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Terminology and nomenclature in capillary electroseparation systems

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Abstract

An outline of the basic theory of capillary electroseparations (CES) is given. This forms the basis for recommended naming of the various techniques and recommended methods for reporting migration and elution data. For those techniques where the separation process is primarily based upon electrophoresis [capillary electrophoresis (CE) and capillary gel electrophoresis (CGE)], the electroosmotic mobility of the electrolyte (if not zero) and the electrophoretic mobilities of the analytes should be reported. For those techniques where separation is primarily based upon partitioning between phases that move at different rates [capillary electrochromatography (CEC) and capillary micellar electrochromatography (CMEC)], the electroosmotic mobility of the electrolyte and the electromigration mobility of any moving secondary phase should be reported, along with the capacity factors (k') or effective capacity factors of the analytes. The band dispersion of CES systems should be measured in terms of the HETP, as in chromatography.

1. Introduction

Capillary electroseparation (CES) methods are characterized by the fact that they are all carried out in essentially the same equipment which consists of the following main components:

(a) a fine capillary, usually of quartz, within which separation occurs (bore 50–200 μm, length 200–1000 mm);

(b) a high-voltage power supply capable of delivering 50 kV at 100 μA;

(c) two electrolyte reservoirs into which the ends of the capillary dip, one connected to the high-voltage supply and the other earthed;

(c) an on-column injection system, usually at the high-voltage end of the capillary;

(d) an on-column detector quantitating analyte within a short segment of the capillary (<1 mm), UV and fluorescence detectors being the commonest;

(e) optionally, a means of pressurizing either or both of the inlet and outlet ends of the capillary;

(f) suitable electronics for managing the above, including means of measuring the current flowing through the capillary;

(g) a suitable Faraday cage to ensure safe operation of the high-voltage section of the equipment;

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(h) preferably, thermostating of the capillary by either forced air or liquid.

2. Capillary electroseparation techniques – names

Currently four distinct CES techniques have been described, as shown in Table 1. They are variously carried out in open or packed tubes, and they can separate both charged and uncharged species through differences in either their electrophoretic mobilities or their partition coefficients between phases that move at different rates.

Capillary electrophoresis was originally called capillary zone electrophoresis [1], which indicated that separated analytes migrated as separate independent non-contiguous zones, and distinguished it from isotachophoresis, but the word "zone" has now fallen out of use and it is recommended that the simpler term capillary electrophoresis (CE) should be universally adopted. The term capillary gel electrophoresis concisely describes the process whereby ionic analytes are separated in a capillary filled with gel (often polyacrylamide). Although molecules such as DNA fragments have very similar electrophoretic mobilities in open solution, they migrate at different rates when their electrophoresis is obstructed by the presence of a gel. The larger species are more seriously obstructed than the smaller species and so move more slowly. There seems every reason to retain the term capillary gel electrophoresis (CGE).

Capillary liquid chromatography in which the liquid is driven along the tube by an electric field rather than pressure [2,3] is basically similar to ordinary liquid chromatography and uses a tube packed with a conventional HPLC stationary phase (although the particles may be much smaller). Analytes are separated primarily because of their different partition ratios between a mobile phase, the electrolyte, and a stationary phase borne by the packing material. As the flow of electrolyte is achieved by electroosmosis (formerly called electroendosmosis), rather than by application of pressure, the technique was originally termed capillary electroendosmotic chromatography. However, this name is unnecessarily complicated and it is recommended that the technique now be called capillary electrochromatography (CEC). The term electrochromatography has also been used by Tsuda [4] to describe an HPLC technique in which pressure is the primary driving force but an electric field is used to achieve additional selectivity; it is proposed that this technique should now be called pressurized electrochromatography.

The elegant technique invented by Terabe and co-workers [5,6], whereby analytes are partitioned between background electrolyte and micelles in a micellar solution, was originally called micellar electrokinetic chromatography. Again, this is unnecessarily lengthy and the

Table 1
Electroseparation techniques

Technique	Open tube	Packed tube
Electrophoresis	CE	CGE
Chromatography	CMEC	CEC

Electrophoresis methods
CE = capillary electrophoresis (ions only)
CGE = capillary gel electrophoresis (ions only)

Chromatographic methods
CMEC = capillary micellar electrochromatography (neutrals, ion pairs, ions)
CEC = capillary electrochromatography (neutrals, ion pairs, ions)

method is now commonly known either as micellar electrochromatography or micellar electrophoresis. As the separation is primarily based on partitioning between two phases, the electrolyte and the micelles, the process is strictly chromatographic and not electrophoretic. Accordingly, it is recommended that the name capillary micellar electrochromatography (CMEC) should be adopted.

In summary, the names of the techniques should include the word "electrophoresis" when the basis for separation is primarily differences in electrophoretic mobility, and should include the word "chromatography" when the basis for separation is primarily differences in the partition ratios of analytes between phases which move at different rates (the rate of movement of one of the phases can of course be zero, as in simple packed column electrochromatography).

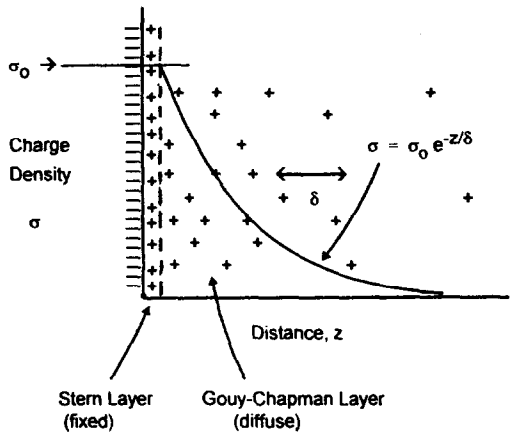

Fig. 1. Diagramatic representation of the electrical double layer showing the negatively charged surface, fixed excess positive ions in the Stern layer and mobile excess positive ions in the diffuse Gouy–Chapman layer. The so-called "thickness of the double layer" is shown as δ.

3. Basic electrochemical phenomena relevant to CES

All CES methods by definition must involve one or both of the primary electrochemical phenomena, electroosmosis and electrophoresis. Some of them also involve chromatographic partitioning between phases.

3.1. The electrical double layer

Both of the primary electrochemical phenomena result from the presence of the electrical double layer which is present at virtually all interfaces, and particularly at interfaces between a solid and an electrolyte. Fig. 1 illustrates, in a highly diagramatic way, the surface of silica in contact with an electrolyte. The surface contains chemically bound $Si–O^-$ groups at its surface, and is therefore permanently electrically charged. When in contact with an electrolyte, these surface ions are balanced by an excess of positive ions within the electrolyte. Owing to the strong electrostatic interactions between the negative ions in the surface and the positive ions in the solution, the layer of excess positive ions in the solution is very thin. It is generally agreed (see, for example, Bockris and Reddy [7]) that this layer of excess positive ions can be separated into two parts. Close to the surface is a layer of ions which are adsorbed on the surface and are essentially fixed. They do not enter into the electrokinetic phenomena. This is called the Stern or Helmholz layer. The remaining ions form a diffuse layer called the Gouy–Chapman layer. The ions in this diffuse layer exchange continuously with those in the rest of the solution, and are indeed indistinguishable from them. The charge density of the excess ions, σ, falls exponentially with distance, z, from the surface according to Eq. 1, as shown in Fig. 1.

$$\sigma = \sigma_0 \exp(-z/\delta) \quad (1)$$

where δ is the so-called "thickness" of the double layer (often denoted by $1/\kappa$), and is given by

$$\delta = [(\varepsilon_0 \varepsilon_r RT)/(2cF^2)]^{1/2} \quad (2)$$

Typical values of δ are given in Table 2. The electrical potential at the boundary between the Helmholtz layer and the diffuse part of the

Table 2
Thickness of the electrical double layer, δ

c (mol l^{-1})	δ (nm)
0.1	1.0
0.01	3.1
0.001	10.0

double layer is called the "zeta potential", ζ, and is of the order of 10–100 mV.

3.2. Electroosmosis

When a potential difference is applied to a surface, as is the case when a field is applied along the length of a quartz capillary, the ions in the diffuse layer experience a force parallel to the surface. Because there is a slight excess of positive ions in this layer, the solvent immediately in contact with the surface experiences a net force towards the negative electrode; this is resisted by the viscosity of the liquid, resulting in shear developing within the double layer which is proportional to the excess charge density at any point in the layer. The result, as shown in Fig. 2, is that the liquid at the solution-side boundary of the double layer moves at a constant velocity, u_{eo}, given by Eq. 3. The process is called electroosmosis, with electroosmotic velocity,

$$u_{eo} = (\varepsilon_0 \varepsilon_r \zeta/\eta)E \quad (3)$$

As the field E is an experimental variable, it is generally more convenient to characterize electroosmotic movement by the electroosmotic mobility, μ_0(m^2 s^{-1} V^{-1}):

$$\mu_{eo} = (\varepsilon_0 \varepsilon_r \zeta/\eta) \quad (4)$$

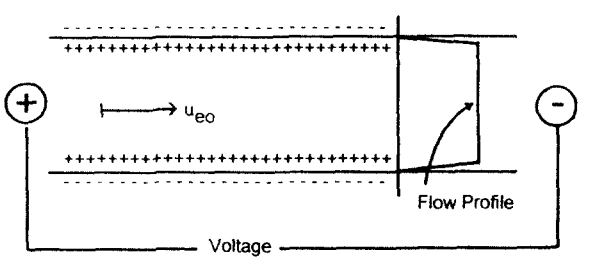

Fig. 2. The phenomenon of electroosmosis in a narrow tube, showing the plug-like flow profile with the very thin shear layer close to the wall of the tube.

In the case of a narrow open tube, the liquid within the tube moves as a plug, so that the velocity of the liquid is constant over more or less the entire tube section except for a very thin layer at the wall which is a few δ's thick. A very important feature of the electroosmotic flow is that its velocity is independent of the bore of the tube in which it occurs, provided that the bore is significantly greater than the thickness of the double layer. In previous papers [2,3] we have shown, using the results of Rice and Whitehead [8], how the mean flow velocity depends on the ratio of the tube diameter, d, to the double layer thickness, δ. Table 3 gives some typical values. With typical values of δ, and an electrolyte concentration of 0.01 M, the minimum bore required to maintain good electroosmotic flow will be around 100 nm. Typical bores used in CES are 500–1000 times larger.

3.3. Electrophoresis

A spherical particles of radius, a, will move in its surrounding electrolyte according to Eqs. 5 and 6. This process is called electrophoresis, and is illustrated in Fig. 3.

$$\text{electrophoretic velocity}, \quad u_{ep} = (\varepsilon_0 \varepsilon_r \zeta/\eta) f(a/\delta) E \quad (5)$$

$$\text{electrophoretic mobility}, \quad \mu_{ep} = (\varepsilon_0 \varepsilon_r \zeta/\eta) f(a/\delta) \quad (6)$$

Table 3
Dependence of mean flow velocity on ratio of tube diameter, d, to double layer thickness, δ

d/δ	u_{av}/u_0
2	0.10
5	0.39
10	0.64
20	0.81
50	0.92
100	0.98

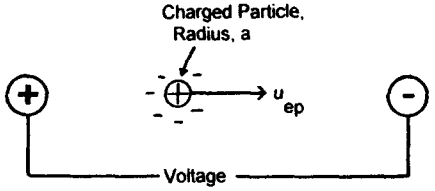

Fig. 3. The phenomenon of electrophoresis showing a positively charged particle of radius a surrounded by its negatively charged ionic atmosphere.

$f(a/\delta) = 1$ for $a/\delta \gg 1$ and 2/3 for $a/\delta \ll 1$. Thus, when the radius of the particle is large compared with δ, the particle moves at the same velocity as if it were subject to electroosmosis, but when the radius is small compared with δ, as for an ion, the velocity is 2/3 that for electroosmosis with the same zeta potential.

3.4. Flow in packed beds

In a bed packed with particles, e.g., dense silica monospheres, the electrolyte will try to flow over these particles at the rate given by Eq. 3 or 5 provided that the particles are large enough. This is shown diagramatically in Fig. 4, where the surfaces of the particles are shown bearing negative charge and the liquid in contact with them bearing excess positive charge. As the particles are fixed in the bed the liquid moves through the bed much in the same way as it moves along a narrow open capillary. Generally, the channels between particles of a packed bed have a mean diameter of about one third that of the particles. Hence, on the basis of Table 3, the flow rate through a packed bed will begin to decrease noticeably only when the particle diameter is less than about 100δ. Reference to Table 2 indicates that with a 0.01 M electrolyte this will occur when the particle diameter is about 0.3 μm.

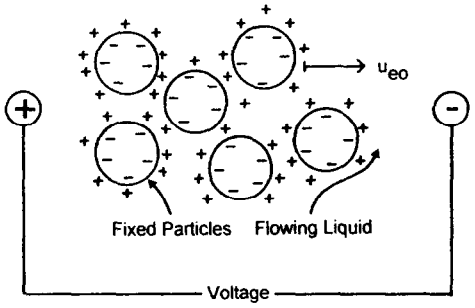

Fig. 4. The phenomenon of electroosmosis in a packed bed showing particles with a negatively charged surface and surrounding liquid containing excess positive charge.

However, when the diameter of the channels in a packed bed is reduced to 2δ, corresponding to spherical particles 6δ in diameter, or 20 nm for a 0.01 M electrolyte, there will be virtually no electroosmotic flow. This would be the case for a column packed, for example, with a gelled silica sol (typical particle diameter 13 nm), and of course for a column packed with a polymer gel as in GCE. In such cases migration can occur only by electrophoresis.

When the particles of a packed bed are porous, the extra-particle flow-rate will be the same as in a bed of impermeable particles but, unless the pore diameter within the particles is very large, there will be virtually no flow within the particles. Accordingly, the mean flow-rate averaged over the entire cross-section of the bed will be reduced below the interparticle flow-rate in the same way as when the flow is pressure driven.

3.5. Molar conductivity, ionic mobility, diffusion coefficient

The molar conductivity, Λ, of a dissolved salt A_nB_m is the conductivity measured between two plates unit distance apart which have between them a perpendicular cylinder containing 1 mol of A_nB_m. This definition is illustrated in Fig. 5.

The individual ionic molar conductivities, λ, of the separate ions of an electrolyte are the contributions to the total molar conductivity from 1 mol of ion (A or B). The molar conductivities are related to the ionic mobilities by the equations

$$\text{ionic molar conductivity, } \lambda = zF\mu \quad (7)$$

$$\text{molar conductivity, } \Lambda = z_{tot}F(\mu_A + \mu_B) \quad (8)$$

where z is the charge on an individual ion and

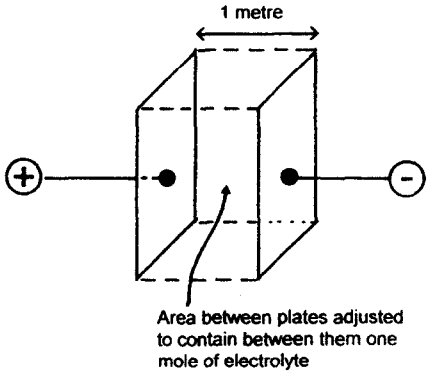

Fig. 5. Illustration of definition of molar conductivity.

z_{tot} is the total charge on the anions (or cations) of the salt A_nB_m.

The molar conductivity of the background electrolyte in a CE system is readily obtained from the resistance per unit length of the capillary, $R(=E/i)$, the molar concentration of the electrolyte, c (mol m^{-3}), and the cross-sectional area of the capillary, A (m^2):

$$\lambda = 1/(RcA) = i/(EcA) \tag{9}$$

The mobility of an ion is also related to its diffusion coefficient. According to Einstein, the diffusion coefficient of a species can be related to its drift velocity in a field (see [7]). For an ion in an electric field the appropriate relation is given by

$$D_m = \mu RT/(zF) \tag{10}$$

If typical values are inserted, e.g., $\mu = 1.0 \times 10^{-7}$ m^2 s^{-1} V^{-1}, $z = 1$, T = 300 K, we obtain

$$D_m = 1.0 \times 10^{-7} \times 8.3 \times 300/96\,500$$
$$= 2.5 \times 10^{-9}\ m^2\ s^{-1}$$

4. Partitioning in CES systems

Whereas ionized species can be separated purely on the basis of their different electrophoretic mobilities, uncharged species can be separated only by differential partitioning between two phases which moves at different velocities. With a background electrolyte necessarily being one of the phases, there are two second phases which have been used: (a) particles fixed in a packed bed as in HPLC and (b) charged micelles which move by electrophoresis within the background electrolyte. Micro-emulsion particles have also been used in place of micelles, and most of the discussion given below also applies equally to CES with microemulsions.

In each instance the extent of partitioning is best represented by the chromatographic capacity factor k', which is defined as

$$k' = \frac{\text{amount of analyte in disperse phase}}{\text{amount of analyte in dispersion medium}} \tag{11}$$

The use of the term "disperse phase" in place of "stationary phase" may not be familiar to chromatographers. However, the former term is more in keeping with the theory of micelles and emulsions. The difference between CEC on the one hand and CMEC on the other is chiefly that in CEC the disperse phase is truly stationary, being in the form of particles packed into a bed, whereas in the CMEC the disperse phase moves with the background electrolyte but at a different speed. The concept of k' nevertheless applies equally to both systems; k' is related to the concentration distribution coefficient, D, through the phase ratio, ϕ:

$$k' = \frac{\text{concentration in disperse phase}}{\text{concentration in dispersion medium}} \cdot \frac{\text{volume of disperse phase}}{\text{volume of dispersion medium}}$$
$$= D\phi \tag{12}$$

It is reasonable to assume that the phase ratio, ϕ, is very nearly independent of temperature and therefore that the temperature dependence of k' is essentially the same as that of D. Accordingly, it provides the enthalpy of exchange between the two phases, ΔH, via the Van't Hoff relationship:

$$\mathrm{d} \ln k'/\mathrm{d}T = \mathrm{d} \ln D/\mathrm{d}T = \Delta H/RT^2 \tag{13}$$

5. Plate efficiency in CES systems

Band dispersion is characterized in CES systems in the same way as in chromatography, and the dispersive processes which can in principle occur are exactly the same. Thus the plate efficiency, N, and the plate height, H, for any analyte are obtained from the standard deviation of its peak profile, σ, and its migration time, t, using the equations

$$N = (t/\sigma)^2 \tag{14}$$

$$H = L/N = L(\sigma/t)^2 \tag{15}$$

Jorgenson and Lukacs [1] were the first to point out that because of the plug flow profile in CE the only contribution to the plate height was that given by the B term of the Van Deemter equation, i.e., the term which provided for axial diffusion. With no packing there was no contribution from the A term (eddy diffusion), and with no retention at the walls of the tube and no variation in flow velocity across the tube there were no contributions from C terms (resistance to mass transfer). The same argument applies to CMEC and CGE because the small size of the micellar particles in the former and the fine reticulation of the gel in the latter make mass transfer between and within the phases so fast as to produce no dispersion.

Accordingly, we can write for the plate height H

$$H = 2D_m/u \tag{16}$$

where u is the overall migration rate of the analyte. With values of D_m around 10^{-9} m^2 s^{-1} for small analytes in water and u values of around 2 mm^{-1} s we can expect plate heights of the order of 1 μm and plate efficiencies of 500 000 for 0.5-m columns. In the case of CGE the diffusion of the analytes (usually DNA fragments) is severely restricted by the presence of the gel, but being multi-charged they still experience a large enough force to provide adequate migration rates. The result is migration rates similar to those in CE, but diffusion coefficients that are 10 or possibly 100 times lower. The plate efficiencies then achieved, as shown in numerous examples, may be as high as 10^7.

The situation with CEC at first sight looks less encouraging, as we now have to contend with the flow and mass transfer problems which we normally encounter in HPLC. If indeed we were restricted to particles of 3 or 5 μm this conclusion would be justified. However, in CEC, it should be possible to work with particle no larger than 0.5 μm [2,3]. The reduced velocity at 1 mm s^{-1} then falls from a typical HPLC value of around unity to around 0.1. Both the A and C term contributions then fall to low values, and it again becomes possible to approach the ideal value of H given by Eq. 12. Current progress on using small particles [9] in electrochromatography shows that with 1.5-μm porous particles it is possible to achieve at least 250 000 plates per metre. Higher efficiencies are achievable, but without retention, using non-porous silica monospheres.

6. Characterization of migration parameters in CE and CGE

Electropherograms are sufficiently similar to chromatograms that it is tempting to think that they can be characterized by quoting effective k' values for the different analytes using a neutral marker as the analogue of an unretained solute. Such a species would migrate at the electroosmotic velocity u_{eo}. Regrettably, this is not an acceptable procedure.

As shown in Figure 6 (top), any ionized species will migrate with an overall velocity, u, given by

$$u = u_{eo} + u_{ep} \tag{17}$$

where u_{eo} and u_{ep} are taken as positive when the movement is towards the cathode (the negative electrode) and negative when in the opposite direction. It is readily shown that the effective k' for any analyte would then be a function of the ratio u_{eo}/u_{ep}. However, u_{eo} and u_{ep} are totally

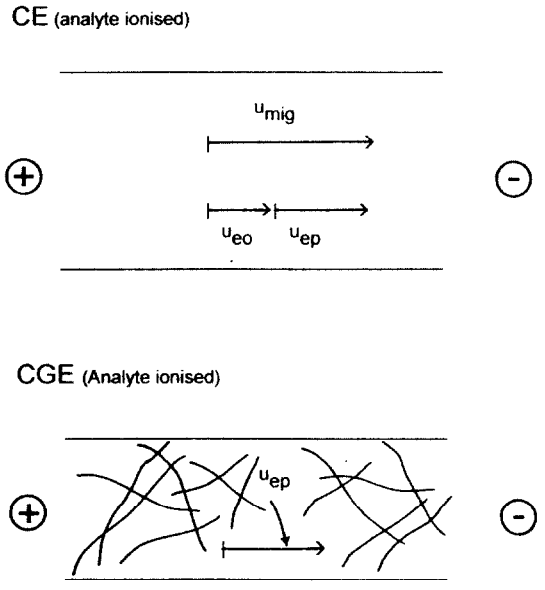

Fig. 6. Key parameters that characterize capillary electrophoresis (CE) (top) and capillary gel electrophoresis (CGE) (bottom).

independent: u_{eo} is a property of the electrolyte/silica surface and u_{ep} is a property of the analyte. Their ratio has no fundamental significance, and is not therefore a valid parameter for characterizing the system. Accordingly, a CE system can only be characterized by quoting both u_{eo} and u_{ep} separately. As both of these are proportional to the field, it is recommended that CE systems should be characterized by quoting the electroosmotic mobility, μ_{eo}, for the electrolyte/capillary, and the electrophoretic mobility, μ_{ep}, of each analyte. μ_{eo} should be established by including a neutral marker in the analyte sample. The two mobilities are obtained from the migration/elution times of neutral and ionized species, t_n and t_{ion}, by the equations

$$\mu_{eo} = (L/E)(1/t_n) \quad (18)$$

$$\mu_{ep} = (L/E)[(1/t_{ion}) - (1/t_n)] \quad (19)$$

For CGE systems there is no electroosmosis (see Fig. 6, bottom), and it is therefore recommended that CGE systems should be characterized by quoting the electrophoretic mobility, μ_{ep}, of each analyte.

7. Characterization of elution parameters in CEC

7.1. Neutral analytes

Fig. 7 illustrates the essential features of the CEC process. The flow in CEC is primarily by electroosmosis, and its velocity is characterized by stating the electroosmotic mobility, μ_{eo} of the electrolyte (eluent) in the packed column. This may be found using an unretained neutral marker by Eq. 18. The degree of retention or capacity factor, k', of the other analytes is obtained directly from the electrochromatogram as in HPLC by measuring retention times of the unretained neutral marker, t_n, and of each analyte, t_R:

$$k' = (t_R - t_n)/t_n \quad (20)$$

7.2. Neutral and ionized analytes

When both neutral and ionized analytes are present there is the added complication that the ions when in the mobile phase will migrate at a

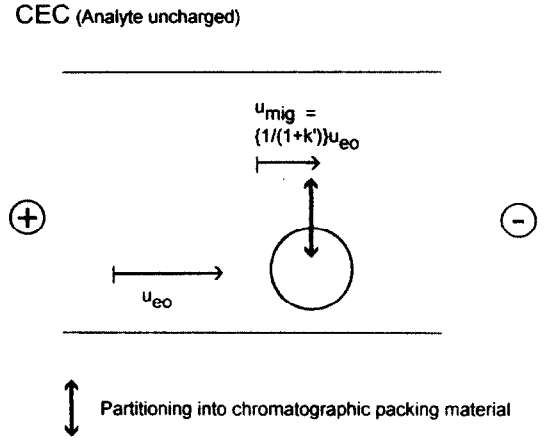

Fig. 7. Key parameters that characterize capillary electrochromatography (CEC).

velocity u_{ion} ($=u_{eo}+u_{ep}$). It is not possible in a single experiment to distinguish between the contributions to the elution time from electrophoresis and partitioning. The two factors can be distinguished only by carrying out a further independent experiment. For example, a pressure-driven chromatogram will provide k' values, whereas a CE experiment will provide electrophoretic mobilities.

In practice, it is likely that electrochromatographic separations will stand on their own, and it will not generally be necessary to separate electrophoretic and partitioning contributions to the elution velocity, so that "effective k' values" will most likely be quoted. It must be noted, however, that irreproducibility from column to column could result from assuming that there will always be a constant relationship between electroosmotic and electrophoretic mobilities for a given type of column packing.

Accordingly, it is recommended that CEC separations should be characterized by quoting the electroosmotic mobility of the electrolyte/packing, μ_{eo}, and the effective k' values for each analyte, having included an unretained neutral marker in the analyte sample to establish t_n. The effective k' values should be obtained from Eq. 20 even when there are ionized analytes present.

8. Characterization of elution parameters in CMEC

8.1. Neutral analytes

Fig. 8 illustrates the essential features of the CMEC process. In CMEC the analytes are partitioned between the background electrolyte and the micelles. The electrolyte moves at a velocity u_{eo}, while the micelles migrate within the electrolyte at a their electrophoretic velocity $u_{ep(mic)}$. The net migration velocity of the micelles in the capillary, u_{mic}, is thus

$$u_{mic} = u_{eo} + u_{ep(mic)} \tag{21}$$

An analyte that is not partitioned into the micelles ($k' = 0$) moves at a velocity u_{eo}, whereas an analyte this is completely partitioned into the micelles ($k' = \infty$) moves at a velocity u_{mic}. There is therefore a window of elution velocity and elution time within which all neutral analytes will emerge from the column. In more detail, the k' value of an analyte, which characterizes its partitioning, is defined as

$$k' = \frac{\text{amount of analyte in micelles}}{\text{amount of analyte in electrolyte}} \tag{22}$$

The proportions of analyte present at any instant within the electrolyte and within the micelles are $1/(1+k')$ and $k'/(1+k')$, respectively. When an analyte molecule is within the electrolyte it moves at a velocity u_{eo}, and when it is within the micelle it moves at a velocity u_{mic}. The mean rate of movement of a band of analyte is therefore

$$u = [k'/(1+k')]u_{mic} + [1/(1+k')]u_{eo}$$
$$= [k'/(1+k')]u_{ep(mic)} + u_{eo} \tag{23}$$

The k' value for any analyte is readily found from Eq. 23 as

$$k' = (u_{eo} - u)/(u - u_{mic}) \tag{24}$$

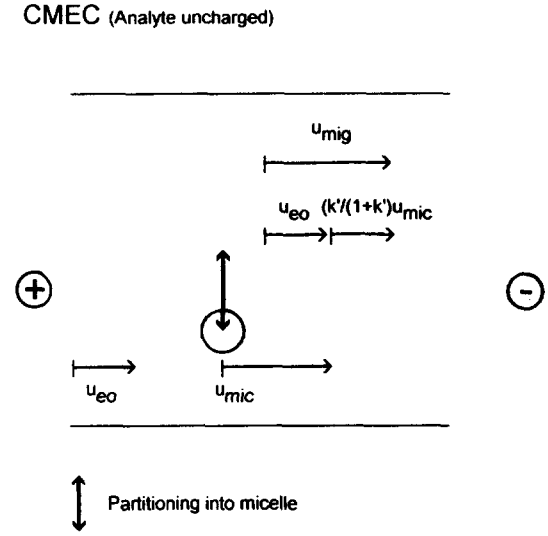

Fig. 8. Key parameters that characterize capillary micellar electrochromatography (CMEC).

In terms of elution times:

$$k' = [(1/t_n) - (1/t_R)]/[(1/t_R) - (1/t_{mic})]$$
$$= [1 - (t_R/t_n)]/[(t_R/t_{mic}) - 1] \quad (25)$$

where t_n is the time of elution of an analyte confined to the electrolyte and t_{mic} is the time of elution of an analyte confined to the micelles; k' varies in a non-linear, but well defined, manner with the retention time t_R, ranging from $k' = 0$ when $t_R = t_n$ to $k' = \infty$ when $t_R = t_{mic}$.

8.2. Neutral and ionized analytes

The situation is again complex, as in CEC. Ionized solutions will migrate while in the electrolyte at their own velocity $u_{ion} = u_{eo} + u_{ep}$. The contributions to the overall elution time arising from electrophoresis and partitioning cannot be separated in a single experiment. It is not possible, as in CEC, to carry out a pressure-driven experiment to establish the k' values of all solutes, so the only possible way to separate the electrophoretic and partitioning components is to vary the degree of partitioning relative to electrophoresis. This can be achieved by carrying out experiments with differing contents of the micellar agent because, as shown by Terabe et al. [6], k' is proportional to the concentration of micellar agent in excess of the critical micelle concentration (cmc).

Following Eq. (23) we can write

$$u = [k'/(1 + k')]u_{mic} + [1/(1 + k')]u_{ion} \quad (26)$$

where u_{ion} now replaces u_{eo}. As k' is proportional to the excess concentration, c, of micellar agent we can replace k' by αc, resulting in

$$u = [\alpha c/(1 + \alpha c)]u_{mic} + [1/(1 + \alpha c)]u_{ion} \quad (27)$$

where u, u_{mic} and c are known but α and u_{ion} are unknown. Rearrangement gives

$$c(u - u_{mic}) = (u_{ion}/\alpha) - (u/\alpha) \quad (28)$$

A plot of $c(u - u_{mic})$ against u will then give a straight line of gradient $1/\alpha$ and intercept u_{ion}/α. This analysis assumes that u_{ion} and u_{mic} do not change with c, the micellar concentration. The assumption seems likely to hold. However, the simplest way to isolate u_{ion} is undoubtedly to carry out the experiment with the micellar agent present at a concentration just below the cmc when $u = u_{ion}$. Under these conditions the zeta potential should be unchanged.

As with CEC, it is probably unnecessary in most practical cases to determine the electrophoretic mobility and capacity factor of an ionized solute separately. Accordingly, it seems best to characterize all capillary micellar electrochromatograms by stating "effective k' values" defined according to Eq. 25 whether the analytes are neutral or ionized. However, it must be noted that analytes with extreme values of u_{ep} may elute outside the window formed by the micellar peak and the unretained peak. In such cases the effective k' values will be negative. Eq. 25 indicates that when an analyte elutes outside the window beyond the micelle (for which $k' = \infty$), it will have an effective k' between $-\infty$ and -1; when it elutes beyond the unretained neutral marker (for which $k' = 0$), it will have an effective k' between 0 and -1.

It is recommended that capillary micellar electrochromatograms should be characterized by stating the electroosmotic mobility of the electrolyte/capillary, μ_{eo}, and the overall electromigration mobility of the micelles, μ_{mic} $[=\mu_{ep(mic)} + \mu_{eo}]$, and by stating the effective k' values for each analyte. Where some of the analytes are ionized, their effective k' values may be negative.

9. Summary of recommendations

It is recommended:

(1) that the four distinct capillary electroseparation methods should be named capillary electrophoresis (CE), capillary gel electrophoresis (CGE), capillary electrochromatography (CEC) and capillary micellar electrochromatography (CMEC);

(2) that, where the primary separation process is electrophoresis (CE, CGE), the separation should be characterized by stating the electroosmotic mobility of the electrolyte and the electrophoretic mobilities of the analytes;

(3) that, where the primary separation processes is partitioning (CMEC, CEC), the system as a whole should be characterized by stating the electroosmotic mobility of the electrolyte and the overall migrational mobility of the micelles (where relevant); the separation itself should be characterized by stating the k' or effective k' values of the analytes;

(4) that band dispersion should be characterized by stating the HETP or plate number, these being measured in the same way as in liquid chromatography.

Symbols

a	Radius of particle or ion
A	Cross-section of capillary
c	Concentration of electrolyte or micellar material
D	Distribution coefficient between phases
D_m	Diffusion coefficient
E	Electric field strength
F	Faraday constant = 96 500 C mol^{-1}
H	HETP
ΔH	Enthalpy of transfer between phases
i	Electric current along capillary
k'	Capacity factor
L	Length of capillary from injector to detector
N	Number of theoretical plates
R	Resistance per unit length of electrolyte in capillary
	Universal gas constant = 8.314 J K^{-1} mol^{-1}
T	Absolute temperature
t	Migration time
$t_{ion}, t_{mic}, t_n, t_R$	Migration time of ion, micelle, neutral solute, analyte
u	Migration velocity
u_{ion}, u_{mic}	Migration velocity of ion, micelle
u_{eo}, u_{ep}	Electroosmotic velocity, electrophoretic velocity
z	Distance from surface,
z, z_{tot}	Charge on ion (in units of electronic charge), or salt
α	Constant
δ	Double-layer thickness
ε_0	Permittivity of vacuum = $8.85 \cdot 10^{-12}$ C^2 N^{-1} m^{-2}
ε_r	Dielectric constant
ϕ	Phase volume ratio
η	Viscosity
κ	Reciprocal of double-layer thickness
λ	Ionic molar conductivity
Λ	Molar conductivity of salt
μ	Mobility (subscripts as for u)
σ	Standard deviation of peak (in time units)
σ, σ_0	Charge density, standard charge density
ζ	Zeta potential

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