In the second edition (1986) of this reference work, the index doesn’t even mention electrophoresis. Traditional electrophoretic methodologies, although they used simple and reliable equipment, were always limited by their low resolution of analytes, low throughput, the need to visualise the separated bands and the qualitative nature of the results. They were, in fact, the electrophoretic equivalent of thin-layer chromatography (TLC) and, like TLC, fell from favour with the uptake of high-performance liquid chromatography (HPLC) during the 1970s.

Since 1986 a new ‘form’ of separation science, namely capillary electrophoresis (CE) has become accepted as both a research and a routine technique for the analysis of a wide variety of analytes, including drugs. Modern capillary electroseparation was introduced by Jorgenson and Lukacs (1981) and was developed rapidly by many other groups of separation scientists. By 1987 the first commercial instrument for performing CE had been introduced. CE now occupies a complementary role to HPLC in drug analysis, and by 2003 some 2200 assays for drugs had been produced, many of which are unique to CE.

The seminal publication of Jorgenson and Lukacs (1981) is usually taken as the origin of modern CE. A period of rapid development from about 1987 to 1995 was followed by a period of acceptance of the technology. During this period many of the HPLC companies that originally entered the field either abandoned commercialisation of CE systems or concentrated on building dedicated systems for techniques such as deoxyribonucleic acid (DNA) analysis or nanotechnologies. Today only two major instrument companies market general-purpose systems, although a number of smaller companies are also active in the field.
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Theoretical outline

The basic principles behind CE differ little from those of traditional electrophoresis in that the separation of two or more charged analytes (ionic compounds) or particles results from their different mobilities (i.e. speed plus direction of movement) when placed in a conducting medium under the influence of an applied direct current (DC) electric field. In electrophoresis the movement is towards the electrode of opposite charge to that of the ion or charged particle. Cations, being positively charged ions, move towards the negative electrode (the cathode). Anions are negatively charged and therefore move to the positive electrode (the anode). Importantly, neutral species do not move under the influence of the electric field, although they may diffuse from the load position or be carried by electro-osmotic flow (EOF).

At its simplest the speed of migration in an electric field of any ion or compound that carries an overall charge at a given pH is considered to be the vector sum of a driving force (the electrical potential) and any resistant forces. In simple solution, ions move freely towards the opposite electrode and the product of the charge on the ion and the applied electric field (V) gives the electric force ($F_{ef}$) experienced by the charged species. The potential gradient down which the ion moves is given by the electric field strength (E).

$$\text{Electric field strength } (E) = \frac{\text{Applied voltage}}{\text{Distance between electrodes}} = \frac{V}{d} \quad (30.1)$$

where $q$ is the total charge on the ion.

However, even a simple ion can be considered as a particle so this movement is opposed by a frictional drag ($F_{fr}$) given by Stokes Law:

$$\text{Friction } (F_{fr}) = 6\pi\eta vr \quad (30.3)$$

where $\eta$ is the viscosity of the medium, $r$ is the ‘radius’ of the molecule and $v$ is the velocity.

On applying a voltage there is a rapid acceleration of all the ionic species and equilibrium is achieved in a few microseconds, at which point the following equilibrium conditions apply:

$$F_{ef} = F_{fr} \quad (30.4)$$

Therefore
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So the velocity

\[ v = \frac{\text{distance moved}}{\text{time taken}} = \frac{qE}{6\pi r \eta} \]  

(30.6)

Electrophoretic mobility (\( \mu \)) is defined as the average velocity with which an ion moves under unit applied electric field under defined conditions:

\[ \mu = \frac{\text{Average migration velocity}}{\text{Electric field strength}} = \frac{v}{E} \]  

(30.7)

Substituting for \( v \) from Equation (30.3):

\[ \mu = \frac{q}{6\pi r \eta} \]  

(30.8)

Mobility can therefore be interpreted as proportional to a charge to size ratio for a molecule in a given buffer at a set pH. The units of \( \mu \) are cm/s divided by volts/cm, that is \( \text{cm}^2\text{V}^{-1}\text{s}^{-1} \). The magnitude of \( \mu \) for typical small ions is of the order of \( 10^{-6} \text{ cm}^2\text{V}^{-1}\text{s}^{-1} \) (e.g. \( \mu \) is \( -5 \times 10^{-5} \text{ cm}^2\text{V}^{-1}\text{s}^{-1} \) for a typical anionic sulfonamide, such as sulfacetamide). Note that differences in the sign of the mobility lead to different directions of movement in traditional electrophoresis.

The above theoretical description, well established by the 1950s, clearly showed that faster analyses should be achieved by using high voltages. However, the limitations to electrophoretic separations brought about by convective mixing caused by the heating effect of the current were also appreciated. This Joule heating seriously limited the speed of separation, since it restricted the voltages that could be used. For macromolecular separations the use of anti-convective gels such as polyacrylamide became, and have remained, the standard approach, but to limit the heat generated, voltages are still low (approx. 200 to 500 V) and runtimes are therefore long (3 to 18 h).

Jorgenson and Lukacs (1981), although not the first to attempt to overcome the problem of Joule heating, demonstrated a very simple solution. They showed that the rate of cooling could be increased substantially by increasing the surface-to-volume ratio of the electrophoresis buffer. This could be done readily using small-bore capillaries, with which the Joule heating was shown to be dissipated efficiently to give very high-resolution separations. The increased rate of cooling dramatically lowered the deterioration in peak efficiency and resolution.
caused by Joule heating. Although Jorgenson originally worked with glass capillaries, these were soon replaced by silica capillaries. In addition, this new format could use the significant EOF of the background electrolyte (BGE) in the capillary to separate cations, anions and uncharged molecules simultaneously, with all analytes usually going towards the cathode at which a single online detector is placed.

Most surfaces, including silica, acquire an intrinsic charge when wetted and attach a layer of solvent when under the influence of an electric field. This causes a movement of liquid towards one of the electrodes (i.e. electro-osmosis). In traditional electrophoresis electro-osmosis is usually detrimental, but in CE it can be of major benefit.

The mobility of the bulk liquid ($\mu_{EOF}$) is given by

$$\mu_{EOF} = \frac{\varepsilon E \zeta}{4\pi \eta}$$  \hspace{1cm} (30.9)

where $\varepsilon$ is the dielectric constant of the solution and $\zeta$ is the zeta potential of the surface.

For silica capillaries the charge on the surface results from ionisation of the silanol groups. The pK$_a$ of silanol groups of silica is variable but is about 4.5.

Zeta potential is given by

$$\zeta = \frac{4\pi \delta e}{\varepsilon}$$  \hspace{1cm} (30.10)

where $\delta$ is the thickness of the double layer and $e$ is the total excess charge per unit of area.

The zeta potential is an inverse function of the square root of the total molarity of BGE (the Debye–Huckel equation), and therefore the EOF decreases as the square root of molarity. Since EOF is generated at the capillary wall, its flow profile is flat rather than parabolic as in pumped flows. This effect contributes to the efficiency of CE.

The addition of reagents that can bind to the surface silanols, such as cationic detergents like tetradecyltrimethyl ammonium bromide (TTAB) to the BGE, can reverse the direction of the EOF. This is beneficial in the analysis of anions such as nitrate and nitrite, but it is essential to saturate all the silanols prior to establishing the separation.
Mobility in capillary electrophoresis

On an electropherogram the mobility ($\mu$) of an analyte is calculated from its migration time ($t$) and the length of the analytical section of the capillary, which is normally less than the total length down which the applied potential is dropped.

$$\mu = \frac{\text{Velocity}}{\text{Potential gradient}} = \frac{t/L}{V/L}$$  \hspace{1cm} (30.11)

where $l$ is the length to detection window and $L$ is the total capillary length.

Separation depends on differences in mobility for the analytes, but $\mu$ is the sum of the mobility of the analyte $\mu_a$ and the EOF $\mu_{EOF}$, and is called the apparent mobility ($\mu_{app}$).

$$\mu_{app} = \mu_a + \mu_{EOF}$$  \hspace{1cm} (30.12)

(The sign of $\mu$ is important.)

Peak efficiency

Jorgenson also developed a simple theoretical understanding of this new separation mode. The separation efficiency of CE is expressed in terms similar to those used in chromatography and are familiar to chromatographers. For a Gaussian peak – this is more likely in CE than HPLC – the number of theoretical plates ($N$) for a peak migrating at time $t$ is given by

$$N = \frac{(l/c)^2}{\sigma^2} \text{or} [5.54 \ (t/w_{1/2})^2]$$  \hspace{1cm} (30.13)

where $\sigma$ is the standard deviation of the peak and $w_{1/2}$ is the width of the peak at half height.

In a capillary the only major contribution to zone dispersion is the time-dependent longitudinal diffusion. This can be characterised by Einstein’s equation for diffusion in liquids

$$\sigma^2 = 2Dt$$  \hspace{1cm} (30.14)
where $D$ is the diffusion coefficient for the analyte. Substituting for time and combining this equation with that for mobility

$$
\sigma^2 = \frac{2DLI}{V\mu_{\text{app}}} \quad (30.15)
$$

Combining this equation with that for efficiency

$$
N = \frac{\mu_{\text{app}}IV}{2DL} \quad (30.16)
$$

From these equations it is apparent that in CE peak efficiency, measured as $N$, increases linearly with the applied voltage. So the higher the voltage, the faster the separation and the narrower the peaks. A high pH, and therefore high $\mu_{\text{EOF}}$, is also advantageous to high efficiency. Analytes with a high mobility are separated efficiently and those analytes with low diffusion coefficients, such as proteins, should have high separation efficiencies.

Typical diffusion coefficients in water at room temperature are given in Table 1.

The generic Giddings’ resolution equation ($R_s$) as expressed in electrophoretic terms:

$$
R_s = \frac{\sqrt{N}}{4} (\Delta \mu/\mu_{av})
$$

can now be rewritten as

$$
R_s = \frac{1}{4} \sqrt{\left(\frac{\mu_{av} + \mu_{\text{EOF}}}{2D}\right) \left(\frac{\Delta \mu}{\mu_{av} + \mu_{\text{EOF}}}\right)}
$$

From this equation two findings are apparent:

Although peak efficiency increases linearly with voltage, resolution requires a four-fold increase to double resolution.

Infinite resolution is possible when $\mu_{\text{EOF}}$ is equal in magnitude, but opposite in direction to $\mu_a$.

Since Jorgenson originally derived these formulae a number of variations have appeared that include corrections for sample injection size, Joule heating variations across the capillary, etc. However, his formulae still offer a
reasonable approximation to the experimental observations. The theory of CE was reviewed recently (Mosher et al. 1992; Poppe 1998)

Modes of capillary electrophoresis

CE is recognised generally as the description appropriate for the whole field of separation science based on electromigration techniques. With the increasing number of variants on the basic technique, more specific definitions are now required, although no International Union of Pure and Applied Chemistry (IUPAC) definitions had been agreed by the time of going to press. The following definitions are the author’s own.

Capillary zone electrophoresis (CZE), in which ionic species are separated according to their mobility and polarity in aqueous solution, is the most frequently used option in CE. CZE does not necessarily require aqueous electrolytes and non-aqueous BGEs (NACE) such as acetonitrile can be used, provided the current can be carried by a suitable soluble electrolyte such as ammonium acetate. CZE does not separate neutral compounds, although they do move towards the detector, travelling with the EOF front. A sub-type of CZE is capillary ion analysis (CIA), which is used to determine simple ionic species in aqueous solution rapidly and usually employs indirect detection. CZE is illustrated by the separation of members of the sulfonamide family shown in Fig.1.

Figure 1: CZE separation of sulfonamides. Separation of twenty compounds, seventeen sulfonamides, levamisole, trimethoprim, and pyrimethamine (all 50 μM); separation with 30 mM sodium dihydrogen phosphate/10 mM...
sodium tetraborate pH 6.75, 15 kV, HD 1.8 s at 50 mbar; hydrodynamic injection, 60(47) × 50 μm, 200 nm. Key: PST, phthal-sulfathiazole; PY, pyrimethamine; SA, sulfanilic acid; SAA, sulfanilamide; SAC, sulfacetamide; SDI, sulfadiazine; SDIM, sulfadimethoxine; SG, sulfaguanidine; SIOX, sulfaisoxazole; SM, sulfameter; SMOP, sulfamethoxypyridine; SMOZ, sulfamethoxazole; SMR, sulfamerazine; SMZ, sulfamethazine; SP, sulfapyridine; SQ, sulfaguanulose; SST, succinyl-sulfathiazole; ST, sulfathiozole; and TRI, trimethoprim.

Other forms might be entitled interaction CE (ICE), in which the analytes’ mobilities are modified by the presence of additives in the BGE that cause:
- Complexation, for example metals and amino acids or borate in the case of sugars
- Inclusion into chemical additives such as cyclodextrins.

A similar mechanism is involved in affinity CE (ACE), in which biospecific interactions, usually with macromolecules, occur. Chiral separations by CE can be obtained using either ICE or ACE methods. A long-established form of ICE is micellar electrokinetic capillary chromatography (MEKC), often also called MECC. This separation mode, introduced in 1984 by Terabe et al., allows the separation of neutral molecules by differential partitioning into migrating charged micelles formed from suitable detergents incorporated into the background CE electrolyte. MEKC is considered to offer an intermediate mechanism between electrophoresis and reversed-phase chromatography in that the hydrophobicity of the analyte can be a dominant factor in the final separation. As well as surfactants, other additives (such as cyclodextrins) can be included in the MEKC electrolyte to enhance separations. MEKC does not prevent the simultaneous separation of charged species in the samples, a factor of some importance in drug analyses, since an uncharged drug often gives rise to charged metabolites. MEKC is illustrated in Fig. 2, in which the metabolism of ibuprofen is being monitored. A more recent, but related, development is microemulsion electrokinetic capillary chromatography (MEEKC), in which a charged surfactant–based microemulsion is formed in the BGE to produce the hydrophobic pseudo-chromatographic phase.
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Figure 2.: MEKC separation of ibuprofen and its metabolites in urine. Conditions: HP3D system; capillary, silica, 47(38.5) cm × 50 μm; buffer, 25 mM sodium tetraborate pH 9.5 that contains 75 mM sodium dodecyl sulfate (SDS) and 6.2 mM sulfated-β-cyclodextrin; sample, normal pooled urine (dilute 1 + 1) or standard; load, HD 2 s at 50 mbar; voltage, 22 kV; temperature, 25°; detection, 195 to 300 nm (195 nm shown).

Other early forms of electrophoresis, such as isoelectric focusing (IEF) and isotachophoresis (ITP), can also be performed in capillary format, known as capillary isoelectric focusing (cIEF) and capillary isotachophoresis (cITP). In cIEF the order of the final separation is according to the isoelectric points of the analytes when they migrate in a pH gradient formed in the capillary using ampholytes. Although IEF is a standard methodology in gel electrophoresis and offers a high resolution of proteins, it has been used relatively little in CE because of the high background absorbance of the ampholytes. In cITP, the separation occurs in an electrolyte system composed of two buffers with widely different mobilities that encompass the mobilities of the analytes. At equilibrium adjacent zones must migrate with equal velocities, which can be achieved only if all the bands contain ions at the same concentration, and therefore cITP can be used to concentrate dilute samples prior to CZE and has proved useful in combination with mass spectrometry (MS).

Another separation mode, capillary sieving electrophoresis (CSE), involves the electromigration of macromolecules through a sieving media to generate a separation based on molecular size. Initially, the media was a porous semi-solid gel, as in slab gel electrophoresis, and was termed capillary gel electrophoresis (CGE). The filling of capillaries with gels has now been supplanted by the use of replaceable entangled viscous polymer solutions, such as 0.5% w/v hydroxyethylcellulose. It may be necessary to minimise electro-osmosis for maximum resolution. CSE is now commonplace in a number of commercial DNA sequencers.

A recent development is capillary electrochromatography (CEC) in which EOF is used to drive eluent through a capillary that contains a stationary phase. The stationary phase may be HPLC phases mechanically packed into the capillary, open-tubular capillaries with a phase chemically bonded to the capillary wall or porous monolithic beds of a phase chemically formed in the capillary. In HPLC the flow resistance afforded by the particle size and narrow bore columns limits column efficiencies, but CEC does not suffer from flow-generated back pressures and can therefore use narrow capillaries packed with sub-micron particles.

Comparison to other analytical separation methods

For many analyses, CE is a complementary technique to HPLC. However, it can offer a number of advantages. It can analyse very small samples; in fact, many CE runs can be made from as little as 10μL of sample and still leave sufficient sample for a HPLC injection. It is less sensitive than HPLC, but it can be used to detect at wavelengths below those normally used in liquid chromatography (LC; e.g. <200 nm). CE can be used with a wider range of
analytes, since anions, cations and neutrals can all be assayed in one CE run, especially when using MEKC. Compared to HPLC, it can be applied readily to a wider range of analytes, from ions to macromolecules. Chiral CE is much simpler and cheaper to investigate than chiral HPLC. The capital costs of the instruments are equivalent for both systems, but CE is significantly cheaper to run and does not require the disposal of large quantities of organic solvent.

Instrumentation for capillary electrophoresis

Jorgenson’s experimental work led to the development of a new type of instrument for analytical separations that was very similar in its operation to HPLC. Most of the major HPLC instrument manufacturers had, by the end of the 1980s, introduced CE systems of various degrees of sophistication. There was also considerable hype that CE was going to replace HPLC for analytical separations. Yet by the end of 2001 only two major instrument companies were still marketing complete CE instruments, at least for the analytical chemistry market.

CE is characterised by its ability to resolve rapidly the components of usually complex aqueous samples using applied dc voltages with field strengths up to 1 kV/cm to give a very high resolution ($N > 250,000$) that measures <10 nL of sample with analytical precision. To maintain efficiency, detection [ultraviolet (UV), fluorescence] is nearly always on-line (i.e. across a window burnt into the polyimide coating of the silica capillary). Detection at the end of the capillary can be achieved using electrochemical detectors or, more importantly, MS.

A typical CE instrument consists of a capillary, detector, high–voltage power source and a recording device (Fig.3). The capillary used is made of fused silica and externally coated with a thin layer of polyimide that prevents surface hydration, so making the capillary flexible. The capillary is usually about 375 μm outside diameter and 10 to 150 μm internal diameter (ID), with 50 and 75 μm the more usual. There is no typical or required capillary length for CE, although in commercial instruments there are minimum lengths that can be used, usually dependent on the position of the detector. Clearly, the combination of aqueous salt solutions and very high voltages means that instruments must be designed with appropriate safety features. It must not be possible to gain access, particularly to the anodic vial, when high voltages are being applied.

Modern commercial instruments are sophisticated computer–controlled instruments capable of unattended reliable operation with capacities of some 100 samples. The capillary temperature must be well controlled to minimise Joule heating and optimise migration–time reproducibility. Detection is by diode array, although other detection options are on offer. The Royal Society of Chemistry has published a check list to aid in the selection of CE instruments (Greenfield et al. 2000).
Power supplies and electrodes

All systems require a power supply able to generate stable voltages up to 30 kV DC at up to 300 mA. The 30 kV is the standard maximum, since voltages much above that cause shorting within the system. The system should be able to operate in both normal and reversed-potential mode, in which the detector end of the capillary becomes the positive electrode. Reversed polarity is useful for both CIA and when using short-end injection. The voltage must be regulated carefully and controls that either cut-off the power or reduce the wattage if the current limits are exceeded must be included in the software. It is often advantageous to be able to ramp the voltage to the operational voltage over a short period (e.g. 10 s at the start of the run). The voltage is conducted via high-voltage cables to the electrodes, which are either simple platinum wires or platinum tubes of about 1 mm bore. Clearly, with such high voltages in the presence of aqueous salt solutions safety is a priority in the design of the commercial instruments and operators must follow the manufacturers instructions when using or maintaining their CE systems.

Capillaries

CE capillaries can be obtained both from instrument manufacturers with a pre-prepared detection window or made in the laboratory from polyimide-coated fused-silica capillary. Relatively few companies supply the base polyimide-coated fused-silica capillary and most instrument manufacturers design their capillary mounts to accommodate the polyimide-coated silica capillary manufactured by PolyMicro Technologies, which has an outer diameter of 375 μm. The capillary must be mounted in the CE instrument’s specially designed holder and loaded into the...
thermostatted capillary compartment of the instrument. In some instruments the ends of the capillary are guided by the cassette through the centre of the platinum electrodes into the electrolyte. In others the capillary merely sits next to a solid electrode wire. To allow detection a small section of polyimide coating has to be removed, which makes a weak point in the capillary.

Although a basic concept in CE is that efficient cooling is generated by the capillary format, in practice additional cooling is built into commercial instruments. Capillaries are either cooled by a forced stream of cooled air or by immersion in a cooling liquid. Using a cooled liquid has been shown to be more efficient than forced air, but in practice there is no difference in performance between the two methods.

Samples and sample injection

As in all analytical methods, correct sample preparation and treatment is essential. Since the capillary ID is small, samples for CE should be free from particulate matter and care should be taken to avoid formation of precipitates.

As in HPLC the sample to be analysed is placed in a small vial, typically a 1.5 mL HPLC vial, in the sample holder of the CE system. Polypropylene vials can often be used in CE since samples are usually in aqueous solution. Micro insert vials are often used, since commonly only nanolitres are injected from very small (<10 μL) samples. In most systems the vials are capped to reduce evaporation, which can be significant when only small volumes of sample are to be used. The cathodic and anodic electrolyte solutions are also usually held in 1.5 mL vials. The sample racks in some instruments are thermostatted to either reduce sample evaporation by cooling or heated to enable reactions such as enzyme digestions to take place.

Most instruments offer two means of injection. Hydrodynamic (HD) injection is the most widely used. The injection end of the capillary is moved into the sample vial and either a controlled pressure (e.g. 50 mbar) is applied to the sample or a vacuum is applied to the other end of the capillary for a few seconds. In this way a few nanolitres of sample enter the capillary. The amount injected is given by Poiseuille’s formula. In HD injection there is no sample bias. In electrokinetic (EK) injection, the injection end of the capillary is moved into the sample vial and analytes are migrated into the capillary under a transient high voltage (e.g. 5 kV for 5 s). EK injection biases the sample injection towards the most electrophoretically mobile species, so it can be used to analyse samples in difficult matrices such as pharmaceutical syrups as well as oligonucleotides in DNA analysis.
Detectors

Although many detectors have been proposed for CE, few are commercially available. The standard detector supplied with commercial instruments is a UV/visible spectrophotometric detector, most often a diode array detector (DAD). The light from the lamp is focussed via suitable optics through a window formed in the capillary. Since the Beer–Lambert law governs detection sensitivity, the micrometre long paths within the capillary mean that optical detection in CE is sensitivity limited by this very reduced path. Various attempts to overcome this limitation have been developed, which include capillaries with bubbles at the detection site and two forms of Z-cell. One design places a bend in the capillary, but this is fragile. The second uses a cell, rather like an HPLC flow cell, coupled to the capillary and gives a light path of 1 mm, but to align this micro plumbing is difficult. However, an advantage of silica is that very low UV wavelengths can be used. It is common practice to detect at 200 nm, or even as low as 185 nm. At such wavelengths a very large number of compounds exhibit significant UV absorbance, although selectivity of detection can be compromised. A confusion can arise because only very small volumes of samples are analysed in CE, typically 1 to 20 nL. This means that the mass sensitivity of CE (i.e. the numbers of molecules detected) is very low, whereas the concentration sensitivity is usually much higher. A modern UV/visible detector for CE operating with clean buffers should be capable of better than 10 mAu full-scale sensitivity with 1 to 2% baseline noise.

A commercial conductivity detector is available for use in CIA, but it is necessary to isolate the detector cell effectively from the high voltages in the system. Although many types of electrochemical detector have been published, there is no commercial model available for CE.

A fluorescence detector based on the designs for HPLC that offers a wide range of excitation wavelengths is also available. At least two manufacturers produce laser-induced fluorescence (LIF) detectors, but the choice of excitation wavelengths is extremely limited. Only commercial laser wavelengths are available (e.g. 325 nm from a helium–cadmium laser and 488 nm from an argon laser) and there is no readily available UV laser. With the best systems a few hundreds of molecules can be measured, which corresponds to picomole detection limits. Pre-, on- and post–capillary reaction schemes have been developed successfully to derivatise analytes with fluorescent reagents. Most derivatisation reagents used with HPLC have been used with CE, especially so with derivatives, such as dansyl chloride, for primary amines. Unfortunately, most derivatives react with the charged groups on analytes which renders them less suitable for CZE and MEKC, for which methods need to be developed, is usually used.

MS is being increasingly used with CE. Samples can be measured off-line if fraction collection on the CE is possible or by collecting a continuous trace of sample on a film. This type of output has been used with matrix-assisted laser desorption ionisation – time of flight (MALDI-ToF) mass spectrometry. Direct interfacing with MS, especially electrospray, is becoming common. Most commercial systems can be linked either via commercial or lab-constructed interfaces to mass spectrometers. It is often necessary both to add make-up solvent and
sometimes to degrade the sharpness of the CE peak to ensure it is not missed during the scan cycle of the MS. Although simple volatile CE buffers, such as formate and acetate, are MS compatible, not all CE electrolytes systems are compatible. In particular, MEKC buffers and cyclodextrin additives should not be sprayed directly into most MS systems.

Indirect detection is often used with CE when it is necessary to detect a non-UV-absorbing species, such as inorganic ions. Indirect detection works by the displacement of a UV-absorbing, fluorescent or electroactive BGE component by the analyte. This leads to reduction of a high background signal level. Important parameters are the background signal noise level, stability and the efficiency of displacement of the detected compound by the analyte.

Some idea of the relative sensitivities of CE detectors towards favoured analytes, expressed both in terms of mass and concentration limit of detection (LOD) is given in Table 30.2. However, the rule of thumb is that CE detection is 10 times less sensitive than that of HPLC.

**Automation and data output**

In modern CE instruments, autosampler technology has improved injection reproducibility, and capillary and sample cooling have improved migration–time reproducibility. Instruments can run unattended, run samples in a pre–programmed order and allow any combination of washes and buffers to be used during analysis. Different methods can be programmed to run sequentially to analyse the same samples for different analytes or completely unrelated samples. Most instruments allow all the instrument parameters mentioned in this chapter and some instruments allow full control of both detection and data collection, which makes the whole system self-contained. Data analysis is the same as for HPLC systems.

**Data acquisition rate and response time**

The narrow peaks inherent in CE necessitate the use of faster detector rise times than is normal in HPLC, and therefore data acquisition rates for CE should be >25Hz. Response time is adjustable on most detectors and is the time it takes for a detector to respond to a peak, defined as the time taken for a detector to change for 10% to 90% of its range. Narrow peaks can be misrepresented if slow response times are used, and very narrow peaks may even be missed. In addition, a high mobility analyte may give a non-Gaussian peak shape, which produces poor quantitative data when low data collection rates are used. The higher data collection rates give a better representation of the true peak shape.
An unusual requirement in CE is the need to work with spatial area rather than the simple integrated area. This is because the area of a peak is dependent on its apparent mobility (i.e. later migrating components move through the detector slower than earlier peaks and so record a larger peak area for the same amount of analyte). This is simply corrected for by dividing the measured area by the migration time to give the spatial area. The calculation of spatial area must be included in the integration software used for CE. It is spatial area that should be used in quantitative calculations.

Operation of capillary electrophoresis systems

Capillary preparation

Capillaries should be cut to the required length with a ceramic cutter or fibre–optic cable cutter and pulled lengthways to break the capillary. A magnifying glass is used to check that the ends of the capillary are clean cut – or they are trimmed until straight. Irregular capillary ends can cause poor peak shapes and lead to carryover of sample. Some workers suggest burning off 1 mm or so of polyimide at the end. Since UV light cannot pass through the polyimide coating a small window must be formed at the required site of the detector, which can be done in various ways (e.g. using a naked flame), but the use of an electrical burner is recommended. The window is burnt at the measured point on the capillary so that the polyimide is charred completely, and then carefully cleaned off with a tissue wetted in methanol. After preparing the window, a magnifying glass is used to check that it is clear of burnt polyimide. The capillary should be mounted in the instrument’s capillary cassette and the cassette loaded into the instrument.

Prior to use it is always necessary to clean and condition a new silica capillary. To do this, rinse the capillary with 0.5 M sodium hydroxide for 30 min at 50°, then rinse immediately with water followed by the chosen buffer for a further 20 min. A less aggressive clean should be used at the start of each day or when the separation becomes poor. Flush for 15 min with 0.1 M sodium hydroxide at 40°, followed by a 2 min wash with water prior to 5 min with the electrolyte. If using CZE, wash the capillary between runs for 2 min with 0.1 M sodium hydroxide followed by a 3 min flush with electrolyte. The electrolyte wash may need to be longer if an acidic buffer is used (note that for coated and filled capillaries it is essential to follow the manufacturer’s instructions, not the rules given here).

Washing between runs is not always necessary with MEKC buffer systems, since they are alkaline and contain protein-solubulising detergents. To prevent ion depletion and pH changes the anodic and cathodic buffers should be replenished approximately every five to ten runs.
Buffers

The correct formulation and adjustment of the CE buffer (or more correctly the BGE) is essential since it is the pH of the buffer more than anything else that controls the separation. Buffers should be prepared using HPLC, analytical grade or better reagents and deionised water. The pH should be adjusted after dissolving all the inorganic species, but prior to the addition of any surfactant, EOF modifier or organic additive to the buffer. Buffer ions should be chosen that form good buffers, that is are to be used within ±1 pH unit of their $pK_a$ (Table 30.3) and carry low currents. The pH meter should be calibrated against the necessary standard solution range (pH 4 and 7 and pH 7 and 10) before pH adjustment is made. The concentration of the BGE is of less importance. Higher molarity buffers suppress EOF and enhance system buffering, but carry higher currents and therefore generate more heat. For most systems EOF is virtually independent of the nature of the buffer salt’s cation for monovalent ions. In the author’s laboratory, 1 mM sodium ethylenediaminetetraacetic acid is routinely added to buffers as this removes some baseline artefacts. Buffers should be degassed by sonication, and when optimal performance is required, filtered through a 0.45μm membrane. De-gassing with helium, as in HPLC, can also reduce baseline noise.

All buffer systems are UV absorbing, particularly in the 190 to 230 nm spectral region, which is the range of UV detection most commonly used in CE. Inorganic buffer systems, such as phosphate and borate, are preferred when low UV detection is used (Table 30.3). Background absorption is one of the causes of baseline noise, so reducing the absorption of the buffer reduces background noise. This reduction can be achieved in various ways. The BGE system can be changed to a lower UV-adsorbing inorganic buffer, HPLC grade usually being better than A.R. grade salts, but this is not always practical. ‘Cleaning’ the aqueous buffer by passage down a C$_{18}$ solid-phase extraction (SPE) cartridge can reduce background noise significantly by removing trace contaminants (Hows et al. 1997).

One important advantage of CE methods is that they not only use very little sample, but also, compared to HPLC, they consume very little in the way of buffers and expensive buffer additives, such as cyclodextrins. Whereas a gradient reversed phase (RP)-LC system might use many litres of organic solvent per week, a similar separation by CE would use less than 100 mL of a usually aqueous buffer. Such savings have cost, health, safety and environmental advantages.

Method development and optimisation

The starting point for developing a CE separation is to first study the literature. There are nearly 10 000 published CE articles, of which some 400 describe drug analyses in biofluids or other biological matrices. If there is no publication on the compound(s) in question, but some basic physicochemical data that include $pK_a$ values are known, then migration by CZE is amenable to simple modelling from first principles. The various modelling
approaches range from the calculation of the change in the degree of ionisation with pH using the Henderson–Hasselbalch equation, possibly corrected for molecular size, performed in a standard spreadsheet to commercial computer simulations. In CZE, compounds are best resolved in a buffer with a pH that is mid-way between their respective pKₐ's. A plot of pH against mobility clearly shows this and enables the selection of the optimum pH for the BGE.

Regardless of whether working with CZE or MEKC, method development should start with the minimum usable length of new 50μm capillary for the instrument under study. Rigorous conditioning of the capillary is described under Capillary preparation. Prepare samples at a concentration of about 1 mg/mL and use HD injection (e.g. 2 s at 50 mbar) followed by detection at about 200 nm for all initial runs.

Fig.4 presents a generalised approach to the selection of a starting position for the analysis of small molecules by CE given a minimum of chemical knowledge about the compounds. It is linked to the generic CE buffer systems listed in Table 30.4. If physicochemical data for the analyte(s) are not available, the CZE optimisation is started with an initial electrolyte of 20 mM sodium tetraborate pH 9.2, as this generates a high EOF that should ensure all peaks migrate towards the detector, as well as being a good buffer system. It is usually helpful to also monitor the EOF in the BGE; this is done by injection of a neutral marker, such as mesityl oxide or acetone. Buffers can then be varied to develop better resolution. Once buffer conditions for a separation are determined, other experimental parameters that can be optimised easily are applied voltage and capillary temperature, since these are usually software controlled.
Figure 4. Scheme for the selection of a CE method based on analyte characteristics
<table>
<thead>
<tr>
<th>Generic buffer</th>
<th>Composition</th>
<th>pH</th>
<th>CE conditions</th>
<th>Analytes</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>25 mM sodium phosphate</td>
<td>2.8</td>
<td>CZE</td>
<td>25–30</td>
<td>25°</td>
</tr>
<tr>
<td>B</td>
<td>20 mM sodium tetraborate</td>
<td>9.2</td>
<td>CZE</td>
<td>20–25</td>
<td>25°</td>
</tr>
<tr>
<td>C</td>
<td>25 mM sodium tetraborate plus 75 mM SDS</td>
<td>9.2</td>
<td>MEKC</td>
<td>15–25</td>
<td>20°</td>
</tr>
<tr>
<td>D</td>
<td>10 mM sodium tetraborate plus 2 mM TTAB + 5 mM sodium chromate</td>
<td>9.0</td>
<td>CIA</td>
<td>25–30</td>
<td>20°</td>
</tr>
<tr>
<td>E</td>
<td>10 mM sodium tetraborate plus 2 mM TTAB + 5 mM imidazole</td>
<td>9.0</td>
<td>CIA</td>
<td>25–30</td>
<td>25°</td>
</tr>
<tr>
<td>F</td>
<td>25 mM sodium tetraborate plus 75 mM SDS plus 6.25 mM sulfated β-cyclodextrin</td>
<td>9.2</td>
<td>CD-modified MEKC</td>
<td>18–22</td>
<td>25°</td>
</tr>
</tbody>
</table>
### Table 30.4. Suggested generic CE buffer systems for drugs and their metabolites

Other typical operating conditions: silica capillary, 50μm i.d. × 44 cm (37 cm to window); injection, 2 s at 50 mbar HD; detection, 195 nm. TTAB, trimethyl tetradecyl ammonium bromide; SDS, sodium dodecyl sulfate.

Increasing the voltage decreases the analysis time. A plot of applied potential against current (Ohm’s law plot) should be made. The voltage at which this plot becomes nonlinear is that at which peak efficiency is compromised because of Joule heating, so it is best to operate at a slightly lower voltage. Temperature can have variable effects in CZE. It changes buffer viscosity, changes the apparent pH and may change analyte solubility, so it needs to be optimised carefully. Optimisation of the amount of sample to inject is also readily investigated, since it is also software controlled, but as a rule of thumb the injection volume should not occupy more than 2% of the total capillary length.

Optimisation of capillary bore and/or length is much more difficult, since many new capillaries would need to be prepared. It is best to optimise a separation on the shortest capillary that the system can accommodate and only increase its length if this is necessary to improve resolution.

The buffer composition is more complex in MEKC so optimisation is more difficult. The pH of the background continues to be fundamentally important to the separation, since it controls not only the EOF, but also the mobility of any charged species in the samples. In addition it is necessary to optimise the concentration of the surfactant and possibly the concentration of any modifier, such as acetonitrile in the BGE. The type and concentration of the buffer salts in the BGE can also influence the formation of micelles. Chemometric methods have been used successfully to optimise these complex MEKC systems.

<table>
<thead>
<tr>
<th>Generic buffer</th>
<th>Composition</th>
<th>pH</th>
<th>CE conditions</th>
<th>Analytes</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>Methanol plus acetonitrile (50:50) plus ammonium acetate (1 mM)</td>
<td></td>
<td>Non-aqueous CZE</td>
<td>20–30</td>
<td>Non-polar analytes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Typical voltage (kV)</td>
<td>Temperature</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>Heptane (0.8% v/v) plus butan-1-ol (6.6% v/v) plus 3.3% w/v SDS in 25 mM sodium tetraborate</td>
<td>9.2</td>
<td>MEEKC</td>
<td>20</td>
<td>Charged and neutral analytes; endogenous compounds</td>
</tr>
</tbody>
</table>

Note: TTAB, trimethyl tetradecyl ammonium bromide; SDS, sodium dodecyl sulfate.
Generic systems for drug assays

Its high resolving power means that a limited number of simple CE electrolyte systems can be used to separate a large number of diverse drug types. A number of authors have developed generic approaches to CE separations. MEKC, especially when modified by the addition of sulfated-β-cyclodextrin, was developed by the author’s laboratory as a powerful generic methodology especially applicable to biological fluid analyses (Alfazema et al. 2000). Table 30.4, based on the methods used in the author’s laboratory, summarises these generic systems.

Quantification and validation

The ultimate goal of any analysis is the identification and, if required, quantification of the analytes in the given sample. Confidence in the results obtained requires a full validation of the assays, and this is no different for CE assays.

Identification can be achieved in a variety of ways:

- Matching of migration and/or retention times with standard compounds. This can suffer from matrix effects with real samples, so ideally real samples should be spiked with standard compounds.
- Matching of relative migration times or mobilities to internal standards or the use of capacity factors and/or retention time in MEKC.
- The use of detectors that provide qualitative data, such as DADs and mass spectrometers.
- The use of enzymatic and/or chemical derivatisation to modify and/or remove peaks from the electropherograms.
- Fraction collection and subsequent use of MS, nuclear magnetic resonance, etc., to provide qualitative and structural data.

Quantitative measurements are the same for CE as for HPLC and gas chromatographyGCin that peak areas or heights should be determined using an integration method. One difference, though, is the need with CE to use spatial areas to correct for velocity differences in the detector. External or internal standardisation or standard addition can be used. Although CE offers higher resolution than HPLC, there is still a possibility of peaks not being resolved, but if a DAD detector is used, then peak purity can usually be determined automatically.

Validation is an important aspect of any separation assay (see Chapter 11). Limit of detection (LOD) is defined as the smallest measured amount from which it is possible to deduce the presence of the analyte; in CE this is
calculated as the apparent content that corresponds to three times the peak-to-peak baseline noise. Reproducibility is defined as the closeness of agreement between n mutually independent tests obtained under identical conditions, where n is typically 10 sequential tests. The measure of this is the relative standard deviation (RSD), also known as coefficient of variation (CV) and is the ratio of the standard deviation to the mean value. The injection reproducibility of commercial CE instrumentation is excellent, about 1% RSD. Linearity is best determined using corrected peak areas because the linear range of peak heights is shorter through band broadening and mobility matching effects in CE. Overall assay reproducibility in CE, particularly for drug assays in biofluids, should not be significantly different from HPLC (i.e. 5 to 10%)

Samples and sample preparation

Sample preparation depends on the drug and matrix to be analysed and ranges from nothing to procedures that are as extensive as those required for other separation techniques. The degree of preparation depends on the matrix, the analyte(s) and their concentration, the detection mode to be employed and the required selectivity and sensitivity. The first ‘obvious’ statement is that the analyte(s) must be in solution, preferably in an aqueous buffer, to be analysed by CE. An analyte soluble only in organic solvents and injected from such a solution can precipitate in the electrolyte and possibly block the capillary, although injection from organic solvents can also generate useful stacking effects.

In CE, ample preparation methods should accomplish at least one but preferably more of the following factors:

- Enhance analyte selectivity and concentration
- Stabilise the analyte(s)
- Control the composition of the final sample, especially with respect to pH
- Enhance sample loading via stacking
- Remove matrix components, such as protein that can change the EOF
- Free analyte(s) bound onto proteins
- Remove particulate material that could block the capillary
- Give high recovery of the analyte(s)
- Avoid excessive dilution
- Work with the very small amounts of sample required in CE (<20 μL).
Methods without extraction for drugs in biofluids

For a protein-free biofluid, such as urine, the minimum preparation usually necessary is to filter the sample through a 0.45 μm membrane, or if small volumes are very limited centrifuge at about 13 000g to remove any particulates that may have formed. For some CZE assays this minimal approach can cause changes in migration times because of the variable salt molarity in some samples, such as urine. These variations can often be overcome by using one or more of the following approaches:

- Diluting all samples approximately 10-fold with water or, better, a 1 + 9 dilution of the running buffer with de-ionised water, since this also controls sample pH
- Injecting less sample
- Changing to another injection mode
- Diluting to a constant salt concentration
- Increasing the molarity of the running electrolyte within the current constraints of the instrument.

If these fail to control migration-time variation, an MEKC-based assay should be tried. It is our experience that SDS-based MEKC is more robust with respect to sample matrix. This robustness of MEKC is such that drugs in plasma, serum and other protein-containing fluids can be assayed without any sample preparation. This approach, introduced by Nakagawa et al. (1988), relies on the ability of SDS to solubilize proteins, which prevents them sticking to the capillary surface and gives the proteins an overall negative charge, so further retarding their electrophoretic migration. At the same time, an MEKC separation of the analyte is developed. Choosing a selective detection wavelength can further enhance the utility of this approach. A representation of this for ibuprofen in serum is shown in Fig. 30.5. Between the broad protein peak and the neutral peak there is a zone in which it is possible to separate and quantify analyte peaks.

Ultrafiltration gives minimal matrix disruption and is very suitable for CE. A 10 kD cut-off ultra-filter gives 10 to 50 μL of protein-free filtrate from 500 μL of serum in 10 to 20 min when centrifuged at about 13 000g. The low sample yield is not a problem for CE analysis. Only free analyte(s) of molecular weight below the cut-off are in the filtrate and recoveries can be very variable.

Extraction based capillary electrophoresis methods

Off-line sample pre-concentration methods prior to CE, such as freeze-drying, liquid-liquid extraction and solid-phase extraction (SPE) are used as for HPLC, can achieve most of the effects listed above. The most
important reason to use such techniques is to remove proteins. Protein-free extracts of serum prepared by most standard methods (e.g. acid precipitation or precipitation with organic solvents) are suitable for both CZE and MEKC. The important aspects are to avoid excessive dilution and interference of the precipitant with the electrophoresis. The final extract must not affect the electrophoresis by being too acidic or too alkaline compared to the separation buffer. Such differences in pH and/or molarity can cause major variations in migration times in CZE. Traces of solvents that remain from liquid-liquid or SPE extracts may disturb MEKC separations.

Unfortunately, trichloroacetic acid, the most efficient protein precipitant, leaves interference peaks that absorb in the low wavelength UV. Therefore, when using CE below 220 nm, perchloric acid (PCA) followed by neutralisation with potassium hydroxide or potassium carbonate and removal of the resultant precipitate is the recommended acid precipitant. 500μL of a 5% v/v aqueous PCA solution precipitates the proteins in 1 mL serum. The use of 10 parts 5% v/v PCA solution precipitates the proteins in one part of tissue when homogenised together.

Another method of sample concentration in the capillary is sample stacking, which is achieved by dissolving the sample in a buffer more dilute than the running electrolyte. The sample is hydrodynamically injected, but when the voltage is applied the electric field strength is greater in the dilute sample than in the running electrolyte. Since electrophoretic velocity is proportional to field strength, the analyte ions move rapidly towards the cathode, but when they reach the higher concentration running electrolyte the field strength drops rapidly and they stack against this boundary in a narrow zone. This suggests that the sample is best dissolved in water, but unfortunately peak narrowing is countered by peak broadening because of mixing in the sample zone and poor solute buffering. The optimal sample buffer concentration for stacking is about one-tenth that of the electrolyte. Although stacking can be used to narrow the final peak width, it is still not advisable to increase the initial sample plug length beyond 1% of the capillary length. A popular and simple extraction method for drugs in CE assays is to extract the plasma and/or serum with acetonitrile and then inject relatively large amounts of the extract, which leads to a degree of sample stacking that enhances sensitivity.

When modifying HPLC methods such as SPE for CE, the aim should be to produce a 10-fold more concentrated final extract. This is often as simple as re-dissolving in only 10% of the suggested final volume. The low injection volumes required by CE, even with dilute analytes, mean it is possible to work with much smaller amounts of final sample and/or extract, provided there is sufficient for the sample injection vial in the instrument. It is routine for CE to use vial inserts of samples when as little as 5μL suffices for many injections.

Automated SPE has become the method of choice for sample preparation prior to HPLC. A number of attempts have been made to automate SPE for use with CE, but these have not yet proved popular. These and related techniques, such as immuno-extraction, have been reviewed by Veraart et al. (1999) and Gilar et al. (2001).
General applications to drug assays

Clearly, CE is very good for separating charged species, whether small molecules or macromolecules. It is necessary to operate in MEKC mode to separate more neutral molecules, such as many drugs. There are now over 8000 publications that describe separations of more than 10 000 compounds by CE. Charged and neutral compounds are all separated easily, but there are too many examples in the literature to even attempt to describe them here, so the following section attempts to offer some concise summaries.

Drugs in formulations

A number of workers have demonstrated that CE can be used readily to analyse drugs in formulations. In such situations the limited sensitivity of CE is usually no problem, since suitable dilutions can be prepared. EK injection can minimise problems caused by viscous solutions and particulates from crushed tablets. Care must be taken when using HD injection and viscous solutions, since differences in viscosity between sample and standard solution can lead to differences in the amount of sample injected and thereby to quantification errors.

Drugs in biofluids

CE has proved valuable for the analysis of drugs and their charged metabolites, such as glucuronide and sulfate conjugates. At present CE separations tend to be of high efficiency, but are relatively slow and drug assays are no exception. Time saving can be achieved by the reduction in sample preparation that can be afforded by the selectivity of CE, both with regard to sample loading and system selectivity. Another advantage is that CE is able to work at low UV-wavelengths (i.e. <210 nm), which enables the detection of drugs without obvious chromophores.

Assays for drugs in biofluids that use CE are, in general, either for drugs taken in relatively high doses (e.g. paracetamol or aspirin) or where sample extract clean-up is employed to increase the final analyte concentration. The majority of assays of this type use a common simple MEKC separation (e.g. 10 mM borate, pH 9 with 50 mM SDS at 30 kV in 50 μm capillaries, method C in Table 30.4). A group at the Mayo Clinic were among the first to demonstrate that to obtain additional pharmacological information from drug assays in biofluids it is necessary to link CE with MS. Pharmacogenetics has become an important area of drug metabolism and therapy in which MEKC is used to probe differences in the metabolism of drugs, such as caffeine, between individuals. Most drug assays are relatively slow (e.g. 10 to 15 min). With current commercial CE equipment samples must be analysed in series so the throughput of samples is maybe 80 to 100 per day. Recently a drug analysis system with multiple parallel capillaries has been introduced. This is well below the throughput achievable with enzyme-linked immunosorbent assays (ELISAs), for example. Much faster analyses of drugs by CE are possible by actively cooling short capillaries.
or using short-end injection and minimising sample preparation.

Identification of drugs separated by capillary electrophoresis

The identification of peaks that migrate in CE is similar in approach to that for HPLC, except that samples move through the detector much faster and the quantities and concentrations are approximately 10 times less. The two most popular CE systems include DADs, which can greatly aid peak identification. However, there is a problem in that the software has usually been written for HPLC and places equal emphasis on all parts of the collected spectra. A DAD for CE is usually programmed to collect spectral data from 190 nm to visible wavelengths.

A routine to help confirm the identification of drugs and their metabolites resolved by CE is as follows:
Determine the analyte's apparent charge from its migration time relative to a neutral marker
Peak parameters:
migration time against external standards
peak shape
relative peak-size pattern recognition
Co-injection (possibly including any extraction steps) with authentic compounds
Vary electrophoretic conditions slightly (e.g. buffer pH, temperature), and repeat (1) and (2) under each condition
Spectral characterisation:
determine absorbance maximum and compare to standards and published data
measure absorbance ratios in two separate runs at two wavelengths, and compare the ratios for the possible standard and the unknown
if scanning UV detection available, determine peak purity and attempt peak identification against a spectral library
Peak shifting by:
incubating with appropriate enzymes (e.g. glucuronidase)
derivatisating with appropriate reagents
Other detection modes include using selective detectors (e.g. fluorescence or electrochemical are helpful but difficult in CE)
Mass spectral identification involves multiple fraction collection (possible on some systems) plus off-line MS or, if available, on-line CE-MS.

Fast assays by capillary electrophoresis

CE can generate a large number of theoretical plates rapidly (e.g. in 1 min a CZE separation can produce more plates than a typical 15 cm HPLC column). To fully utilise such capabilities short capillaries are needed. On some instruments short capillaries <25 cm can be used by by-passing parts of the capillary cassette, while on others very short paths are possible by running the system in reverse [i.e. using what is normally the cathode end as the injection (anode) end]. This is often referred to as short-end injection. When working with the high potential gradients used in such rapid analyses it is necessary to avoid too much Joule heating. This may be achieved by reducing the applied voltage, which can defeat the goal of the rapid system. It is beneficial, if the instrument allows, for the capillary to be held at a sub-ambient temperature and so actively cool the capillary. Using such techniques, the analysis time for some assays may be reduced to less than 1 min per sample. Fig. 30.6 shows such a short-end injection applied to the separation of normal human urine by MEKC for which the total analysis time
was less than 3 min. Combining such rapid analyses with CE's abilities to work with minimal sample preparation could mean that sample throughput and ease of analysis rivals that of ELISAs.

Assay of chiral drugs in biofluids

CE has proved to be an adaptable method to determine enantiomeric drug purity and is widely used for this purpose in the pharmaceutical industry. Many types of electrolyte additives can be employed to resolve enantiomers; these range from proteins to chiral surfactants. However, the most commonly employed method is to include native cyclodextrins in the electrolyte. Native β-cyclodextrin (CD) is the most commonly employed, since its internal cavity matches well to the largest number of drug molecules. More recently, chemically modified cyclodextrins, especially the sulfated derivatives of β-CD have been shown to give separations of chiral compounds with spectacular resolution between the enantiomers. As a starting point for developing enantiomeric separation methods, a 25 mM phosphate pH buffer (approx. pH 2.5) with highly sulfated-β-CD, hydroxypropyl-β-CD, heptakis-(2,6-O-dimethyl)-β-CD or heptakis-(2,3,6-O-trimethyl)-β-CD has been shown to form a BGE combination capable of resolving over 90% of the chiral drug compounds.

Although most commonly used with standard preparations of drugs for quality-control purposes, chiral CE has also been used to study enantiomeric metabolism in humans and other species. A variety of drug classes have been resolved chirally and quantified in samples that range from urine to hair extracts. Table 30.6 summarises details of the CE assays for chiral drugs in biofluids up to 2003 for which resolution of enantiomers was reported. Technical aspects of such methodologies are reviewed in detail in Zaugg and Thormann (2000).

Conclusions and future directions

In comparison to HPLC, CE achieves better resolution than both isocratic and gradient HPLC using simpler instrumentation. CE is not a preparative technique, although it has been used as a micro-preparative system to isolate very small amounts of protein for sequencing. It is less sensitive than HPLC by about an order of magnitude. There is little difference in terms of quantitative data and analytical precision. Sample preparation probably needs to be better controlled and understood than it does for HPLC. At present, CE instrumentation is more expensive than HPLC, although running costs are considerably lower. CE uses considerably less sample and reagents than does HPLC. Waste disposal problems are reduced considerably. The high resolving power of CE means that a relative simple CE system can resolve as many peaks as a gradient HPLC system in a shorter time, with greater throughput and lower running costs. Today, CE is therefore a very complementary technique to other separation methods.
CE has found a role in many laboratories and offers useful complementary separations to HPLC and is clearly an established technique with a long-term future in the separation of pharmaceuticals, in both simple preparations and biofluids. Future developments in pharmaceutical analysis may see miniaturisation developing even further, with the commercialisation of the experimental systems for chemical analysis on a chip (Lab-on-Chip), since such devices are already available for macromolecules such as DNA.