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# Influence of the electrical double-layer on electroosmotic flow in capillary electrochromatography

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## Abstract

An intensive investigation into the electroosmotic flow in capillary electrochromatography has been performed on  $C_{18}$  packed columns. Linear velocities obtained when varying voltage and electrolyte characteristics were comparable to those found in capillary electrophoresis. Only at low ionic strength was a deviation from the expected relationship observed; this was attributed to double-layer overlap in the particle pores and turbulent flow. Van Deemter studies on  $C_{18}$  phases confirmed that linear velocity was unrelated to particle size, but did not support current theory that smaller particles will result in improved efficiency. The role of particle porosity remains ambiguous; lower flow-rates were generated on a  $C_{18}$  material with 30 nm pores compared to an 8 nm pore material, yet silica did not display the same trend. Our results suggest that high efficiencies can be obtained on 3  $\mu\text{m}$  particles although the columns may be poorly packed. Reproducible separations are most likely to be achieved on well-packed columns of larger particle size. Studies on silica highlighted problems in column manufacture; duplicate columns gave comparable flows but not separation parameters. New procedures for the use of very small particles (1.5  $\mu\text{m}$ ) are desirable. © 1999 Elsevier Science B.V. All rights reserved.

**Keywords:** Electroosmotic flow; Electrochromatography

## 1. Introduction

Capillary electrochromatography (CEC) is generally thought of as having its origins in the 1970s, when Pretorius et al. [1] first demonstrated the use of electroosmotic flow (EOF) as a “pump” to transport a liquid through a chromatographic column. Despite the advantages of a significant reduction in plate height and the absence of any notable pressure drop, the potential of the technique was largely not realised. It was not until the 1990s that the scientific community began to take a renewed interest, mainly as a result of the theoretical and experimental studies

published by Knox and co-workers [2–5]. Since then its popularity has been on the increase, as reflected in the number of scientific publications dealing with all aspects of the technique.

For CEC to gain acceptance as an analytical technique, a great deal of attention will have to be paid to the understanding of the fundamental processes governing the EOF. The EOF is important not only as a transport mechanism for the mobile phase, but also for increasing peak efficiency; under conditions of plug-flow, the flow-velocity profile should give reduced band dispersion. The electrolytes (or mobile phases) are typically selected to control the EOF. Generally they comprise an aqueous buffer component and an organic solvent (or modifier) component; the more important variables are pH, ionic strength and the type and proportion of organic

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modifier. Many studies have been performed on the effect of these factors [6–12], yet differing results have been obtained. Sometimes, the experimental work can account for the diversity: for example, on some occasions there has been no attempt to control ionic strength when studying the effect of pH. Even so, it must be recognised that to gain a thorough understanding of the processes involved in CEC is not a trivial exercise. A lot of factors have yet to be taken into account, such as the individual contribution of the open and packed sections of a column [13,14]; to date, their influence upon the more fundamental aspects have largely been overlooked.

At the heart of the separation is the column, which typically consists of a fused-silica capillary (50–100  $\mu\text{m}$  I.D.) packed with stationary phase particles ( $\leq 3 \mu\text{m}$ ); the characteristics of these particles (type, size and porosity) are varied to suit the analytical problem. The majority of stationary phases utilised in CEC have been silica-based particles, although new columns incorporating monoliths [15], cellulose particles [16] and polyacrylamide–poly(ethylene glycol) gels [17] have also been reported. Monolithic columns of controlled porosity [18] may be of particular interest in view of the advantages of larger pore materials [19]: Li and Remcho found that pore sizes ranging up to 400 nm gave increased loadability and efficiency, and were far easier to work with. Theory [2] predicts that as particle sizes are reduced, increased peak efficiency should result. Whilst high efficiencies have been obtained on particle sizes  $\leq 1.8 \mu\text{m}$  [4,20–22], the use of small particles has not been without problems and may well become greater as the particles are reduced further.

The work reported in this paper presents a comprehensive study into factors affecting the EOF, where every attempt has been made to keep the variables constant, bar the one under investigation. Thus it was hoped to keep interaction effects to a minimum, and obtain meaningful data. Results from open-tubular work, performed under the same conditions, are shown for comparative purposes. The effect of particle size and porosity was studied on  $\text{C}_{18}$  materials ranging from 1.5–10  $\mu\text{m}$  diameter, and on 5  $\mu\text{m}$  silica; the 5  $\mu\text{m}$  phases were of both 8 and 30 nm porosity. Each set of experiments was typically performed on duplicate columns. Finally, attention is given to the practical considerations of employing small particle materials.

## 2. Experimental

### 2.1. Reagents and materials

Capillary electrophoresis (CE) and CEC separations were carried out on either open- or laboratory-packed fused-silica capillaries (Composite Metal Services, Worcester, UK), of 100  $\mu\text{m}$  I.D.  $\times$  375  $\mu\text{m}$  O.D. Stationary phases of varying type (Waters Spherisorb ODS1, Spherisorb silica or high purity silica), particle size and porosity were all provided by Phase Separations (Deeside, UK). Ethylparaben, thiourea (99+%) and naphthalene were obtained from Sigma–Aldrich (Poole, UK), and HPLC-grade acetonitrile from Merck (Poole, UK). All other chemicals (reagent grade) were purchased from Fisher Scientific (Loughborough, UK), Sigma–Aldrich or Vickers Labs. (Pudsey, UK). Water purified with an Elgastat UHQII (Elga, High Wycombe, UK) was used throughout, and all mobile phases filtered prior to use through a 0.2- $\mu\text{m}$  Whatman Anotop syringe filter (Phase Separations).

Aqueous buffers of constant ionic strength (10 mM) were prepared in the manner detailed by Perrin [23]; for the pH range of interest, the buffers required were KCl–HCl (pH 2.9), succinate (pH 3.6–6.0), phosphate (pH 6.5–7.5), borate (pH 9.0–9.5) and carbonate (pH 9.6–10.5). For electrolytes containing acetonitrile, the electrolytes were prepared in a similar manner. The ionic strength was not expected to remain constant due to a change in the dissociation constant of the buffer components: dissociation constants ( $K_a$ ) of weak acids decrease with a decrease in the dielectric constant of the solvent [24]. However, every attempt was made to ensure that analogous aqueous and mixed solvent systems contained the same buffer concentration. Throughout the text, the stated electrolyte pH and ionic strength refers to that of the aqueous buffer solution it is based on. For most of the studies, the electrolytes were variations of the basic 10 mM phosphate, pH 7.5, containing 70% (v/v) acetonitrile.

The EOF was determined by measuring the mobility of a neutral and unretained ( $t_0$ ) marker, typically 0.5 mM thiourea in water and 1–2 mM thiourea in  $\text{CH}_3\text{CN}$ –water (60:40, v/v) for CE and CEC, respectively. During the Van Deemter studies, equimolar mixtures of the  $t_0$  marker and analyte (2.5 mM of each in  $\text{CH}_3\text{CN}$ –water, 50:50, v/v) were

used; these were varied to suit the column being tested. For columns packed with ODS1 materials the mixture contained thiourea as the marker and either ethylparaben or naphthalene as the analyte; the latter was used for 1.5  $\mu\text{m}$  ODS1 columns since ethylparaben was not retained. For silica packing materials, thiourea was the analyte and naphthalene was the marker.

## 2.2. Preparation of CEC columns

Capillaries (approximately 50 cm length) were packed using a Shandon HPLC column packer (Hypersil, Runcorn, UK) at 300 bar, using acetone as both the slurry and packing solvent. One end of the capillary was connected to a Valco union (Phase Separations) containing a metal screen (2  $\mu\text{m}$  pores), thus eliminating the need to make an initial frit, whilst the other was connected to a reservoir containing the stationary phase slurry (80–100 mg  $\text{ml}^{-1}$ ). Although the capillary packed within the first few minutes, the sonication and pressure was maintained for an additional 15 min to complete the packing procedure. Short sections (17 cm) of the packed capillary were conditioned on an HPLC pump with  $\text{CH}_3\text{CN}$ –water (80:20, v/v) (1 h at 35 bar) followed by water (2 h at 35–70 bar) to facilitate frit and window manufacture, and remove excess packing. A hot filament device was used to manufacture the frits and the windows. The final columns were about 13 cm in length with a packed bed of 8.5 cm. Each was coupled to a separate section of fused-silica capillary (100  $\mu\text{m}$  I.D.  $\times$  375  $\mu\text{m}$  O.D.) via a PTFE sleeve (10–15 mm of 1/16 in. PTFE tubing, drilled to 340  $\mu\text{m}$  I.D.) to give a combined total length of around 33 cm for installation in the instrument cartridge (1 in. = 2.54 cm).

CEC columns were conditioned with the relevant electrolyte prior to use by initially flushing on an HPLC pump (1.25 h at 35–55 bar) then installing in the instrument for voltage conditioning (10 kV at 30 min) with pressurisation (2 bar on each vial). For subsequent changes in electrolyte, voltage conditioning (as detailed above) was employed.

## 2.3. Instrumentation and operating procedure

Experiments were performed on a HP  $^{3\text{D}}$ CE system (Hewlett-Packard, Cheadle Heath, UK) using

HP ChemStations software for system control, data acquisition and data analysis. CE separations were performed with an untreated fused-silica capillary of total length 33 cm (length to detector 25 cm), which had initially been conditioned with a rinse cycle of 5 min NaOH (0.1 M), 10 min water, and 20–30 min electrolyte. Prior to each injection it was rinsed with electrolyte (1.5 min). Thiourea was loaded by a 2-s pressure injection (10 mbar) at the anode and separated under normal polarity conditions using a voltage of 10 kV. CEC separations were carried out on coupled columns under reverse polarity conditions (length to detector = packed length) with detection through the frit. Samples were injected electrokinetically (5 s at 5 kV) at the anode, and separated using an applied voltage of typically 10 kV. A pressure of 2 bar was applied to the electrolyte vials throughout the run. For both CE and CEC, the external temperature of the capillary/column was thermostatted at 20°C, and detection was carried out at 254, 240 and 210 nm with a bandwidth of 10 nm and a detector response of 0.3 s. Results are generally presented as the average of two columns unless otherwise stated.

## 3. Results and discussion

### 3.1. Open-tubular studies

Initial studies were performed by CE to confirm theoretical relationships already well discussed in the literature [25–27]. Since CEC separations are typically performed using a mobile phase containing a high organic solvent content [28], comparisons were made between aqueous and mixed-solvent media. The majority of the work was carried out using variations on a phosphate electrolyte, pH 7.5 that has an ionic strength of 10 mM when prepared in water. For ease of reporting it has been assumed that the incorporation of an organic solvent into the electrolyte has no effect on either the resultant pH or ionic strength, and there are no changes in pH with ionic strength, i.e., pH and ionic strength are constant unless directly varied.

In CE, the magnitude of the EOF generated is proportional to the zeta potential,  $\zeta$ , developed by the double-layer of counterions close to the capillary wall (Eq. (1)):

$$\text{Mobility, } \mu_{\text{EOF}} = \frac{\epsilon_0 \epsilon_r \zeta}{\eta} \quad (1)$$

where  $\epsilon_0$  represents the permittivity of a vacuum,  $\epsilon_r$  the dielectric constant, and  $\eta$  the viscosity. Since  $\zeta$  is governed by the surface charge of the capillary,  $\sigma$ , and the double-layer thickness,  $\delta$ , (Eq. (2)) then factors influencing these, such as applied field strength, pH, ionic strength and viscosity of the running electrolyte, can be used to manipulate the EOF.

$$\text{Zeta potential, } \zeta = \frac{\sigma \delta}{\epsilon_0 \epsilon_r} \quad (2)$$

### 3.1.1. pH

Fig. 1 shows the EOF profiles obtained over the pH range 3.6–10.5 in both aqueous electrolytes and those containing 70% (v/v) CH<sub>3</sub>CN. The observed trends were generally in accordance with CE theory; the reduced EOF shown by the mixed solvent systems is mainly the result of acetonitrile having a lower dielectric constant than water.

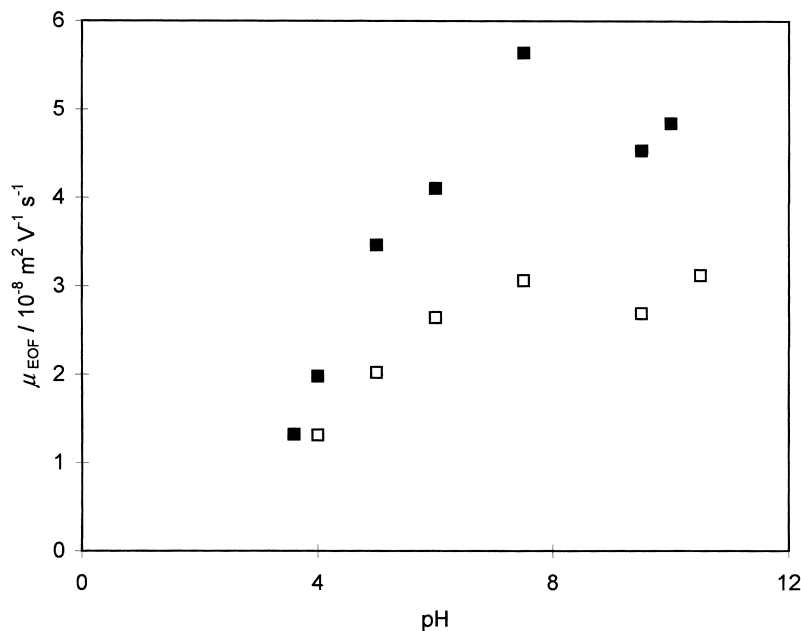


Fig. 1. Comparison of EOF profiles obtained for CE using aqueous (■) and mixed solvent (□) electrolytes. Conditions: capillary, 33 cm (25 cm effective length) × 100 μm I.D.; temperature, 20°C; pre-injection rinse, 1.5 min electrolyte; pressure injection, 2 s at 10 mbar; applied voltage, 10 kV; ramp, 0.01 min; detection, UV absorbance at 240 and 210 nm with a bandwidth of 10 nm and a detector response of 0.3 s; electrolytes (mobile phases) of varying pH, aqueous systems 10 mM ionic strength, and similar solutions containing 70% (v/v) acetonitrile; analyte concentration, 0.5 mM thiourea in water.

Table 1

Approximate  $\epsilon_r/\eta$  ratios for binary mixtures with water at 25°C

Solvent	$\epsilon_r/\eta$ (cP <sup>-1</sup> ) for varying % organic		
	0%	50%	100%
Acetone	88	23	68
Acetonitrile	88	75	105
Methanol	88	37	60
2-Propanol	88	15	7

### 3.1.2. Type and proportion of organic solvent

CE experiments were performed using the basic phosphate electrolyte, containing 0–96% (v/v) organic modifier. Four modifiers of different solvent nature were investigated, namely acetonitrile, methanol, acetone and 2-propanol. Assuming no contribution from the buffer component, the ratios of dielectric constant to viscosity for 0, 50 and 100% organic were predicted [29,30]. From these (Table 1) and Eq. (1), an indication of the expected EOF behaviour with increasing modifier content can be obtained: for the electrolyte containing 2-propanol a decrease in

EOF would be expected as the proportion is increased, whilst for the other modifiers a minimum in the EOF should occur. Experimental findings (Fig. 2) are in good agreement, with acetone, acetonitrile and methanol all showing an EOF minimum around 50–70% organic, although the increase observed when using even higher acetonitrile content was far greater than anticipated. Wright et al. [31] have also witnessed this behaviour for acetonitrile–water systems without supporting electrolyte; they have suggested it can be explained by changes in solvent polarity and hydrogen bond donor ability. However, without the phosphate buffer, we found that mixtures containing above 80% acetonitrile were unable to support EOF. Subsequently acetonitrile was selected as the organic solvent for the CEC work since it gave the highest EOF and a comparatively stable EOF region (60–80% acetonitrile) within which the effect of solvent evaporation should be minimal.

No visible signs of phosphate precipitation were experienced during this experimentation, although there was some resistance to filtration at higher solvent concentrations. At a later date some problems were encountered with the stability of the phosphate electrolyte: on a minor scale this was

observed as a slight opalescence in the solution, but in more extreme cases precipitation and even separation into a bilayer system resulted. This behaviour has been partially attributed to the phosphate inducing changes in the polar nature of the acetonitrile, thus affecting the solubility. Since the changes often appeared reversible, this may explain the lack of information presented in the literature on this subject. Our own communications with external collaborators have produced widely differing reports on usage. We ourselves have used up to 20 mM (ionic strength) phosphate in 80% acetonitrile, but have also experienced problems at as low as 10 mM in 80% and 15–20 mM in 70% acetonitrile.

### 3.1.3. Ionic strength

From theory, an increase in the ionic strength,  $I$ , will cause a decrease in the magnitude of the EOF. This is generally due to a reduction in the double-layer thickness, see Eq. (3) where  $F$ ,  $R$  and  $T$  are the Faraday constant, universal gas constant and temperature, respectively.

$$\text{Double-layer thickness, } \delta = \left( \frac{\epsilon_0 \epsilon_r RT}{2F^2 I} \right)^{0.5} \quad (3)$$

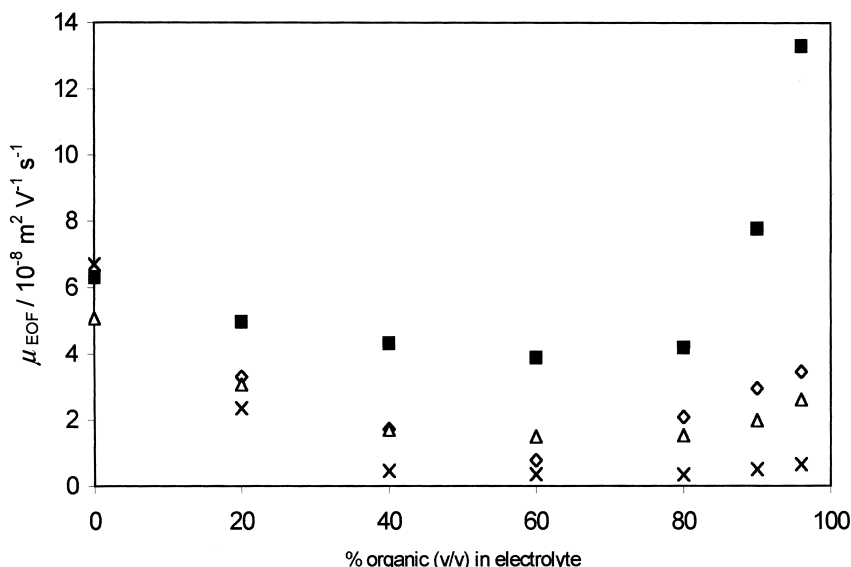


Fig. 2. Effect of modifier type and concentration upon the EOF obtained in CE. Electrolytes were based upon the pH 7.5, 10 mM ionic strength phosphate aqueous system, and contain 20–80% (v/v) acetone (◇), acetonitrile (■), methanol (△) and 2-propanol (×). All other conditions as in Fig. 1.

Table 2

Calculated double-layer thickness ( $\delta$ ) for aqueous and CH<sub>3</sub>CN–water (70:30, v/v) phosphate solutions (pH 7.5) of varying ionic strength

Double-layer thickness (nm)	<i>I</i> (mM)					
	1.0	2.5	5.0	10.0	15.0	20.0
$\delta$ (aqueous)	9.6	6.1	4.3	3.0	2.5	2.2
$\delta$ (70:30)	7.6	4.8	3.4	2.4	2.0	1.7

The converse also applies, i.e., as *I* is decreased so  $\delta$  also increases and gives greater EOF. In practice, the EOF profile depends on the capillary internal diameter, *d*, and  $\delta$ . Rice and Whitehead [32] proposed that for conditions of plug flow, *d* would need to be far greater than  $\delta$ ; it has been suggested from this that the EOF is acceptable when  $d \geq 10\delta$  [2]. As *d* and  $\delta$  approach then double-layer overlap begins to occur until finally, when they are of similar magnitude, parabolic flow results.

Some values for the double-layer thickness, calculated from Eq. (3) for a temperature of 293 K and assuming  $\epsilon_r$  values of 80 [33] and 50 [30] for aqueous and CH<sub>3</sub>CN–water (70:30, v/v) systems, respectively, are shown in Table 2. The double-layer thickness at low ionic strengths is generally of little

concern in CE: capillary diameters are rarely less than 20  $\mu\text{m}$  whilst  $\delta$  is typically in the nm range. However, under CEC conditions overlap is likely to occur in pores etc., thus reducing the driving electrolyte flow. Experimental results for the effect of ionic strength in CE are displayed graphically in Fig. 3. Generally the relationship expected between EOF and ionic strength (Eq. (3)) holds, although at low ionic strength ( $\leq 5$  mM) a deviation from linearity is seen. This was more evident for the aqueous systems, and is presumably due to such causes as the effect of dilution on pH and low buffering capacity.

### 3.1.4. Voltage

From the simple theoretical relationship between linear velocity,  $v_{\text{EOF}}$ , mobility and electric field strength, *E*, it is evident that the EOF velocity is directly proportional to the field strength (or voltage, *V*, if the capillary length, *L*, is kept constant).

$$v_{\text{EOF}} = \mu E = \mu \frac{V}{L} \quad (4)$$

The EOF mobility should be constant, but from a practical point of view there are generally deviations from linearity at high field strengths as a result of

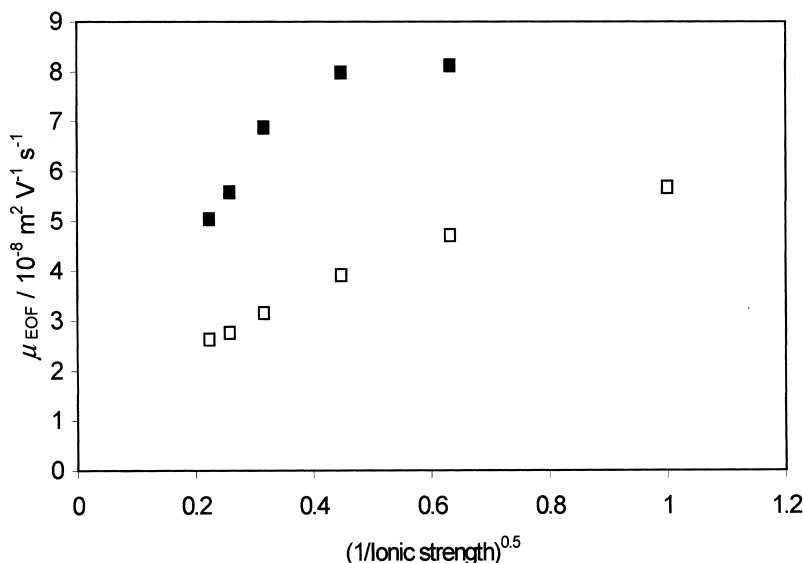


Fig. 3. Effect of ionic strength in CE for separations performed using aqueous (■) and acetonitrile (□) containing electrolytes. General conditions as in Fig. 1; electrolytes, pH 7.5 phosphate with ionic strength ranging from 1.0–2.0 mM. Mixed solvent systems prepared similarly but contain 70% (v/v) acetonitrile.

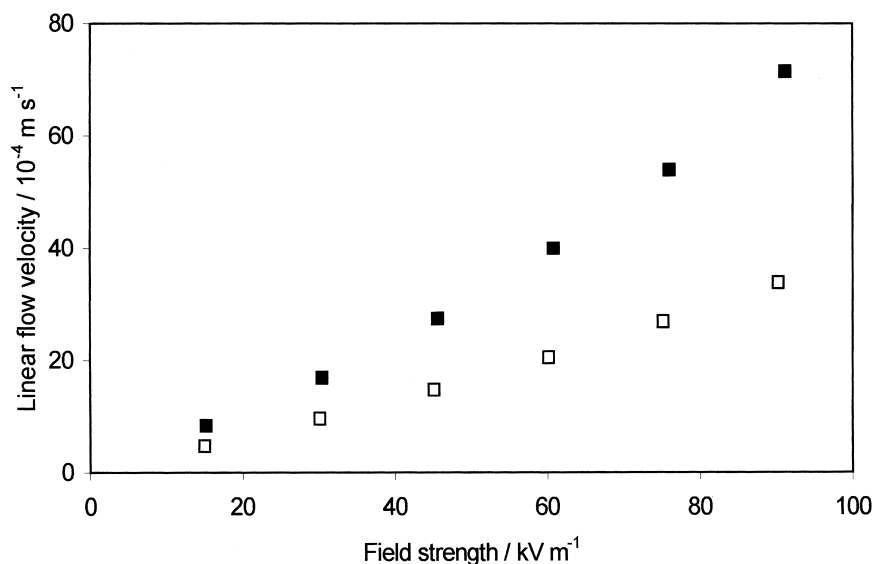


Fig. 4. Effect of field strength upon EOF velocity in CE for aqueous (■) and 70% acetonitrile (□) systems. Phosphate, pH 7.5 (10 mM ionic strength) electrolyte used, all other conditions as in Fig. 1.

Joule heating. However, for mixed solvent systems it is typically far less than that observed in similar aqueous solutions, as illustrated in Fig. 4.

### 3.2. Packed column work

As stated in Section 3.1, the majority of the work was carried out using variations on a phosphate electrolyte, pH 7.5 of 10 mM ionic strength when prepared in water.

#### 3.2.1. pH

The effect of pH in CEC was studied on ODS1 (3  $\mu\text{m}$ ) and silica (5  $\mu\text{m}$  Spherisorb) columns using thiourea and naphthalene as the respective EOF markers. Fig. 5 illustrates the change in EOF velocity with pH for the different types of column. Both the silica and ODS1 columns followed a similar trend to that obtained in CE, although the EOF is greater. During this set of experiments, results at pH 2.9 for the open capillary and ODS1 packed column were not obtained on a regular basis. This is in direct contrast to Dittman and Rozing [8] who reported a far higher EOF than expected on  $\text{C}_{18}$  columns at low pH.

#### 3.2.2. Proportion of organic solvent

The effect of acetonitrile content was studied over the range 20–80% (v/v) using ODS1 (3  $\mu\text{m}$ ) columns; experiments at higher percentages of acetonitrile were not performed due to solubility constraints. From Fig. 6 it is evident that there is little difference between the EOF velocities in CE and CEC at  $\geq 40\%$  acetonitrile, and both techniques appear to follow the same trend. The implication is that far higher EOFs could be generated for use in CEC if the problems associated with high acetonitrile content could be overcome.

#### 3.2.3. Ionic strength

The effect of ionic strength was studied over the range 1–20 mM phosphate using various ODS1 columns. As the ionic strength is reduced the EOF is expected to increase unless double layer overlap occurs. For packing materials of pore size 8 nm, double layer overlap in the pores should occur at ionic strengths of  $\leq 2.5$  mM where the double layer is at least 4 nm (see Table 2). Fig. 7 illustrates the effect of ionic strength on columns of varying particle size and porosity. The increases in EOF for CE and CEC are generally similar at the higher ionic strengths, but below 5 mM the EOF in CEC typically

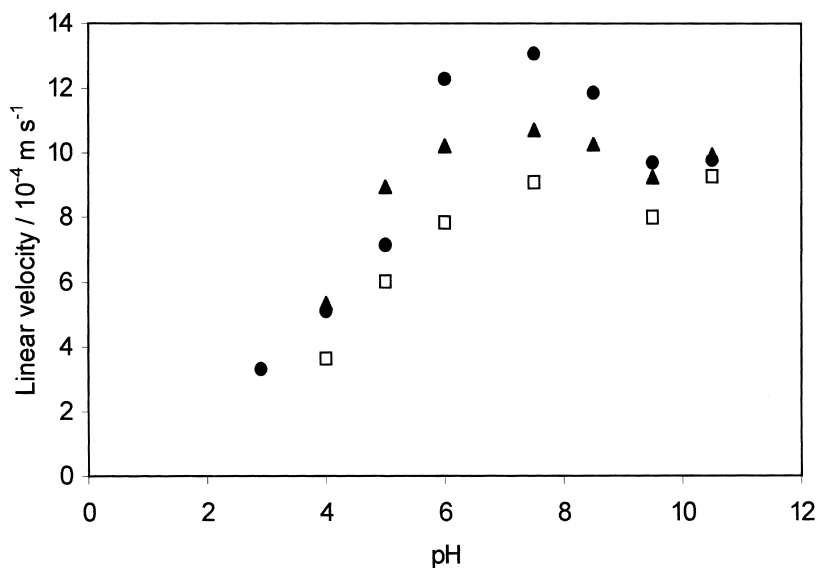


Fig. 5. Comparison of EOF profiles obtained in CE (□) and in CEC utilising ODS1 (△) and silica (●) columns. CEC conditions: coupled capillaries, 33cm [packed length (and length to detector) 8.5 cm] × 100 μm I.D.; electrokinetic injection, 5 s at 5 kV; reverse polarity; applied voltage, 10 kV; ramp, 0.10 min; run pressure, 2 bar on both electrolyte vials; detection through the packing material; electrolytes of varying pH, ~10 mM ionic strength containing 70% (v/v) acetonitrile; sample concentration, 1–2 mM thiourea in CH<sub>3</sub>CN–water (60:40, v/v). CE and all other general conditions as in Fig. 1.

starts to level off and drop slightly. By contrast, in CE the EOF continued to rise. This marked change in the EOF at lower ionic strengths would suggest the presence of double layer overlap within the pores

of the packing material. Stevens and Cortes [34] observed a fall in EOF velocity as the particle size was reduced from 50 μm to 10 μm, a factor they attributed to double layer overlap within interparticle

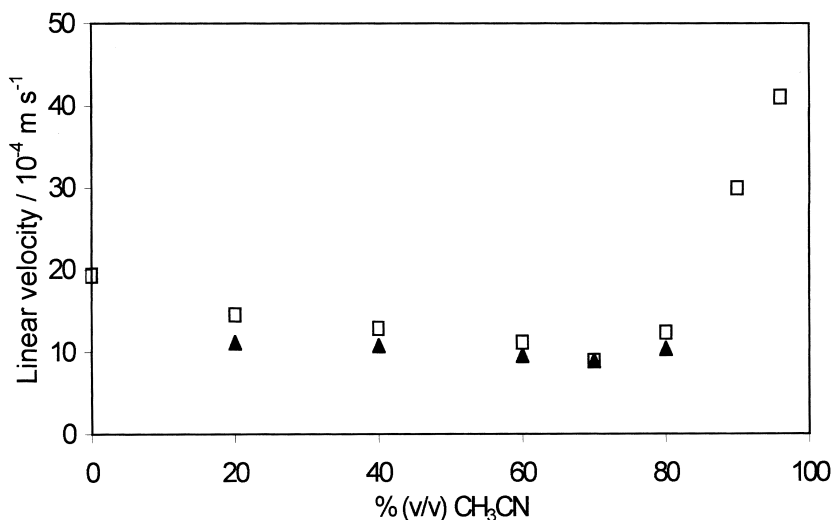


Fig. 6. Effect of acetonitrile content on linear velocities obtained in CE (□) and CEC utilising ODS1 (▲) columns. Electrolytes: phosphate, pH 7.5, ~10 mM ionic strength, containing 20–80% (v/v) acetonitrile. All other conditions as in Figs. 1 and 5.



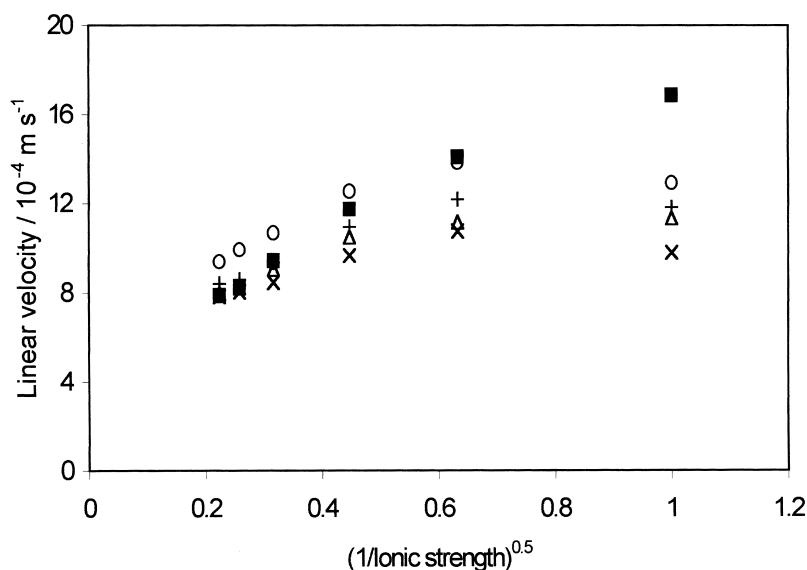


Fig. 7. Comparison of the effect of ionic strength in CE (■) and in CEC with columns of varying particle size and porosity. The CEC columns studied were 3 ( $\Delta$ ), 5 ( $\circ$ ) and 10 (+)  $\mu\text{m}$  ODS1 material with 8 nm pore size, and 5  $\mu\text{m}$  ODS1 with 30 nm pore size ( $\times$ ). CE and CEC conditions as in Figs. 1 and 5, respectively; electrolyte, phosphate, pH 7.5,  $\sim 10$  mM ionic strength, containing 70% (v/v) acetonitrile.

channels. However, both theoretical and experimental treatments by Knox and Grant [2,4] have indicated that full EOF velocity should be maintained for particle sizes down to 0.5  $\mu\text{m}$  in 1 mM electrolytes. From our results the relationship between ionic strength and velocity would appear to be unrelated to either particle size or porosity. The linear velocities generally followed the trend 5  $\mu\text{m}$  > 10  $\mu\text{m}$   $\approx$  3  $\mu\text{m}$  for the 8 nm pore material, with the 30 nm pore material (5  $\mu\text{m}$  X) showing the least flow. Although overlap was unlikely to occur in the pores of this material, the observed flow could still be reduced as a result of turbulence and a less than-plug-like flow.

### 3.2.4. Voltage

The effect of voltage was studied up to 20 kV; at voltages greater than this problems were experienced with columns drying out due to self-heating, and loss of electrolyte from the vials. No significant difference was observed between the EOF velocities generated in the ODS1 (3  $\mu\text{m}$ ) columns and open capillaries. The relationship between linear velocity and applied voltage appeared linear with regression lines having the following equations; slopes and intercepts are given with 95% confidence limits.

CE, column 1  $y = (1.1 \pm 0.1)x - (8.4 \pm 19.8) \cdot 10^{-1}$ , correlation coefficient,  $r = 0.9991$ ; CE, column 2  $y = (1.0 \pm 0.1)x - (7.0 \pm 13.0) \cdot 10^{-1}$ , correlation coefficient,  $r = 0.9995$ ; CEC, column 1  $y = (1.1 \pm 0.1)x - (8.3 \pm 6.7) \cdot 10^{-1}$ , correlation coefficient,  $r = 0.9988$ ; CEC, column 2  $y = (1.1 \pm 0.1)x - (9.0 \pm 7.8) \cdot 10^{-1}$ , correlation coefficient,  $r = 0.9984$ .

Further investigations with voltage effects at different ionic strengths on an ODS1 column gave no real evidence of deviations of the velocity from linearity. As expected the gradients of the regression lines increased with decreasing ionic strength: the gradient changed from  $0.79 \cdot 10^{-4} \text{ m s}^{-1} \text{ kV}^{-1}$  for a 20 mM electrolyte solution to  $1.26 \cdot 10^{-4} \text{ m s}^{-1} \text{ kV}^{-1}$  for a 1 mM electrolyte.

## 3.3. The role of column particle size and porosity

### 3.3.1. Effect of particle size

The EOF velocity,  $v_{\text{EOF}}$ , can be described by the following equation:

$$v_{\text{EOF}} = \frac{\epsilon_0 \epsilon_r \zeta E}{\eta} \quad (5)$$

The analogous equation for pressure-driven flow,  $v_{PR}$ , is

$$v_{PR} = \frac{d_p^2 \Delta P}{\phi \eta L} \quad (6)$$

where  $\Delta P$  is the pressure drop,  $d_p$  is the particle diameter and  $\phi$  is a dimensionless flow resistance factor. By comparison of the two equations it is evident that the electroosmotic velocity is independent of particle size [35].

The experimental findings presented in Fig. 8 for EOF velocity versus applied voltage essentially confirm the theory: average linear velocity values for 3  $\mu\text{m}$ , 5  $\mu\text{m}$  and 10  $\mu\text{m}$  ODS1 columns are extremely similar. Since any difference between the column types is observed at the higher voltages, it is probable that the variations in linear velocity are related to Joule heating and not particle diameter. However, we can surmise that the dissipation of Joule heating is linked to packing efficiency, which may indirectly depend on particle diameter. Studies in which the composition of the electrolyte, i.e., ionic strength (Fig. 7) and the proportion of acetonitrile (Fig. 9), is changed suggest that the 5  $\mu\text{m}$  particle

size may generate a higher EOF under certain conditions.

### 3.3.2. Van Deemter profiles

During a chromatographic separation, thermodynamic and kinetic processes can occur on the column which contribute to band broadening. The extent of band broadening and the factors that affect it are commonly expressed in terms of the column efficiency [36,37]. The abbreviated version of the Van Deemter equation (Eq. (7)) relates the efficiency, via the plate height  $H$ , to the  $A$ ,  $B$  and  $C$  terms which represent “eddy diffusion”, longitudinal diffusion and mass transfer, respectively.

$$H = A + \frac{B}{\bar{v}} + C\bar{v} \quad (7)$$

The average mobile phase linear velocity,  $\bar{v}$ , is used because it can be directly related to the speed of analysis, whereas flow-rate is dependent on the cross sectional area of the column and the volume occupied by the stationary phase. The composite curve of  $H/\bar{v}$  is typically used to diagnose the condition of a column without determining the constants  $A$ ,  $B$  and  $C$ . For CEC, higher efficiencies are theoretically

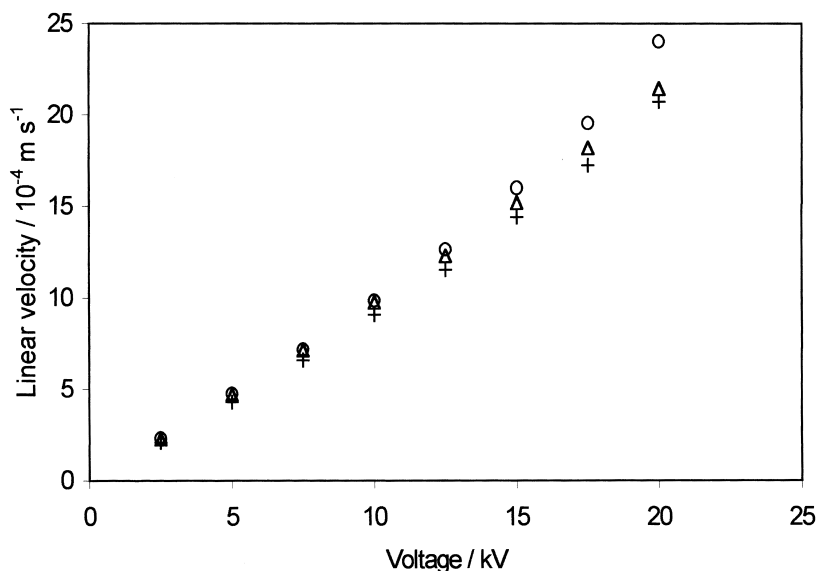


Fig. 8. Effect of voltage in CEC using columns of differing particle size: 3 ( $\Delta$ ), 5 ( $\circ$ ) and 10 (+)  $\mu\text{m}$  ODS1 material (8 nm pore size). CEC conditions as in Fig. 5; electrolyte, phosphate, pH 7.5,  $\sim 10$  mM ionic strength, containing 70% (v/v) acetonitrile; applied voltage, 2.5–20 kV.

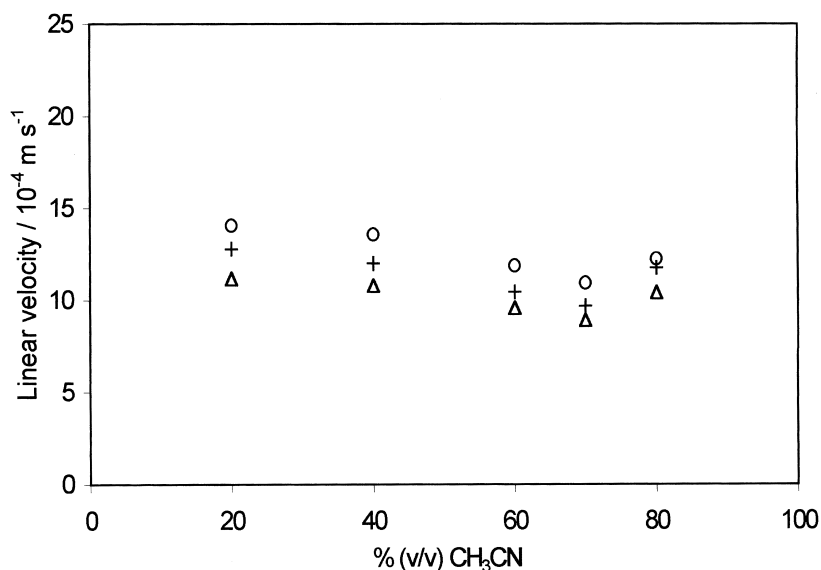


Fig. 9. Effect of changing acetonitrile content in CEC for 3 ( $\Delta$ ), 5 ( $\circ$ ) and 10 ( $+$ )  $\mu\text{m}$  ODS1 (8 nm pore size) columns. CEC conditions as in Fig. 5; electrolyte, phosphate, pH 7.5,  $\sim 10$  mM ionic strength, containing 20–80% (v/v) acetonitrile.

attainable as a result of a significant reduction in the  $A$  term; the EOF is considered to be plug-like in character, whilst in HPLC the flow is parabolic since it is pressure driven.

Van Deemter plots were constructed for each type of packing material, namely 3–10  $\mu\text{m}$  ODS1 (all 8 nm pore size), by measuring the peak efficiency of a slightly retained compound (ethylparaben) at various linear velocities. The mobile phase velocity was controlled by varying the applied voltage in the range 2.5–20 kV, and calculated from the migration time of thiourea. Fig. 10a depicts the average  $H/\bar{v}$  plots obtained for the three particle sizes. The curves can essentially be divided into three regions which correspond to the  $A$ ,  $B$  and  $C$  terms. The contribution of longitudinal diffusion to plate height ( $B$ ) only becomes significant at mobile-phase velocities less than the minimum, and is indicated by the initial drop in the curve. The minimum that follows at intermediate velocities represents the contribution of “eddy diffusion” ( $A$  term); lower values of  $H$  are more favourable. Finally, at the higher velocities the mass transfer ( $C$  term) dominates, the sharper the minimum the higher  $C$  is. Whilst higher efficiencies are expected from smaller particle sizes, Fig. 10a shows that on average the 3 and 5  $\mu\text{m}$  columns

exhibit similar characteristics, although both are more efficient than their 10  $\mu\text{m}$  counterpart. However, at high velocities it is evident that the mass transfer contribution towards the plate height is least for the 10  $\mu\text{m}$  columns. This is in direct contrast to work reported by Li and Remcho [19], who showed both increased efficiency and decreased mass transfer with decreasing particle size of a Nucleosil  $C_{18}$  packing.

Fig. 10b displays the same results plotted in terms of the reduced plate height,  $h$ , versus the linear velocity, where  $h$  states the number of particle diameters that constitute one plate height.

$$h = \frac{H}{d_p} \quad (8)$$

Thus the reduced plate height is independent of the particle diameter, and the constants are dependent on how well the column is packed. From Fig. 10b it is apparent that the columns become progressively less well packed as the particle diameter is decreased, minimum reduced plate heights for the individual columns are: 1.0 and 1.1 for the 10  $\mu\text{m}$ , 1.4 and 1.7 for the 5  $\mu\text{m}$ , and 2.6 and 2.9 for the 3  $\mu\text{m}$  particles. Most likely this is due to the particle

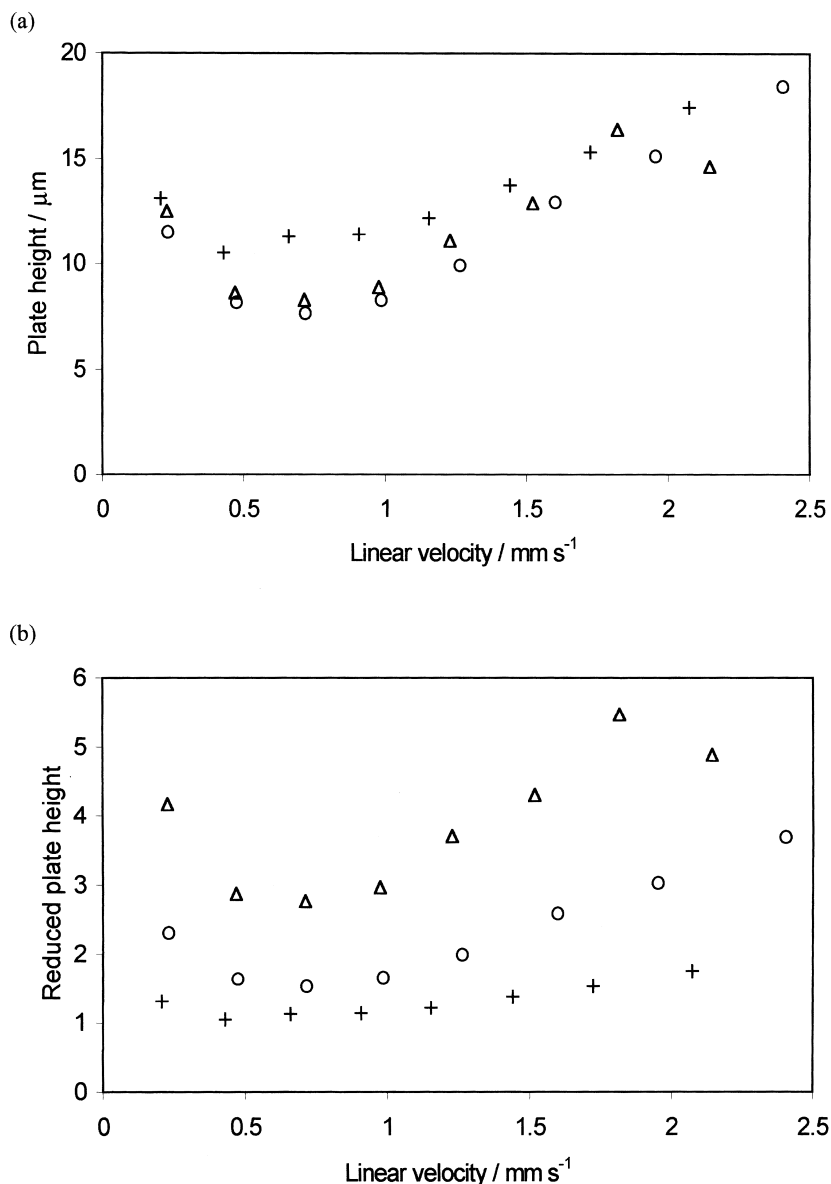


Fig. 10. Van Deemter plots for the separation of ethylparaben on 3 ( $\Delta$ ), 5 ( $\circ$ ) and 10 (+)  $\mu\text{m}$  ODS1 (8 nm pore size) columns, where thiourea was the linear velocity marker: (a) plate height vs. linear velocity and (b) reduced plate height vs. linear velocity. CEC conditions as in Fig. 5; electrolyte, phosphate, pH 7.5,  $\sim 10$  mM ionic strength, containing 70% (v/v) acetonitrile; applied voltage, 2.5–20 kV; analyte mixture, 2.5 mM of each component in  $\text{CH}_3\text{CN}$ –water (50:50, v/v).

size distribution which becomes more difficult to control as the particle size is decreased and will vary according to how it is measured [35]. Although there are three methods by which particle size distributions can be characterised, namely number, area and

volume, manufacturers typically do not stipulate which has been used. A common observation when a number distribution has been employed is the presence of fine material ( $< 2 \mu\text{m}$ ), which is thought to impede the packing process. Consequently it would

appear that high efficiencies can be generated on small particles even if the columns are poorly packed. However, the impact of poor column-to-column variation for small particle sizes may far exceed the benefit of high peak efficiencies, and for the sake of method robustness some peak efficiency may have to be sacrificed.

The retention factor,  $k'$ , is a measure of the relative times spent by the solute in the stationary and mobile phases for a given set of operating parameters. It is calculated from the migration times of the solute,  $t_r$ , and a neutral and unretained compound,  $t_0$ , (for which  $k'=0$ ).

$$k' = \frac{t_r - t_0}{t_0} \quad (9)$$

The average retention factors for ethylparaben on each type of column used have been calculated over the voltage range studied (Table 3); thiourea was used as the  $t_0$  marker. From Table 3 it is apparent that whilst the retention factors remain relatively constant over the range of voltages employed, there is a slight change in retention of ethylparaben with

Table 3  
Average retention factors calculated for ethylparaben on ODS1 columns during the van Deemter study

Voltage (kV)	Average retention factor			
	3 $\mu\text{m}$	5 $\mu\text{m}$	10 $\mu\text{m}$	5 $\mu\text{m X}$
2.5	0.30	0.34	0.23	0.14
5.0	0.30	0.35	0.24	0.14
7.5	0.30	0.35	0.24	0.15
10.0	0.30	0.35	0.23	0.17
12.5	0.30	0.35	0.23	0.15
15.0	0.27	0.35	0.22	0.14
17.5	0.30	0.36	0.22	0.14
20.0	0.30	0.38	0.22	0.14

Identical experimental conditions used throughout. With the exception of the 5  $\mu\text{m X}$  (30 nm pore) column, all stationary phases were of 8 nm pore size.

CEC conditions: coupled capillaries, 33 cm [packed length (and length to detector) 8.5 cm]  $\times$  100  $\mu\text{m}$  I.D.; electrokinetic injection, 5 s at 5 kV; reverse polarity; applied voltage, 2.5–20 kV, ramp, 0.10 min; run pressure, 2 bar on both electrolyte vials; detection through the packing material; electrolyte, phosphate, pH 7.5,  $\sim$ 10 mM ionic strength, containing 70% (v/v) acetonitrile; sample concentration, 2.5 mM of each component (thiourea and naphthalene) in  $\text{CH}_3\text{CN}$ -water (50:50, v/v).

ODS1 particle size and porosity. The greatest difference is observed on the 5  $\mu\text{m}$  material which exhibits the highest and lowest retention factors for the 8 and 30 nm pore size, respectively.

Attempts to perform a similar study on different types of silica, but the same particle size, using thiourea as the solute and naphthalene as the neutral and unretained marker were largely unsuccessful. The exercise highlighted problems in the column preparation, for example frit manufacture, and whilst the linear velocities were comparable for duplicate columns the separation parameters were not. Columns of 5  $\mu\text{m}$  Spherisorb silica gave minimum reduced plate heights of around 3.1 and 5.2 with corresponding velocities of 0.84 and 0.89  $\text{mm s}^{-1}$ , compared to minimum reduced plate heights of 1.4 and 1.7 with velocities of 0.70 and 0.72  $\text{mm s}^{-1}$  for 5  $\mu\text{m}$  ODS1 columns. Although baseline resolution of thiourea and naphthalene was generally obtained, it was felt that the low retention factors did not aid the investigation; these ranged from 0.1 to 0.3 for the different types of column. Worst affected was the 30 nm pore material, which also showed signs of peak splitting at voltages typically at 15 kV and above. No significant difference was observed in the linear velocities generated between the silica and ODS1 columns over the range of voltage applied.

### 3.3.3. Effect of particle porosity

The majority of stationary phases utilised within this project have had a standard pore size of 8 nm. As we have already seen, double layer overlap at low ionic strengths ( $<5$  mM) should predominate in the pores and result in a lower EOF; 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 mM, respectively (Table 2). With a larger pore size, such as 30 nm, although double layer overlap should not occur, the EOF was less than that obtained for the corresponding 8 nm material (see Fig. 7). A comparison of linear velocities achieved on 5  $\mu\text{m}$  stationary phases (ODS1 and a high purity silica) of 8 and 30 nm pore sizes, under various conditions, is summarised in Table 4.

Comparing similar stationary phase types, it can be seen that for the ODS1 material linear velocities generated by the 8 nm pore material are consistently higher than those measured for the 30 nm pore. This

Table 4  
Comparison of linear velocities generated by 5  $\mu\text{m}$  ODS1 and high purity silica with 8 or 30 nm pores

Variable	$\nu_{\text{ODS1}}$ ( $\cdot 10^{-4}$ m s $^{-1}$ )		$\nu_{\text{silica}}$ ( $\cdot 10^{-4}$ m s $^{-1}$ )	
	8 nm	30 nm	8 nm <sup>a</sup>	30 nm
<i>Voltage (kV)</i>				
2.5	2.3	2.0	1.4	1.9
5.0	4.8	4.1	3.6	4.0
7.5	7.2	6.1	5.7	6.2
10.0	9.9	8.4	8.9	8.6
12.5	12.7	10.7	11.2	11.1
15.0	16.0	13.4	14.2	13.8
17.5	19.6	16.0	17.0	16.6
20	24.1	19.0	20.2	19.9
<i>CH<sub>3</sub>CN (%)</i>				
20	14.0	11.5	11.3	13.0
40	13.6	10.7	11.3	11.8
60	11.9	9.1	10.3	10.2
70	11.0	8.8	9.6	9.3
80	12.2	10.6	10.5	11.1
<i>Ionic strength (mM)</i>				
1.0	12.9	9.8	9.6	10.6
2.5	13.8	10.8	9.6	11.1
5.0	12.6	9.7	10.8	10.4
10.0	10.7	8.5	9.2	8.7
15.0	9.9	8.0	8.9	8.4
20.0	9.4	7.8	7.9	8.4

Default conditions: electrolyte, phosphate, pH 7.5, 10 mM, 70% (v/v) CH<sub>3</sub>CN; voltage, 10 kV.

<sup>a</sup> Results for single column only.

trend is not observed for the high purity silica columns, which show comparable velocities regardless of pore size, albeit of similar magnitude to the 30 nm ODS1 material. A Van Deemter plot of plate height versus linear velocity for the 5  $\mu\text{m}$  ODS1 columns (Fig. 11) does not provide sufficient evidence to support the theory of enhanced efficiency arising from perfusive transport as reported by Li and Remcho [19].

### 3.4. Use of small diameter (<3 $\mu\text{m}$ ) particles

A theoretical treatment of separation procedures by Knox and Grant [2] predicted that highly efficient CEC should be attainable on submicron particles, since losses in EOF velocity should only become appreciable with particle diameters below 1  $\mu\text{m}$ . To

date, although the majority of publications in CEC have dealt with 3 or 5  $\mu\text{m}$  particles, there have been several reports demonstrating the applicability of very small particles. Typically these have been in the range 0.5–1.8  $\mu\text{m}$ , and have covered both nonporous [4,20] and porous [21,22] materials. However, whilst the results obtained on these columns have been extremely promising, they must be looked at in the context of previous comments concerning the nature of particle size measurements. Our own experiences with 1.5  $\mu\text{m}$  ODS1 material (porous and solid) have not supported the consensus of opinion that submicron particles are the way forward, instead they have highlighted practical difficulties in both the column construction and operation.

The preparation of small particle columns has been found to be extremely time-consuming and largely unsuccessful; it can take hours to pack a small section of capillary [21], and just as long to remove excess packing to reach the desired length. Despite sonication of the material prior to and during the packing process, when viewed under a microscope the particles have typically formed agglomerates which are capable of blocking the capillary; a sure sign of this is a discontinuous packed bed where large sections of open capillary predominate. However, small particles themselves are capable of blocking the frit or sinter, thus impeding solvent flow through the capillary and this, coupled with increasing back pressure as the bed is formed, accounts for much of the timely nature of the process. During initial operation, the porous column was found to stabilise far quicker than the nonporous one, and could tolerate a higher ionic strength electrolyte; it is presumed that the smaller volume of liquid present for the nonporous columns was more susceptible to localised heating effects. However, under the microscope the porous bed showed visible signs of void formation, which could account for the inconsistency in the few results obtained and its rapid collapse. The use of far lower ionic strength electrolytes (typically 1 mM) extended the life of the solid column, but since only low voltages could be employed (<10 kV) limited results were obtained for most studies. Linear velocities generated at the low ionic strengths were similar to those for other particle sizes, but showed a marked reduction as the ionic strength was increased (Table 5).

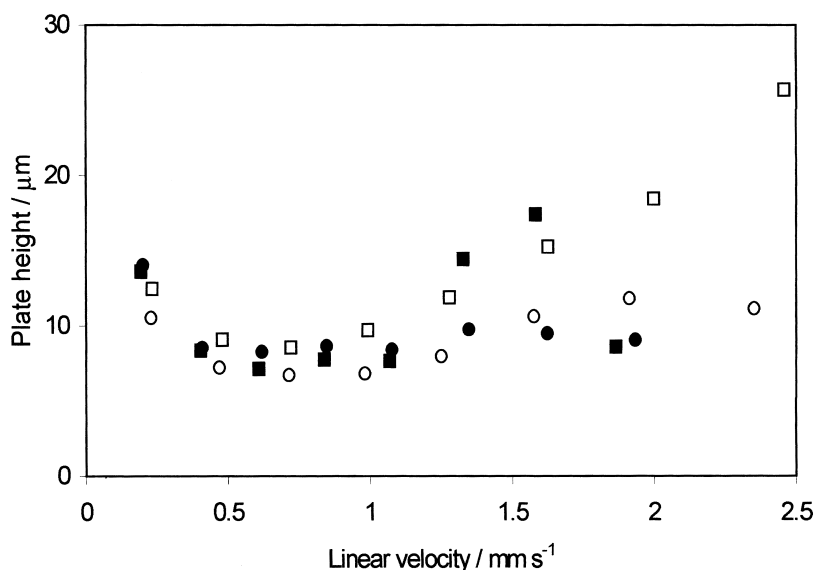


Fig. 11. Effect of porosity on the separation of ethylparaben from thiourea (linear velocity marker). Results are shown for individual columns prepared from 5  $\mu$  ODS1 of 8 nm ( $\square$ ,  $\circ$ ) and 30 nm ( $\blacksquare$ ,  $\bullet$ ) pore size. CEC conditions as in Fig. 5; electrolyte, phosphate, pH 7.5,  $\sim$ 10 mM ionic strength, containing 70% (v/v) acetonitrile; applied voltage, 2.5–20 kV; analyte mixture, 2.5 mM of each component in  $\text{CH}_3\text{CN}$ -water (50:50, v/v).

Attempts to run the column under a different acetonitrile composition were unsuccessful: apart from irreproducible results, relative standard deviations were at best 7%, the current was extremely unstable and the column prone to drying out. A partial Van Deemter study carried out on one column only over the voltage range 1–8 kV, gave a reduced plate height of 10 for naphthalene (Fig. 12); ethylparaben was not retained as in previous studies,

presumably due to the lower carbon loading associated with solid material. A typical separation is shown in Fig. 13. Our results did not suggest any benefit in moving towards small particle sizes, and it should be questioned whether this approach is really practicable.

#### 4. Conclusions

Our study has demonstrated that there is still a significant way to go in understanding the role of factors that govern the EOF in CEC. Results have suggested discrepancies in new theories and possible flaws in existing ones, all of which need to be substantiated further. In view of the diversity of stationary phases of similar type, the industry should agree to and implement a standard characterisation procedure. Until then, it remains a distinct possibility that the lack of comparable results between researchers arises from the column packing itself; in addition, the quality of the column packing and frit manufacture should be addressed. However, even if the column characteristics could be readily defined and reproduced, there is still a great need to understand

Table 5

Comparison of linear velocities generated under varying ionic strength conditions for ODS1 columns of different particle size

Ionic strength (mM)	Linear velocity ( $\cdot 10^{-4}$ m s $^{-1}$ )		
	1.5 $\mu\text{m}$ solid <sup>b</sup>	3 $\mu\text{m}$ <sup>a</sup>	5 $\mu\text{m}$ <sup>a</sup>
1.0	6.08	5.67	6.46
2.5	5.09	5.58	6.92
5.0	3.24	5.25	6.28
10.0	2.54	4.52	5.34

Values quoted for 5 kV measurement where <sup>a</sup> denotes that the velocity has been corrected for voltage.

<sup>b</sup> Results for single column only.

CEC conditions as in Table 3 with the following exceptions: applied voltage, 5 or 10 kV; electrolyte, phosphate, pH 7.5, 1.0–10 mM ionic strength, containing 70% (v/v) acetonitrile; sample concentration, 1–2 mM thiourea in  $\text{CH}_3\text{CN}$ -water (60:40, v/v).

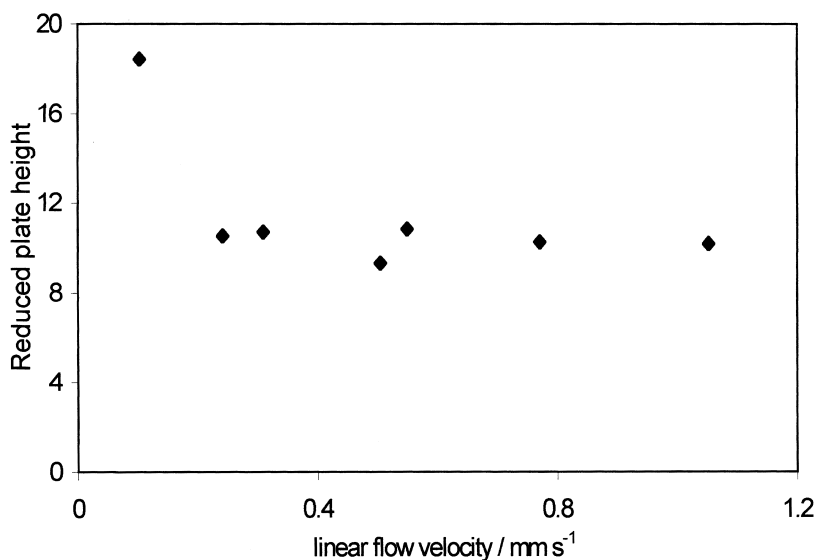


Fig. 12. Van Deemter plot for the separation of naphthalene on a 1.5  $\mu\text{m}$  ODS1 solid column, using thiourea as the linear velocity marker. CEC conditions as in Fig. 5; electrolyte, phosphate, pH 7.5,  $\sim 1.0$  mM ionic strength, containing 70% (v/v) acetonitrile; applied voltage, 1.0–8.0 kV; analyte mixture, 2.5 mM of each component in  $\text{CH}_3\text{CN}$ –water (50:50, v/v).

the behaviour of buffers in mixed-solvent systems. It is essential that these problems are resolved if CEC is to be accepted as an analytical technique, and follow the natural progression towards miniaturisation.

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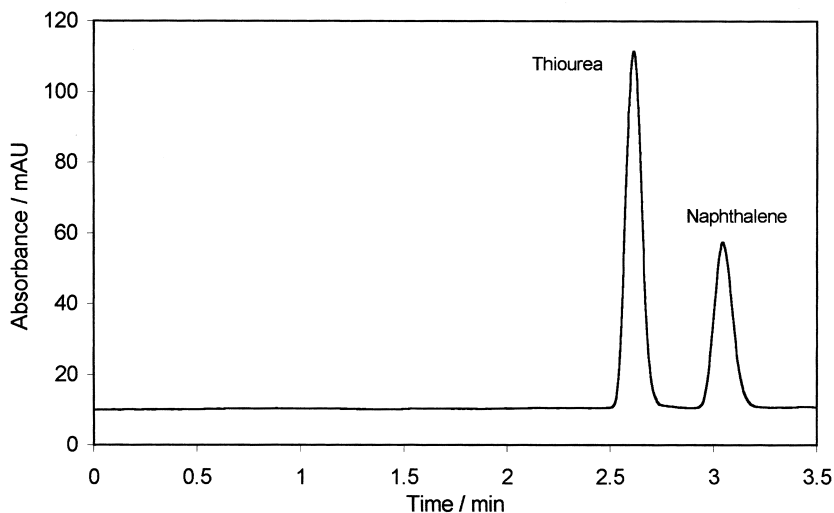


Fig. 13. Typical chromatogram obtained for the separation of an equimolar mixture of thiourea and naphthalene (2.5 mM of each component in  $\text{CH}_3\text{CN}$ –water, 50:50, v/v) on a 1.5  $\mu\text{m}$  ODS1 column. CEC conditions as in Fig. 5 except; electrolyte, phosphate, pH 7.5,  $\sim 1.0$  mM ionic strength, containing 70% (v/v) acetonitrile; applied voltage, 5.0 kV.



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