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Attempt to define the role of the length of the packed section in capillary electrochromatography

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Abstract

The problems of memory effects when using ion-exchange columns have been addressed. Studies on capillaries packed with a strong cation-exchange (SCX) material have shown voltage conditioning to be sufficient to produce an equilibrated column and reliable results. Storage in acetonitrile–water (80:20, v/v) for six weeks had no detrimental effect on the column performance. Columns used for capillary electrochromatography typically consist of an open and packed section, each contributing to the electroosmotic flow (EOF). Studies performed on SCX columns of varying proportions of packed section (25–100% of the total length) showed that at pH 7.5 the field strengths are similar in both sections and the linear velocity changes little with the packed section length. In contrast, at pH extremes the field strengths were greater in the packed section and the velocity decreased with length. A comparison of the conductance ratios for C_{18} and SCX columns indicated little difference between them at high pH. However, at low pH results were not routinely obtained for the C_{18} column, whereas the short SCX column gave rise to far higher EOF than expected.

Keywords: Electrochromatography; Electroosmotic flow; Capillary columns; Packed length

1. Introduction

The renewed interest in capillary electrochromatography (CEC) over the last 10 years has largely emanated from the theoretical and experimental studies published by Knox and co-workers [1–4]. Since then, reviews on the technique and its application have been published on a regular basis: the more recent of these include general papers by Cikalo et al. [5], Robson et al. [6], Colón and co-workers [7,8] and Altria et al. [9]; an article on column technology [10]; and more theoretical treatments by Ståhlberg [11] and Rathore and Horváth [12,13]. However, there still remains a long way to go if the mechanisms operating in CEC are to be fully understood.

For CEC to become widely accepted as an analytical technique, capable of achieving reproducible separations, then a comprehensive understanding of the generation and control of the electroosmotic flow (EOF) is essential. This is complicated by the fact that chromatographic columns are likely to behave differently according to their type and packed structure. An important parameter, which has been largely overlooked, is the column conductivity. Since this is primarily a function of the column porosity and tortuosity, it can be used to characterise the structure of packed columns and provide an indication of the flow permeability [14]. Relative conductivities, determined experimentally, appeared to confirm the theory that they were essentially a structural constant

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and independent of particle size, column dimensions and field strength. Although EOF can contribute to the observed conductivity, by transporting excess charges in double layers, theoretical calculations have shown that this can be largely ignored since it typically accounts for less than 5% of the bulk conductivity [15]. More comprehensive studies have been performed by Choudhary and Horváth [16] on columns of varying inner wall charge packed with charged and uncharged stationary phases. In addition they considered the effect of column architecture. To date the majority of CEC columns have comprised a long packed section and a much shorter open section through which detection is usually performed. Each section has its own conductance and consequently different voltage drop and electric field strength since the current is conserved across the whole length of capillary. From the mass conservation law, the volumetric flow-rate must be the same in both segments of capillary. Thus the EOF velocity will decrease on going from the packed to the open section as a result of the increase in available cross-sectional area. Results presented by Choudhary and Horváth [16] indicated abrupt changes in the electric field strength at the packed/open interface, and the existence of differential temperature fields in the two sections. In their conclusions they emphasised the need for further investigations into the role of the open and packed segments, and highlighted that the dual nature of the column could be responsible for several problems associated with the measurement of EOF velocity in CEC. However, we have noticed problems in terms of reporting the magnitude of the EOF: for mobility calculations, which require knowledge of the voltage drop, it is often assumed that the voltage is over the packed section only whilst linear velocities are often reported without the relevant voltage data.

The interface between the open and packed sections of a CEC column is the major source of discontinuities in parameters such as conductivity, electric field strength and flow velocity. This presents a problem in measuring and interpreting them, and makes comparison of data from different sources difficult. To evaluate the electrochromatographic parameters in the two column segments, Rathore and Horváth [17] have presented a set of equations that utilises readily obtainable experimental data. Although their study has only focused on the interface between the open and packed section, it should be remembered that discontinuities may also arise from changes in the property of the packing such as frits. The work presented here has attempted to define the role of the length of the packed section for untreated fused-silica capillaries packed to different lengths with either a C_{18} (ODS1) or strong cation-exchange (SCX) packing material (propylsulphonic acid). The effect of electrolyte pH on the resistivities and conductance ratios of the columns is also investigated.

2. Experimental

2.1. Reagents and materials

Electroseparations were carried out on 100 μm I.D.×375 μm O.D. untreated fused-silica capillaries (Composite Metal Services, Harlow, UK), either open or packed to different lengths with 3 μm (mean pore size 8 nm) Waters Spherisorb SCX and ODS1 materials (Phase Separations, Deeside, UK). Thiourea (99+%) was obtained from Sigma–Aldrich (Poole, UK) and HPLC-grade acetonitrile from Merck (Poole, UK). All other chemicals (reagent grade) were purchased from Sigma–Aldrich, Fischer Scientific (Loughborough, UK) or Vickers Labs. (Pudsey, UK). Distilled water was used throughout, and all mobile phases filtered prior to use through a 0.2-μm Whatman Anotop syringe filter (Phase Separations).

Electrolytes were based on the constant ionic strength (10 m*M*), spectrophotometric buffers described by Perrin [18]. Buffers of a high (carbonate, pH 10.5), intermediate (phosphate, pH 7.5) and low (KCl–HCl, pH 2.9) pH were selected, but were prepared in acetonitrile–water (80:20, v/v) systems such that the buffer concentrations of the final solutions were identical to that found in analogous aqueous systems. Although attempts were made to maintain a constant ionic strength, it was expected to vary from 10 m*M* on account of the mixed media used: dissociation constants (K_a) of weak acids decrease with a decrease in the dielectric constant of the solvent [19]. Throughout the text, the electrolyte pH stated refers to the pH of the aqueous buffer

solution it is based on, and not the ''apparent'' pH of the mixed solvent system, which was not measured. Generally, it has been assumed that the incorporation of acetonitrile in the electrolyte has no effect on either pH or ionic strength. The EOF was determined by measuring the mobility of a neutral and unretained marker, i.e., 0.5 m*M* thiourea in water and 1–2 m*M* thiourea in acetonitrile–water (60:40, v/v) for capillary electrophoresis (CE) and CEC, respectively.

2.2. Preparation of CEC columns

Capillaries (typically of 40 cm length) were packed using a Shandon HPLC column packer (Hypersil, Runcorn, UK) at 300 bar, against a Valco union (Phase Separations) containing a metal screen (2 μm pores). The stationary phase slurry was prepared in acetone (80–100 mg ml^{-1}), and sonicated prior to and throughout the packing process; acetone was also employed as the packing solvent. Once the capillary had packed the pressure was held for an additional 15 min. The columns were conditioned on an HPLC pump with acetonitrile–water (80:20, v/v) (1 h at 35 bar) followed by water (2 h at 35–70 bar); at this stage the frits and window were made using a hot filament device and excess packing removed. Columns were prepared with approximately 25, 50, 75 and 100% of the total length packed. When the column was installed in the instrument cartridge, the position of the frit at the packed/open section interface was often stressed and subject to breakage. In many of these cases it was necessary to couple the packed section to a separate section of fused-silica capillary (100 μm I.D.×375 μm O.D.) via a PTFE sleeve (10–15 mm of PTFE tubing, 1.59 mm O.D., drilled to 340 μm I.D.).

CEC columns were conditioned with the relevant electrolyte prior to use by one of two methods. In the first method, the capillary was flushed on an HPLC pump (1–2 h at 35 bar) then installed in the instrument for voltage conditioning (10 kV) with pressurisation (2 bar on each vial) until a steady current was obtained. In the second method, the capillary was installed in the instrument and a suitable voltage (10 or 20 kV) applied with pressurisation as before for 30 min.

2.3. Instrumentation and operating procedure

Experiments were performed on a HP ^{3D}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). The capillary was conditioned before use with a rinse cycle of 5 min NaOH (0.1 *M*), 10 min water and 20–30 min electrolyte, then with electrolyte (1.5 min) prior to each injection. 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 (20 kV at low pH). CEC separations were carried out on capillaries of total length around 33 cm (length to detector 8 or 25 cm depending upon whether the instrument was run under normal or reverse polarity conditions) with varying lengths of packed section. Thiourea was injected electrokinetically (15 s at 5 kV) at the anode. The applied voltage was either 10 or 20 kV, and 2 bar pressure 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 thiourea peaks were detected at 240 and 210 nm with a bandwidth of 10 nm and a detector response of 0.3 s. Detection in CEC was performed through the packing material. Unless otherwise indicated, the data presented typically represents an average of results obtained on two columns.

3. Results and discussion

3.1. Effect of column conditioning procedure in CEC

Two conditioning procedures for CEC have been outlined in the experimental section; one relies predominantly on pressure-driven flow, whereas the other utilises electrodrive. We have observed that columns conditioned initially on an high-performance liquid chromatography (HPLC) system cannot be installed in the instrument and used immediately since the currents generated are typically very unstable. In addition, repeated removal from the instrument and connection to the pump shortens the life of

the column. On the other hand, columns are often difficult to voltage condition from storage since they are not sufficiently ''wet'', and at low pH the EOF may not be sufficient to guarantee adequate conditioning within a reasonable time. The best option is to first ''wet'' the column on an HPLC pump, then to condition with each subsequent electrolyte by applying a voltage. However, there still remains the chief concern that when using ion-exchange materials as stationary phases, the column may not be at an ionic equilibrium and there may be memory effects as a result of retained ions from the previous analysis. The linear velocities generated on a SCX column (8 cm packed length) at different pH values are shown in Table 1. The trend observed for the change in linear velocity with electrolyte pH has been reproduced despite changing the running order of the electrolytes. The two sets of values are comparable and suggest that the approach adopted when changing electrolytes, i.e., voltage conditioning (10 kV for 30 min with 2 bar pressure on both vials), is sufficient to generate an equilibrated column even at low pH.

Table 1
Effect of column conditioning on the average linear flow velocities measured, at 10 kV, for a short SCX column at various electrolyte pH values[a]

Electrolyte ''pH''	Linear velocity ($\cdot 10^{-4}$ m s^{-1})	RSD (%) ($n=10$)
In order of pH		
10.5	5.60	5.5
7.5	2.80	2.2
2.9	8.97	2.0
Random order		
10.5	5.80	2.6
2.9	9.17	2.6
7.5	2.97	4.3

[a] CEC conditions: coupled capillary 33 cm (length to detector and packed length 8.5 cm)×100 μm I.D.×375 μm O.D.; temperature, 20°C; electrolytes of varying pH, ~10 m*M* ionic strength containing 80% (v/v) acetonitrile; electrokinetic injection, 15 s at 5 kV at anode; applied voltage, 10 kV reverse polarity, ramp 0.10 min; run pressure, 2 bar on both electrolyte vials; detection, UV absorbance through the packing material at 240 and 210 nm, bandwidth 10 nm and detector response of 0.3 s; sample concentration, 1–2 m*M* thiourea in acetonitrile–water (60:40, v/v).

3.2. Effect of column storage in CEC

The average linear velocity was measured at three pH values on a fully packed SCX column (total length=length packed). After flushing with acetonitrile–water (80:20, v/v) the column was stored with its ends in water; previous studies have highlighted that acetonitrile or acetonitrile–water mixtures attack the polyimide coating on fused-silica capillary, thus rendering the column more susceptible to breakage. After six weeks the column was re-conditioned by the initial HPLC then voltage procedure and the experiments repeated. The results shown in Table 2 indicate that storing a column in acetonitrile–water (80:20, v/v) would appear to have no detrimental effects on its subsequent performance.

3.3. Effect of length of packed section

3.3.1. Calculation of voltage drop in the packed section

A capillary column comprising a packed and an open section can effectively be considered as two resistors connected in series. By varying the ratio of the packed to open section, it should be possible to calculate the voltage drop in each section, and thus determine the individual contributions to the total EOF. Two methods were used to calculate the voltage drop over the packed section, V_p. In the first,

Table 2
Effect of column storage in CEC[a]

Electrolyte ''pH''	Linear velocity ($\cdot 10^{-4}$ m s^{-1})	RSD (%) ($n=10$, *$n=5$)
Before storage		
10.5	3.12	0.7
7.5	2.92	*1.8
2.9	2.71	2.2
After storage		
10.5	3.20	0.6
7.5	2.99	1.0
2.9	2.82	3.8

[a] Average linear velocities were measured at 10 kV on a fully packed SCX column. CEC conditions: as for Table 1 except fully packed column used, total length=length packed=33 cm (length to detector 8 cm).

the resistance of a fully open capillary gives the resistance of the open and subsequently packed section, R_o and R_p, respectively, whilst the second method uses the resistance of a fully packed capillary to directly calculate the resistance of the packed section. Throughout the calculations it has been assumed that the relationship

$$R = \frac{\rho L}{A} \quad (1)$$

also holds true for semi- and fully packed columns. L and A are the capillary length and cross-sectional area, respectively, whilst the proportionality constant, ρ, is known as the resistivity. The inverse of resistance is the conductance, G, and the inverse of the resistivity is the conductivity, κ.

3.3.1.1. Method (i)

Applying a voltage, V, across an open capillary of length L_T gives a measurable current, I, from which the resistance, R, is calculated using Ohm's law. Since resistance is proportional to length then the resistance, R_o, of an open section of length L_o, in a packed capillary is given by

$$R_o = R(L_o/L_T) \quad (2)$$

To maintain conservation of the current it follows that

$$I = \frac{V_o}{R_o} = \frac{V_p}{R_p} = \frac{V}{R_{o+p}} \quad (3)$$

Thus the total resistance of the packed column is given by

$$R_{o+p} = V/I = R_o + R_p \quad (4)$$

Knowing the applied voltage and resultant current when using this column gives R_{o+p}, from which the resistance in the packed section, R_p, can be determined. The voltage across the packed section is then given by

$$V_p = IR_p \quad (5)$$

3.3.1.2. Method (ii)

In a similar approach to Method (i), the resistance of a fully packed capillary of length L_T is used to provide R_p. For a packed capillary containing both open and packed sections, the proportionality of resistance to length has been assumed to be valid, and R_p is calculated in a like manner to Eq. 2. The voltage across the packed section is then given by

$$V_p = I(L_p/L_T)R \quad (6)$$

and

$$R_o = R_{o+p} - R_p \quad (7)$$

Both methods share the same major limitations: they rely on the measurement of the current generated in the partially packed capillary, which is typically in the low μA range, and they assume a linear relationship exists between resistance and length for packed and partially packed columns. This could potentially lead to a high degree of uncertainty in the calculations. In addition, the frits will have their own contributions to the resistance; for ease they have been assumed to be part of the column. The two methods of calculation did not give good agreement for values of V_p calculated for SCX packed columns (see Table 3), and sensible numbers were not always obtained. Technically, the second approach should be more valid since it takes into consideration any conductive nature of the particles. Calculating V_p and V_o independently is also subject to

Table 3
Variation of V_p for SCX packed capillaries at ''pH 7.5'' according to the method of calculation

Average length packed (%)	Calculated V_p (kV)	
	Method (i)	Method (ii)
73.5	7.5	6.4
74.1	9.5	27.9
72.0	3.8	14.7
51.5	4.6	5.4
47.4	3.1	5.7
25.8	3.5	2.1
25.1	−1.5	3.6
24.9	−0.4	3.2
25.9	−1.1	3.6

problems since the combined total voltage often exceeds that applied during the separation.

3.3.2. Effect of pH on column resistivity

For a given electrolyte, the resistivity of the packed and open sections should be constant regardless of their length. This is evident in Table 4 where the resistivities for column of varying length have been calculated for electrolytes of different pH. At the high pH both types of column exhibit similar resistivity in the packed sections, whereas at ''pH 7.5'' the resistivity is greater for the ODS1 packed column. At the low pH, results were not routinely obtained on the ODS1 column so a comparison cannot be made. The ratio of the resistivities of the packed and open sections provides information on the relative field strengths experienced. Combining Eq. 1 and Ohm's law with the electric field strength, *E*, gives for a packed column

$$E_p = \frac{V_p}{L_p} = \frac{I\rho_p L_p}{L_p A} = \frac{I\rho_p}{A} \quad (8)$$

It follows that, assuming conservation of current and identical cross-sectional areas in the two sections,

$$\frac{E_p}{E_o} = \frac{\rho_p}{\rho_o} \quad (9)$$

At the extremes of pH range it would appear that the electric field strength is far greater in the packed section, especially at the low pH. However, at the intermediate pH, the field strengths are similar in both sections.

3.3.3. Conductance in open and packed capillaries

In calculating conductance ratios, to minimise the effect of uncertainty in the current data, R_o and R_p were calculated independently (as opposed to calculating one then subtracting from R_{o+p}) then summed to give the total column resistance. G_T/G_o represents the ratio of the conductance of the whole column to that of the open section, and G_p/G_o represents the ratio of the conductances of the packed and open sections. Whilst positive values were obtained in all cases (Table 5), this approach neglected any interactions. At the high pH, the two types of column packing material showed practically identical behaviour. Although differences between them appeared as the pH was dropped, there was no reliable data for the ODS1 columns at the low pH to make a full comparison. The higher conductance at the low pH can be attributed to the presence of H^+, K^+ and Cl^- ions. With the exception of ''pH 7.5'' data, the total calculated conductance decreased as the length of the packed section was increased. The relationship is less defined though, if just the experimental voltage and current are used to calculate the conductance (Table 6). The conductance ratios G_T/G_o and G_p/G_o indicate that as the bed length is increased, the conductance of the open section becomes greater whilst that of the packed section

Table 4
Comparison of resistivities for open and packed sections of SCX and ODS1 packed columns at different pH values

Length packed (%) (approx.)	pH	3 μm SCX			3 μm ODS1		
		$10^{-9}\ \rho_p$ ($\Omega\ m^{-1}$)	$10^{-9}\ \rho_o$ ($\Omega\ m^{-1}$)	ρ_p/ρ_o	$10^{-9}\rho_p$ ($\Omega\ m^{-1}$)	$10^{-9}\ \rho_o$ ($\Omega\ m^{-1}$)	ρ_p/ρ_o
25	10.5	8.37	3.47	2.41	8.34	3.47	2.40
50		8.37	3.47	2.41			
75		8.37	3.47	2.41	8.34	3.47	2.40
25	7.5	6.84	7.37	0.93	10.01	7.37	1.36
50		6.84	7.37	0.93			
75		6.84	7.37	0.93	10.01	7.37	1.36
25	2.9	5.38	1.24	4.32			
50		5.38	1.24	4.32			
75		5.38	1.24	4.32			

Table 5
Summary of conductance and conductance ratios for capillaries packed to different lengths with either 3 μm SCX or 3 μm ODS1 material

Length packed (%) (approx.)	pH	3 μm SCX			3 μm ODS1		
		G_T ($\cdot 10^{-9}$ S)	G_T/G_o	G_p/G_o	G_T ($\cdot 10^{-9}$ S)	G_T/G_o	G_p/G_o
0	10.5	0.87			0.87		
25		0.65	0.55	1.22	0.63	0.56	1.26
50		0.52	0.30	0.42			
75		0.44	0.14	0.17	0.44	0.13	0.14
100		0.36			0.36		
0	7.5	0.41			0.41		
25		0.42	0.76	3.16	0.37	0.68	2.16
50		0.43	0.52	1.11			
75		0.44	0.28	0.40	0.33	0.20	0.26
100		0.44			0.30		
0	2.9	2.42					
25		1.33	0.41	0.68			
50		0.92	0.19	0.24			
75		0.72	0.07	0.08			
100		0.56					

Table 6
Comparison of total conductance values for 3 μm SCX columns[a]

Approx. length packed (%)	pH	G_T ($\cdot 10^{-9}$ S)	
		Calc.	Expt.
0	10.5	0.87	0.87
25		0.65	0.72
50		0.52	0.50
75		0.44	0.94
100		0.36	0.36
0	7.5	0.41	0.41
25		0.42	0.72
50		0.43	0.50
75		0.44	0.55
100		0.44	0.44
0	2.9	2.42	2.42
25		1.33	1.33
50		0.92	0.92
75		0.72	1.27
100		0.56	0.56

[a] Calculated data (Calc.) differs from the results obtained directly from the experimental data (Expt.) in that the resistances of the open and packed sections were determined independently then summed to give the total resistance.

becomes less. However, at ''pH 2.9'' the G_p/G_o ratios are approximately half of those at ''pH 10.5'', which suggests that the packed section contributes far less to the conductance; a factor we have attributed to unionised silanol groups on the packing material. The results at ''pH 7.5'' show that the length of the packed section has little impact on the total conductance of the column, and that the conductance of the open section does scale approximately with its length. At this pH the contributions of the open and packed sections are roughly equal when the capillary is half packed. However, the same effect at ''pH 10.5'' is only apparent when the capillary is about one quarter packed. Fig. 1 depicts the different trends observed in CEC for the effect of bed length according to how the results are presented. With the exception of results generated at low pH, it would appear that the EOF in a packed column is generally less than that observed in an open capillary. In terms of mobility, the effect of the bed length is unclear: a lot of scatter in the data points is evident, and there are no obvious trends. However, for linear velocity the EOF is fairly consistent at ''pH 7.5'', whilst at the extreme pH values the velocity decreases with length of packed bed. It is interesting to note that the EOF generated at the low pH is typically greater than

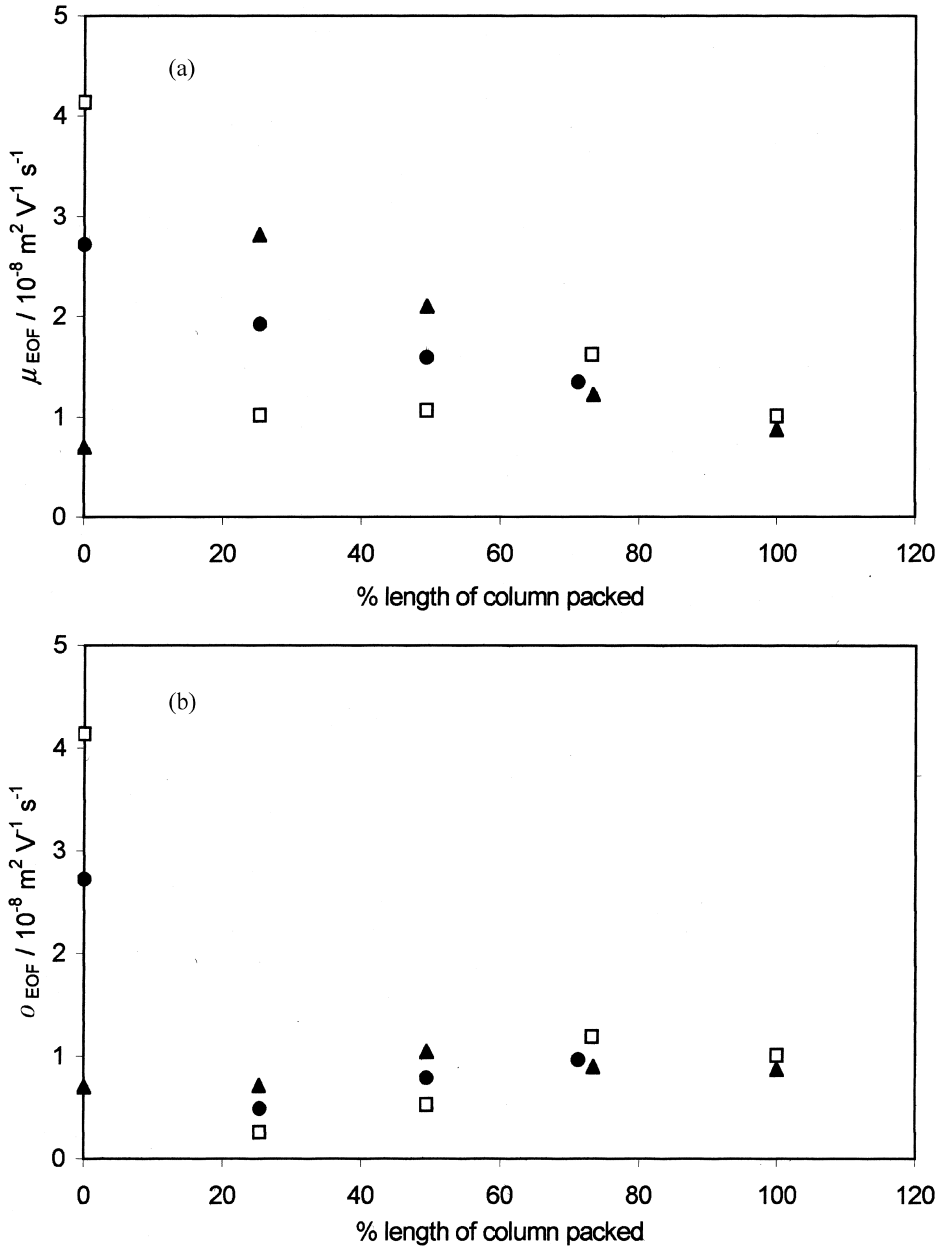
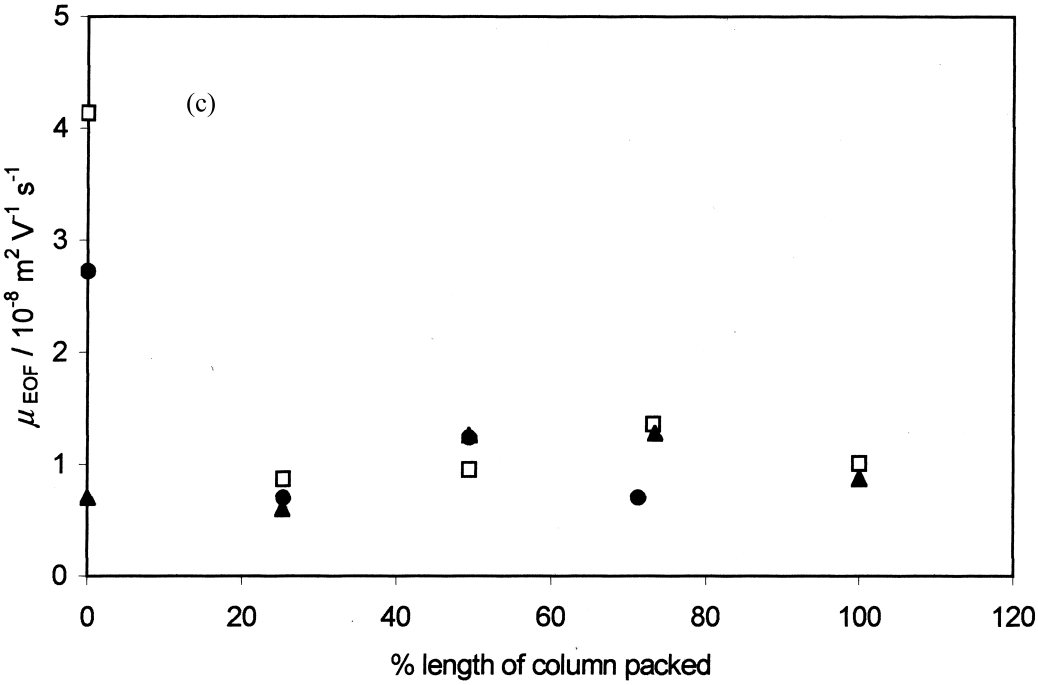


Fig. 1. Effect of length of SCX packed bed on the EOF at pH 10.5 (●), 7.5 (□) and 2.9 (▲). μ_{EOF} values calculated using the assumption that the voltage drop is (a) over the total length of capillary and (b) over the packed section only. For (c) the calculated voltage drop, V_p, over the packed section is used, whilst (d) shows the linear velocity values at 10 kV. CE conditions (i.e., 0% packed capillary): capillary, 33 cm (25 cm effective length)×100 μm I.D.×375 μm O.D.; pre-injection rinse, 1.5 min electrolyte; pressure injection, 2 s at 10 mbar; applied voltage, 10 kV (20 kV at low pH), ramp 0.01 min; analyte concentration, 0.5 m*M* thiourea in water. CEC conditions: capillaries (100 μm I.D.×375 μm O.D.), total length 33 cm (length to detector 8 or 25 cm depending upon instrument polarity), packed length variable; electrokinetic injection, 15 s at 5 kV; applied voltage, 10 kV, ramp 0.10 min; run pressure, 2 bar on both electrolyte vials; detection, through the packing material; sample concentration, 1–2 m*M* thiourea in acetonitrile–water (60:40, v/v). For both CE and CEC the capillary was thermostatted at 20°C; electrolytes of varying pH, ~10 m*M* ionic strength containing 80% (v/v) acetonitrile; detection, UV absorbance at 240 and 210 nm with a bandwidth of 10 nm and a detector response of 0.3 s.



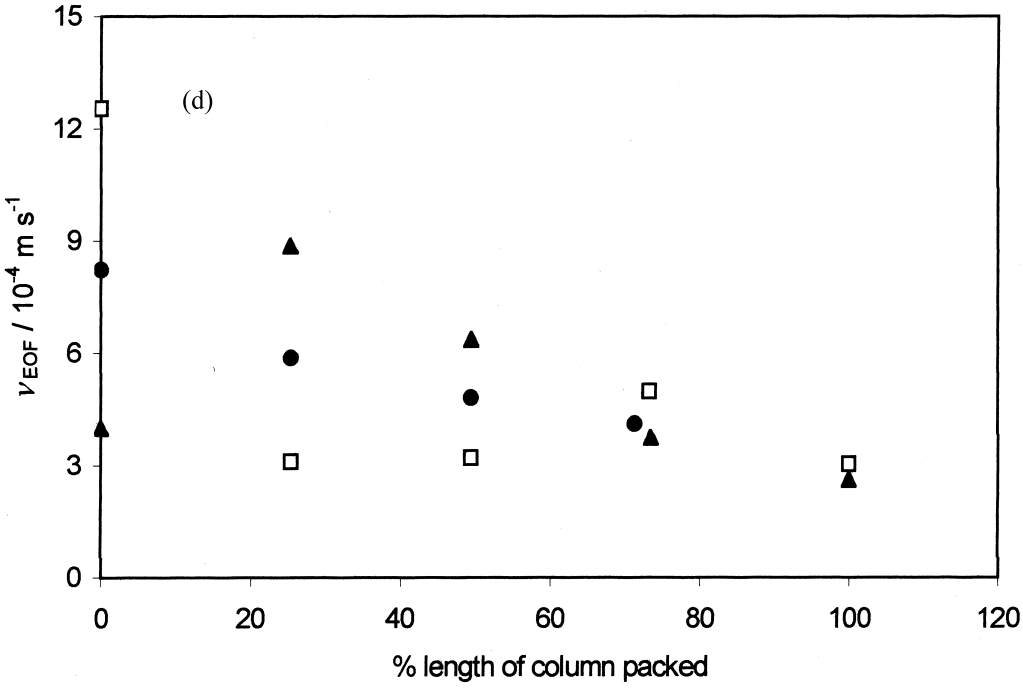


Fig. 1 (*continued*)

at the other pH values, yet there is little difference between the EOF generated within fully packed columns. This unexpectedly high EOF generated by an ion-exchange packing material has been predicted by Dukhin [20] and confirmed by Baran et al. [21]. Their model suggested that in columns packed with conductive particles, ''electroosmosis of the second kind'' could occur at high electric field strengths, as a result of polarization of the double layer. One of the conditions necessary to satisfy this model was that the conductivity of the packing material should be greater than the electrolyte. Since resistivity is the reciprocal of conductivity then the ratio ρ_p/ρ_o is equivalent to κ_o/κ_p. From Table 4, at low pH the conductivity ratio for the SCX column is 4.32; this means that the conductivity of the electrolyte is greater than the packing material, and the high EOF velocity cannot therefore be attributed to ''electroosmosis of the second kind''.

4. Conclusions

This study has shown that a greater understanding of the contributions of both packed and open sections is essential if the EOF is to be controlled in a reliable manner. The results obtained have demonstrated that widely differing packing materials can behave in a similar fashion under the right conditions, and that the length of the packed section is likely to become an important variable in CEC. However, the work has also highlighted discrepancies between theoretical and experimental observations for which there is no adequate explanation at present. Further investigations are to be encouraged in this area; the future of CEC may well depend upon them.

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References

[1] J.H. Knox, I.H. Grant, Chromatographia 24 (1987) 135.
[2] J.H. Knox, Chromatographia 26 (1988) 329.
[3] J.H. Knox, I.H. Grant, Chromatographia 32 (1991) 317.
[4] J.H. Knox, K.A. McCormack, Chromatographia 38 (1994) 279.
[5] M.G. Cikalo, K.D. Bartle, M.M. Robson, P. Myers, M.R. Euerby, Analyst 123 (1998) 87R.
[6] M.M. Robson, M.G. Cikalo, P. Myers, M.R. Euerby, K.D. Bartle, J. Microcol. Sep. 9 (1997) 357.
[7] A.L. Colón, G. Yong, A. Fermier, Anal. Chem. 69 (1997) A461.
[8] A.L. Colón, K.J. Reynolds, R. Alicea-Maldonado, A. Fermier, Electrophoresis 18 (1997) 2162.
[9] K.D. Altria, N.W. Smith, C.H. Turnbull, Chromatographia 46 (1997) 664.
[10] J.J. Pesek, M.T. Matyska, Electrophoresis 18 (1997) 2227.
[11] J. Ståhlberg, Anal. Chem. 69 (1997) 3812.
[12] A.S. Rathore, Cs. Horváth, J. Chromatogr. A 743 (1996) 231.
[13] A.S. Rathore, Cs. Horváth, J. Chromatogr. A 781 (1997) 185.
[14] Q.-H. Wan, J. Phys. Chem. B 101 (1997) 8449.
[15] Q.-H. Wan, J. Phys. Chem. B 101 (1997) 4860.
[16] G. Choudhary, Cs. Horváth, J. Chromatogr. A 781 (1997) 161.
[17] A.S. Rathore, Cs. Horváth, Anal. Chem. 70 (1998) 3069.
[18] D.D. Perrin, Aust. J. Chem. 16 (1963) 572.
[19] R.A. Robinson, R.H. Stokes, Electrolyte Solutions, Butterworths, London, 1965.
[20] S.S. Dukhin, Adv. Colloid Interface Sci. 35 (1991) 173.
[21] A.A. Baran, Y.A. Babich, A.A. Tarovsky, N.A. Mischuk, Colloids Surf. 68 (1992) 141.