High Voltage Capillary Zone Electrophoresis: Operating Parameters Effects on Electroendosmotic Flows and Electrophoretic Mobilities

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Key Words

Zone electrophoresis
Electrophoresis in capillaries
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Summary

In High Voltage Capillary Zone Electrophoresis a field is applied across a narrow bore capillary filled with electrolyte solution. An electroendosmotic (EEO) flow is generated within this capillary which sweeps solutes along the tube.

An absolute method of flow estimation is described, along with some operating parameter effects on the solute mobility. System parameters enabling flow direction reversal and a zero flow are described. The use of several capillaries simultaneously and the effect of pH on EEO flow rates are also shown. Effects of various operating parameters on solute resolution are also detailed.

Introduction

High Voltage Capillary Zone Electrophoresis (HVCZE) is a rapidly developing, highly efficient separation technique in which a large potential drop is applied across a narrow bore capillary. Charged species will migrate electrophoretically towards the appropriate electrode and are separated by differences in their electrophoretic mobilities. In addition, under the influence of the applied field, electroendosmotic (EEO) flow is generated in the capillary which transports solutes molecules towards the detecting system independent of their individual charge. The characteristics of the EEO flow is such that minimal zone broadening occurs [1], as the solute travels along the capillary, which keeps zones sharp. Efficiencies of up to one million theoretical plates have been reported [2].

Solute migration times are dependent upon both EEO flow rate and solute electrophoretic mobility. Clearly, a precise measure of EEO flow rate would enable accurate determination of electrophoretic mobilities. Previous work in this area [2] has largely depended upon measurement of uncharged marker solute elution times, which are carried through the system under EEO flow only. Whilst this method is satisfactory, problems may arise due to adsorption or conductivity effects. Here we describe a method of estimating the EEO flow, which is free from any anomalous effects.

Theory

The hydrated surface of silica consists of ionized silicic acid groups which are neutralised by hydronium ions (hydrated cations). These cations arrange themselves into two layers. There is a closely bound layer held by electrostatic forces which is termed the "compact layer". Thermal motion causes some of the hydronium ions to diffuse away from the compact layer and form a loosely held layer termed the "diffuse layer".

If an electric field is imposed tangentially to the surface, the ions in the diffuse layer migrate towards the oppositely charged electrode entraining the water of solvation resulting in a flow of the solution [3]. This movement of solution is known as electroendosmosis (EEO) and its direction and magnitude depends upon both the substance of the containing capillary and the nature of the solvent used [4]. If the bulk solution flows tangentially to the surface a "plane of shear" is established at, or just above, the interface between the compact and diffuse layers. The potential at this plane of shear is termed the zeta potential (ζ) and is affected by the nature of any added electrolyte, in terms of its concentration and valency, i.e. the composition of the solvent used and the material used to construct the capillary.

The flow profile observed for EEO is virtually "plug" flow compared with that observed with "normal" laminar flow in capillaries. This plug flow profile reduces the band broadening processes that occurs as a solute travels along the tube; thereby minimizing zone broadening due to kinetic effects. Electroendosmotic flow has been exploited for both electrophoretic and chromatographic separations [1].

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The velocity of the EEO flow (U_{EEO}) is given by:

$$U_{EEO} = (D/4\pi\eta)E\zeta = \mu_{EEO}E \quad (1)$$

where:

E = Electric field gradient (V/cm)
D = Dielectric constant
η = Viscosity of the medium
ζ = Zeta potential
μ_{EEO} = Coefficient of electroendosmotic flow, (may be taken to be analogous to electrophoretic mobility)

The electrophoretic migration velocity of an ion (U_{EP}) is given by:

$$U_{EP} = \mu_{EP}V \quad (2)$$

V = Applied voltage
μ_{EP} = Electrophoretic mobility ($cm^2 sec^{-1} V^{-1}$)

And the net migration velocity of an ion (U_{net}) in the presence of EEO flow may be obtained from:

$$U_{net} = (\mu_{EP} + \mu_{EEO})V \quad (3)$$

remembering that mobilities are signed quantities.

Migration time data may be used in order to ascertain μ_{EP} values if precise μ_{EEO} measurements have been made.

$$T = L^2/V(\mu_{EP} + \mu_{EEO}) \quad (4)$$

L = Length of capillary to optical centre of detector (cm)
T = Migration time (sec)

Experimental

A dual polarity 0–60kV power supply (Brandenburg, Croydon, Surrey, Model No. 2928) was used. Fused silica capillary was supplied by Scientific Glass Engineering, Milton Keynes. A modified Perkin Elmer Model No. PE-2000 fluorimeter detector was used throughout. A diagram of the experimental system is shown in Fig. 1. Sample injection was by the "electromigration" technique as described elsewhere [1]. EEO flows were measured by weighing the mass of buffer transferred from the anode to the cathode (or vice versa) over a timed period using a Mettler Digital balance Model No. AE160, as previously described [5]. Data presented here are the means of duplicate measurements.

All the reagents used were analytical grade and supplied by British Drug Houses, Poole, Dorset, England. Solutions were prepared by weight in degassed, near conductivity water obtained from a Elgastat Spectrum Still, Model No. SC1, High Wycombe, Bucks, England.

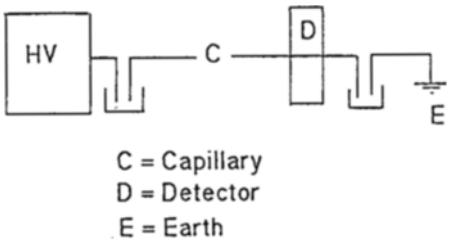

Fig. 1
Diagram of system apparatus

Results and Discussion

An absolute method of estimating electroendosmotic flows has been developed [5] based on weight measurements. Precisions of 1.3% R.S.D. have been obtained from 10 replicate measurements; which compares favourably with previously described methods of EEO flow estimation.

Weights of solvent transferred per second were transformed into EEO flow rates ($cm\ sec^{-1}$) using eq. (5):

$$\text{EEO flow rate} = cm\ sec^{-1} = \{(m/\rho)/t\}/CSA \quad (5)$$

ρ = Density
CSA = Capillary cross sectional area (cm^2)
t = time of weight transfer (sec)
m = mass transferred (g)

The coefficient of EEO flow, μ_{EEO}, is calculated from eq. (6).

$$\mu_{EEO} = cm^2 sec^{-1} V^{-1} = \text{EEO flow rate}/E \quad (6)$$

Previous methods used to estimate EEO flow rates include the use of migration time data from neutral solutes. These solutes, possessing a zero mobility, are swept to the detector solely by the EEO flow. Migration times are obtained subject to there being no ionization of the solute in the solvent, or any adsorption onto the capillary walls. Flow rate measurements obtained using neutral species may be time consuming as the migration times tend to be long.

Problems of solute adsorption can be pronounced in HVCZE [2] which have led to attempts to deactivate the walls by silanization. However, silanization affects the zeta potential [1, 7] and therefore alters the EEO flow rate. High solute concentrations have been shown to severely distort the conductivity profile [2]. Therefore, careful consideration must be taken when selecting an appropriate "neutral" marker solute.

Another method adopted for flow rate estimation has been to place a u.v. absorbing species into the high voltage buffer reservoir and then monitor the u.v. absorption of the earth reservoir [3]. This involved periodic stopping of the voltage and taking sample for testing. This method gave precisions in the order of 6% R.S.D. Apparent problems with this technique include molecular diffusion of the solute occurring whilst the voltage is switched off. Additional errors will occur due to the intervals between absorbance checks. Monitoring the time required for the level in one of the reservoir to be raised a specific height has also been used [4] to estimate EEO flows.

Solute migration time is related to both solute mobility and EEO flow rate (eq. (4)). Good reproducibility (1.5% R.S.D.) of migration times has been observed from 10 replicate runs. Reproducibility is often poor in conventional forms of electrophoresis; largely due to problems of gel to gel variation. However, in HVCZE the system parameters are more readily controlled leading to good

precision. These results are in accordance with those recently published (8) concerning reproducibility in electrokinetic chromatography; an associated technique.

The electrophoretic mobility of an ion can be found directly from eq. (4) if the exact EEO flow rate for a particular capillary, under certain conditions, is first established.

Conditions for Zero EEO Flow

Use of a 0.02 M s-benzyl thiouronium chloride (BTC) solution (pH 4.5) in HVCZE has been found to produce a zero EEO flow. Under these conditions mobilities may be directly calculated from migration times using eq. (4), as the U_{EEO} term is zero i.e.

$$T = L^2/V(\mu_{EP}) \quad (7)$$

This property of zero flow is an advantage when studying parameter effects on mobilities; as there is no EEO flow term.

A decrease in BTC concentration from 0.02 M resulted in a concentration dependent increase in flow rate (Table I). It was impossible to operate at higher concentrations than 0.02 M due to problems of joule heating.

Application of a negative potential to a capillary containing 0.02 M s-benzyl thiouronium chloride also resulted in a zero flow. Therefore this electrolyte can be used for the analysis of either anions or cations by a simple reversal of the power supply polarity.

Relationship Between Applied Voltage and Mobility

Mobilities of two fluorescent solutes, quinine and quinacrine, were monitored, with respect to applied voltage. The results are shown in Fig. 2 below. Results were obtained applying voltages across a 75 μm wide, 100 cm long capillary filled with 0.02 M phosphate buffer.

Table II shows that mobilities were found to be effectively constant below 15 KV, but above this level increased linearly with applied voltage. At these higher voltages the capillary was unable to dissipate efficiently all the joule heat evolved; thus leading to a rise in temperature. Solvent viscosity decreases with rise in temperature, and since electrophoretic mobilities are inversely proportional to viscosity this decrease in viscosity above 15 KV leads to higher mobilities. This explains the deviation from linearity with increase in applied voltage.

Table I. Flow rate dependence on BTC concentration

Concentration (M)	Flow rate (cm sec^{-1})
0.02	0
0.002	0.049

Table II. Relationships of mobility with applied voltage

Solute	Equation of the line	Correlation
Quinine	y = 2.10E-4 + 7.14e-6x	1.00
Quinacrine	y = 2.73E-4 + 7.98e-6x	1.00

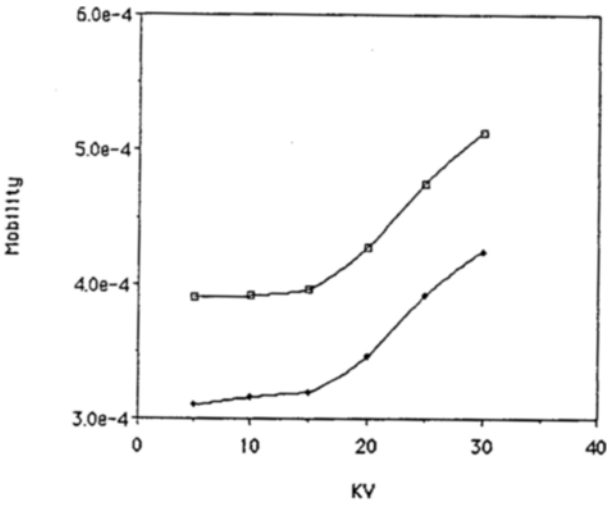

Fig. 2
μE ($cm^2 sec^{-1} V^{-1}$) versus applied voltage

Effect of pH on EEO Flow Rate

It has been previously shown [3] that EEO flow rates were linearily dependent on pH. However, this work covered only a limited range of pH from 5 to 8. It has been found that with a high pH, fast flows are observed but as pH conditions are varied through neutral and then acidic, the flow is greatly reduced, ceases and then reverses its direction (Fig. 3). These results may be explained as occurring due to neutralisation of surface anions, such as siloxy ions, by hydrogen ions from within the bulk solution. The positive flow values observed using acidic solutions may be explained by some protonation of surface silanols which would result in a positively charged surface.

The solutions used to produce actual pH conditions are shown in Table III and were all 0.02 M concentration. All measurements were made applying + 30 KV across a 75 μm bore, 100 cm long capillary.

Effect of pH on Mobility

The mobility of quinine has been studied over a range of pH values. Mobilities were calculated using eq. (4). The EEO flow rates varied as in Fig. 3. The pH dependency of quinine is shown in Fig. 4.

Quinine is known to have pKa values of 4.11 and 8.00. Thus effect of pH variation is that at low (acidic) pH's, below the first pKa, quinine exists as a doubly charged

Table III. Electrolyte solutions used in EEO versus pH experiment

pH	0.02 M solution	pH	0.02 M solution
12.1	NaOH	4.5	s-Benzylthiouronium Chloride
11.05	$NaHCO_3$	2.8	Thiodiglycollic Acid
6.8	Phosphate buffer		

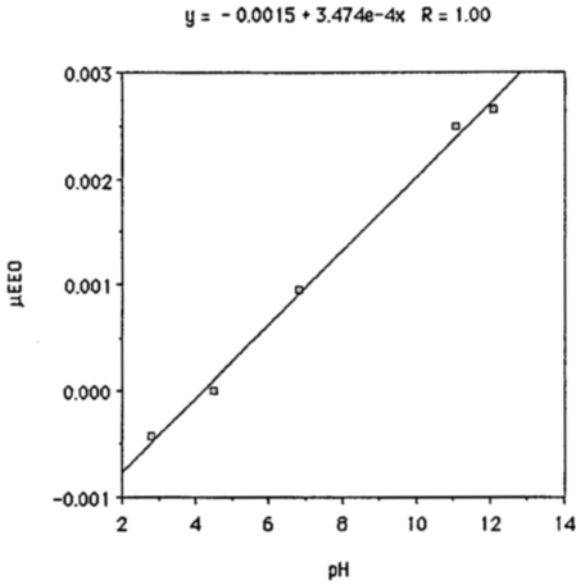

Fig. 3
μEEO ($cm^2 sec^{-1} V^{-1}$) versus pH

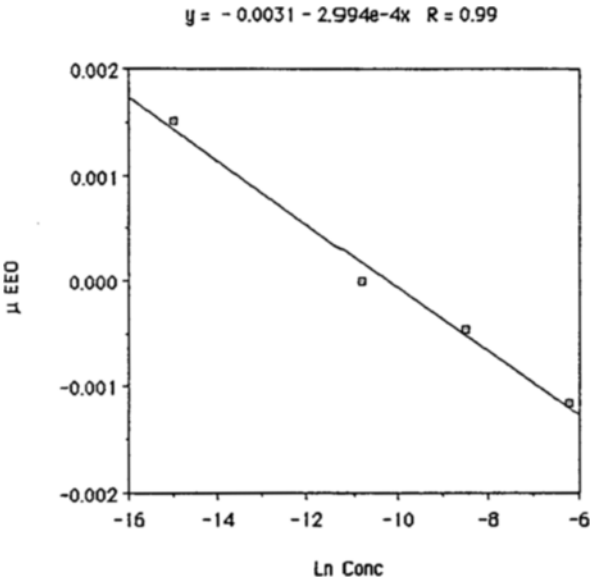

Fig. 5
μEEO ($cm^2 sec^{-1} V^{-1}$) versus Ln CTMAB concentration (M)
30KV applied across a 75μm X 100cm capillary

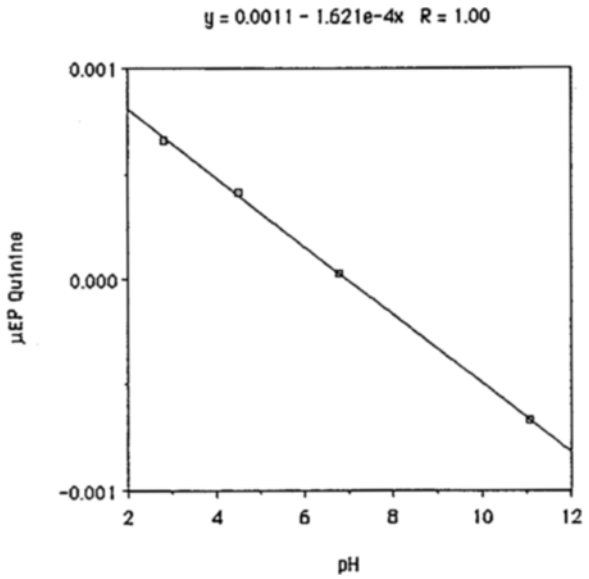

Fig. 4
μEP ($cm^2 sec^{-1} V^{-1}$) of quinine versus pH
30KV applied across a 75μm X 100cm capillary

cation with an associated strongly positive mobility. When operating at a pH between the two Pka's, quinine will exist as a singly charged cation; with a diminished positive mobility. Above pH 8.00 quinine exists in the anionic form with a negative mobility.

Reversal of EEO Flow Direction

A reversal in the direction of "normal" EEO flow (i.e. towards the earth electrode) may be produced using solutions of cetyltrimethylammonium bromide (CTMAB). The flow rate became more positive as the CTMAB concentration was reduced; as shown in Fig. 5. Due to limited water solubility, concentrations in excess of 0.002M were not used. The graph was extrapolated back to include a flow rate measurement performed on pure distilled water.

A "normal" flow direction is produced when applying a negative potential to a solution of CTMAB. This combination of both negative applied potential and solutions of CTMAB may be useful for anion analysis. Migration of anions would, under these operating conditions, be in the flow direction, instead of against it; as is normally the case.

The magnitude of flows produced using CTMAB solutions has been shown to be pH independent over the range pH 4.5 to 11.0. This affords a means whereby it may be possible to operate at pH extremes whilst still maintaining a constant flow rate. It may be beneficial to operate at pH extremes when attempting analysis of components with high or low pKa values. Increasing the ionic strength of a 0.002M CTMAB solution by the addition of sufficient potassium chloride, to make the solution 0.02M in KCl, had no effect on the EEO flow rate. The zeta potential should be influenced by the addition of any added electrolyte. However, the current carried by the solutions increased due to the increased conductivity of the carrier electrolyte.

These findings are currently under investigation [9].

Flow Rate Manipulation by pH Control

The elution order usually obtained in HVCZE, when applying a positive potential, is that of cations (migrating by EEO and their electrophoretic mobilities), neutrals and lastly anions. Cations are eluted rapidly allowing little time for separation to occur. However, anions have long elution times as their electrophoretic migration direction is against the EEO flow. It is not possible to slow down the flow rate sufficiently to allow separation of cations

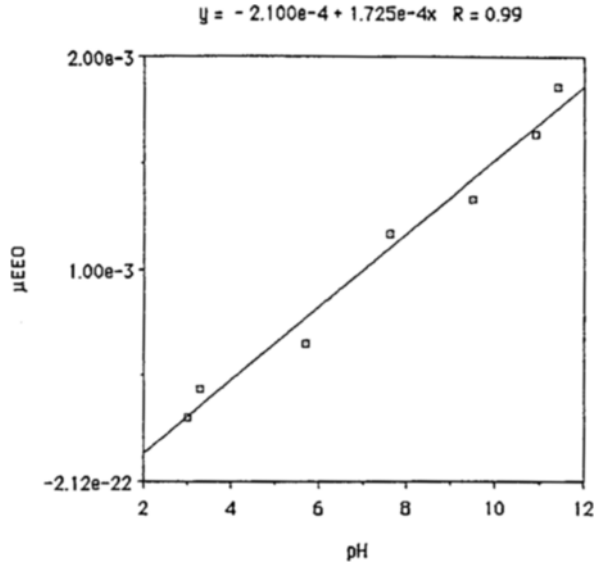

Fig. 6
μEEO ($cm^2 sec^{-1} V^{-1}$) versus pH
30KV applied across a 75μm X 100cm capillary

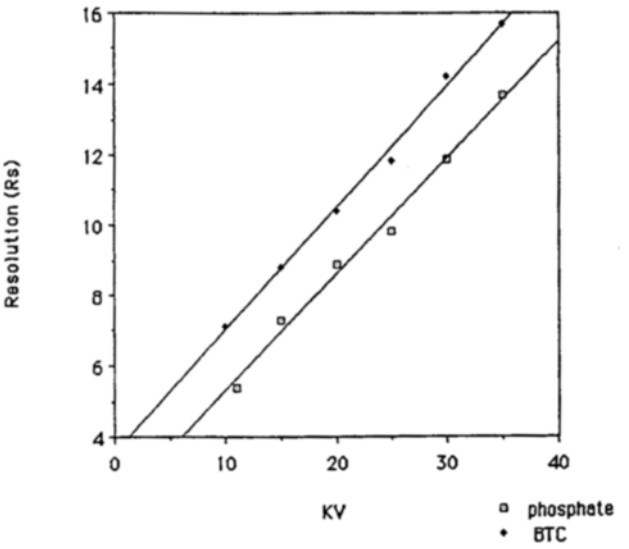

Fig. 7
Resolution (Rs) versus applied voltage
Voltage applied across 75μm X 100cm capillary

Table IV. Effect of NaOH additions to buffer solutions on pH and EEO flow rate

Addition of NaOH (ml)	pH	EEO flow rate
0	3.0	3.02e-4
0.2	3.3	4.44e-4
1.55	5.7	6.54e-4
3.18	7.6	11.73e-4
4.35	9.5	13.33e-4
5.06	10.9	16.34e-4
5.86	11.4	18.61e-4

Table V. Resolution and migration time dependency on applied voltage

i) Migration times (mins) using 0.02M BTC			
Applied voltage	Quinine	Quinacrine	ΔT
10KV	34.6	28.0	6.6
30KV	8.8	7.1	1.7
ii) Relationships between Rs and applied voltage			
Electrolyte	Equation of the line		Correlation
0.02M BTC	y = 3.55 + 0.346x		1.00
0.02M phosphate	y = 2.00 + 0.330x		1.00

without excessively extending anion migration times. This problem may be solved by pH manipulation of EEO flow rates. Aliquots of 0.02M NaOH were added to several 100ml portions of a universal buffer [10] which had an initial pH of 3.0 (Table IV). Flow rate measurements were performed on these solutions. These alkali additions caused the flow rate to increase proportionately as shown in Fig. 6.

A graph of addition of NaOH (ml) versus μEEO gave an linear relationship with a correlation factor of 0.99.

At the initial, low pH values, the minimal flow rates observed would allow time for separations of cations to occur. However, an increase in pH would raise the flow rate in order to elute the neutral species. At higher pH values i.e. in excess of pH 7, the fast flow would be suffice to sweep the anions to the detector within manageable lengths of time.

Computer control of alkali addition to the buffer reservoir may present a method that may be of considerable use for the separation of complex mixtures of anions, neutrals and cations. This will be reported subsequently.

Multiple Capillary Applications

Use of a single power supply for simultaneous multiple separations has also been demonstrated. Two or more capillaries have been operated in parallel from the same buffer reservoir. The current doubled as the current carrying capacity of the system doubled. Flow rates were found to be identical when voltage was applied across several identical capillaries at the same time. Thus it may be possible to construct a device capable of performing several electrophoretic separations simultaneously with the resultant saving in analysis time and, in addition, this facility could enable preparative separations to be undertaken, without overloading any one of the capillaries with loss in resolution. Individual capillaries would have to be separately loaded with sample.

Effect of Applied Voltage upon Resolution

The effect of varying the applied voltage upon solute resolution has been studied and the results are shown in Fig. 7 and Table V. The solutes used were quinine and quinacrine. The quinacrine was eluted first in all electro-

pherograms. A resolution factor (Rs) between two components [1] can be calculated from eq. (8). Two carrier electrolytes were used, 0.02M phosphate buffer (pH 6.8) and 0.02M BTC (pH 4.5).

$$Rs = 0.177(\mu_1 - \mu_2)[V/D(\mu av + \mu_{EEO})]^{0.5} \quad (8)$$

Fig. 7 (Table V) shows that the resolution was observed to increase dramatically with a rise in applied voltage (Table V).

Conclusions

Estimation of EEO flow rates by the weighing method has been shown to be precise and uncomplicated. EEO flow rates have been shown to be linearily related to pH over a large range and mobilities have been shown to be linearily related to pH and voltage (above 15KV). EEO flow rate manipulation by pH control has been demonstrated, as has the operation of multiple capillaries in parallel. The zero EEO flow rate of 0.02M BTC solutions provides a useful means for the direct calculation of mobilities.

The flow reversal of CTMAB solutions has been shown to be concentration dependent and this is under investigation. Further studies of parameter effects upon resolution are currently being performed.

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