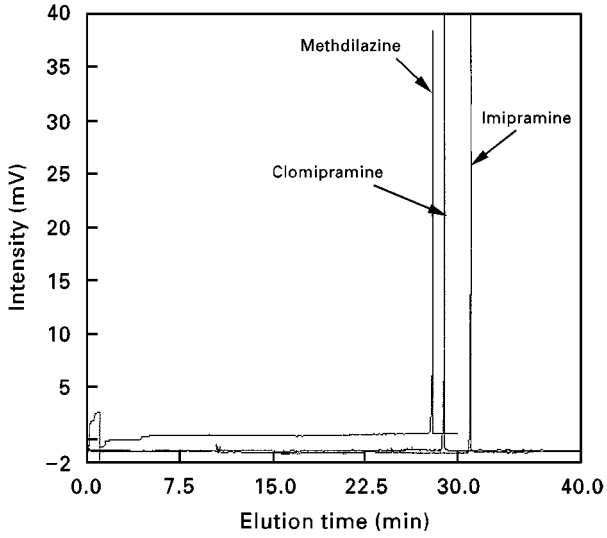

Figure 20 CEC separation of three tricyclic antidepressants on an SCX column with the SO_3H group attached to the silica via a phenyl group. Packed length = 40 cm, total length = 56 cm; voltage, 30 kV; detection at 210 nm; 0.02 aufs; carrier, 70% acetonitrile/30% 0.01 mol L^{-1} NaH_2PO_4, pH 2.3; injection, 0.4 min at 2 kV.

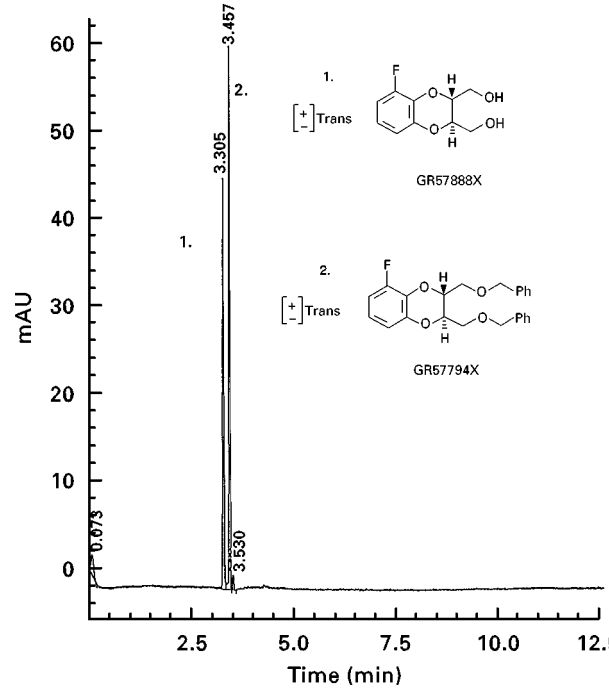

Figure 21 CEC separation on a nonporous phase, Micropell-C_{18}. Voltage, 30 kV; temperature, 30°C; detection at 210 nm; carrier, 70% acetonitrile/30% 2 mmol L^{-1} Na_2HPO_4, pH 7.0; injection, 10 s at 2 kV.

would normally be too strongly retained on conventional porous reversed-phase packings. Micropell C_{18}, made by Horváth (Yale, USA), has been used successfully for CEC with relatively small molecules even though there is little retention because of the very small surface area. **Figure 21** shows the separation of the neutral diol, GR5788X, from the dibenzyl compound, GR57994X, in less than 4 min on a 40 cm capillary packed with this 2 μm nonporous phase.

Conclusion

Capillary electrochromatography is a highly promising technique that couples the advantages associated with capillary electrophoresis with those of HPLC. Theory predicts a 2- to 3-fold increase in efficiency on going from an HPLC pressure-driven system to a CEC electrically driven system. Because there is no pressure drop across the capillary column in CEC, it is possible to use long columns that would require prohibitively high pressures if used in pressure-driven systems. Examples have been given of separations by CEC on 40 cm long capillaries giving efficiencies of 4×10^5 plates m^{-1}. This is equivalent to 160 000 effective plates, which is a factor of about 15 times greater than that currently available in HPLC.

The development of stationary phases that allow CEC to be used over a wider pH range will allow the analysis of mixtures containing neutral and charged species and will offer a viable complementary technique to HPLC and capillary electrophoresis.

See also: **II/Chromatography: Liquid:** Column Technology; Mechanisms: Reversed Phases; Detectors for Capillary Electrophoresis; Micellar Electrokinetic Chromatography. **III/Chiral Separations:** Capillary Electrophoresis.

Further Reading

Dittman MM and Rosing GP (1996) Capillary electrochromatography – a high efficiency micro-separation technique. *Journal of Chromatography A* 744(1–2): 63–74.

Knox JH and Grant IH (1987) Miniaturisation in pressure and electroendosmotically driven liquid chromatography: some theoretical considerations. *Chromatographia* 24: 135–143.

Knox JH and Grant IH (1991) Electrochromatography in packed tubes using 1.5 to 50 μm silica gels and ODS bonded silica gels. *Chromatographia* 32: 317–328.

Pretorius V, Hopkins BJ and Schieke JD (1974) Electroosmosis – a new concept for high speed liquid chromatography. *Journal of Chromatography* 99: 23–30.

Smith NW and Evans MB (1995) The efficient analysis of neutral and highly polar pharmaceutical compounds using reversed-phase and ion-exchange electrochromatography. *Chromatographia* 41: 197–203.