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Review

Theory of capillary electrochromatography

Keith D. Bartle*, Peter Myers

School of Chemistry, University of Leeds, Woodhouse Lane, Leeds LS2 9JT, UK

Abstract

The present state of the theory of capillary electrochromatography (CEC) is reviewed. Emphasis is placed on electroosmosis and the electrical double layer, and the generally good understanding of the factors affecting the electroosmotic flow in CEC columns. The relation of CEC to other electrically driven separations are described, along with band broadening, and the influence of column temperature in CEC. The theoretical potential of CEC is assessed from the standpoint of current and future column technology, and likely future application areas are described. © 2001 Published by Elsevier Science B.V.

Keywords: Electrochromatography; Electroosmotic flow; Mobile phase composition; Temperature effects; Band broadening

Contents

*Corresponding author. Tel.: +44-113-2336-490.
E-mail address: k.d.bartle@chem.leeds.ac.uk (K.D. Bartle).

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1. Introduction

Capillary electrochromatography (CEC) is a recently developed analytical separation technique combining, in principle, advantages of high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) [1–5]. To date, its most frequent applications have been mainly as a variant of HPLC in which the flow of mobile phase through the column is maintained by an electric field rather than by applied pressure. This flow, the so-called electroosmotic flow (EOF), is generated by a large voltage applied along the length of the column; in the presence of an added electrolyte, positive ions accumulate in the electrical double layer of the particles of column packing, and move towards the cathode dragging the liquid phase along with them. CEC resembles CE in that small (50–100 μm) internal diameter columns are employed to maximise surface area-to-volume ratio and conduct away ohmically generated heat and thus minimise thermal gradients which might increase band dispersion. The separating principle in CEC is most usually partition between the liquid and solid phases, although in applications to charged analytes, the effect of different rates of electromigration may be superposed.

Driving the flow by electroosmosis results in a number of important advantages for CEC over HPLC. Since the EOF velocity is independent of the size of the particles in the packed bed, in contrast to HPLC, smaller particles and longer columns may be employed with consequent increase in column efficiency and resolving power for mixtures. A further advantage of electrodriven flow is that the velocity profile of the EOF reduces dispersion of the band of solute passing through the column, with a further improvement in column efficiency.

The combined effect of reduced particle size, increased column length, and plug flow is the possibility of a resolution domain for CEC similar to that of capillary gas chromatography (GC), and currently not approached by HPLC. Whereas selectivity is most often the basis of separation in HPLC, in CEC it becomes possible to separate complex mixtures by using the higher available plate numbers.

In this review, the theoretical basis of CEC and its practice is developed, with emphasis on the EOF, and the factors which influence it, on band broadening in CEC, and the influence of temperature on this and other performance factors. Finally, the theoretical potential of CEC is discussed in the light of the above considerations.

2. Electroosmosis, the electrical double layer, and electroosmotic flow

Under the influence of an applied electric field, a liquid containing an electrolyte will move relative to a stationary charged surface, a process known as electroosmosis [6]. The surface charge is usually acquired as a result of ionisation; for example silanol groups on the surface of fused-silica capillary are ionised to give rise to a surface with negative charge. The distribution of nearby ions in solution is affected, and counter-ions are attracted to the surface to maintain the charge balance, whilst ions of like charge are repelled. A double layer of electric charge is formed (Fig. 1), described by a modified version of the Gouy–Chapman model [6–8] in which the counter-ions are arranged in ''fixed'' and ''diffuse'' layers, with a surface of shear located just beyond the interface. The electric potential drop between the silica wall and the surface of shear is the zeta potential, ζ, which falls exponentially in the diffuse layer eventually to zero, but by a factor e^{-1} over a distance δ, known as the double layer thickness. The zeta potential depends on the product of δ and the surface charge σ according to [6]:

$$\zeta = \frac{\sigma\delta}{\epsilon_0\epsilon_r} \tag{1}$$

where ϵ_0 is the permittivity of a vacuum and ϵ_r is the dielectric constant of the electrolyte solution. The double layer thickness also depends on ϵ_r and on I, the ionic strength of the electrolyte solution [9]:

$$\delta = \left(\frac{\epsilon_0\epsilon_r RT}{2F^2 I}\right)^{0.5} \tag{2}$$

When a potential difference is applied across the column length, the solvated cations in the diffuse layer move towards the cathode, taking the solvent molecules with them. The resulting electroosmotic

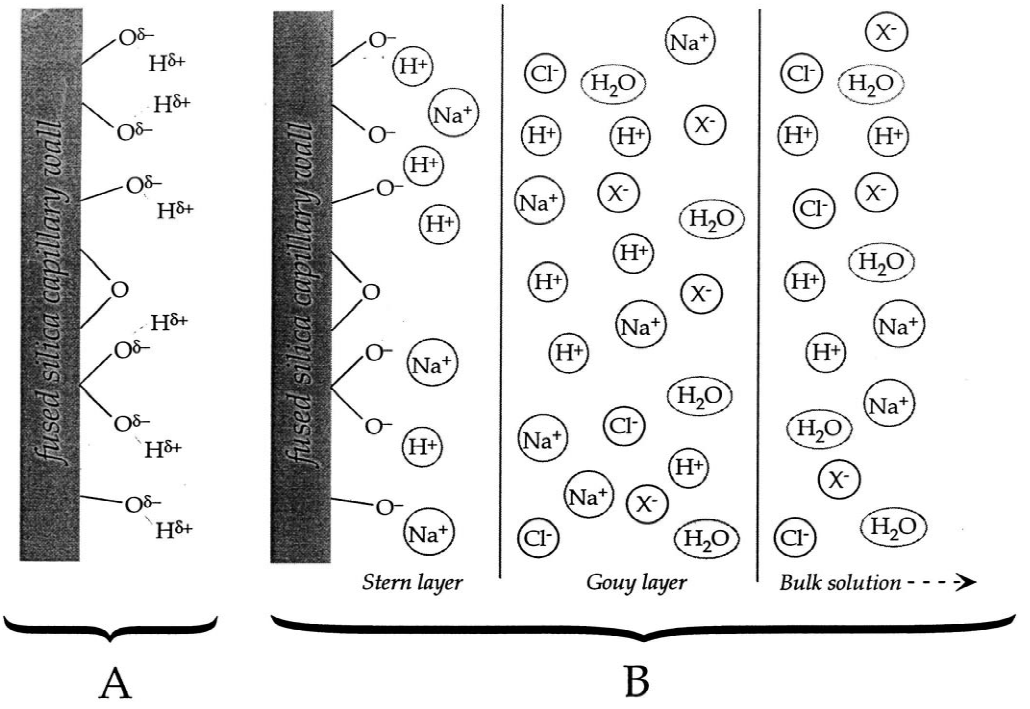

Fig. 1. (A) Representation of the surface of fused-silica tubing. (B) Formation of an electrical double layer near the surface of fused-silica tubing (courtesy of V.T. Remcho and P.T. Vallano).

mobility, μ_{eof} is then related to ζ and to η the viscosity of the solution by:

$$\mu_{eof} = \frac{\epsilon_0 \epsilon_r \zeta}{\eta} \quad (3)$$

and the linear velocity, $u_{eof,}$ is described by the Smoluchowski equation [10]:

$$u_{eof} = \frac{\epsilon_0 \epsilon_r \zeta E}{\eta} \quad (4)$$

where E is the electric field strength.

The double layer thickness, δ has typical values for 1 and 100 nM aqueous 1:1 electrolyte solutions of 10 and 1 nm, respectively. The velocity of electroosmotically generated plug flow is independent of the open-tube internal diameter, d_c, provided that d_c is greater than about 50δ. At this condition, double layer overlap occurs, and the flow velocity is reduced. Flow profiles for such cases have been calculated by Rice and Whitehead [11] and are shown in Fig. 2 for various d_c/δ ratios; a marked

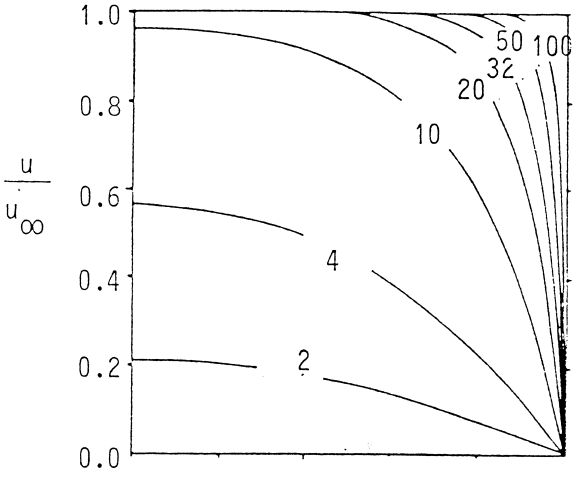

Fig. 2. Velocity profiles from the Rice and Whitehead treatment where double layer overlap occurs: r/a is fractional distance from centre of column ($r/a=0$ at centre, $r/a=1$ at the wall). The ratio of d_c/δ is shown on each line (After Ref. [12]).

decline in electroosmotic flow is clearly apparent when $d_c/\delta \leq 50$, and particularly when $d_c/\delta \leq 10$. For a 40% reduction in velocity over that in a tube of infinite diameter, considered by Knox and Grant [12] to be the acceptable limit, d_c exceeds 10δ.

In a packed tube, the mean channel diameter replaces the open-tube diameter, and may be calculated from the ratio of values of the value of ϕ, the dimensionless flow resistance parameter, for a bed of randomly-packed impermeable spheres ($\phi \approx 500$) to the value for an open tube ($\phi = 32$). It follows [12] that:

$$\frac{\text{mean channel diameter}}{\text{particle diameter}} = \left(\frac{32}{500}\right)^{1/2} \approx 0.25$$

and for slurry-packed columns a significant loss in EOF velocity is expected when $d_p \leq 40\delta$.

While much CEC work has been carried out in open fused-silica columns, the majority of reported studies have involved fused-silica capillaries packed with bonded silica particles or containing a monolithic packing based either on silica or on a polymer carrying charge. In packed-column CEC, both the capillary wall and the column packing can sustain electroosmosis [13–15], but there are a greater number of ionisable groups in the packing which has a far larger surface area in comparison with the internal fused-silica wall. In fact, the packed column may be regarded as an array of spherical particles, with the EOF originating in the channels between the particles, with average diameter between a fifth and a quarter of the particle diameter [16].

3. Relation of capillary electrochromatography to other electrically driven separation methods

While in CE the charged analytes are separated because of different rates of migration in an electric field, in HPLC, and in CEC of neutral species, the analyte and the mobile phase move in the same direction, and the sample components emerge in sequence of their retardation by the stationary phase; this situation is described by case A in Fig. 3. However, if the solute is charged, there are [17] three possible operational modes depending on the direction and magnitude of electrophoretic migration with respect to the EOF (cases B, C and D in Fig. 3):

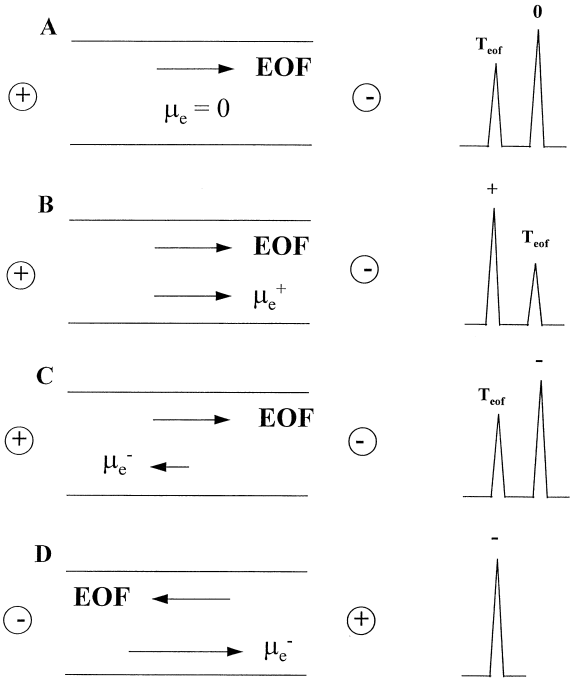

Fig. 3. Capillary electrochromatography of charged species.

(B) co-directional, where the migration velocities of charged species are always greater than that of the EOF marker. The components emerge before the EOF marker;

(C) counter-directional, where the EOF velocity is greater than the electrophoretic velocity of a charged component, which emerges after the EOF marker;

(D) counter-directional, where the EOF velocity is less than the electrophoretic velocity, and detection of a charged component is only possible with instrument polarity reversed. In this case, the EOF marker is not detected.

The sequence of emergence of analytes from the CEC column depends on the magnitudes of their different retardation and migration velocities and can be divided [18] into components that are separative (selective interactions with a stationary phase) or non-separative (migration not contributing to separation). The CEC retention factor k_c is given by:

$$k_c = k + kk_e + k_e \quad (5)$$

where the HPLC retention factor:

$$k = \frac{t_R - t_0}{t_0} \quad (6)$$

and the CE velocity factor:

$$k_e = \frac{t_{eof} - t_m}{t_m} \quad (7)$$

In Eqs. (6) and (7), t_R and t_m are, respectively, the elution and migration times, t_0 is the retention time of an unretained marker, and t_{eof} is the retention time of a solute moving only under the influence of the EOF.

The product kk_e is the consequence of simultaneous chromatography and electrophoresis. If $k_e = 0$ then only HPLC operates and $k_c = k$; if $k = 0$, then the only process is CE since $k_c = k_e$.

The principle of separation of micellar electrokinetic chromatography (MEKC) – the partition of analytes between the EOF-driven electrolyte and micelles moving in the opposite direction – means that all electrically neutral compounds have migration times between t_0 and t_{mc}, the migration time of the micelle. CEC offers the advantage that the time window for separation is unlimited. A hybrid of MEKC and CEC has been proposed by Knox [19]; in colloidal sol electrochromatography, a colloidal sol is used as the moving fluid. If the colloidal particles are charged, they move relative to the eluent, and partition between two phases occurs and results in separation.

4. Effect of variables on the electroosmotic flow

4.1. Contribution of packed and open sections

As discussed in Section 2, it might be concluded that the column packing is mainly responsible for the generation of EOF, even if the velocity of the EOF in a CEC column is most likely to be reduced compared to that in an open tube because of the tortuosity and porosity of the packed bed, although there do not appear to be any adverse effects resulting from packing irregularities.

While there is no convincing underlying theory governing the contributions of packed and open sections of fused-silica capillary, investigations [13] based on electrical conductivity measurements allowed the voltage drop in each section to be calculated, and hence the individual contributions total EOF. The column was considered as two resistors in series, corresponding to the packed and open sections. The currents flowing during CEC were measured for fully and partially (25–100%) packed capillaries, and hence the electrical field strengths in both sections and individual contributions to the total EOF were derived. The results showed that at extremes of mobile phase pH (2.9 and 10.5), field strengths were greater in the packed section, but at intermediate (7.5) pH, field strengths were similar in both sections. It follows that, at pH extremes, the EOF linear velocity decreases with the length of the packed bed, while at pH values most commonly employed in CEC, EOF velocity changes little with packed length. Substantial contributions to the EOF from the capillary wall are evident.

4.2. Applied voltage

From the theoretical relationship (Eq. (4)) between the EOF velocity and the electric field strength, u_{eof} should be directly proportional to E, or to the applied voltage, V, if the column length, L, is kept constant since:

$$E = \frac{V}{L} \quad (8)$$

Deviations from linearity in graphs of u_{eof} versus V have been observed at high field strength because of ohmic heating which reduces viscosity and hence increases EOF velocity (Eq. (4)). Eq. (4) is obeyed [2] up to 20 kV for mixed solvent systems (e.g., Fig. 4).

For a given E, values of u_{eof} for particles with different diameters are similar (Fig. 4). While small differences are observed at higher electric field strengths, it is likely that any variations are a consequence of ohmic heating and resultant changes in viscosity (see Section 6), since dissipation of heat depends on packing efficiency, which depends on particle diameter. Since u_{eof} depends on E, an increase in column length must be accompanied by an increase in voltage. Most CEC experiments so far have been carried out with applied voltages up to 30 kV, but equipment allowing V up to 90 kV has been reported [20].

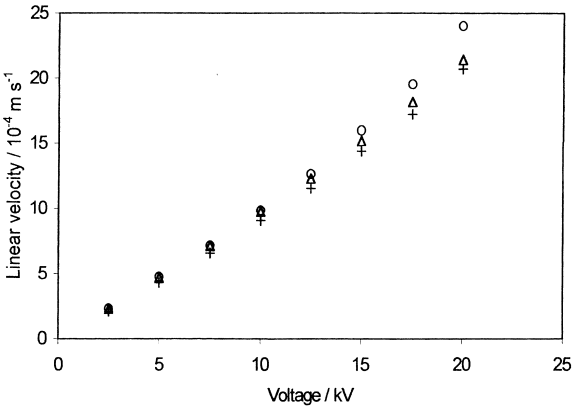

Fig. 4. Effect of voltage in CEC using columns with different particle size: 3 (△), 5 (○) and 10 (+) μm ODS1 material (8 nm pore size). (From Ref. [2]).

4.3. Mobile phase pH

The dependence of EOF mobility on mobile phase properties has been well explored theoretically and in many experiments on CEC columns packed with silica based particles; comparisons have also been made with results in open-tubular (CE) columns. The reduction in μ_{eof} with decreasing pH is readily explained by the expected decrease in silanol group ionisation at lower pH; the zeta potential, ζ depends on surface charge (Eq. (1)), and μ_{eof} on ζ (Eq. (3)). In applying this apparently simple approach, it is, however, important to keep constant all variables except the one in question. In the case of changed pH, the concomitant change in ionic strength could also be influential (Section 4.4); low pH buffers are often prepared by adjusting a higher pH buffer with acid, hence increasing the ionic strength. Moreover, it is well known [21,22] in CE that adding organic solvents to an electrolyte shifts the pK_a values of surface silanol groups to higher values. In fact, at pH values >9, where all the surface silanols should be dissociated, the μ_{eof} is little changed; but at low pH there is still a substantial EOF suggesting that there is still significant silanol ionisation. This result has allowed many applications of CEC in the separation of acidic compounds in ion-suppressed mode at low pH.

4.4. Ionic strength of mobile phase

Eq. (2) shows how the double-layer thickness and hence the zeta potential and μ_{eof} are proportional to $I^{-0.5}$. The increase of μ_{eof} with $I^{-0.5}$ is similar for both CE and CEC at higher ionic strength (Fig. 5), but below a concentration of 5 m*M* phosphate in acetonitrile–water the CEC mobility levels off and begins to drop for silica packings with pore size of 8 nm, while continuing to rise in CE. This result is a consequence of double-layer overlap within the pores of packing material; the double layer thickness is about 4 nm for $I \cong 2.5$ m*M*. Care is also necessary in assuming that a variation of I does not influence investigations of the type and proportion of organic solvent in the mobile phase (see Section 4.7).

4.5. Packing particle diameter

Equation (4) shows how the particle diameter (d_p) should not affect the mobile phase velocity. This is in marked contrast to the pressure-driven flow velocity $\bar{v}$ described [23] by the Kozeny–Carman equation:

$$\bar{v} = \frac{\epsilon^2}{180(1-\epsilon)^2} \cdot \frac{d_p^2}{\eta} \cdot \frac{\Delta p}{L} \tag{9}$$

where ϵ is the porosity and Δp the pressure drop. Clearly, for pressure-driven flow a decrease in d_p

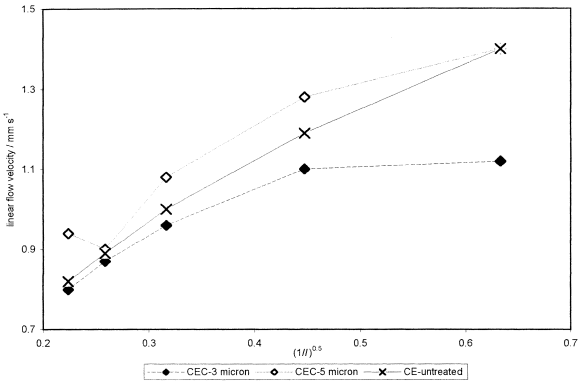

Fig. 5. Comparison of the effect of ionic strength (I) in CE and CEC. (Data from Ref. [2]).

must be compensated for by a large increase in Δp thus limiting particle sizes for conventional HPLC. A number of groups have, however, demonstrated CEC on columns packed with small diameter particles [24,25].

The applicability of Eq. (4) in CEC has been demonstrated for monodisperse packings [26], but in work with columns packed with conventional materials with more usual particle-size distributions a reduction in mean particle diameter from 5 to 3 μm for ODS1 silica reduced by 60% the mobile phase velocity for the same voltage and buffer concentration [27]. This may result from double layer overlap in a bed with smaller interstitial channels consequent on the presence of more very small particles in the nominal 3 μm packing.

4.6. Organic groups bonded to the packing

The most commonly used stationary phases for CEC are silica particles with bonded groups, usually alkyl groups, with octadecylsilyl (C_{18}) groups preeminent. As discussed in Section 4.1, the flow of mobile phase towards the anode arises as a result of the electrical double layer which originates from the accumulation of positive ions from the buffer next to the unbonded $Si–O^-$ groups of the particle and column wall. CEC separations on a number of other silica-bonded phases have been made [27,28] (e.g., Fig. 6) but if these cannot carry a charge, there is no appreciable affect on EOF velocity, although there may be improvements in the chromatographic behaviour of the column; for example, phenyl bonded silica has been shown to reduce the tailing of peaks from basic compounds, because, it was claimed, of the steric shielding by the bulky organic group of the analyte from the $Si–O^-$ and Si–OH groups on the packing [29].

A number of attempts have been made [28,30] to increase the magnitude of the EOF by bonding negatively charged groups to the silica, especially sulfonic acid groups in either strong cation exchange (SCX), or so-called mixed mode phases which incorporate both sulfonic acid and alkyl groups (Table 1).

4.7. Solvent composition of the mobile phase

The type and proportion of organic solvent in the mobile phase is predicted to influence the EOF mobility through the ratio of permittivity to viscosity, ϵ_r/η, in Eq. (3). Typical values [2] of ϵ_r/η for mixtures of water with a variety of organic solvents are listed in Table 2, calculated with the assumption

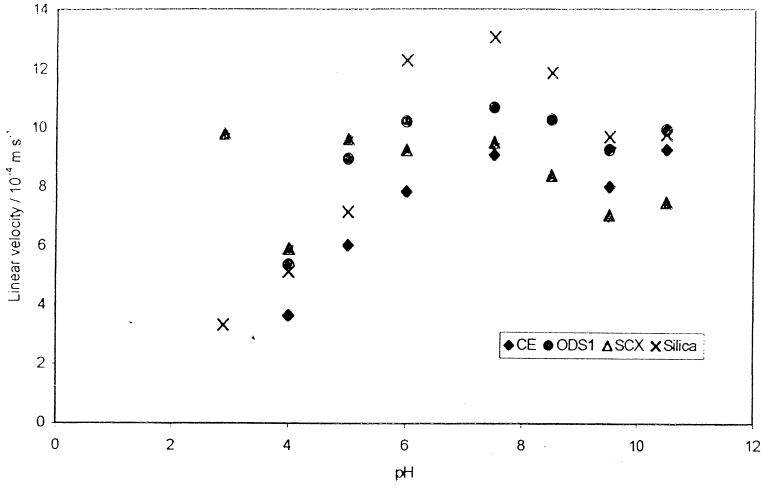

Fig. 6. Dependence of EOF linear velocity on pH in CEC and CE. Electrolytes of varying pH of approximately 10 m*M* ionic strength containing 70% acetonitrile. (From Ref. [2]).

Table 1
Bonded groups employed in studies of pH dependence of EOF in CEC

Group	Structure
Octadecylsilyl	C_{18}
Strong cation exchange (SCX)	Propyl sulfonic acid
Strong anion exchange (SAX)	*N*-Propyl-*N*,*N*,*N*-trimethyl ammoniun
Aminopropyl	C_3

of no contribution from the buffer component. Experimental findings for experiments in open tubes are in good agreement, with acetone, acetonitrile and methanol all showing an EOF minimum around 50–70% organic, although the increase observed when using a higher acetonitrile content was far greater than that anticipated. Wright et al. [31] also witnessed this behaviour for acetonitrile–water systems without supporting electrolyte, and explained it by changes in solvent polarity and hydrogen-bond donor ability. Acetonitrile–buffer is generally selected as mobile phase in CEC. However, many recommended reversed-phase HPLC methods employ methanol–water as mobile phase, and the reduced EOF velocity consequent on the much smaller values of ϵ_r/η for this solvent system mean that direct transfer of HPLC to CEC may not be possible. For example, on aminopropyl bonded silica (Table 1), the EOF is reversed by changing the pH [32]; below pH $\cong$ 4 the NH_2 groups become positively charged and overcome the influence of the $Si–O^-$ groups, offering the possibility by changing the buffer of augmenting a separation based on partition by an electromigration. A strong anion exchange (SAX) packing (Table 1), however, has a reversed EOF which is virtually independent of pH because the NR_4^+ groups maintain their positive charge over a wide pH range [32].

Use of alternative solvents such as acetonitrile–buffer may, however, be possible. In HPLC the principle of isoeluotropy is well known – the reproduction of retention behaviour for mobile phases of similar eluotropic strengths. Isoeluotropy has been demonstrated for neutral solutes in CEC on ODS bonded silica [32,33]. As discussed above, retention times are much longer for a methanol–buffer (80:20) mobile phase than for acetonitrile–buffer (70:30) which has similar eluotropic strength, but retention factors are much more similar (Fig. 7). Deviations from unit gradient may arise from the choice of thiourea as retention marker.

For HPLC, the retention factor, *k*, is related to the percentage organic in the aqueous mobile phase, *p*, via:

$$\ln k = \ln k_0 - ap \tag{10}$$

where k_0 is the retention factor for water eluent, and *a* is a constant. In CEC experiments, as the percentage of acetonitrile in the mobile phase was increased, there was a linear fall in ln *k* (Fig. 8), suggesting that well-established theories used in HPLC method development should be equally applicable to the separation of neutral molecules in CEC [32,33].

Table 2
Approximate ϵ_r/η ratios for binary mixtures with water at 25°C

Solvent	ϵ_r/η (cP^{-1}) for varying % organic		
	0%	50%	100%
Acetone	88	23	68
Acetonitrile	88	75	105
Methanol	88	37	60
2-Propanol	88	15	7

From Ref. [2].

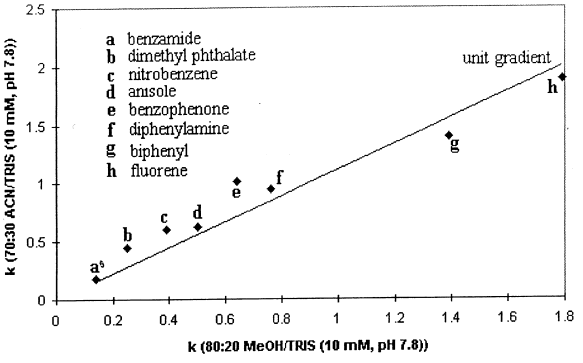

Fig. 7. CEC retention factors for mobile phases isoeluotropic in HPLC. (From Ref. [32]).

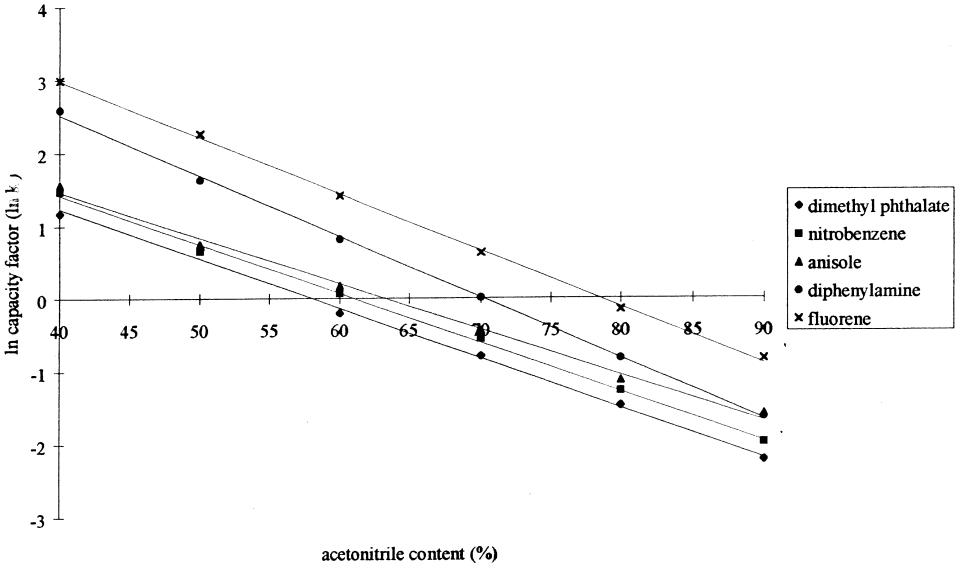

Fig. 8. Effect of acetonitrile content of the mobile phase on the logarithm of the capacity factor, *k*, on ODS1 stationary phase. (From Ref. [27]).

5. Band-broadening in capillary electrochromatography

In column chromatography, a number of processes [23] bring about the broadening of solute bands; (a) eddy diffusion, originating from the variety of flow paths through the packed bed; (b) axial molecular diffusion; (c) resistance to mass transfer in the mobile and stationary phases; and (d) system effects, such as those arising from dead volumes. The smaller theoretical plate heights (H) and hence greater plate numbers N ($=L/H$) in CEC in comparison with conventional pressure driven HPLC arises from reduced contributions to H from factors (a) and (c) above.

Differences in flow velocity profiles in the packed bed are evident in CEC. Clearly, the plug flow profile of CEC substantially reduces the eddy diffusion (or multipath) term in comparison with the parabolic flow profile of HPLC. Since this term is also proportional to the column particle diameter, the use of smaller particles should further reduce the contribution to H; the contribution from slow mass transfer in the mobile phase, $C_m\bar{u}$ where $\bar{u}$ is average linear mobile phase velocity, is proportional to d_p^2.

The use of EOF to drive the mobile phase flow gives rise to a plug-flow profile in the channels between the particles. This also reduces the mass transfer contribution to H by a factor which can be estimated by considering such a channel as an open tube, of diameter d_c, for which C_m is given by the Golay equation [34]:

$$C_m = \frac{f(k)d_c^2}{D_m} \tag{11}$$

where D_m is the diffusion coefficient of the solute in the mobile phase. For parabolic flow, as in HPLC:

$$f(k) = \frac{1 + 6k + 11k^2}{96(1 + k)^2} \tag{12}$$

But for plug flow, in CEC [35]:

$$f(k) = \frac{k^2}{16(1 + k)^2} \tag{13}$$

It follows that, for a given k, and the same d_c and D_m, the contribution to H from this source for CEC is about half that in capillary HPLC.

The above treatment may be extended by use of the model developed [36,37] by Horváth and Lin to

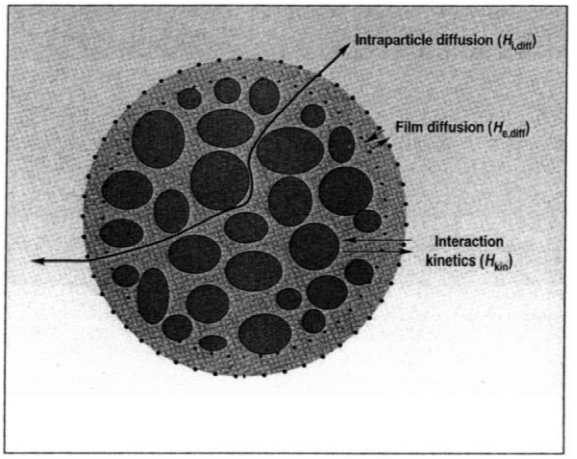

Fig. 9. Sources of dispersion in liquid chromatography. (From Ref. [38]).

describe band broadening in HPLC which was applied to CEC by Dittmann et al. [38].

With the assumption that the different contributions to peak dispersion are additive, the overall plate height in both HPLC and CEC is given by:

$$H = H_{\text{disp}} + H_{\text{e,diff}} + H_{\text{i,diff}} + H_{\text{t,diff}} + H_{\text{kin}} \qquad (14)$$

where H_{disp} is the plate-height increment caused by axial dispersion of the solute in the interstitial space; $H_{\text{e,diff}}$ is the plate height contribution from film resistance at the particle boundary; $H_{\text{i,diff}}$ is the contribution from intraparticle diffusion; $H_{\text{t,diff}}$ is the contribution from transchannel mass transfer; and H_{kin} is the contribution of solute–stationary phase interaction kinetics. The sources of band-broadening increments $H_{\text{e,diff}}$, $H_{\text{i,diff}}$ and H_{kin} are shown in Fig. 9, and the equations for these and H_{disp} are given in Table 3. Dittmann et al. [38] assumed that all the contributions to plate height in the Horváth model are independent of flow profile except $H_{\text{t,diff}}$, arising from diffusion across the channels between particles. Expressions analogous to Eqs. (11) and (12) were written for $H_{\text{t,diff}}$, and all the contributions to plate height were combined and graphs of H versus $\bar{u}$ were hence plotted (Fig. 10). The substantial contributions to the eddy diffusion term evident for pressure driven flow (HPLC) were considerably reduced in electrodriven mode, with the resulting overall dispersion approximately 50% lower than when the mobile phase is driven hydraulically. It follows that a HPLC column packed with 5 μm particles operated in CEC mode will generate double the number of theoretical plates. Minimal reduced plate heights h $(=H/d_{\text{p}})$ near unity are thus predicted, and this has generally been borne out by experiment. The small increase in H with $\bar{u}$ at $\bar{u}$ beyond $\bar{u}_{\text{opt}}$, the mobile phase velocity at the minimum value of H, leads to the expectation that efficient CEC should be possible at high values of $\bar{u}$, thus shortening analysis times.

Horváth and Lin [37] showed how Eq. (14) can be simplified to:

$$h = B/\upsilon + A\upsilon^{1/3} + C\upsilon \qquad (15)$$

where υ is reduced velocity. Eq. (15) is identical with the empirical so-called Knox equation which is very commonly used to monitor the performance of HPLC columns. Fitting of Eq. (15) to empirical data gives representative values of h in HPLC near 2;

Table 3
Contributions to dispersion in a packed bed

Plate height contribution by axial dispersion in a packed bed	$H_{\text{disp}} = \dfrac{2\gamma D_{\text{m}}}{u} + \dfrac{2\lambda d_{\text{p}} u^{1/3}}{u^{1/3} + \omega(D_{\text{m}}/d_{\text{p}})^{1/3}}$
Plate height contribution by film resistance	$H_{\text{e,diff}} = \dfrac{\kappa(\Psi + k + \Psi k)^2 d_{\text{p}}^{5/3} u^{2/3}}{D_{\text{m}}^{2/3}(1+\Psi)^2(1+k)^2}$
Plate height contribution by intraparticle diffusion	$H_{\text{i,diff}} = \dfrac{\theta(\Psi + k + \Psi k)^2 d_{\text{p}}^2 u}{30 D_{\text{m}} \Psi (1+\Psi)^2 (1+k)^2}$
Plate height contribution by solute interaction kinetics	$H_{\text{kin}} = \dfrac{2k^2 u}{(1+\Psi)(1+k)\beta k_2}$

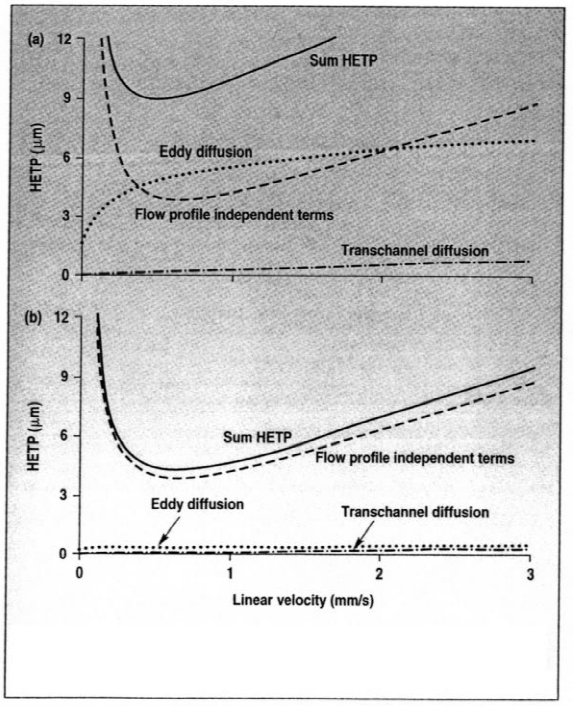

Fig. 10. Van Deemter plots for (a) HPLC (pressure drive), and (b) CEC (electrodrive). (From Ref. [38]).

Knox [39] has pointed out how it has often been claimed erroneously that this represents the theoretical minimum value of *h*. In fact while there are established expressions for *B* and *C*, there is no good theoretical value for *A*. For pressure driven flow in well packed columns, a value of $A \approx 1$ appears to hold, but for electrodrive A may have a value considerably less than 1, thus leading to *h* well below 2 and as low as 1.

Experimental confirmation of the importance of the A term in defining the advantages of CEC has been addressed [40] by Tallarek et al., who used pulsed magnetic field gradient nuclear magnetic resonance to study flow field dynamics, and found an *A* term smaller by a factor of 2.5 in CEC than in microHPLC on the same column; Wen et al. [41] reached similar conclusions from chromatographic measurements, and also showed a smaller reduction in the *C* term.

The *A* term is clearly a major contributor to band broadening, but depends on what Knox refers [39] to the ''goodness'' of packing of the column. Correspondingly, Horváth and Lin have pointed out that in their expression corresponding to *A* for the contribution arising from diffusion in the column interstitial space, the parameters λ and ω relate to column packing material and procedure as well as tube dimensions and vary significantly from column to column.

The majority of CEC separations so far reported have been reversed-phase experiments carried out on HPLC stationary phases with 3 μm particle diameter. Great differences have been observed for the CEC separation of polycyclic aromatic hydrocarbons (PAHs) on nominal 3 μm ODS packings [3] (Table 4). These differences could most probably originate from two sources: (a) the packing procedure, for reasons discussed above; and (b) particle-size distribution.

A number of procedures have been suggested [42–47] for the packing of silica particles into capillaries to produce columns for CEC; electrokinetic [44] and centrifugal [45] packing have been proposed, although the use of liquid slurries with concurrent ultrasonication [46] has been most common. Very high plate numbers have also been found for columns packed with a supercritical fluid carrier with ultrasonication [47]. Comparisons [42] of columns packed with the same material by different methods suggested that there were no significant differences in EOF velocity, migration time or retention factor of neutral analytes under the same conditions of buffer and voltage. However, differences in column performance were found to originate in random variations between replicate columns packed by the same method.

A further reason for the wide divergence in CEC column plate numbers revealed by Table 4 is the

Table 4
Efficiencies obtained for isocratic CEC of PAHs using HPLC stationary phases

Stationary phase	Range of efficiencies (plates m^{-1})
3 μm Spherisorb ODS1	200 000–240 000
3 μm Nucleosil 100 C_{18}	91 000–147 000
3 μm Spherisorb C_{18} PAH	Up to 260 000
3 μm Synchrom	102 000–138 000
3 μm Vydac C_{18}	More than 160 000
3 μm Hypersil	240 000–280 000

From Ref. [3].

particle size distribution, which is unlikely to be uniform even though the packings are all listed as having 3 μm diameter. The distribution, moreover, will vary with the measurement procedure. Number, area, or volume distribution may be used to characterise particles for column packing, and this is seldom stipulated by manufacturers. If number distributions are compared, fine material below 2 μm is evident in virtually all nominal 3 and 5 μm materials, and this is both likely to cause difficulties with packing and to be difficult to remove via the usual air classification used by manufacturers to produce different particle sizes.

The above discussion suggests, in keeping with the conclusions of Knox [39], that substantial improvement in column packing is all important and that significant problems are involved in packing smaller particles as well as larger ones. Better particle fractionation, or the use of monodisperse particles is required for the production of narrow bore columns for both HPLC and CEC. Knox has also pointed out [39] that reduced plate heights, *h*, below unity can be obtained in HPLC for larger particles, whereas the practical minimum for 3 and 5 μm articles is nearer 2. Our CEC results on ODS 1 bonded silica showed [2] that, while *h* was approximately 1.0 for 10 μm particles, it was significantly increased for 5 and 3 μm particles (Fig. 11) presumably because of less uniform packing. These conclusions may have a significant influence on the results of CEC experiments with small-diameter

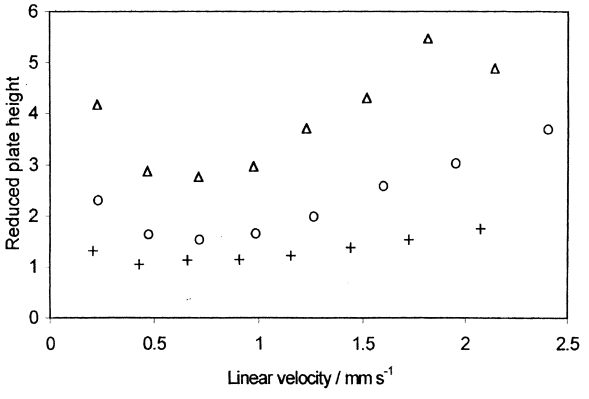

Fig. 11. Van Deemter plots for ethylparaben on 3 (△), 5 (○) and 10 (+) μm ODS1. Applied voltage: 2.5–25 kV. Electrolyte: phosphate, pH 7.5 containing 70% acetonitrile. (From Ref. [2]).

particles. The early theoretical work or Knox and Grant showed [48] how very highly efficient CEC should be attainable on sub-micron particles, but there have been conflicting reports on the practical applicability of small non-monodisperse (<2 μm) particles in CEC. In our hands, the preparation of columns packed with such materials was largely unsuccessful [2], in spite of the use of ultrasonication. Agglomeration of packing material and discontinuities in the packed bed were evident, leading to poor column durability and performance, in stark contrast to results with 3 μm particles. Monodisperse particles in the range 0.2–0.3 μm have been successfully packed into columns for CEC by Adam et al. [24].

As has been observed [49] in previous HPLC work on packed capillary columns, better efficiencies were observed [27] for CEC on very small internal diameter columns. For example, 3 μm ODS1 particles packed into 30 μm I.D. fused-silica tubing gave columns with reduced plate height below 1. It is not yet clear, however, whether previous explanations [49] based on a ''wall effect'' are relevant in the case of plug flow.

The porous nature of silica particles which make up many CEC columns would suggest that they should support electroosmosis through the particle. This should result in a considerable improvement in plate height by eliminating stagnant mobile phase reservoirs into which analyte molecules may diffuse and thus reducing the contribution of slow mass transfer [50]. The EOF cannot be supported if there is double-layer overlap, and for electrolytes containing 70% (v/v) acetonitrile the double layer thickness is typically around 5 and 8 nm for ionic strengths of 2.5 and 1.0 m*M* so that no through-particle perfusion is expected in many conventional stationary phases which have a standard pore size of 8 nm.

Investigations with 30 nm pore size material showed little improvement, but Li and Remcho studied [51] wide pore material with pore size up to 400 nm, and found that above 100 nm the phases were capable of supporting through-particle electroosmosis with considerably reduced plate heights (Fig. 12). Support for the hypothesis of electroosmotic perfusion has been provided [41] by Wen et al. who found only a small decrease in the *A* and *C*

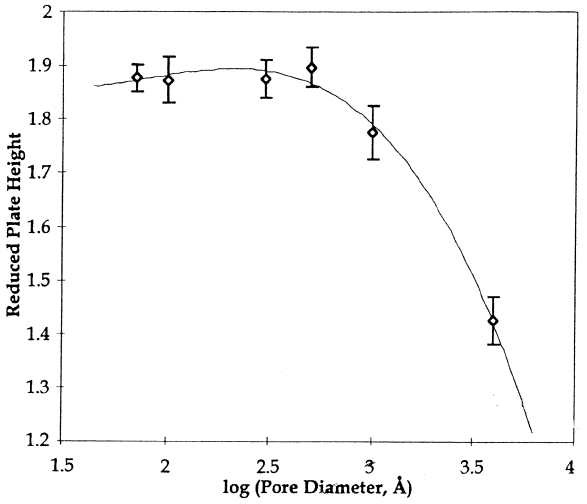

Fig. 12. Effect of increasing pore diameter on plate height in CEC. Columns (75 μm inner diameter) packed with 7 μm spherical, porous C_{18} derivatised silica. Mobile phase acetonitrile–100 m*M* phosphate (pH 6.9) (80:20). (From Ref. [51]).

terms for 30 nm pore ODS particles in comparison with the same material with 8 nm pores: for particles with average pore diameter up to 100 nm it was concluded, however, that through-pore electroosmosis brought about a further decrease in the *C* term in comparison with HPLC.

6. Influence of temperature in capillary electrochromatography

6.1. Retention

The small heat capacity of CEC columns means that column temperature is easily changed. Increased temperature, *T*, reduces mobile phase viscosity via the exponential relation [52]:

$$\eta \propto \exp(\text{constant}/RT) \tag{16}$$

where *R* is the gas constant, and hence increases μ_{eof} (Eq. (3)) so that, for a given voltage, more rapid analysis is possible. Fig. 13 illustrates the effect on retention of a modest increase in column temperature during the CEC separation of capsaicin from some of its derivatives [53].

Retention factors are also influenced by increasing column temperature because of the increased partition into the mobile phase; Van't Hoff plots of ln *k* versus reciprocal absolute temperature are linear (e.g., Fig. 14). The slopes of such plots may differ sufficiently for column separation selectivity to be changed by temperature variation [32]. For example, Fig. 15, shows electrochromatograms of a number of diuretics on ODS bonded silica at temperatures between 15 and 60°C. The resolution of chlorothiazide and hydrochlorothiazide increases with decreasing temperature, and the relative retention of chlorthalidone and hydroflumethazide is reversed with increasing temperature. Temperature programming in CEC [54] may be effective.

Djordjevic et al. [55] used Van't Hoff plots to compare the entropies of solute transfer, ΔS, between mobile and stationary phases in CEC and pressure driven microHPLC on the same column. ΔS was found to be more negative for electrodrive, a difference attributed in part to ohmic heating which brings about differences between set and actual column temperature. It follows that CEC retention factors may vary with electric field strength and hence applied voltage; such variations can be minimised by carrying out CEC at elevated temperature. The accurate control and measurement of column temperature in CEC is vital.

6.2. Injection

Knox and McCormack [56] showed how ohmic heating of the electrolyte in CEC, or indeed in any capillary electroseparation technique, causes the liquid to expand with consequent loss of all, or at least part, of the injected sample if the rate of expansion exceeds the rate of migration of the slowest moving analyte. To avoid this effect, the voltage ramp rate, dV/dt must be controlled, subject to a maximum given by:

$$dV/dt < \left(\frac{\mu R}{\gamma_e \alpha}\right)/1 + d_v(\Delta T) \tag{17}$$

where μ is the overall mobility of the analyte (the sum of μ_{eof} and the electrophoretic mobility of an ionic analyte), *R* is the column resistance per unit length, γ_e is the coefficient of expansion of the injected solution, α is the power–temperature coeffi-

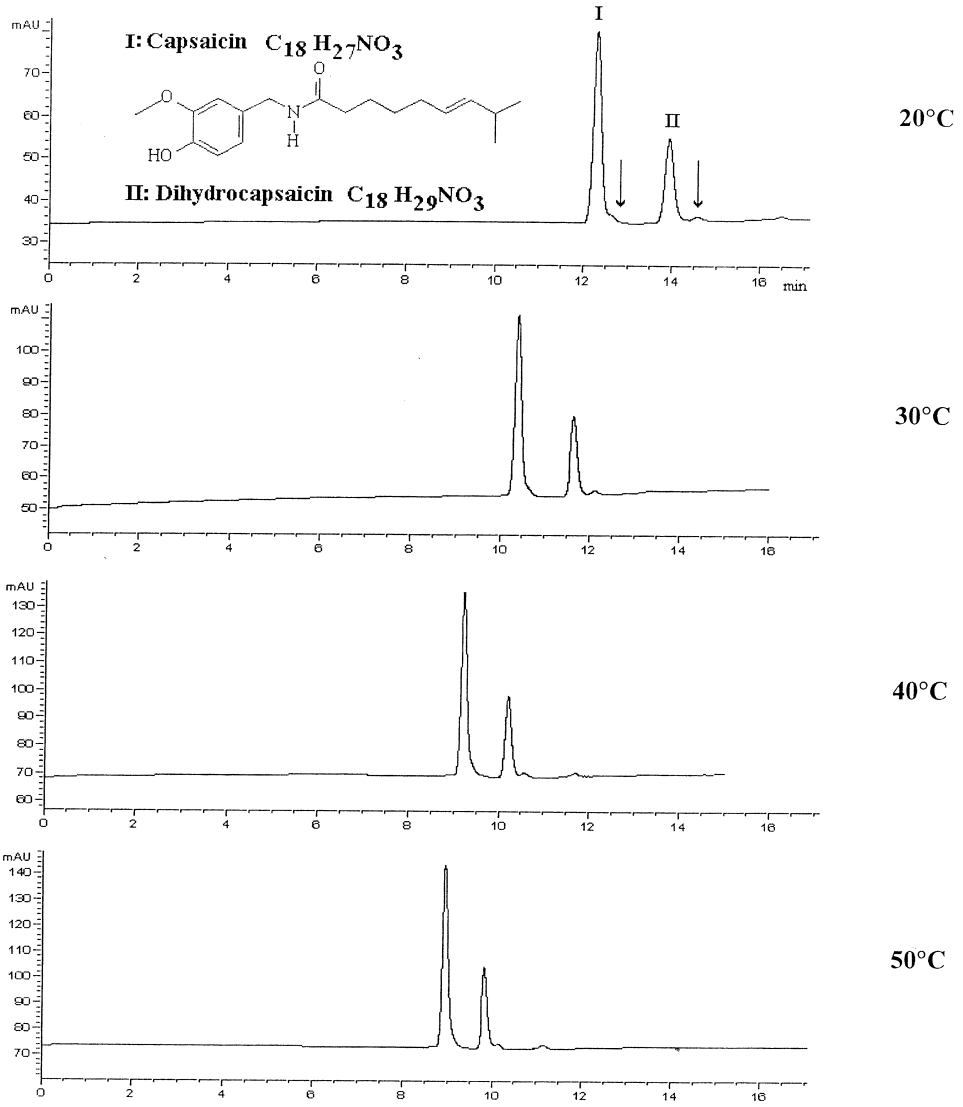

Fig. 13. Effect of temperature variation on the analysis of capsaicin. Column 25 cm×50 μm I.D. packed with 3 μm ODS1. Mobile phase acetonitrile–water (80:20) with 50 m*M* Tris. (From Ref. [53]).

cient of the capillary, and d_v is the temperature coefficient of the viscosity. Necessary values of the parameters in Eq. (17) under the desired operating conditions are obtained as follows: μ and R from V, E and I and the migration velocity of the slowest moving analyte. For a typical capillary α is about 12 KmW^{-1}, while for an aqueous buffer at between 10 and 70°C, δ is around $2.5 \cdot 10^{-2}$ K^{-1}. ΔT is obtained from:

$$\Delta T = \alpha E^2 / R \quad (18)$$

dV/dt can thus be calculated, and it was recommended that control of ramp-up be incorporated in electrodriven separation systems.

6.3. Peak dispersion

In CEC a further factor in addition to those

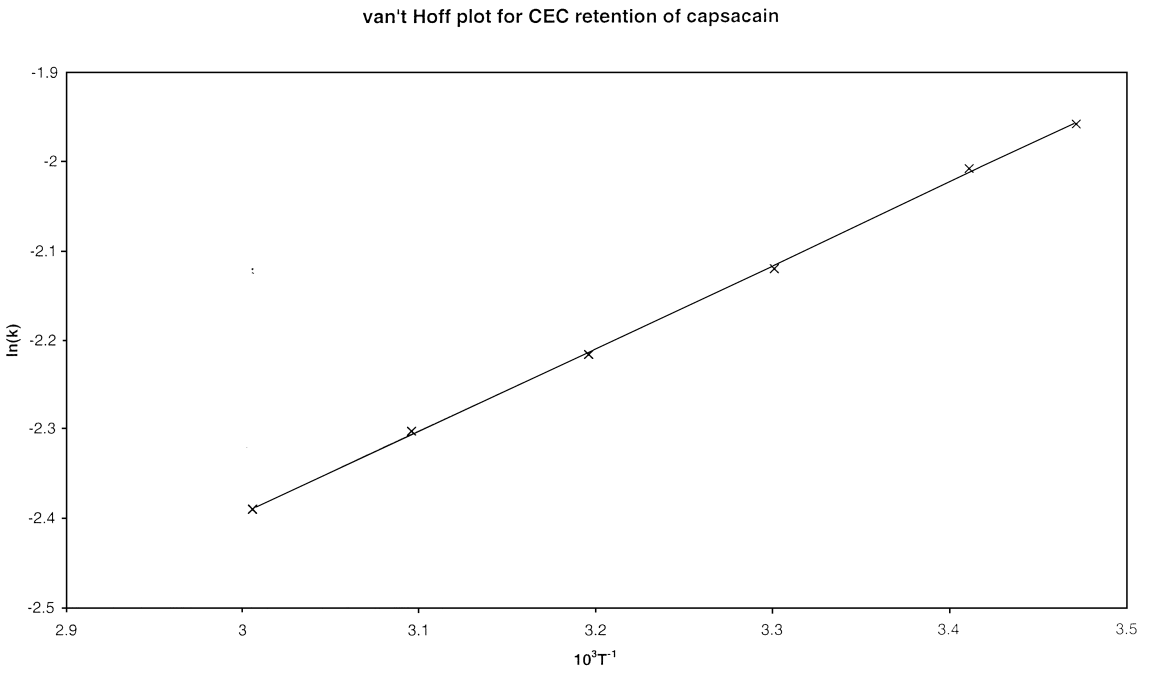

Fig. 14. Van't Hoff plot for CEC retention of capsaicin. Conditions as in Fig. 13.

discussed in Section 5 which may influence column efficiency is ohmic heating in the capillary. Boughtflower et al. [46] recommended the use of zwitterionic buffers such as tris(hydroxymethyl)-methylamine (Tris) to reduce self heating, but there will inevitably be temperature differences between the capillary and the surroundings and the parabolic temperature profile within the core of the capillary (Fig. 16). The first of these will result in a change in analyte diffusion coefficient D_m, but is minimised by forced air cooling; the second will bring about a migration rate profile across the column, superposed on the main migration velocity of the whole band [10].

Knox showed [10] how the temperature excess, θ_{core}, within the core of the tube (i.e., the difference between the temperature of the axis of the tube and the inner wall) is given by:

$$\theta_{core} = (\epsilon\lambda c E^2) \cdot (d_c^2/16K) \quad (19)$$

where λ is molar conductivity, c is concentration of electrolyte, ϵ is bed porosity, and K is thermal conductivity. The plate height, H, then depends on θ_{core} according to:

$$H = \frac{(0.015\theta_{core})^2 d_c^2 u_{eof}}{96 D_m} \quad (20)$$

And the thermal contribution to the plate height is given by:

$$H_{TH} = 10^{-8} \cdot \frac{\epsilon_0 \epsilon_r \delta}{D_M \eta K^2} \cdot E^5 d_c^6 \lambda^2 c^2 \quad (21)$$

H_{TH} can now be compared with the plate height arising from axial diffusion in the column, and Table 5 lists the electric field strengths and electrolyte concentrations for which the plate height contribution from thermal effects is less than 10% of the plate height contribution from axial diffusion.

7. Theoretical potential of capillary electrochromatography

7.1. Application areas of capillary electrochromatography

In the past years, a large number of published

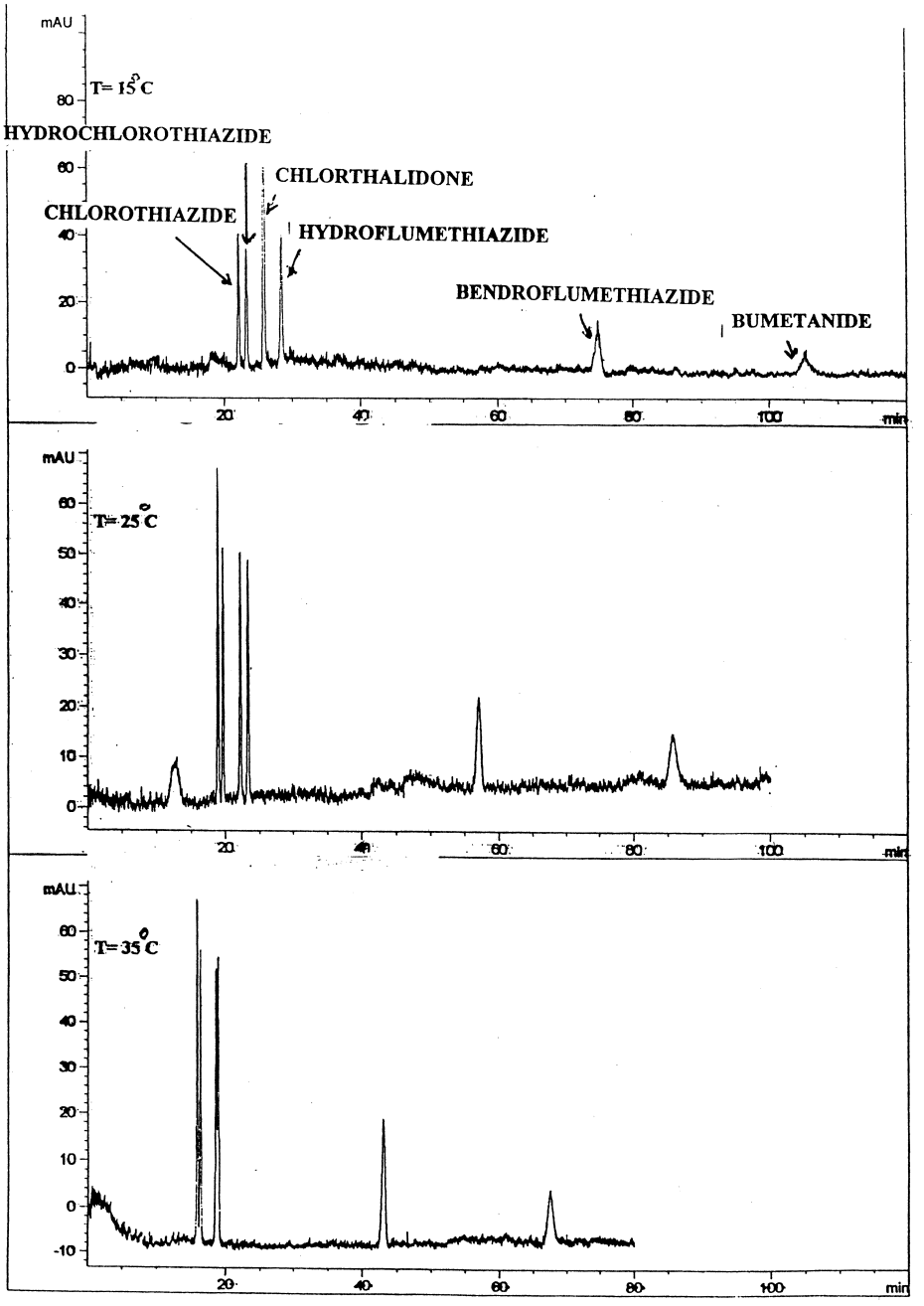

Fig. 15. Temperature effects in the CEC of diuretics. (From Ref. [32]).

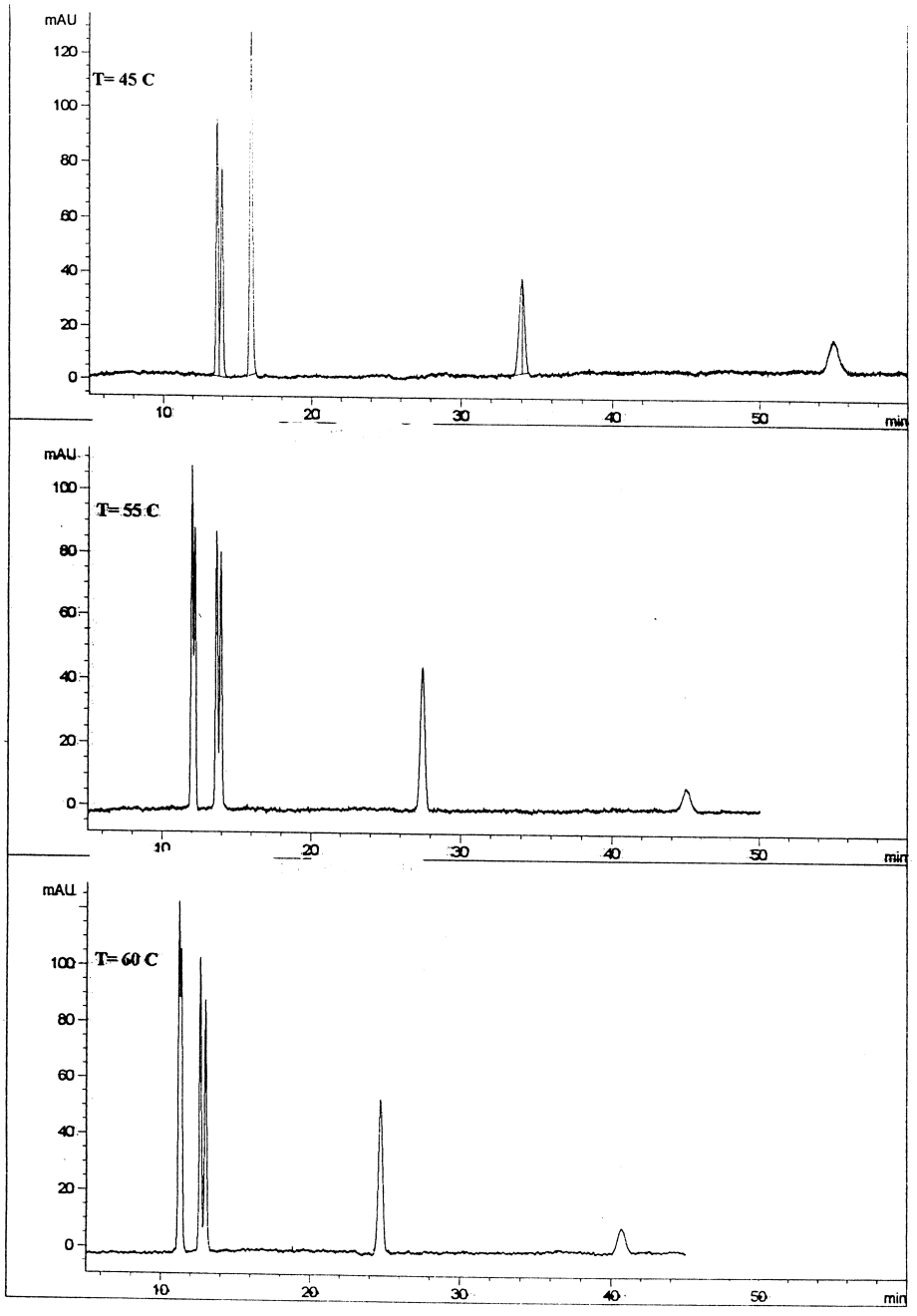

Fig. 15. (*continued*)

applications has appeared [1,3–5], with principal growth areas in pharmaceuticals, natural products and chiral compounds. The driving force behind these applications has been plate number. The now-classic separation [57] of tipredane diastereoisomers by a ''brute force'' high-resolution CEC was an early result of the availability of columns with up to 100 000 theoretical plates, 40 cm long columns

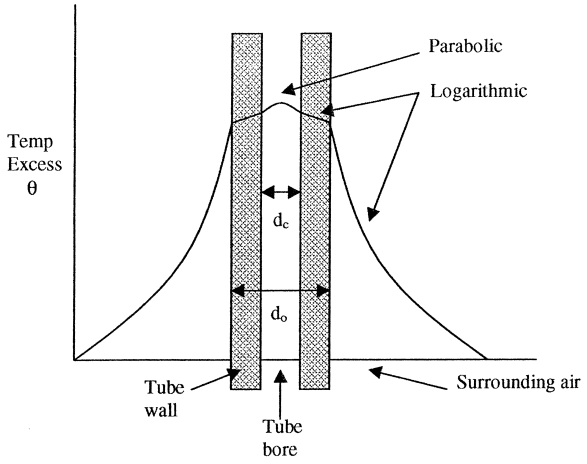

Fig. 16. Representation of the temperature profile across a capillary tube containing electrolyte heated by the passage of an electric current. (From Ref. [10]).

packed with 3 μm particles and now readily prepared or commercially available. With such high efficiencies, CEC should deliver real advantages in terms of the column peak capacity, *P*, the number of peaks which can be separated in a chromatogram between realistic retention factor limits:

$$P = 1 + \frac{N^{1/2}}{4} \cdot \ln(1 + k) \quad (22)$$

Even though peak capacity may represent an overestimate of resolution when applied to real mixtures, it still allows comparisons of separation methods. For example, Table 6 compares values of *P* available in capillary GC and HPLC with those theoretically possible in CEC. Very substantial improvements over HPLC are possible with currently available columns packed with 3 μm particles, and more especially if the full capability of 1.5 μm particle columns can be realised. For a reduced plate height of 1, *N* now equals $2 \cdot 10^5$ and for $k = 10$, *P* is >250 taking CEC into a resolution domain similar to that possible in GC, and which conventional HPLC cannot approach.

Most HPLC separations are currently achieved on the basis of selectivity, but the much higher plate numbers of CEC may offer substantial advantages for very complex mixtures of natural products and biological compounds. Already, CEC has been shown [58–60] to be a promising technique for the separation of mixtures of triglycerides, carotenoids and flavanoids, with substantial improvements over HPLC, and of peptides and protein digests [61,62].

Table 5
Maximum tube diameter (μm) for which plate height contribution from thermal effects is less than 10% that of axial diffusion contribution

	E (kV m^{-1})	10	20	50	100
Electrolyte concentration (m*M*)	1	1500	750	300	150
	10	700	350	140	70
	100	320	160	60	30

Data from Ref. [10].

Table 6
Available peak capacities[a] in gas, liquid, and capillary electrochromatography

	Column length	Theoretical plates	Peak capacity
GC	50 m	200 000	260
HPLC	25 cm	25 000	90
CEC	25 cm (3 μm particles)	60 000	140
	50 cm (3 μm particles)	120 000	200
	50 cm (1.5 μm particles)	200 000	260

[a] Calculated from Eq. (22) with $k = 10$.

The analogy here is with the progress of GC, where the advent of fused-silica capillary columns offered the resolution necessary to make routine the analysis of complex mixtures of fossil fuel and environmental origin.

7.2. Potential of capillary electrochromatography

The discussion in Section 7.1 is based on currently available, or nearly available column technology. In fact, as early as 1987, Knox and Grant explored [12] the question of open-tube or particle dimensions for CEC. Using the arguments presented in Section 2, they listed particle and tube diameters at which significant loss of EOF velocity would occur (Table 7). For typical mobile phase ionic strengths of 1 to 10 m*M* it should be possible to employ particle sizes as low as 0.5 μm with little sacrifice of EOF, with realistic expectations, as long as columns can be packed, of plate numbers $>500\,000$ per column, leading to peak capacities above 400.

More recently, Knox has pointed out [63] that diffusion considerations should also apply and that maximum efficiency can be derived from a version of the Knox equation for CEC (Eq. (15)):

$$H = 1.5 \cdot \left(\frac{D_m}{\bar{u}}\right) + d_p \cdot (\bar{u} d_p / D_m)^{1/3} \qquad (23)$$

where D_m is the analyte diffusion coefficient in the mobile phase. For readily attainable (with $E \approx 10$ V cm^{-1}, $\zeta = 30$ mV) values of $\bar{u}$, the minimum value of H is obtained when $d_p \leq 1$ μm, so that the d_p value at the diffusion limit is just above the minimum d_p imposed by considerations of double layer overlap. However, as previously discussed in Section, such materials must necessarily be monodisperse if columns are to be successfully packed with lengths sufficient to generate plate numbers beyond 500 000.

Luo and Andrade [64] re-examined the mechanism of EOF by comparing the conclusions of Rice–Whitehead theory on the determination of minimum d_p with those consequent on more recent treatments of the velocity profile in electroosmotically driven flow. They concluded, again, that for ionic strength of 10 m*M* the particle size can be less than 1 μm. A version of Eq. (23) was again used to calculate diffusion limited plate numbers in the region of 500 000–1 000 000 (Table 8). It was pointed out, however, that the local values for porosity for particle packing are very variable and unpredictable, citing the recognition by Giddings [23] that no satisfactory mathematical description of pore structure exists, and any understanding of porosity depends on ''inexact and intuitive'' concepts. Accordingly, Luo and Andrade concluded [64] that the full potential of CEC would not be realised for randomly packed particle beds, and proposed packings based on (a) many bundles of rigid fibres, or (b), and more easily fabricated, a continuous polymeric bed with submicron channels.

Several column systems have been proposed [65–67] based on manufacture by polymerisation of either silica or monomers inside a fused-silica tube to produce a monolithic bed which is then derivatised. Other monolithic columns have been produced in a single step co-polymerisation process which allows fine control of the porous properties of the final column [68,69] Alternatively, etching of channels

Table 7
Particle and tube diameters at which significant[a] loss of EOF velocity will occur (data from Ref. [12])

Electrolyte concentration[b] (m*M*)	δ (nm)	Minimum diameter (μm)	
		Open tube $d_c = 10\delta$	Slurry packed column $d_p = 40\delta$
0.01	100	1.0	4.0
0.1	31	0.3	1.2
1	10	0.1	0.4
10	3	0.03	0.12
100	1	0.01	0.04

[a] 40% loss.
[b] 1:1 electrolyte in aqueous solution.

with appropriate dimensions on plates may be the way forward [63].

8. Nomenclature

α	Power–temperature coefficient
β	Phase ratio
γ_e	Coefficient of expansion
γ	Obstruction factor
δ	Double layer thickness
ϵ	Column packing porosity
ϵ_0	Permittivity of vacuum
ϵ_r	Dielectric constant of electrolyte solution
ζ	Zeta potential
σ	Surface charge
η	Viscosity
θ	Tortuosity factor
θ_{core}	Temperature excess in core of tube
κ	Structural packing parameter
λ	Structural parameter related to flow inequalities in the bed, molar conductivity
μ	Overall analyte mobility
μ_{eof}	Electroosmotic mobility
$\tilde{\nu}$	Reduced mobile phase velocity
$\bar{v}$	Pressure driven flow velocity
ϕ	Flow resistance parameter
ψ	Ratio of the intraparticle volume to the interstitial volume
ω	Structural parameter related to particle porosity
Δp	Pressure drop
ΔS	Entropy of solute transfer
ΔT	Temperature rise
a	Constant
c	Concentration of electrolyte
d_c	Capillary column diameter
d_p	Particle diameter
h	Reduced plate height
k	Chromatographic retention factor
k_c	CEC retention factor
K	Thermal conductivity, Kelvin
P	% organic content of mobile phase
t	Time
t_R	Elution time
t_m	Migration time
t_0	Retention time of unretained marker
t_{eof}	Retention time of solute moving only with EOF
t_{mc}	Retention time of micelle
u	Average linear mobile phase velocity
u_{opt}	Average linear mobile phase velocity at minimum H
D_m	Diffusion coefficient of solute in mobile phase
E	Electric field strength
F	Faraday constant
H	Plate height
H_{disp}	Plate height increment from axial dispersion of solute
$H_{e,diff}$	Plate height contribution from film resistance
$H_{i,diff}$	Plate height contribution from intraparticle diffusion
$H_{t,diff}$	Plate height contribution from transchannel mass transfer
H_{kin}	Plate height contribution of solute stationary phase interaction kinetics
I	Ionic strength
L	Column length
R	Gas constant, column resistance
T	Absolute temperature
V	Applied voltage

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