14 High-performance capillary electrophoresis

KEYPOINTS

Principles

Separation is carried out by applying a high potential (10–30 kV) to a narrow (25–75 μm) fused silica capillary filled with a mobile phase. The mobile phase generally contains an aqueous component and must contain an electrolyte. Analytes migrate in the applied electric field at a rate dependent on their charge and ionic radius. Even neutral analytes migrate through the column due to electro-osmotic flow, which usually occurs towards the cathode.

Applications

- An accurate and precise technique for quantitation of drugs in all types of formulations.
- Particular strength in quality control of peptide drugs.
- Highly selective and is very effective in producing separation of enantiomers.
- Very effective for impurity profiling due to its high resolving power.
- Very effective for the analysis of drugs and their metabolites in biological fluids.

Strengths

- Potentially many times more efficient than HPLC in its separating power.
- Shorter analysis times than HPLC.
- Cheaper columns than HPLC.
- Negligible solvent consumption.

Limitations

- Currently much less robust than HPLC.
- Sensitivity lower than HPLC.
- More parameters require optimisation than in HPLC methods.

Introduction

Electrophoresis

Capillary electrophoresis (CE) is the most rapidly expanding separation technique in pharmaceutical analysis and is a rival to HPLC in its general applicability. The instrumentation is quite straightforward, apart from the high voltages required, but the parameters involved in optimising the technique to produce separation are more complex than those involved in HPLC. The technique is preferred to HPLC where highly selective separation is required.

Separation of analytes by electrophoresis is achieved by differences in their velocity in an electric field. The velocity of an ion is given by the formula:

$$v = \mu_e E \qquad \textbf{[Equation 1]}$$

where v is the ion velocity, μ_e is the electrophoretic mobility and E is the applied electric field.

The electric field is in volts/cm and depends on the length of the capillary used and strength of the potential applied across it. The ion mobility is given by the relationship shown below:

$$\mu_e = \frac{\text{Electric force } (F_E)}{\text{Frictional drag } (F_v)}$$

$$F_E = qE$$

where q is the charge on the ion and E is the applied electric field, i.e. the greater the charge on an ion the more rapidly it migrates in a particular electric field

For a spherical ion:

$$F_v = -6\pi\eta r v$$

where η is the viscosity of the medium used for electrophoresis, r is the ion radius and v is the ion velocity.

When the frictional drag and the electric field experienced by the ion are equal:

$$qE = -6\pi\eta r v$$

substituting this expression into Equation 1:

$$\mu_e = \frac{q}{6\pi\eta r} \qquad \textbf{[Equation 2]}$$

If the applied electric field is increased beyond the point where the drag and electric field are equal, the ion will begin to migrate. From Equation 2 it can be seen that:

(i) The greater the charge on the ion the higher its mobility.
(ii) The smaller the ion the greater its mobility. Linked to this, since Equation 2 applies to a spherical ion, the more closely an ion approximates to a sphere, i.e. the smaller its surface area, the greater its mobility. This effect is consistent with other types of chromatography.

Thus the mobility of an ion can be influenced by its p*K*a value; the more it is ionised the greater its mobility and its molecular shape in solution. Since its degree of ionisation may have a bearing on its shape in solution, it can be seen that the behaviour of analytes in solution has the potential to be complex. For many drugs

the manipulation of the **pH** of the electrophoresis medium should have a marked effect on their relative mobilities. Thus one would predict that the electrophoretic separation of the two bases (morphine and codeine), which are of a similar shape and size but have different p*K*a values,would increase with pH. If we assume that morphine and codeine possess the same mobilities at full charge, then Figure 14.1 indicates how their mobilities vary with pH. As can be seen in Figure 14.1, the biggest numerical difference in mobility is when the pH = p*K*a of the weaker base although the ratio of the mobilities goes on increasing with pH, e.g. at pH 8.9 the ion mobility of codeine is *ca* two times that of morphine.

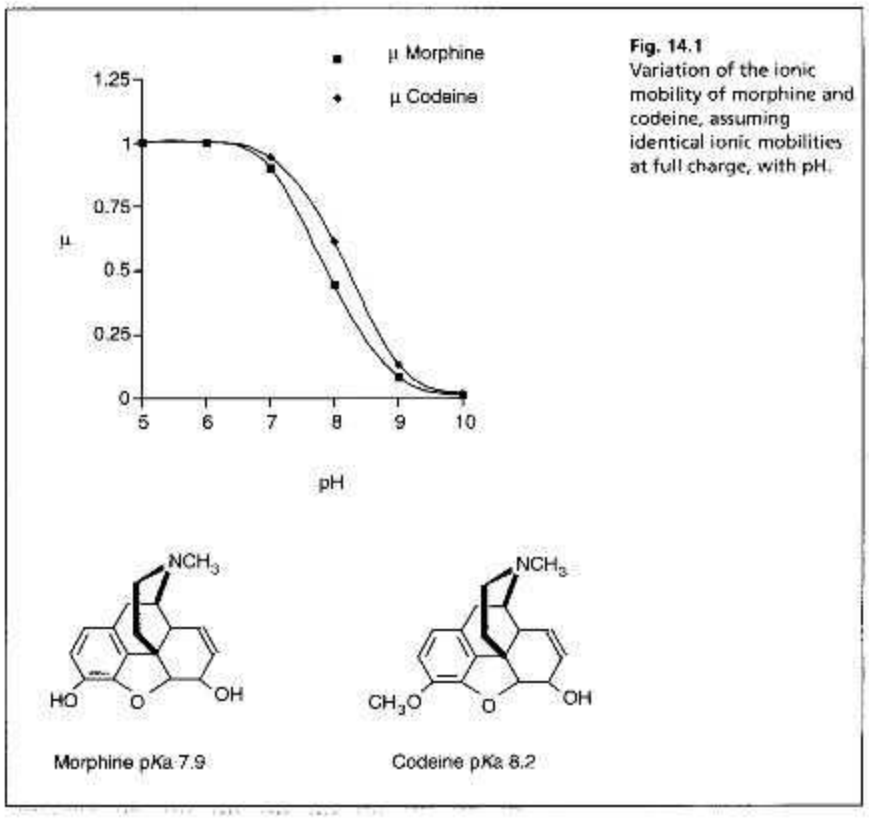


Fig. 14.1
Variation of the ionic mobility of morphine and codeine, assuming identical ionic mobilities at full charge, with pH.

Variation of ion mobility with pH is only part of the story with regard to separation by capillary electrophoresis – the other major factor is electro-osmotic flow (EOF).

EOF

The wall of the fused silica capillary can be viewed as being similar to the surface of silica gel and at all but very low values the silanol groups on the wall will bear a negative charge. The p*K*a of the acidic silanol groups ranges from 4.0–9.0 and the amount of negative charge on the wall will increase as **pH** rises. Cations in the running buffer are attracted to the negative charge on the wall resulting in an increase in positive potential as the wall is approached. The effect of the increased positive potential is that more water molecules are drawn into the region next to the

wall (Fig. 14.2). When a potential is applied across the capillary the cations in solution migrate towards the cathode. The concentrated layer of cations near to the capillary wall exhibit a relatively high mobility (conductivity) compared to the rest of the running buffer and drag their solvating water molecules with them towards the cathode creating EOF. The rate of EOF is **pH** dependent since the negative charge on the silanol groups increases with pH, and between a pH of 3 and 8 the EOF increases about 10 times. The EOF decreases with buffer strength since a larger concentration of anions in the running buffer will reduce the positive potential at the capillary wall and thus reduce the interaction of the water in the buffer with the cations at the wall.

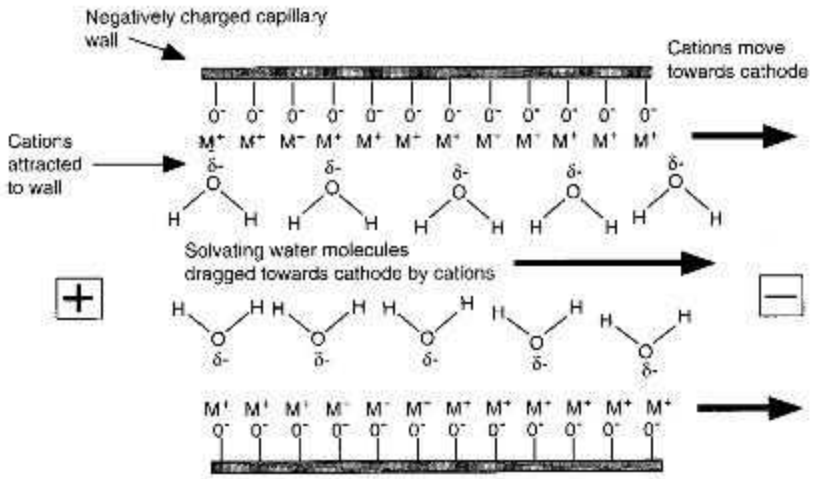
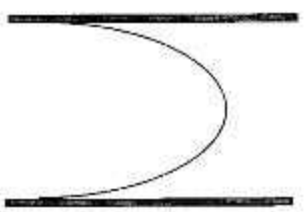
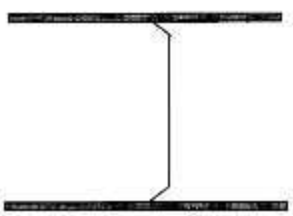


Fig. 14.2
Electro-osmotic flow.

The flow profile obtained from EOF is shown in Figure 14.3 in comparison with the type of laminar flow shown in HPLC. The flat flow profile produces narrower peaks than are obtained in HPLC separations and is a component in the high separation efficiencies obtained in capillary electrophoresis (CE).



Laminar flow caused by drag on the walls of an HPLC column



Electro-osmotic flow

Fig. 14.3
Flow profiles obtained in CE and HPLC.

Migration in CE

The existence of EOF means that all species regardless of charge will move towards the cathode. In free solution, cations move at a rate determined by their ion mobility + the EOF. Neutral compounds move at the same rate as the EOF and anions move at the rate of the EOF – their ion mobility, the rate of EOF towards the cathode

exceeds the rate at which anions move towards the anode, by approximately ten times. A typical separation could be viewed as shown in Figure 14.4. The cations in solution migrate most quickly with the smaller cations reaching the cathode first; the neutral species move at the same rate as the EOF and the anions migrate most slowly with the smallest anions reaching the cathode last. The EOF is useful in that it allows the analysis of all species but it adds complexity to the method in that it needs to be carefully balanced against ion mobility. Table 14.1 shows how EOF can be controlled using different variables and illustrates some of the complexity of CE relative to HPLC.

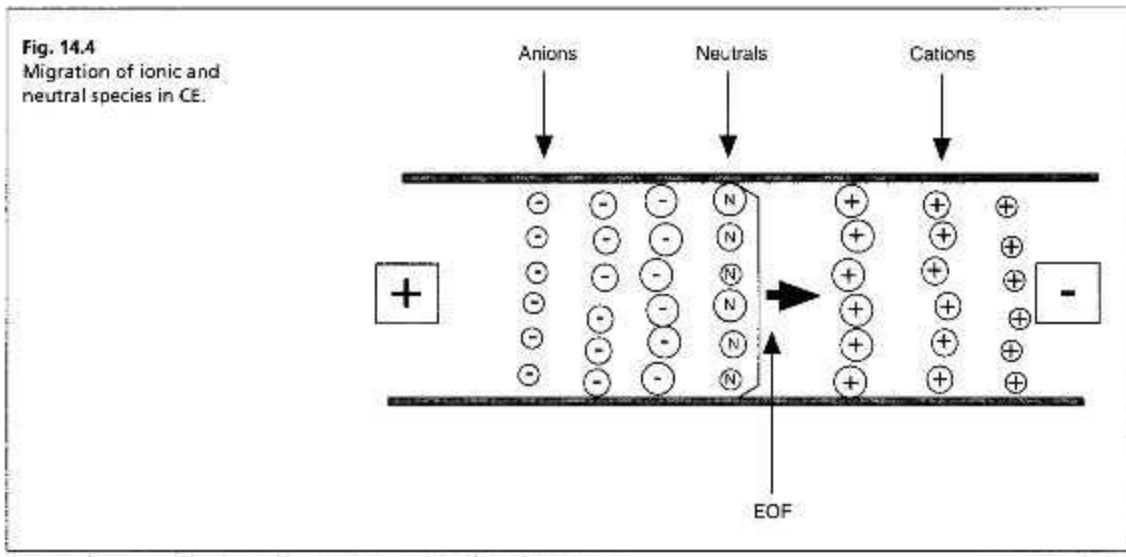


Fig. 14.4
Migration of ionic and neutral species in CE.

Table 14.1 Variables affecting EOF

Variable	Effects on EOF	Comments
Buffer pH	EOF increases with pH	Most convenient method for controlling EOF but has to be balanced against effects on the charge on the analyte
Buffer strength	EOF decreases with increasing buffer strength	(i) Increased ionic strength means increased electric current flow through the capillary, which can cause heating (ii) At low ionic strength more sample absorption onto the capillary walls occurs (iii) Low buffer concentrations reduce sample stacking following injection
Electric field	Increased electric field increases EOF	Lowering the applied electric field may reduce separation efficiency and raising the field strength may cause heating
Temperature	Increased temperature decreases viscosity and thus increases flow	Easy to control
Organic modifier	Changes potential at capillary wall – the dielectric constant of the running buffer and the viscosity. Usually decreases EOF	Complex effects – can be useful but best determined experimentally

Table 14.1 Variables affecting EOF (*Cont.*)

Variable	Effects on EOF	Comments
Surfactant	Absorbs onto the surface of the capillary wall	(i) Cationic surfactants have a high affinity for the silanol groups and thus block access by the smaller cations in solution reducing EOF. At high concentration they form a double layer giving the capillary wall an effective positive charge and causing EOF to reverse flow towards the anode (ii) Anionic surfactants reduce the access of the smaller ions in the running buffer to the positive potential at the wall thus increasing the zeta potential and thus EOF
Covalent wall coating	Can raise or lower EOF depending on the coating	(i) Neutral coatings reduce negative charge of the capillary wall thus reducing EOF (ii) Ionic coatings will have marked effects on EOF

Instrumentation

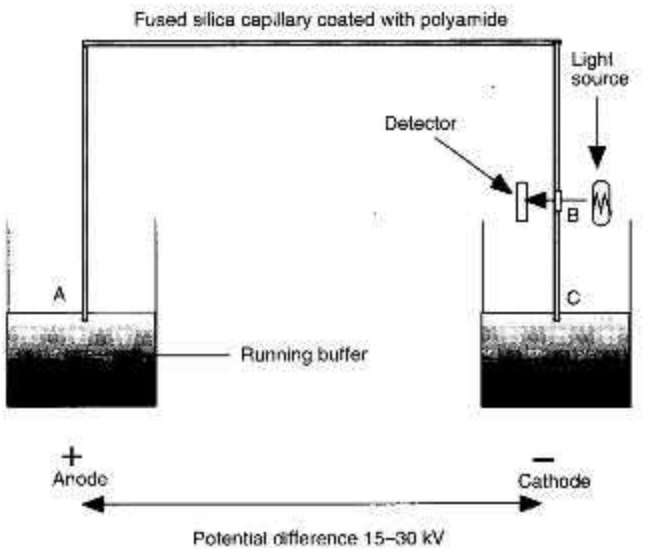


Fig. 14.5
Schematic diagram of a capillary electrophoresis apparatus.

A schematic diagram of a capillary electrophoresis instrument is shown in Figure 4.5. The fundamentals of the system are as follows:

(i) Injection is commonly automated and is usually accomplished by pressuring the vial containing the sample with air.

(ii) Having loaded the sample the capillary is switched to a vial containing running buffer. The flow rate of the running buffer through the capillary is in the low nanolitres/min range.

(iii) The capillaries are like those used in capillary gas chromatography with a polyamide coating on the outside. The length of the capillaries used is 50–100 cm with an internal diameter of 0.025–0.05 mm. They are generally wound round a cassette holder so that they can simply be pushed into place in the instrument.

(iv) At the detector end the capillary has a window burnt into it so that it is transparent to the radiation used for detection of the analyte.
(v) The most commonly used detector is a diode array or rapid scanning UV detector although fluorometric, conductimetric and mass spectrometric detectors are available.

Control of separation

Migration time

As discussed earlier, cations move most quickly towards the point of detection and time has to be allowed for separations to develop and the EOF should not exceed the cationic mobility by an amount which is incompatible with achieving separation. The factors which can be used to control EOF have been discussed earlier. Another factor in allowing separation to develop which is simply controlled is the length of the capillary; however, the longer the capillary in relation to a fixed applied potential the lower the electric field which is in volts/cm. Since the detection system is mounted before the column outlet, it is important that the distance between the detector and the outlet is not too great since the effective length of the capillary is reduced.

Dispersion

Longitudinal diffusion

This is generally the most important cause of peak broadening in CE because of the absence of mass transfer and streaming effects seen in other types of chromatography. Thus to some extent CE resembles capillary gas chromatography but with less mass transfer effects and lower longtitudinal diffusion since the sample is in the liquid phase. Longitudinal diffusion depends on the length of time an analyte spends in the capillary and also on the diffusion coefficient of the analyte in the mobile phase. Large analytes such as proteins and oligonucleotides have low diffusion coefficients and thus CE can produce very efficient separations of these types of analyte.

Injection plug length

The capillaries used in CE have narrow internal diameters. For a 100 cm × 50 μm i.d. capillary an injection of 0.02 μl would occupy a 1 cm length of capillary space. Automatic injection can overcome difficulties in reproducible injection of such small volumes but often detection limits require that larger amounts of sample are injected. Typically the injection is accomplished by applying pressure at the sample loading end of the capillary. An important element in accomplishing efficient sample loading, particularly if detection limits are a problem and a larger volume of sample has to be loaded, is stacking. A simple method for achieving stacking is to dissolve the sample in water or low conductivity buffer. The greater resistance of the water plug causes a localised increase in electrical potential across the plug width and the sample ions dissolved in the plug will migrate rapidly until the boundary of the running buffer is reached. By using this method, longer plugs up to 10% of the capillary length can be injected, resulting in an increase in detection limit.

Joule heating

The strength of the electric field which can be applied across the capillary is limited by conversion of electrical energy into heat. Localised heating can cause changes in

the viscosity of the running buffer and a localised increase in analyte diffusion. Heat generation can be minimised by using narrow capillaries where heat dissipation is rapid and by providing a temperature-controlled environment for the capillary.

Solute wall interactions

Analytes may absorb onto the wall of the capillary either by interaction with the negatively charged silanol groups or by hydrophobic interaction. High ionic strength buffers block the negative charge on the capillary wall and reduce the EOF but also increase heating. If only analysis of cations is required, the pH of the running buffer can be lowered, e.g. to pH 2. The low pH suppresses the charge on the silanol groups, reduces EOF to a low level but ensures full ionic mobility of the cations, which will migrate to the cathode without the aid of the EOF. Full ionisation of the analytes does not allow for differences in p*Ka* to be used in producing separation.

Electrodispersion

The mobility of the running buffer has to be fairly similar to the mobility of the ions in the sample zone. If the mobility of the analyte ions is greater than the mobility of the buffer ions, a fronting peak will result since the ions at the front of the sample zone tend to diffuse into the running buffer solution where they experience a greater applied electric field (due to the higher resistance of the buffer compared with the sample) and accelerate away from the sample zone. This effect will be less if the concentration of the running buffer is much greater than that of the sample. Conversely, if the mobility of the sample ions is lower than that of the running buffer ions, a tailing peak will be produced because the ions in the rear of the sample zone will tend to diffuse into the buffer where they will experience a lower applied electric field (due to the lower resistance of the buffer compared with the sample) and will thus lag further behind the sample zone. This effect will be less if the concentration of the running buffer is much lower than that of the sample zone.

Applications of CE in pharmaceutical analysis

In its simplest form capillary electrophoresis is termed 'capillary zone electrophoresis'. The conditions used in this type of analysis are relatively simple and the mobile phase used consists of a buffer with various additives. Many applications focus on critical separations which are difficult to achieve by HPLC. In many cases it is difficult to explain completely the types of effects produced by buffer additives.

Separation of atenolol and related impurities predominantly on the basis of charge

The β-blocker atenolol is shown in Figure 14.6 with its principal known impurities. These impurities are not readily separated from atenolol by HPLC because of their close structural similarity.[1]

The separation was carried out using a 0.05 mm × 50 cm capillary at 15 kV with a phosphate/borate running buffer. Figure 14.6 shows separation at the optimal pH of 9.7 of atenolol (50 μg/ml) from its impurities spiked into solution at concentrations of 5 μg/ml. The elution order is as would be predicted from the ionisable groups in the molecules. Atenolol (AT) elutes first since it bears a positive charge on the basic secondary amine group (p*Ka* 9.6). The dimer (TA) also carries one positive charge

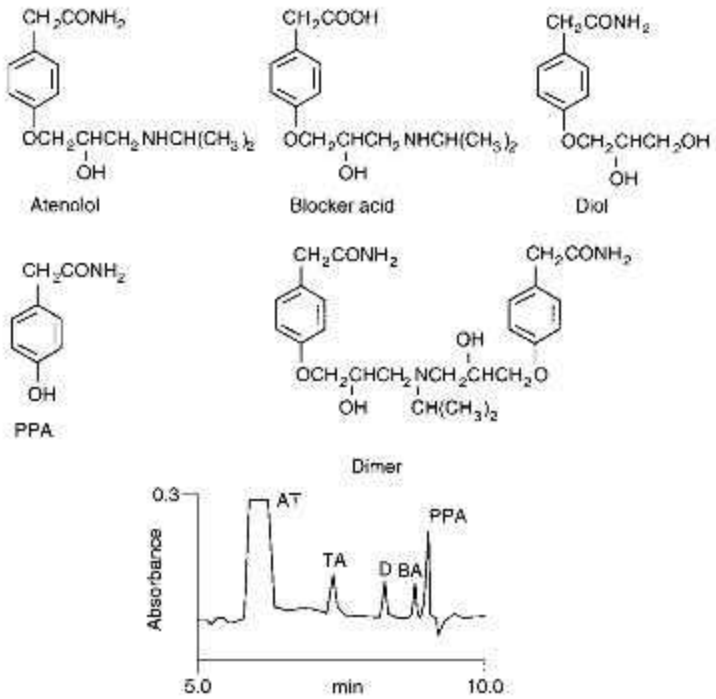


Fig. 14.6
Separation of atenolol and its principal impurities by CE. Reproduced with permission from J. Pharm. Biomed. Anal. (see Reference 1).

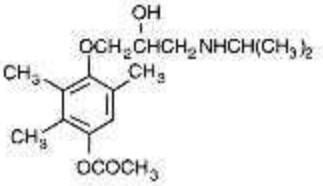
but it is a tertiary amine and has a lower p*K*a than atenolol; it is also a larger ion thus its mobility will be less than that of atenolol (size was sufficient to cause separation of these two molecules at pH 6.5 where both atenolol and the dimer would be fully charged). The diol (D) is a neutral compound and thus should elute at the same rate as the EOF, which will increase with pH. However, in the paper under discussion the elution time of the diol increased with pH; this may be due to complex formation with the borate in the running buffer, which will tend to form a negatively charged complex with a diol. The blocker acid (BA) bears both a positive and a negative charge, which more or less neutralise each other over a quite wide pH range, as the pH rises towards the p*K*a of the amine group (*ca* 9.5) the negative charge of the acidic group becomes predominant; although the molecule will still bear some positive charge, the overall negative charge will cause the molecule to lag behind the EOF. Finally the phenol (PPA) is neutral until its p*K*a value (*ca* 9.7–10) is approached and at higher pH values it will develop a negative charge slowing down its rate of migration; this is consistent with Figure 14.6.

Separation predominantly on the basis of ionic radius

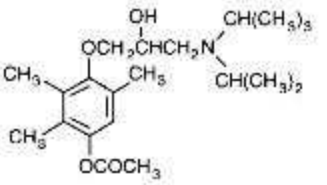
Very small changes in molecular structure can lead to quite marked differences in retention time in CE. An impressive separation of the experimental anti-depressant drug GR50360 from a number of impurities was achieved using isopropanol/0.01 M phosphate buffer pH 7.0 (1:4).[2] In this case the separation is due largely to molecular size or shape since at pH 7.0 the drug and its impurities will be charged to a similar extent.

Self-test 14.1

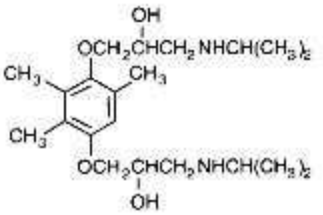
Metipranolol and its possible manufacturing impurities are separated using CE in a buffer at pH 9.5. Suggest the likely order of elution for the components in the mixture.



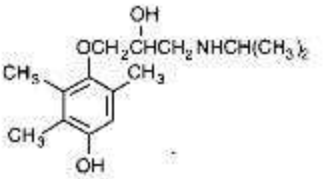
1 Metipranolol p*Ka* 9.5



2 Tertiary amine p*Ka* 9.1



3 Diamine p*Ka* 9.5



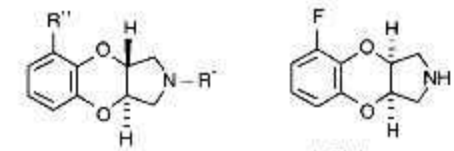
4 Phenol p*Ka* 9.5, 10.0

Answer: Order of elution: 3, 1, 2, 4

Figure 14.7 shows the separation of all six components by CE. The *cis* isomer of GR50360A has a completely different molecular shape from the *trans* isomer, resulting in a smaller ionic radius and thus it runs earlier than the *trans* isomer. Otherwise the compounds elute in order of molecular size, the desfluorocompound being the first of the derivatives of GR50360A to elute. The presence of the isopropanol in the mobile phase slows down the EOF sufficiently for separation to develop.

Analysis of non-steroidal anti-inflammatory drugs (NSAIDs) by CE and separation of anions on the basis of ionic radius

NSAIDs generally contain a carboxylic acid group and when ionised they are anions. In CE using an unmodified capillary the EOF is towards the cathode and the overall mobility of anions is given by the EOF – the mobility of the anions which is towards the anode. In this example[3] the running buffer used in the analysis was carefully designed with respect to its ionic content to avoid electrodispersion. Glycine was found to have a suitable mobility for the analysis of this class of compound because, although it is a small molecule with a carboxylic acid group which is completely ionised at the pH of the analysis (9.1), it also bears a partial positive charge reducing its overall mobility towards the anode and giving it a mobility similar to those of the large lipophilic NSAID acids. The cationic component in the buffer was also found to have an important effect on resolution of the components in a mixture containing four NSAIDs. Triethanolamine was found to be the best cationic component since it reduced EOF because of its relatively low ion mobility and also through increasing the viscosity of the running buffer. Figure 14.8 shows the separation of a mixture containing five NSAIDs: sulindac (S), indomethacin (I), piroxicam (P), tiaprofenic



2 R'' = H R' = H
3 R'' = F R' = H (GR50360A)
4 R'' = F R' = CH_3
5 R'' = F R' = C_2H_5
6 R'' = F R' = C_3H_7

1 *Cis* isomer

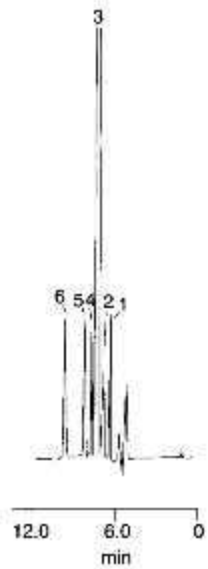


Fig. 14.7
Separation of GR50360 from its related impurities. Reproduced with permission from J. Pharm. Biomed. Anal. (see Reference 2).

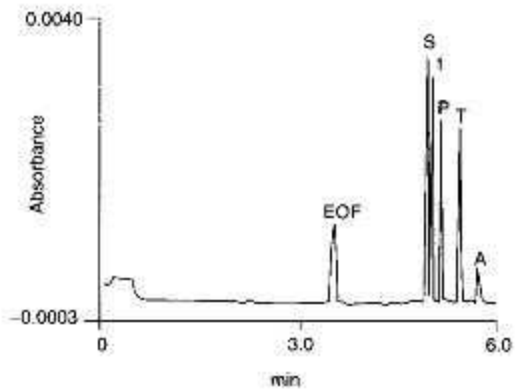


Fig. 14.8
Separation of a mixture of five NSAIDs using a buffer consisting of 0.075 M glycine adjusted to pH 9.1 with triethanolamine. Reproduced with permission from J. Pharm. Biomed. Anal. (see Reference 3).

acid (T) and aclofenac (A). All the drugs are fully charged at pH 9.1 and the separation was achieved more or less according to molecular weight with aclofenac, the smallest molecule, migrating most rapidly in the opposite direction to the EOF.

Separation of peptides

A particular strength of CE is its ability to separate peptides. The use of therapeutic peptides is increasing rapidly and their large size and polarity present particular problems in producing separations. Because peptides usually bear two or more charges, the most important factor to optimise in peptide separations is the pH and concentration of the running buffer. The p*I*-value of a peptide is the pH where its positive and negative charges are balanced. An example is provided by the separation of adrenocorticotrophic hormone (ACTH) from three of its fragments.[4]

Table 14.2 shows the molecular weights and p*I*-values of ACTH and three of its fragments. The p*I*-value gives some indication of the relative number of acidic and basic groups in the peptide; a high p*I*-value indicates a peptide with a large number of basic residues such as lysine and arginine, while a low p*I*-value indicates that the balance is in favour of acidic residues such as glutamic and aspartic acids. In this particular example, conditions (pH 3.8) were chosen where the charge on the basic residues was predominant although at this pH, acidic residues will still bear an appreciable negative charge inhibiting migration towards the cathode. In the current example the separation is consistent with the balance of basic and acidic character in the peptide. The most basic peptide (fragment 1) elutes first whereas the least basic peptide elutes last. Thus in this case the degree of positive charge on the peptides predominates over ionic radius in determining the rate of migration since ACTH migrates more quickly than fragment 3 despite having a much higher molecular weight. The separation was optimised by increasing buffer strength, as can be seen from Figure 14.9 and increased buffer strength gave increased migration time through its effects in reducing EOF. Another important effect of the increased buffer strength in this case is the reduction of the interaction of these highly basic peptides (particularly fragments 1 and 2 and ACTH) with the silanol groups on the capillary wall thus resulting in better peak shape.

Table 14.2 Characteristics of ACTH and its peptide fragments

Peptide	p*I*-value	Molecular weight	No. of amino acids	Calculated mobility	Migration order
Fragment 1	11.69	1652	14	0.0431	1
Fragment 2	10.05	2934	24	0.0379	2
ACTH	9.24	4567	39	0.0216	3
Fragment 3	7.55	1299	10	0.0151	4

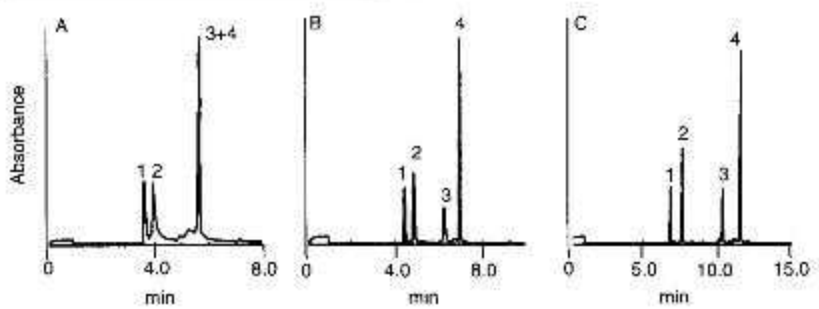


Fig. 14.9
Separation of ACTH and related peptides by CE in buffer at pH 3.8. Buffer strength: (A) 20 mM; (B) 50 mM; (C) 100 mM. Reproduced with permission from J. Chromatogr. (see Reference 4).

In this elegant study it was concluded that for all the peptides studied the best separations were achieved in buffers of medium to high strength (0.05–0.1 M), thus allowing manipulation of EOF without moving away from the optimal pH for the running buffer. It was also concluded that acidic pH values in the range of 2.2–3.8 were best for analysis of basic and neutral peptides whereas acidic peptides were best run at around pH 7.0.

Use of additives in the running buffer

Additives in the running buffer can produce greater selectivity where separation in simple free solution is not possible.

Applications of cyclodextrins in producing improvements in separation

Cyclodextrins are neutral compounds which migrate at the same rate as the EOF. They have large hydrophobic cavities in their structures into which molecules can fit. The ease with which a molecule fits into the cavity of the cyclodextrin is dependent on its stereochemistry. Cyclodextrins have been used as additives both in chiral, where opposite enantiomers form transient diastereomeric complexes with the optically active cyclodextrins, and non-chiral separations where the cyclodextrins affect diastereoisomers to a different extent.

Separation of pilocarpine from its epimer

Pilocarpine (P), a drug used in treating glaucoma, can potentially contain its epimer, isopilocarpine (I) as an impurity. In a study it was not possible to completely separate pilocarpine and isopilocarpine by variation of the pH of the running buffer. The optimal pH for separation should be 6.9 where both compounds are *ca* 50% ionised but even at this pH separation was incomplete.[5]

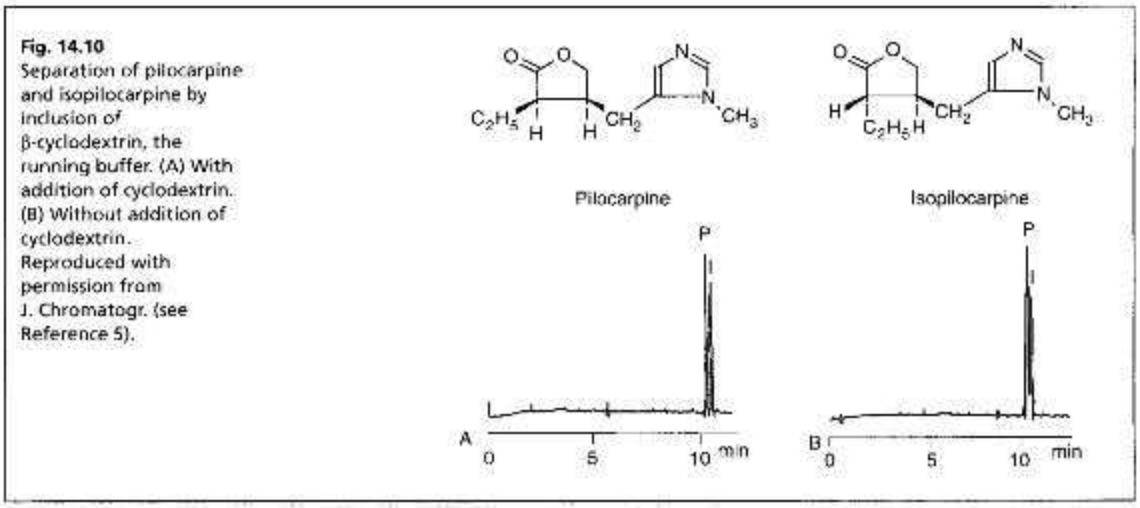


Fig. 14.10
Separation of pilocarpine and isopilocarpine by inclusion of β-cyclodextrin, the running buffer. (A) With addition of cyclodextrin. (B) Without addition of cyclodextrin. Reproduced with permission from J. Chromatogr. (see Reference 5).

Inclusion of 0.01 M β-cyclodextrin in the running buffer resulted in baseline separation of the diastereoisomers. Figure 14.10A shows the separation of the two epimers achieved following addition of the cyclodextrin to the running buffer. In this

example capillaries were used where the silanol groups on the capillary wall had been partially blocked by coating, reducing the negative charge on the wall and thus reducing the EOF and allowing more time for separation to develop. In the present example the separation is achieved by the different degree of complexation of the β-cyclodextrin additive with the two diastereoisomers.

Separation of chiral local anaesthetics

Cyclodextrins are used in GC and HPLC to effect separation of enantiomers and they are also very effective in CE applications. The application of CE to chiral separations will undergo rapid growth in the next few years because of the high efficiencies that can be achieved in such separations using this technique and because of the cheapness of the chiral additives compared to the cost of chiral GC and HPLC columns. A series of enantiomers of local anaesthetics was separated by CE using a phosphate buffer at pH 3.0 containing triethanolamine as a cationic additive and 10 mM of a dimethyl β-cyclodextrin.[6] The addition of the cationic additive reversed the EOF (see Table 14.1) towards the anode, however, the analytes still migrated towards the cathode, having an overall mobility in this direction greater than the EOF towards the anode. This allowed increased time for interaction of the analytes with the cyclodextrin which migrates towards the anode with the EOF. The use of methylated β-cyclodextrin increases the interaction of lipophilic analytes with this chiral selector compared with β-cyclodextrin itself.

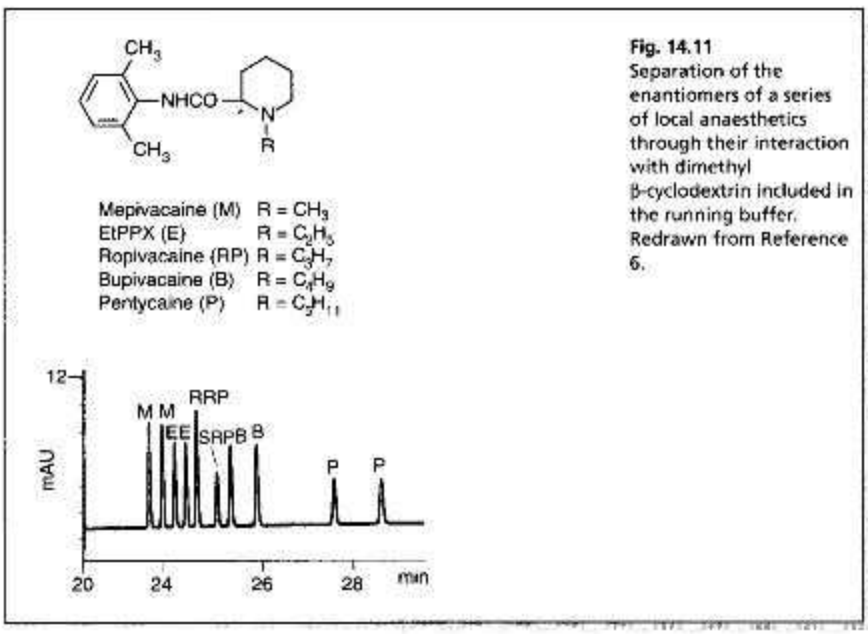


Fig. 14.11 Separation of the enantiomers of a series of local anaesthetics through their interaction with dimethyl β-cyclodextrin included in the running buffer. Redrawn from Reference 6.

Figure 14.11 shows the separation of R and S isomers of a series of structurally related local anaesthetics. Wide separations were achieved for the compounds in this series where it was proposed that the fit of the hydrophobic portion of the analyte into the cyclodextrin was optimal when one of the substituents at the chiral centre was able to interact with the chiral hydroxyl groups on the rim of the cyclodextrin cavity. Table 14.3 shows the association constants calculated for the interaction of the enantiomeric pairs with the dimethylcyclodextrin.[6] The larger the value of K, the

more the enantiomer is retarded by the selector, which in this case is migrating towards the anode. The values in the table also show that the calculated mobilities for each analyte in free solution decrease with the bulk of the N-alkyl substituent.

Table 14.3 Association constants of some enantiomers of some local anaesthetics with dimethyl β-cyclodextrin and their mobilities in free solution

Compound	K_r (L mol^{-1})	K_s (L mol^{-1})	μ (10^{*} m^{2}s^{-1}V^{-1})
Mepivacaine	18	24	1.96
Ropivacaine	18	26	1.82
Bupivacaine	16	26	1.77

Micellar electrokinetic chromatography (MECC)

This extension of the basic CE technique allows the separation of neutral components to be carried out, but it has also been widely used in achieving separations of ionic species. In MECC, a surfactant is added to the mobile phase at a concentration above its critical micelle concentration. The surfactants used can be anionic, cationic or neutral. The micelles act in a manner analogous to the stationary phase in HPLC. Anionic micelles migrate in the opposite direction to the usual EOF, which is towards the cathode. Cationic micelles migrate with the EOF and neutral micelles migrate at the same rate as the EOF. The presence of the surfactant in the running buffer also has an effect on the rate and direction of EOF via interaction with the capillary wall so that the final basis for separation in MECC may be due to a number of mechanisms. The interaction of the analyte with the micelles may be modified using organic solvent additives in the running buffer which reduce the partitioning of the analyte into the micelle; at the same time such organic modifiers tend to reduce EOF. The formation of micelles is illustrated in Figure 14.12.

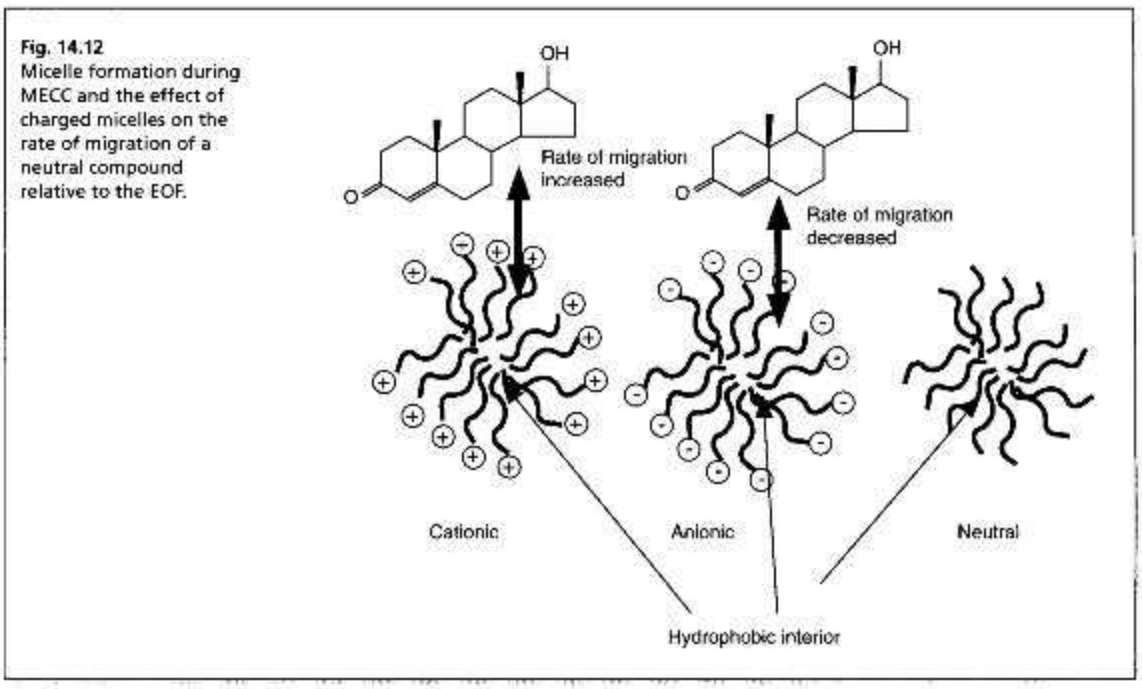


Fig. 14.12 Micelle formation during MECC and the effect of charged micelles on the rate of migration of a neutral compound relative to the EOF.

Separation of cefotaxime from related impurities.

Penicillins and cephalosporins are reactive compounds and may contain a number of degradants; the high selectivity of CE can be advantageous where separation of complex mixtures is required. Some of the impurities may be neutral and separation of neutral impurities from each other requires partitioning into charged micelles which migrate at a different rate from the EOF. In this particular application sodium dodecyl sulphate (SDS), an anionic surfactant, was used to conduct MECC.[7] The pH of the running buffer was 7.2, which was low enough to avoid promoting the degradation of cefotaxime, which is unstable to alkali. Cefotaxime and its related impurities are shown in Figure 14.13.

Figure 14.14A shows the MECC trace obtained from C which was spiked with 0.2% w/w of each impurity and Figure 14.14B shows an unspiked sample of C. The slowest migrating compound was the neutral lactone compound L, which should have the most affinity for the negatively charged and hydrophobic SDS. The other impurities are carboxylic acids which will be fully charged at pH 7.2, thus bearing a negative charge, which will cause some degree of repulsion between the analytes and the negatively charged micelles. The anti-isomer of C is late eluting because of its stereochemistry, hence its ionic radius and partition coefficient are quite different

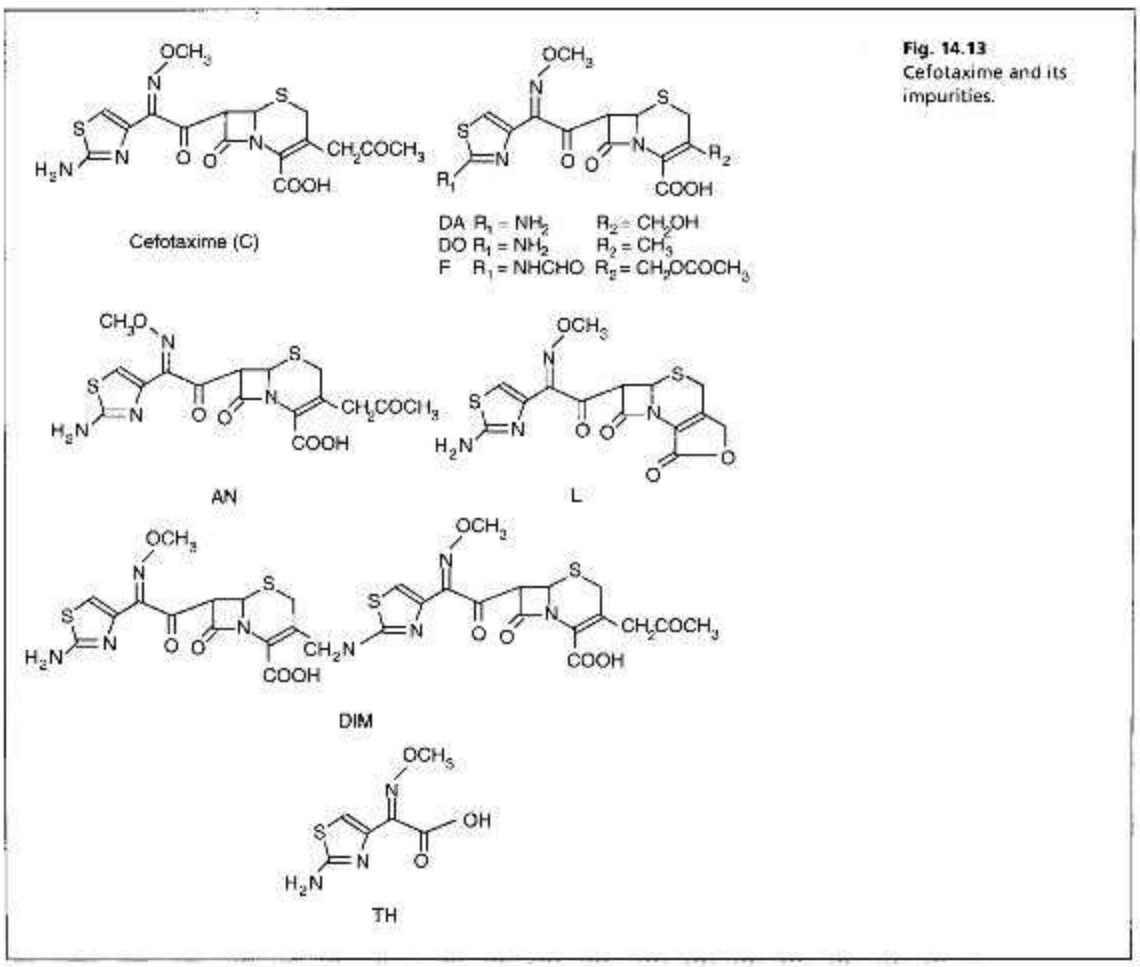


Fig. 14.13 Cefotaxime and its impurities.

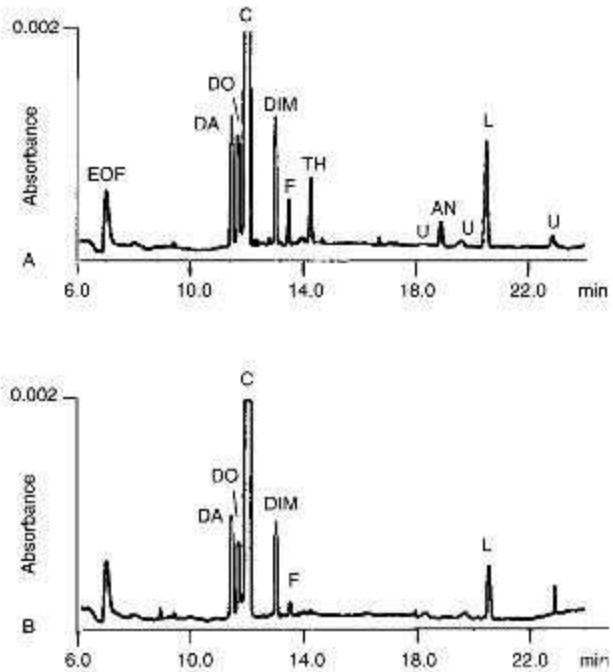


Fig. 14.14
Separation of cefotaxime and related impurities. (A) Sample spiked with impurities. (B) Unspiked sample. U = unknown. Reproduced with permission from Chromatographia (see Reference 7).

from those of C (this is consistent with a lack of antibiotic effect for the anti-isomer). The MECC method was capable of producing separation of all seven impurities from C at the 0.2% level; it gave precision comparable to a previously developed HPLC method and was more rapid than the HPLC method.

Analysis of flavonoids by MECC

Flavonoids are natural products which occur in certain popular herbal medicines such as *Ginkgo biloba*. They are phenols and are not charged until the pH of the running buffer is high. Separation by MECC was carried out using 0.04 M SDS in a 0.02 M borate running buffer at pH 8.2.[8] At this pH the flavonoids studied are more or less uncharged and in the absence of differential partitioning would migrate at the same rate as the EOF. The presence of SDS in the running buffer slows down the rate of migration of these compounds according to how strongly they partition into the SDS micelles, which are moving towards the anode while the EOF is towards the cathode. Figure 14.15 shows the closely related structures of the flavonoids whilst Figure 14.16 shows the separation achieved for a model mixture of these compounds.

The method gave good precision and rapid separation of the mixture. Chromatography of these types of compounds normally requires the use of gradient HPLC with long elution times.

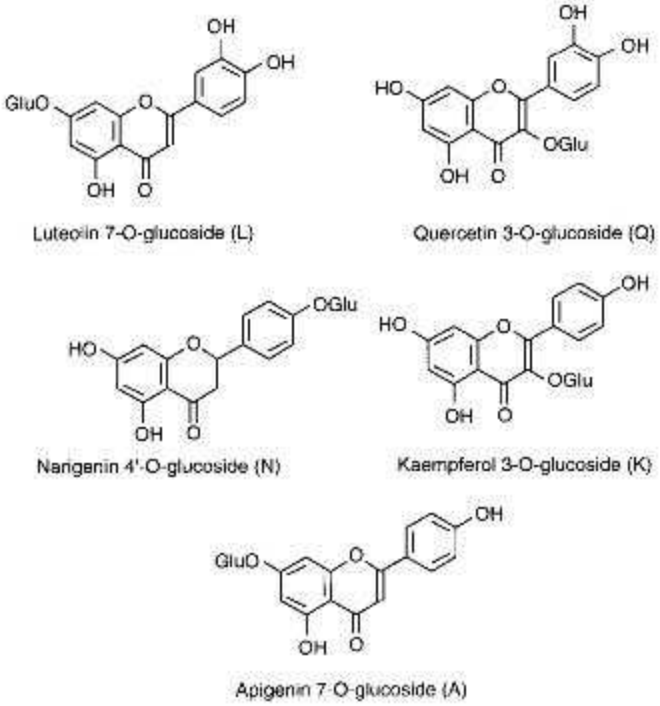


Fig. 14.15
Some flavonoid structures.

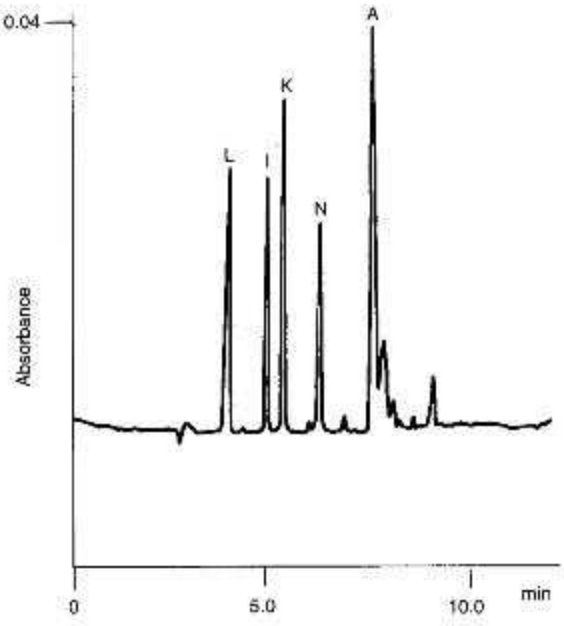


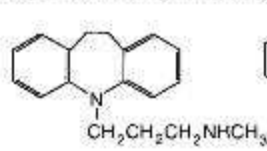
Fig. 14.16
Separation of flavonoids by MECC. Reproduced with permission from J. Pharm. Biomed. Anal. (see Reference 8).

Additional problems

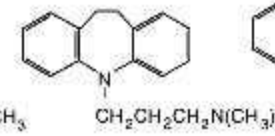
1. Select the most suitable running buffer from those given below to accomplish efficient separation of the following mixtures:
   (i) Two geometrical isomers of a basic drug pKa 9.7.
   (ii) A mixture of neutral corticosteroids.
   (iii) A mixture of opium alkaloids with pKa values in the range 7.5–8.5.
   (iv) Two enantiomers of a local anaesthetic pKa 8.0.
   (v) Two proteins both of ca 20 000 MW, one with a pI-value of 5.5 and the other with a pI-value of 7.1.
   (vi) Human and porcine insulin – human insulin differs from porcine insulin by one amino acid having a more polar threonine residue in place of an alanine residue and their pI-values are the same.
   a. 0.05 mM phosphate buffer pH 7.5 containing 0.05 mM SDS.
   b. 0.02 mM borate buffer pH 9.5 containing 0.01 mM β-cyclodextrin.
   c. 0.05 mM phosphate buffer pH 6.5.
   d. 0.05 mM phosphate buffer pH 8.0.
   e. 0.02 mM borate buffer pH 8.0 containing 0.01 mM propylcyclodextrin.

Answers: (i) b; (ii) a; (iii) a or d; (iv) e; (v) c (vi) a

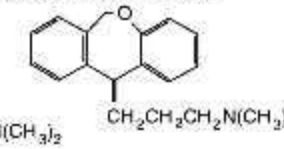
2. Predict the order of elution of the the following tricyclic anti-depressants from a CE system with the following running buffer: 0.5 mM buffer pH 9.55/methanol (84.6:15.4).



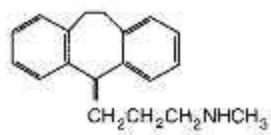
Desipramine pKa 10.2 and < 3



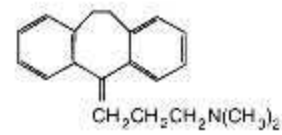
Imipramine pKa 9.5 and < 3



Doxepin pKa 9.0



Nortriptyline pKa 9.7



Amitriptyline pKa 9.4

Answer: desipramine, nortriptyline, imipramine, amitriptyline, doxepin*

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