Characterisation of electroosmotic flow in capillary electrochromatography columns

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Abstract

Currently available capillary electrochromatography (CEC) instrumentation using UV–Vis detection dictates the use of duplex columns. Due to discontinuities (electric field strength and conductivity) that arise at the boundary between the packed and open sections in these columns, the determination of the electroosmotic flow (EOF) is complicated. Thiourea has been found to be an accurate EOF marker under the conditions employed in this study. By injecting this compound onto a fully packed column and comparing the obtained mobilities with those calculated from measured zeta potential values a value for tortuosity has been obtained. The use of laser Doppler velocimetry (LDV) for the measurement of zeta potential has been found to be the most direct and rapid method of characterising silica support materials in terms of electroosmotic mobility. The open section in duplex CEC columns has been shown to influence the actual column flow-rate. The EOF measured using duplex columns of varying packed and open section lengths have been compared with those obtained for a fully packed column. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

To attain rapid, accurate and reproducible separations, it is imperative that the generation and control of the electroosmotic flow (EOF) in capillary electrochromatography (CEC) columns packed with suitable stationary phases is well understood. Currently available CEC instrumentation dictates that the columns employed contain both a packed and open

section with a detection window at the junction (see Fig. 1). Such duplex columns experience abrupt changes in electric field strength, EOF and conductivity at this boundary. The presence of such discontinuities complicates the calculation of EOF and capacity factor values in duplex columns.

A number of groups have attempted to derive theoretical and practical models to enable comprehensive understanding of the flow characteristics in CEC columns [1–5]. Wan [2] applied electrical conductivity values for open and packed columns as a diagnostic tool for determining the porosity of CEC columns. More recently reports [3,4] detailing the

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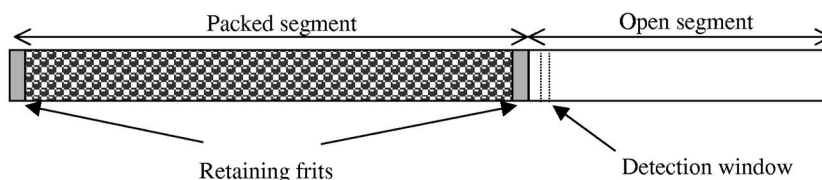*E-mail address:* n.w.smith@ic.ac.uk (N.W. Smith).

Fig. 1. A duplex CEC column consisting of a packed and open segment.

use of mercury porosimetry to determine silica particle pore diameter have enabled a more accurate measurement of flow type, i.e., interparticle and intraparticle. Liapis and Grimes [5] constructed and solved a mathematical model to describe the velocity of the EOF in packed CEC columns. They obtained good agreement between theoretical and practically obtained values of EOF.

In order to get a true understanding of the mechanism of flow through a packed bed, a fully packed column is recommended for the measurement of the EOF. This essentially eliminates the discontinuities associated with duplex columns. The configuration of CEC with mass spectrometry (MS) in our laboratory allows the use of fully packed columns, as the open section after the retaining frit is absent [6]. In addition, bubble formation that occurs at the interface in duplex columns is eliminated and therefore pressurisation of the column is unnecessary.

Conventionally, the EOF is calculated from the migration time of an electrically and chromatographically neutral marker. Various components have been used with thiourea being the most popular choice. Discrepancies have been reported for the EOF when changing the proportion of organic modifier. In the literature many reports have indicated an increase in mobility with increasing acetonitrile content [7–9]. This compliments the behaviour of the bulk properties; viscosity and dielectric constant. In contrast to this, other groups have reported a decrease in mobility with increasing acetonitrile content, for both packed columns [10,11] and open fused-silica capillaries [12]. The latter concluded that the zeta potential itself changes, either by variation of the dielectric constant in the double layer or by a change in adsorption of potential determining ions upon addition of organic modifier. The reason for these contradicting observations is not clear. It may be due

to the chosen EOF marker being retained in the mobile phase being investigated, although in the case of thiourea this is thought to be unlikely. This would therefore indicate that the choice of a neutral marker should take into account the stationary phase of interest and the eluent properties that are to be employed throughout the analysis. The discontinuities mentioned above that are present in duplex columns might also be an additional factor inducing contrasting observations of EOF mobility.

An alternative approach to determining the zeta potential is through the use of laser Doppler velocimetry (LDV), which is a well-established technique used in engineering for the study of fluid flow in a wide variety of situations. It is the velocity of tiny particles within the fluid streams moving at the velocity of the fluid that are measured. LDV is therefore well placed to determine the zeta potential, calculated from the velocity, of particles moving through a fluid under an applied electric field. Luedtke et al. [13] used laser Doppler anemometry to determine the zeta potential by measuring the electrophoretic mobility of silica particles over a wide range of mobile phase pH values.

By accurately measuring the zeta potential (ζ) of the stationary phase dispersed in the desired eluent of known viscosity (η) and dielectric constant (ε_r), the EOF mobility (μ_{eo}) can be calculated using the von Smoluchowski equation as shown below [14]:

$$\mu_{eo} = \frac{\varepsilon_o \varepsilon_r \zeta}{\eta} \tag{1}$$

where ε_o is the permittivity of a vacuum (8.85×10^{-12} C^2 N^{-1} M^{-2}). This calculated value of μ_{eo} using experimentally obtained values for zeta potential does not take into consideration the tortuosity of the packed bed. It therefore follows that by directly comparing these mobility values with the measured mobility on a fully packed column using a

neutral marker, a value for tortuosity can be deduced. In addition, by comparison of these two methods of measuring the EOF it will be apparent whether thiourea is an accurate EOF marker for the conditions employed in this study.

A third method of measuring the EOF in duplex CEC columns has been given by Rathore and co-workers [15–17]. They evaluated the conductivity ratio for various stationary phases by measuring the current generated in a fully packed column and an open capillary [17]. They suggested that the calculation of the 'actual' EOF mobility using this conductivity ratio takes into account the effect of tortuosity. Eq. (2) gives this 'actual' EOF mobility [17] for a neutral marker through a fully packed column:

$$\mu_{\text{eo(actual)}} = \frac{L_e^2}{t_o V} \qquad (2)$$

where L_e is defined as the length of the actual flow path followed by a neutral marker, t_o is the migration time of the neutral marker and V is the applied voltage used.

L_e is calculated using the following equation:

$$L_e = L(\sqrt{i_{\text{open}}/i_{\text{packed}}}) \qquad (3)$$

where i_{open} and i_{packed} are the currents in an open capillary and fully packed column, respectively, and L is the overall column length.

The zeta potential can be calculated from the migration time of a neutral component on a fully packed column using Eqs. (1)–(3). By comparison of this calculated zeta potential with those measured it should be evident whether the inclusion of a conductivity factor yields the same zeta potential values.

The work performed in this investigation compares two methods for characterising the EOF in a 3-μm Waters Spherisorb ODS-1 fully packed column:

(1) from the migration time of a t_o marker (thiourea); and

(2) from the measured zeta potential values using the von Smoluchowski equation.

Each procedure was performed over a range of ionic strengths and organic modifier concentrations. In addition, measured LDV zeta potential values of 3-μm Waters Spherisorb ODS-1 were compared with those calculated using the methods described by Rathore et al. (Eqs. (2) and (3)). In order to evaluate the effect of the open section after the retaining frit on the EOF, duplex columns of varying packed and open section lengths were employed. The effect of ionic strength and organic modifier concentration were also investigated. These measurements of flow were then compared with those obtained for a fully packed column in order to assess the magnitude of the effect of an open section in columns employed for CEC.

2. Experimental

2.1. Chemicals

Thiourea was purchased from Aldrich (Gillingham, UK). Ammonium acetate and acetic acid were of analytical grade and purchased from BDH (Poole, UK). Acetonitrile, methanol and acetone were all of HPLC grade and also purchased from BDH (Poole, UK). Buffer solutions were prepared using deionized water from an Elga Maxima water purifier and the pH adjusted to pH 7.0 with acetic acid. The mobile phase consisted of acetonitrile–ammonium acetate (60:40%, v/v) (25 mM) unless otherwise stated. The mobile phase used to study the organic modifier concentration was prepared to give a fixed ionic strength of 25 mM over the whole range investigated.

2.2. Columns

CEC columns were prepared using a method previously detailed by Carter-Finch and Smith [18]. Capillaries were supplied by Composite Metals (Worcs, UK) and the column packer obtained from Shandon Instruments (Runcorn, UK). Windows and frits were fabricated using an Innovatech ACF electrical burner (Stevenage, UK). The 3-μm Waters Spherisorb ODS-1 material was purchased from Phase Separations (Watford, UK). Column dimensions were as follows:

(1) fully packed column (30-cm packed×100-μm I.D.);

(2) long packed length duplex column (30-cm packed×100-μm I.D. (total column length 45 cm));

(3) short packed length duplex column (15-cm packed×100-μm I.D. (total column length 45 cm)).

2.3. Instrumentation

The fully packed column experiments were performed by CEC–MS on a Sciex API 150MCA single quadrupole Mass Spectrometer (PE BioSystems, Warrington, UK), using a micro-electrospray CEC–MS interface [19–21]. Injections were performed with an automated injection system (Innovatech, Stevenage, UK) [6,22]. The spray voltage was set at −4.5 kV. The sheath flow composition was methanol–water (70:30%, v/v)+0.1% acetic acid and was supplied at a flow-rate of 2.0 μl/min by a Harvard syringe pump. Injections and separations were performed using a high-voltage power supply unit (Spellman, Hauppauge, USA). The separation voltage applied was +20 kV in all the experiments (+15.5 kV overall). Injections were performed at +10 kV for 5 s.

Duplex column studies were performed on a Unicam Lauer Labs Prince instrument (Emmen, The Netherlands) with an applied voltage of +15.5 kV. The Unicam was modified in-house to allow pressurisation of both the inlet and outlet vials. This CEC instrument was equipped with a variable wavelength detector operated at 214 nm.

2.4. Measurement of zeta potential

Zeta potential measurements were performed on a Zetasizer, Malvern instruments (Worcs, UK). Five mg of 3-μm Waters Spherisorb ODS-1 was added to 100 ml of the mobile phase to give an opaque suspension. Previous studies performed over a period of 1 day to 1 week indicated that an equilibration period was necessary in order to allow full wetting of the silica. The zeta potential of the silica suspensions were consequently measured 1 week subsequent to preparation. Ten sequential measurements were performed for each sample.

3. Results and discussion

3.1. Comparison of calculated zeta potential values (using the equations derived by Rathore and co-workers) with measured values for a fully packed column

Zeta potential values for 3-μm Waters Spherisorb ODS-1 were calculated from the 'actual' EOF mobility (Eqs. (2) and (3)) and the von Smoluchowski equation (Eq. (1)) using thiourea as the t_0 marker. The following experiments were conducted:

(1) the effect of ionic strength (for a mobile phase containing acetonitrile–ammonium acetate (60:40%, v/v) pH 7.0) and

(2) the proportion of organic modifier (with a fixed ionic strength of 25 mM ammonium acetate at pH 7.0).

An example calculation for the zeta potential obtained from the Rathore equations for a mobile phase containing acetonitrile–ammonium acetate (60:40%, v/v) (pH 7.0) with an ionic strength of 5 mM is as follows.

Initially, the $\mu_{eo\ (actual)}$ was calculated using Eqs. (2) and (3) where: $L = 300$ mm, $i_{(open)} = 5$ μA, $i_{(packed)} = 2$ μA, $t_o = 299.4$ s and $V = 15\ 500$ V. $i_{(open)}$ and $i_{(packed)}$ were the current measurements obtained in an open capillary (length 300 mm) and fully packed column (length 300 mm), respectively, using the same mobile phase employed for the fully packed column study. This gave a $\mu_{eo\ (actual)}$ value of 48.48 k V^{-1} mm^2 s^{-1}. This value was then inserted into Eq. (4), which is the von Smoluchowski equation rearranged for the zeta potential:

$$\zeta = \frac{\mu_{eo}\eta}{\varepsilon_o\varepsilon_r} \tag{4}$$

where $\eta = 0.5515$ kg s^{-1} M^{-1}, $\varepsilon_o = 8.85\times10^{-12}$ C^2 N^{-1} M^{-2} and $\varepsilon_r = 41.42$. The values for η and ε_r were obtained from the literature [23,24]. This gives a value for ζ of 72.94 mV for 3-μm Waters Spherisorb ODS-1 in a mobile phase of acetonitrile–ammonium acetate (60:40%, v/v) (pH 7.0) with an overall ionic strength of 5 mM. These calculated values of zeta potential at varying ionic strength and acetonitrile concentration were then plotted and compared with the measured LDV zeta potentials. The results are shown in Figs. 2 and 3. Fig. 2 shows
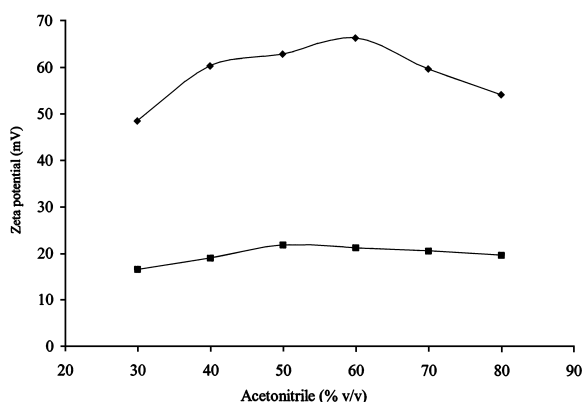
Fig. 2. The effect of acetonitrile (%, v/v) on the zeta potential values of 3-µm Waters Spherisorb ODS-1. (♦) Calculated values from Rathore equations, (■) measured values. Conditions for on-column measurements: column: 30-cm×100-µm I.D. fused-silica capillary packed with 3-µm Waters Spherisorb ODS-1, total column length, 30 cm; eluent, appropriate proportions of acetonitrile plus ammonium acetate, pH 7.0, mixed to give an overall strength of 25 mM; applied voltage, 15.5 kV; temperature, 25°C.

the effect of acetonitrile (%, v/v) with a fixed ionic strength of 25 mM on the zeta potential of 3-µm Waters Spherisorb ODS-1. The values calculated using the equations derived by Rathore et al. show a steady increase up to 60% (v/v) acetonitrile and then
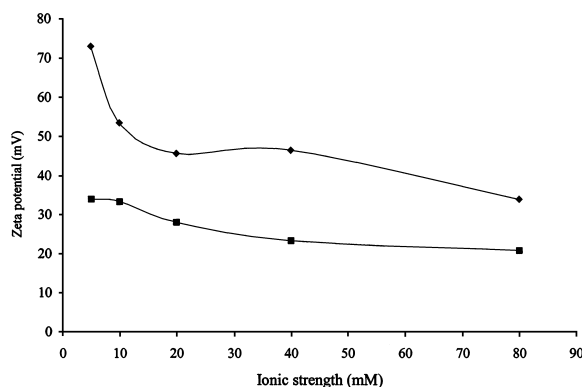

Fig. 3. The effect of ionic strength on the zeta potential values of 3-µm Waters Spherisorb ODS-1. (♦) Calculated values from Rathore equations, (■) measured values. Conditions for on-column measurements: column, 30-cm×100-µm I.D. fused-silica capillary packed with 3-µm Waters Spherisorb ODS-1; total column length, 30 cm; eluent, actonitrile–ammonium acetate, pH 7.0 (60:40%, v/v) ionic strength variable; applied voltage, 15.5 kV; temperature, 25°C.

begin to drop off. The measured LDV zeta potentials, in comparison, remain relatively constant. Overall, the calculated values yield a much higher zeta potential range than those determined by LDV.

The effect of ionic strength on the zeta potential is shown in Fig. 3. Both the measured and calculated values reflect the data published in the literature [25,26] in that the zeta potential decreases with increasing ionic strength as a result of the compressed electrical double layer. The calculated values from the Rathore equations, however, give much higher values of zeta potential, as seen previously in Fig. 2. In addition, they show a steep decrease between 5 and 20 mM.

It is thought that the discrepancies observed between the calculated and measured zeta potential values are due primarily to the principle assumptions and simplifications of any theoretical calculation. Because the understanding of flow mechanisms in CEC columns remains a challenging area of research a method of calculating the true EOF is still very difficult to derive. The equations formulated by Rathore et al. attempt to define the EOF characteristics in CEC columns, but it is the ability to measure these characteristics directly, i.e., using zeta potential, that ultimately present us with an accurate assessment of electroosmotic flow.

Fig. 4 shows the ε_r/η dependence on the percentage of acetonitrile [12]. As depicted by the von Smoluchowski equation, an increase in ε_r/η should result in an increase in EOF. Following a steady
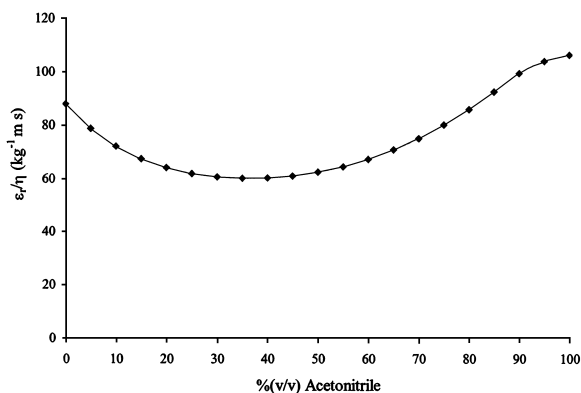

Fig. 4. Variation of the ratio of the dielectric constant and viscosity, ε_r/η, with percentage acetonitrile at 25°C.
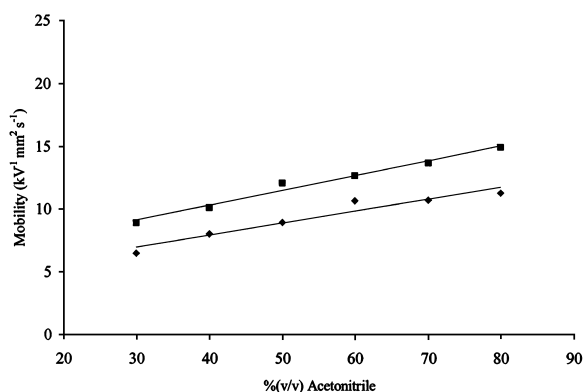
Fig. 5. The effect of acetonitrile (%, v/v) on the mobility of 3-μm Waters Spherisorb ODS-1. (■) Mobility calculated from measured zeta potential values, (♦) mobility calculated from a t_o marker (thiourea). Conditions for on-column measurements: column, 30-cm×100-μm I.D. fused-silica capillary packed with 3-μm Waters Spherisorb ODS-1; total column length, 30 cm; eluent, appropriate proportions of acetonitrile plus ammonium acetate, pH 7.0, mixed to give an overall strength of 25 m*M*; applied voltage, 15.5 kV; temperature, 25°C.

decrease in the range 0–40% (v/v), there is a steady increase in the ratio of ε_r/η.

Fig. 5 shows the effect of acetonitrile concentration on the mobility in a 3-μm Waters Spherisorb ODS-1 column. It is apparent from Fig. 5 that the mobility increases with increasing acetonitrile concentration.

It can therefore be assumed that the mobility is not dependent on the charge of the silica surface alone. By comparing Figs. 2, 4 and 5 it becomes apparent that the mobility in CEC is dependent on both the zeta potential and ε_r/η. It is thought that for proportions of acetonitrile less than 40% (v/v) the zeta potential on the Spherisorb particles essentially dictates the flow velocity. At acetonitrile proportions greater than 50% (v/v) this zeta potential is seen to drop off but as shown by Fig. 5 not with a coincidental decrease in mobility. It must therefore be assumed that the mobility at acetonitrile proportions >50% (v/v) are essentially governed by the ε_r/η ratio of the mobile phase.

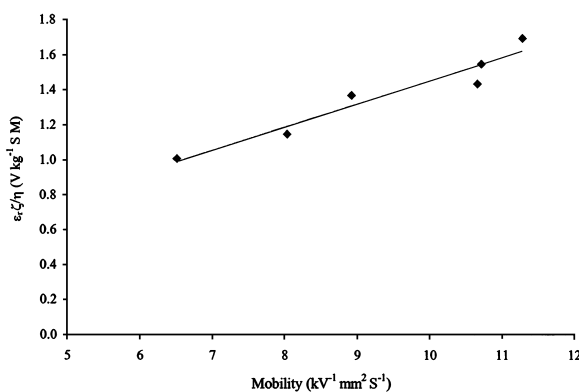Fig. 6 clearly demonstrates the combined effects of dielectric constant, viscosity and zeta potential with mobility.

Fig. 6. Variation of mobility with dielectric constant and zeta potential/viscosity ratio, $\varepsilon_r\zeta/\eta$, at 25°C.

3.2. Calculation of a tortuosity value for 3-μm Waters Spherisorb ODS-1

The electroosmotic mobility in a fully packed column was calculated for 3-μm Waters Spherisorb ODS-1 over a range of ionic strengths and acetonitrile concentrations. These were calculated using two methods:

(1) from the migration time of a t_o marker (thiourea) and

(2) from the measured LDV zeta potential values using the von Smoluchowski equation.

The resultant plots are shown in Figs. 5 and 7.

Theoretically, it would be reasonable to assume that the highest values of mobility would be calculated from the measured LDV zeta potentials, as these do not include the tortuosity associated with a packed column bed.

It is to be expected that the porosity of the silica determines to some extent the mobility of silica particles and hence the zeta potential. Therefore it is assumed that porosity is included in the calculation of mobility from the measured zeta potential. Tortuosity impedes the flow through the column and thus results in a reduced EOF velocity. The values of mobility calculated from the migration time of the neutral unretained marker thiourea, do exhibit this effect. It can therefore be assumed that the difference between these EOF measurements will yield a value for tortuosity. This value for 3-μm Waters
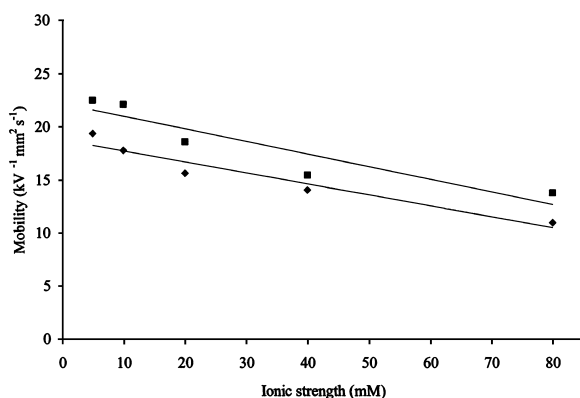
Fig. 7. The effect of ionic strength on the mobility of 3-μm Waters Spherisorb ODS-1: (■) mobility calculated from measured zeta potential values; (♦) mobility calculated from a t_o marker (thiourea). Conditions for on-column measurements: column, 30-cm×100-μm I.D. fused-silica capillary packed with 3-μm Waters Spherisorb ODS-1; total column length, 30 cm; eluent, acetonitrile–ammonium acetate (60:40%, v/v), pH 7.0, ionic strength variable; applied voltage, 15.5 kV; temperature, 25°C.

Spherisorb ODS-1 is calculated to be 0.808 (average of 0.773 for Fig. 5 and 0.842 for Fig. 7). When this tortuosity (τ) effect is included in the von Smoluchowski equation (Eq. (1)) for calculating the linear velocity along with the accurately measured zeta potential values, a true representation of the EOF velocity through a fully packed column will be given (see Eq. (5)):

$$\mu_{eo} = \frac{\varepsilon_o \varepsilon_r \zeta \tau}{\eta} \qquad (5)$$

where τ denotes the tortuosity of the packed bed. The inclusion of tortuosity into the von Smoluchowski equation was first proposed by Grant [27], which replaces τ with γ, a dimensionless factor, with a value estimated to be in the range 0.4–0.7, which accounts for the tortuosity and porosity of the packed bed.

In terms of the discrepancies reported in the literature for using thiourea as a t_0 marker [7–12], results from this work indicate that thiourea is an accurate t_0 marker for a fully packed column at eluent conditions employed in this investigation. Figs. 5 and 7 show that the mobility of thiourea

mirrors the mobility calculated from the zeta potential measurements, as predicted by Eq. (5). It is unlikely that this neutral marker is truly inert for all separation conditions on any stationary phase. At present, it is also not fully understood what the effect is of the open section in a duplex column on the EOF.

3.3. An investigation into the effect of the open section in duplex CEC columns

In order to assess the effect the open section of the CEC column has on the EOF, two duplex columns of varying packed and open lengths were investigated. For comparison, a fully packed column was also used to measure the EOF:

long packed length column (30-cm packed×100-μm I.D. (total column length 45 cm));

short packed column (15-cm packed×100-μm I.D. (total column length 45 cm));

fully packed column (30-cm packed×100-μm I.D. (total column length 30 cm)).

The effect of the ionic strength and organic solvent concentration (with an overall ionic strength of 25 mM) on the migration time of a neutral unretained component (thiourea) was studied for both duplex column dimensions. The mobile phase compositions used were exactly the same as those employed for the fully packed column in order to minimize variability. The EOF generated for both duplex columns was compared with that obtained for a fully packed column using thiourea as the neutral marker. It should be pointed out that during these experiments two short packed length and two long packed length columns were used due to column breaking and failure. The results for each column are shown in Figs. 8 and 9. The results are plotted as mobilities, which corrects for the column length and applied field strength.

As shown in both plots the column with the shortest bed length and longest open section gives the fastest flow. The slowest flow is exhibited by the fully packed column. It is clear that the open section has a dramatic influence on the flow in CEC columns.
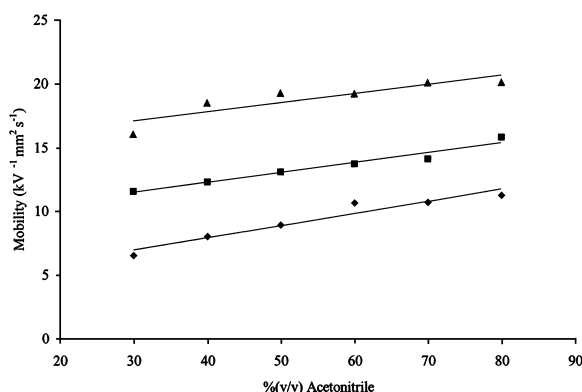
If we compare the fully packed column with the

Fig. 8. The effect of acetonitrile (%, v/v) on the mobility of 3-μm Waters Spherisorb ODS-1 duplex and fully packed columns. (▲) Short packed length column, 15-cm packed×100-μm I.D. (total column length 45 cm); (■) long packed column length, 30-cm packed×100-μm I.D. (total column length 45 cm); (♦) fully packed column, length 30 cm. Conditions for on-column measurements; eluent, appropriate proportions of acetonitrile plus ammonium acetate, pH 7.0, mixed to give an overall strength of 25 mM; applied voltage, 15.5 kV; temperature, 25°C; detection, 214 nm.

duplex column possessing the same packed length, essentially the only difference between the two is the existence of the 15-cm open section. The effect of
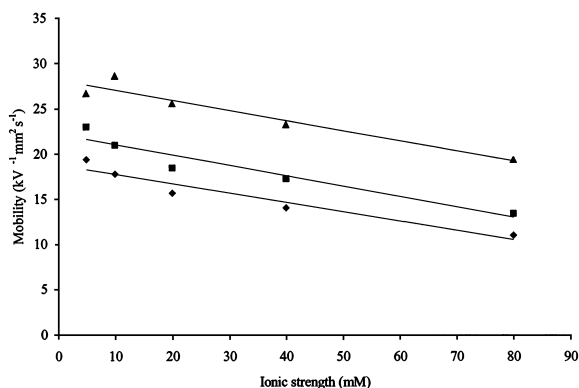

Fig. 9. The effect of ionic strength on the mobility of 3-μm Waters Spherisorb ODS-1 duplex and fully packed columns. (▲) Short packed length column, 15-cm packed×100-μm I.D. (total column length 45 cm); (■) long packed column length, 30-cm packed×100-μm I.D. (total column length 45 cm); (♦) fully packed column, length 30 cm. Conditions for on-column measurements; eluent, acetonitrile–ammonium acetate (60:40%, v/v), pH 7.0, ionic strength variable; applied voltage, 15.5 kV; temperature, 25°C; detection, 214 nm.

this section is calculated to increase the EOF by 33.3% (average of 46.6% from Fig. 8 and 19.9% from Fig. 9). The presence of the open section that possesses an extremely high EOF will essentially dictate, to a certain degree, the rate of flow at which the mobile phase leaves the packed bed. This will also influence the bulk flow-rate through the rest of the column. It may be possible that the effect the open section has on the EOF through the packed bed only becomes significant towards the outlet end of the column. At present this effect is very difficult to ascertain.

4. Conclusions

This study has allowed us to gain further insight into CEC column behaviour and the effects that essential flow dictating parameters have on the EOF mobility. It has been shown that thiourea is a true EOF marker for 3-μm Waters Spherisorb ODS-1 columns under the conditions used in this investigation. From the EOF mobility measured from this component it was also found that the charge on the packing material surface (ζ) alone does not dictate the observed EOF. It is the ε_r/η ratio of the mobile phase plus the ζ that explains the obtained mobility, as depicted by the von Smoluchowski equation. From comparison of the two methods used for measuring the EOF mobility in fully packed columns, a value of tortuosity of 0.808 for 3-μm Waters Spherisorb ODS-1 is given.

The measurement of ζ by LDV is the most direct and rapid method of gaining an insight into the behaviour of silica support materials in a given mobile phase. It therefore essentially eliminates the need to pack CEC columns when characterising the mobility generated by stationary phases.

The open section in duplex CEC columns has been shown to influence the actual column flow-rate. Because the majority of work published to date is performed on a duplex column format it is impossible to eliminate the effects that the open section presents. However, certain steps could be taken in an attempt to minimalise these effects such as employing neutrally coated capillaries [28], e.g., poly(vinyl alcohol) (PVA).

Acknowledgements

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