

Capillary electrochromatography: effect of electrolyte concentration on electroosmotic flow and column efficiency

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Abstract

In current practice of capillary electrochromatography it is commonly assumed that the electroosmotic flow profile in a packed column is identical to that in an open tube. However, this assumption may not be valid in many cases due to thermal and double layer overlap effects. In this work, the dependence of electroosmotic mobility and plate height upon electrolyte concentration was determined for open tubular and packed column electrochromatography. As the electrolyte concentration increases over the range 0.04–1 mM, the electroosmotic mobility falls off steadily and the plate height shows a slight improvement in the open tube. In the packed column, by contrast, the electroosmotic mobility is almost unchanged and the plate height improves dramatically with increasing electrolyte concentration. The anomalous behavior of the packed column is interpreted as showing the thermal and double layer overlap effects, probably occurring in the densely packed segments of the column. © 1997 Elsevier Science B.V.

Keywords: Electrochromatography; Electroosmotic flow; Column efficiency; Mobile phase composition; Electric double layer overlap; Thermal effects

1. Introduction

The past several years have seen intensified efforts to develop the methodology of capillary electrochromatography (CEC), a new separation method based on combined principles of chromatography and electrophoresis [1–8]. In this method, electroosmotic flow induced by an electric field is used to transport the solutes through an electrolyte filled capillary column. It is generally assumed that the electroosmotic flow in CEC retains its plug profile even in the presence of chromatographic packing within the column, thus an unparalleled column efficiency is possible with this method. Several studies [5,8] have demonstrated that reduced plate height, a measure of column efficiency, as low as

unity is attainable for polycyclic aromatic hydrocarbons using columns packed with conventional silica gels. Despite enormous efforts, however, such high efficiencies have not been achieved on a routine basis in the current practice of CEC. This leads us to speculate that the electroosmotic plug flow may be perturbed in many cases due to experimental constraints such as thermal and double layer overlap effects.

Thermal effects are a result of Joule heating arising from an electric current passing through the electrolyte solution within the capillary tube [9,10]. Slow dissipation of heat from the tube wall to the surrounding air causes a radial temperature gradient within the capillary that results in a viscosity gradient in the liquid within the tube. This viscosity

gradient, in turn, causes radial variations in the electroosmotic flow profile. The mean electroosmotic flow-rate is increased in this case since electroosmotic velocities are inversely proportional to fluid viscosity. The thermal effects are most pronounced with high electrolyte concentrations because the heat production is directly proportional to the electrolyte concentration.

Double layer overlap is a phenomenon which may occur in very fine flow channels [3–5,11,12]. Under the conventional conditions that the flow channel is much greater than the double layer thickness, the electroosmotic velocity is independent of the flow channel diameter. However, when the flow channel diameter is small enough compared to the double layer thickness then the double layers begin to overlap in the center of the channel, resulting in radial variations in the electroosmotic flow profile and decreases in the mean electroosmotic flow-rate. In contrast to the thermal effects as mentioned above, the double layer overlap effects may become appreciable only when very low electrolyte concentrations are involved. This is because the double layer thickness is expanded in response to a decrease in electrolyte concentration in the bulk solution.

From the above considerations, it becomes evident that both thermal and double layer overlap effects may be reduced or even eliminated by proper choice of the electrolyte concentration. Thus optimization of this operating parameter in CEC is essential to remove factors interfering with the electroosmotic plug flow and to achieve further enhancement of separation performance. In this work, we used an untreated fused-silica capillary and a capillary column packed with octadecyl bonded silica to investigate the effect of the electrolyte concentration on the electroosmotic flow and column efficiency. In the open tubular capillary with its hydrophilic surface, no solute retention is expected for neutral analyte and, thus, the experiment may be viewed as a special case of electrochromatography where retention factor is zero. We have determined the plate heights and linear velocities at three different concentrations and compared the results from the open tube with those from the packed column. It is anticipated that if the electroosmotic plug flow is not disturbed to a marked extent, both columns should show comparable chromatographic and flow characteristics. Thus a com-

parison of the electrolyte concentration effects would allow the distinction of different factors contributing to flow perturbation and efficiency deterioration.

2. Experimental

2.1. Apparatus

Open tubular electrochromatography was conducted with a Model 270A-HT capillary electrophoresis system (Applied Biosystems, Foster City, CA, USA). A bare fused-silica tubing of 70 cm in length and 100 μm I.D. (Polymicro Technologies, Phoenix, AZ, USA) was used with an effective length (i.e., the distance between the inlet end and the UV detection window) of 50 cm.

Packed capillary electrochromatography was carried out with a laboratory-constructed system, which consisted of a Model R603/05P high-voltage power supply (Wallis, Worthing, UK), a Model 200 UV detector (Linear Instruments, Reno, NV, USA) and a Model 3394 integrator (Hewlett-Packard, Avondale, PA, USA).

2.2. Chemicals

Chemicals used as the test compounds and eluent components were of analytical grade purity or higher and used as received.

Eluents used throughout this work were prepared by mixing HPLC grade acetonitrile with aqueous sodium phosphate solutions (pH 7.0) in a ratio of 75:25 (v/v). Sample solutions were obtained by dissolving test compounds in appropriate eluents so that the same electrolyte concentration as that of corresponding eluent was expected.

2.3. Column preparation

The packed column used in this study was prepared by the following method. Firstly, a porous frit was made at the outlet end of a fused-silica tubing (34 cm \times 200 μm I.D.). The column end was downward tapped into a pile of 25–30 μm Zipax ODS spheres (DuPont, Wilmington, DE, USA). Once the particles were packed into the column to 0.5–1 mm, they were moistened with a dilute solution of sodium

silicate. This step was completed by heating the moistened particles at around 300°C for 30–60 s to give a strong sintered frit. Secondly, the capillary column was packed with 7 μm Zorbax BP C₈ spheres (DuPont) using the slurry packing technique. The slurry was prepared by dispersing the packing particles into acetonitrile in a ratio of 1:5 (w/w). After being transferred into a slurry chamber, which consisted of a stainless-steel tube (5 cm×2 mm I.D.), the slurry was pumped through the capillary column using a Model 303 LC pump equipped with a Model 803 manometric module (Gilson, Villiers, France) at constant pressure of 350 bar. The packing process was visually monitored under a microscope and mechanical shaking was applied to the slurry chamber using a Model V-74 electric engraver (Burgess, Grayslake, IL, USA) once a partial blockage appeared to occur. When the column was fully packed the pump was switched off and the pressure was allowed to release slowly by leakage. The column was then disconnected from the slurry chamber and the second frit was made at the inlet end of the column in the same way as for the outlet frit. Finally, fused-silica tubing (24 cm×100 μm I.D.) was connected to the outlet end of the packed column to facilitate UV detection at 254 nm. By using a capillary connector (Faculty of Chemistry, University of Amsterdam, Amsterdam, The Netherlands) as shown in Fig. 1, the packed column and the open tube were joined together in the narrow part of the connector. The connector was then sealed with an epoxy resin (Ciba-Geigy, Basel, Switzerland).

The column thus prepared was ready for testing when the resin was completely hardened.

2.4. Electrochromatographic measurements

Prior to carrying out electrochromatography, the column should be filled with an electrolyte of chosen concentration. For the open tube, the electrolyte solution in the inlet vial was forced through the column by applying a negative pressure to the outlet vial. However, for the packed column, a Model 414 LC pump (Tegimenta, Rotkreuz, Switzerland) was used to pump the eluent through the column continuously until no bubbles emerged from the outlet over an extended period of time. The columns filled with the eluent were installed into the electrochromatography systems with precaution so that no bubbles were introduced during this procedure. Both ends of the column were kept in contact with the eluent in two vials. The column was preconditioned with applied voltages between 10 and 20 kV until reproducible retention times for test compounds were obtained. The sample injection was made by an electromigration method in a usual manner.

Electroosmotic mobility (μ) and plate height (H) were used in this work to evaluate the effect of electrolyte concentration on electroosmotic flow and column efficiency. The electroosmotic mobility is defined as

$$\mu = v/E \quad (1)$$

where v is the linear velocity at the applied electric field strength E .

The linear velocity (v) is calculated from the migration time (t_0) of an unretained neutral solute by Eq. (2):

$$v = L_{\text{nom}}/t_0 \quad (2)$$

where L_{nom} is the nominal column length by which the solute travels, given as the distance between the inlet end and the detection window of the column.

The plate height (H) is calculated from the theoretical plate number (N) for a retained or unretained solute as follows:

$$H = L_{\text{eff}}/N \quad (3)$$

where L_{eff} is the effective column length by which electrochromatographic separation occurs. L_{eff} is the

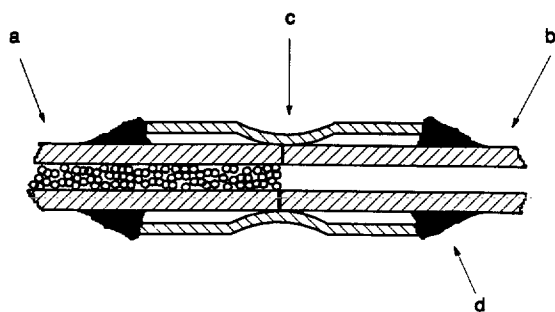


Fig. 1. Illustration of connection made between a packed column and an open tube. (a) packed column; (b) open tube; (c) capillary connector; (d) epoxy resin.

same as L_{nom} in the case of open tube but it is somewhat smaller than L_{nom} in the case of packed column where L_{eff} is the actual length of the packed column.

The plate number (N) is defined by

$$N = 5.54(t_{\text{R}}/W_{1/2})^2 \quad (4)$$

where t_{R} is the migration time of the solute, $W_{1/2}$ is the peak width at half peak height.

3. Results and discussion

3.1. Dependence of linear velocity upon applied field strength

With acetone as the unretained marker, the variations of linear velocity (v) with applied field strength (E) were determined for open tubular and packed column electrochromatography. Fig. 2 shows the results obtained with three different electrolyte concentrations in the range 0.04–1.0 mM. Attempts to work with an expanded concentration range were unsuccessful because problems such as bubble formation at higher concentrations and electric discharge at lower concentrations were encountered with the packed column used in this work.

It can be seen from Fig. 2a that a linear relation exists between the linear velocity and applied field strength in the open tube, which appears to be unaffected by the variation of electrolyte concentrations over the range investigated. This observation conforms to a theoretical expectation that in the absence of thermal and double layer overlap effects, the plot of linear velocity versus field strength ought to be straight, as shown in Eq. (5):

$$v = \epsilon_0 \epsilon_r \zeta E / \eta \quad (5)$$

where ϵ_0 is the permittivity of the vacuum, ϵ_r is the relative permittivity of the medium, η is the viscosity of the medium and ζ is the zeta potential of the flow channel. From the linear plot, it is evident that both the thermal and double layer overlap effects on electroosmotic velocity are insignificant under the conditions employed.

The variation of the linear velocity with the field strength for the packed column is shown in Fig. 2b.

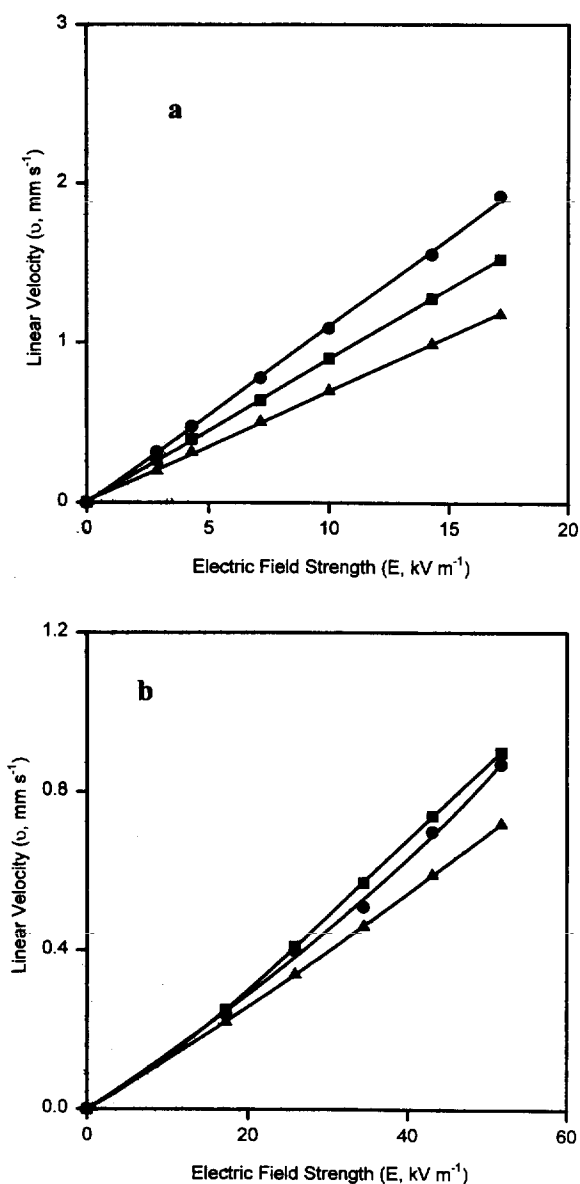


Fig. 2. Dependence of linear velocity upon electric field strength for open tube (a) and packed column (b). Electrolyte concentrations: (●) 0.04 mM; (■) 0.20 mM; (▲) 1.00 mM.

The overall trend is the same for all three different concentrations: a steady increase in the linear velocity with an increasing field strength. In contrast to that observed with the open tube, all the plots covering a range of field strength up to 52 kV m⁻¹ are curved. This curvature has been noted by Knox

and Grant [5] and was explained in terms of thermal effect. As can be seen from Eq. (6), the heat production (Q) in a packed column is influenced not only by the electrical properties but also by the packing structure:

$$Q = E^2 \lambda c \epsilon \gamma^2 \quad (6)$$

where λ is the equivalent conductivity, c is the electrolyte concentration, ϵ is the column porosity and γ is the tortuosity factor. Thus, the local heating effect can be more pronounced than the average due to varied packing densities of the column.

3.2. Dependence of electroosmotic mobility upon electrolyte concentration

The electrolyte concentration exerts its influence on the electroosmotic mobility mainly through the zeta potential (ζ) of the flow channel. According to electrokinetic theory [13], the electroosmotic mobility in part depends on the zeta potential which, in turn, depends on the electrolyte concentration. In the absence of double layer overlap effect, the electroosmotic mobility is given by

$$\mu = \epsilon_0 \epsilon_r \zeta / \eta \quad (7)$$

Comparison of Eqs. (5) and (7) indicates that unlike linear velocity, mobility is independent of the field strength applied and thus serves the purpose of evaluating the effects of electrolyte concentration at various field strengths.

As noted by several workers [14,15], the zeta potential can be related to the electrolyte concentration by an empirical equation:

$$\zeta = a - b \log c \quad (8)$$

where a and b are the empirical constants.

Combining Eqs. (7) and (8) then gives the electroosmotic mobility as a function of the electrolyte concentration:

$$\mu = \epsilon_0 \epsilon_r (a - b \log c) / \eta \quad (9)$$

From the above equation, it follows that plotting μ versus $\log c$ should yield a straight line.

However, the linear relationships mentioned above may not be observed in practice due to thermal and double layer overlap effects. It is a well known fact that the thermal effect causes a reduction in solvent

viscosity and consequently a positive deviation from the otherwise linear plots of μ versus $\log c$. On the other hand, a theoretical analysis [12] of the double layer overlap effect in CEC indicates that the electroosmotic velocity is reduced and therefore a negative deviation from the linear plot of μ versus $\log c$ is expected once the double layer overlap occurs in the flow channels. The thermal and double layer overlap effects operate in an opposite sense with respect to their influence on the electroosmotic mobility. In theory, it is thus possible to identify the dominant effects by examining the shape of the curve describing the dependence of the electroosmotic mobility upon the logarithm of electrolyte concentration.

In Fig. 3, the observed electroosmotic mobility is plotted against the logarithm of electrolyte concentration for open tubular and packed column electrochromatography. The plots display two distinctive trends. As the logarithm of molar concentration decreases, the electroosmotic mobility in the open tube increases linearly whereas the mobility in the packed column has a maximum value at an intermediate concentration, confirming an observation by Knox and Grant with drawn packed capillary columns [5]. The linear plot for the open tube is predicted by Eq. (9), suggesting that both thermal

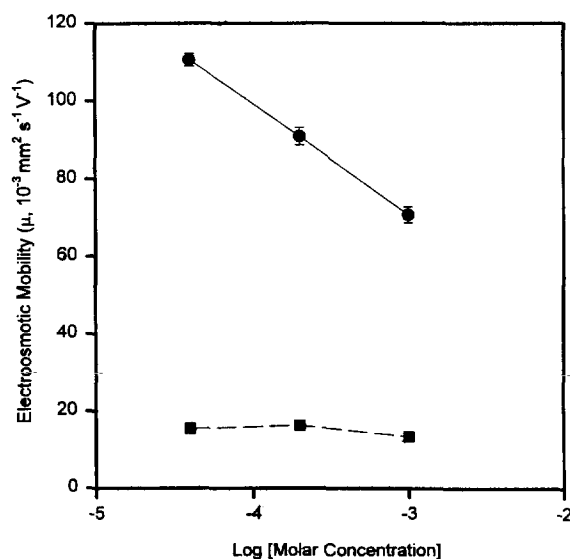


Fig. 3. Comparison of effect of electrolyte concentration on electroosmotic mobility for open tube (upper) and packed column (lower).

and double layer overlap effects are negligible over the range of electrolyte concentration investigated. On the other hand, the anomalous behavior of the packed column is interpreted as showing that the double layer overlap effect is operative in low electrolyte concentrations, giving rise to a fall in electroosmotic mobility with decreasing electrolyte concentration. Due to the heterogeneous nature of the packing, [16] double layer overlap is expected to occur only in the segments of the packing with highest packing densities where the flow channels are considerably smaller than the average channel diameters calculated from the mean particle size of the packing.

3.3. Dependence of plate height upon linear velocity

To a first approximation, the plate height (H) in all forms of chromatography can be given by the well known van Deemter equation [9]:

$$H = A + B/v + Cv \quad (10)$$

where A , B and C are constants corresponding to contributions from flow dispersion, molecular diffusion and mass transfer resistance, respectively. In open tubular electrochromatography the plate height equation for an unretained solute is simplified due to the absence of the contributions from the flow dispersion and mass transfer resistance. Thus, we have

$$H = B/v \quad (11)$$

The plate height decreases steadily with increasing linear velocity in this case. However, once the thermal or double layer overlap effects set in, a mass transfer resistance term must be included in the plate height equation:

$$H = B/v + Cv \quad (12)$$

Fig. 4 shows plate heights determined for acetone in the open tube and nitrobenzene in the packed column, with eluents containing sodium phosphate at a concentration in the range 0.04–1.0 mM. It can be seen from Fig. 4a that the observed plate height in the open tube decreases rapidly with an increase of linear velocity up to about 1.0 mm s^{-1} . Afterwards the change is slower, and even reversed as seen from

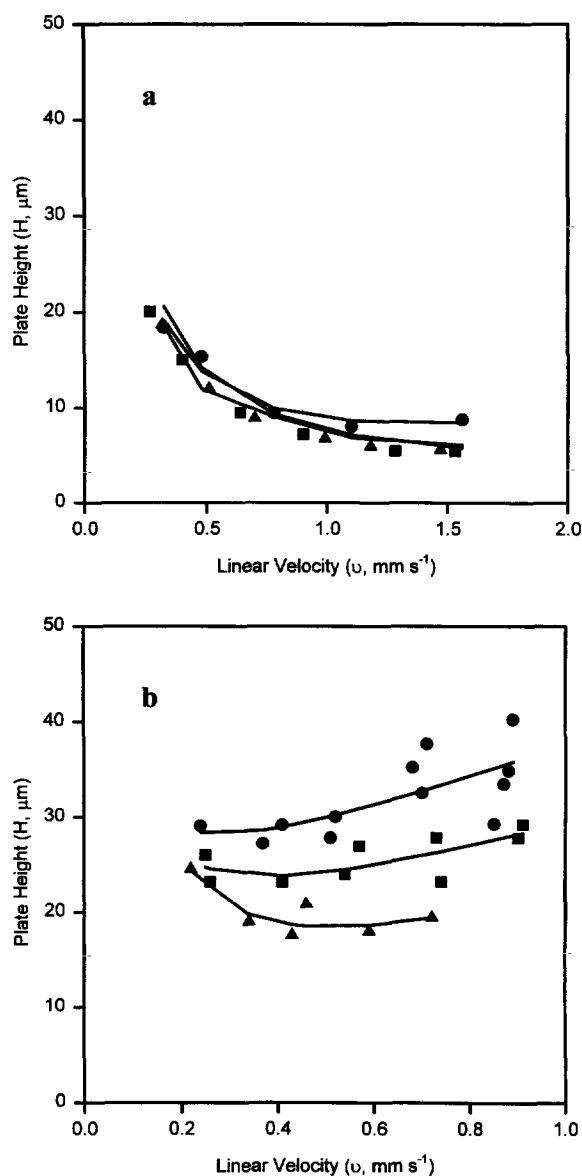


Fig. 4. Dependence of plate height upon linear velocity for open tube (a) and packed column (b). Electrolyte concentrations: (●) 0.04 mM; (■) 0.20 mM; (▲) 1.00 mM.

the upper curve for the electrolyte concentration of 0.04 mM. It is not yet clear what is the factor that causes the anomalous plate height curve for the lower electrolyte concentration. Nevertheless, the general velocity dependence of plate height is qualitatively in agreement with the trend predicted by Eq. (11), suggesting that the electroosmotic plug flow

within the open tube is largely undisturbed, especially for the velocities $v < 1 \text{ mm s}^{-1}$.

Fig. 4b shows that the plate height in the packed column varies with the linear velocity in a usual manner with the minimum occurring in the velocity range $0.4\text{--}0.7 \text{ mm s}^{-1}$. It is noted that the shape of the plate height curve shows slight variation for three different concentrations, though the data are increasingly scattered with decreasing electrolyte concentration.

With the observed plate height values, the constants A , B , C in Eqs. (10) and (12) were determined using a curve-fitting program provided by SigmaPlot for Windows (Jandel Scientific, San Rafael, CA, USA) and the results are given in Table 1. From these data it can be seen that the molecular diffusion term determines the plate height in the open tube whereas the flow dispersion and mass transfer resistance are the dominant factors in the packed column.

3.4. Dependence of plate height upon electrolyte concentration

Although the role of electrolyte concentration in influencing column efficiency is not immediately clear from the plate height equations for packed column and open tubular electrochromatography, its importance can be borne out by noting the fact that the plate height is strongly dependent upon the electroosmotic flow profile which, in turn, is dependent upon the electrolyte concentration due to thermal and double layer overlap effects. In the absence of these unfavorable effects, identical plate heights at the same linear velocity are expected for different electrolyte concentrations. In this case, the electrolyte concentration plays no part in determining the column efficiency.

In order to show the dependence of the plate

Table 1
Constants A , B , C in plate height equations determined by fitting to the experimental data

Concentration (mM)	Open tube		Packed column		
	B	C	A	B	C
0.04	5.93	2.97	17.76	1.49	18.45
0.20	5.48	1.19	12.57	2.11	14.79
1.00	5.86	0.97	2.65	4.02	15.58

height upon the electrolyte concentration, the plate heights at the same linear velocities which are close to optimal velocities, namely, 1.5 mm s^{-1} for the open tube and 0.5 mm s^{-1} for the packed column, were calculated from the respective plate height equations with the parameters given in Table 1. The results are shown in Fig. 5 as plots of plate height versus logarithm of molar concentration. It is evident that the electrolyte concentration has a much greater effect on the plate height for the packed column relative to the open tube. As the electrolyte concentration increases by a factor of 5 over the range $0.04\text{--}1.0 \text{ mM}$, the plate height decreases by approximately $5 \mu\text{m}$ for the packed column. On the other hand, the plate height decreases about $3 \mu\text{m}$ corresponding to the concentration increasing from 0.04 to 0.2 mM and remains unchanged with a further increase in concentration for the open tube.

These results indicate that the mechanisms by which the electrolyte concentration affects the column efficiency in CEC are more complicated than previously thought. We cannot offer a firm explanation for the observed dependence of the plate height upon the electrolyte concentration for the open tube, but we believe that the stronger dependence showing up for the packed column is at least in part due to a

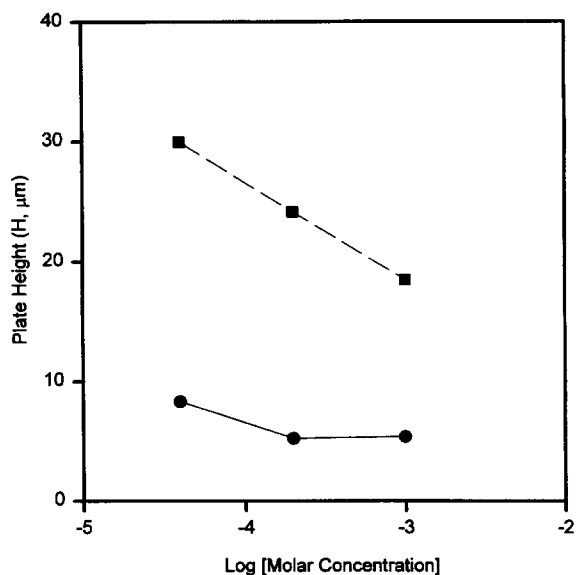


Fig. 5. Comparison of effect of electrolyte concentration on plate height for open tube (lower) and packed column (upper).

combination of thermal and double layer overlap effects as they both cause an increase in the plate height. As mentioned earlier, the thermal and double layer overlap effects are liable to occur in the densely packed segments of the column, precautions must be taken to ensure that there is no partial blockage occurring during the column preparation and operation in order to attain the electroosmotic plug flow and the high column efficiency in CEC without compromise.

3.5. Electrochromatography of neutral solutes

An example of the chromatograms obtained with the packed column is shown in Fig. 6. Using an eluent containing 75% acetonitrile and 1 mM sodium

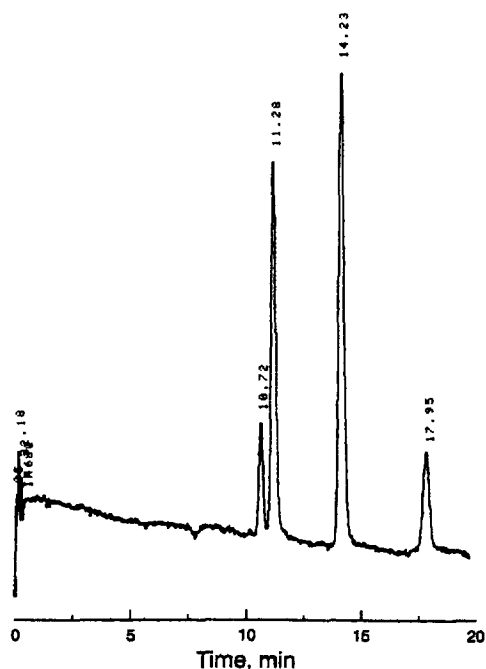


Fig. 6. Separation of neutral solutes by packed capillary electrochromatography. Conditions: column, 34 cm \times 200 μ m I.D. fused-silica tubing packed with 7 μ m Zorbax BP C_{18} , connected with 24 cm \times 100 μ m I.D. fused-silica tubing for UV detection (the length between the inlet end and detection window = 58 cm); eluent, acetonitrile–water (75:25) containing 1 mM sodium phosphate (pH 7.0); field strength, 44 kV m^{-1} ; detection, UV 254 nm. Solutes in elution order: (1) acetone; (2) phenol; (3) nitrobenzene; (4) toluene.

phosphate buffer (pH 7.0), a test mixture of acetone, phenol, nitrobenzene and toluene were nearly baseline separated with excellent peak symmetry. The analysis time was about 18 min at the applied electric voltage of 25 kV which produces a linear velocity of 0.6 $mm\ s^{-1}$. The average reduced plate height was calculated and found to be around 2.5. This column efficiency is indeed moderate as compared to unity for a well prepared column operated under comparable conditions, pointing to the possibility that the electroosmotic plug flow in this column is perturbed by heating or double layer overlap effects.

4. Conclusions

Theoretical considerations concerning the effects of electrolyte concentrations in CEC indicates that in the absence of thermal and double layer overlap effects, the electroosmotic mobility should decrease steadily with increasing electrolyte concentration, and that the column efficiency is essentially unaffected by varying the electrolyte concentration over a reasonable range. The experimental results from this work have proved that it is apparently the case with open tubes. However, a large deviation from these predicted trends has been observed with packed columns. Due to the heterogeneous nature of chromatographic packing, the flow channels within a packed column may vary widely, particularly with the packing materials of poor particle size distribution. Thus thermal and double layer overlap effects are likely to occur in the segments of higher packing densities, giving rise to a reduction in electroosmotic flow and column efficiency.

As has been demonstrated previously, CEC is capable of attaining extremely high column efficiency but this requires that the plug electroosmotic flow is essentially undisturbed even in the presence of the packing. From the above considerations, it follows that homogeneous packing structure comprised of monodispersed spherical particles is desirable, which will allow the development of an interference-free CEC system and a further enhancement of chromatographic performance.

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