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Towards the ultimate minimum particle diameter of silica packings in capillary electrochromatography

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Abstract

Porous silica beads with an average particle diameter between 0.2 and 3 μm have been applied as packing material in capillary electrochromatography (CEC). The experiments were directed to investigate whether it is really feasible and as promising as expected to use such small particles. In CEC, plate heights of $H \approx 1-2 d_p$ can be achieved which is smaller than the plate heights usually attained in high-performance liquid chromatography. Using a capillary packed with 0.5 μm silica beads we achieved a plate height of $H = 3 d_p$ indicating the presence of dispersive effects like Joule heating. Calculations demonstrate that at a field strength of about 800 V cm^{-1} one third of the plate height can be lost by Joule heating effects if the heat is not removed by a cooling system. Additionally, the $H(u)$ curve is still descending at the maximum electroosmotic flow (EOF) velocity we generated with the modified capillary electrophoresis instrument. To fully exploit the potential of submicron size silicas higher field strengths, i.e., higher EOF velocities, must be attained. To study the influence of the kind of packing on the EOF porous as well as nonporous silicas have been applied. The experiments clearly indicate that the EOF of porous and nonporous silicas is the same. Since the EOF is more or less exclusively generated by the packing material the zeta potential of *n*-octyl bonded 0.5 μm silica has been determined. The dependence of the zeta potential on the pH is identical to the dependence of the EOF on the pH in a packed capillary. The point of zero charge of the silica is at pH 2–3. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Silica packings; Electrochromatography; Particle diameter

1. Introduction

Capillary electrochromatography (CEC) has been a subject of intensive research to fully elucidate the separation potential of this hybrid technique. Combining a number of attractive features of the parent methods, micro-high-performance liquid chromatography (μ -HPLC) and capillary electrophoresis (CE), CEC requires a rational understanding in developing a separation protocol. Firstly, one has to take into account that the electroosmotic flow (EOF) transporting the analytes is not only dependant on the

applied electrical field strength but is also affected by the stationary and mobile phase properties. Secondly, the retention of analytes can be governed by surface–solute interactions as well as by electromigration. Depending on their charge, ionized compounds can elute ahead of or after the EOF marker. As a consequence the elution order of charged analytes in CEC can vary significantly from the elution order in HPLC.

The major focus of current research and development in CEC is directed towards the packing design, the column bed structure and the efficiency and stability of packed capillaries. Crosslinked polymers as well as bonded silicas are employed as packings

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in CEC [1]. From the users point of view 3 μm C₁₈ bonded silicas are preferred because there is hardly any porous silica commercially available with an average particle size, d_p , less than 3 μm . Recently, novel synthesis pathways have been proposed which enables one to manufacture microsize silica beads with $d_p \ll 3 \mu\text{m}$ [2]. The average particle size of these beads is sufficiently narrow that no further classification is required. The synthesis of the microsize silicas is based on a modified Stober method using templates as pore structure directing agents [3]. The adjustment of the reaction conditions offers a reproducible variation of the average particle diameter, d_p , in the range of 0.1 to 2.0 μm and the control of the specific surface area, the specific pore volume and the average pore diameter of the silica. In contrast to ordinary HPLC, microsize packing materials can be applied in CEC because there is no backpressure limit.

In this paper we report on the application of the above mentioned porous microsize silica beads with a particle diameter smaller than 3 μm . We also included *n*-octadecyl bonded nonporous 1.5 and 3 μm silica packings (MICRA Scientific) in this study. The experiments were directed to answer the following questions:

1. To what extent does the EOF change when the particle size of the reversed-phase packing is decreased from 3 to 0.2 μm ?
2. How large is the difference in the EOF velocity between CEC capillaries that are packed with porous and with nonporous reversed-phase packings?
3. What is the ultimate efficiency of capillaries packed with micron and submicron size reversed-phase packings?
4. What kind of dispersive phenomena contribute most to the total plate height of CEC capillaries?
5. How should the column design look in CEC when micron and submicron silica beads are applied?

2. Experimental

The laboratory-made silicas were synthesized and silanized as described elsewhere [4]. The nonporous packing materials were a donation from the former MICRA Scientific, Northbrooke, IL, USA. The

fused-silica capillaries (100 μm I.D. \times 360 μm O.D., CS-Chromatographie Service, Langerwehe, Germany) were packed either by the slurry technique (laboratory-made porous particles) or using supercritical carbon dioxide (nonporous silicas). Both packing techniques are also described in detail elsewhere [4,5]. The measurements were performed with a modified HP ^{3D}CE instrument. An external pressure of 10 bar was applied at both capillary ends to avoid degassing of the mobile phase during the experiments. The dimensions of the cartridge holder required a minimum capillary length of 33 cm.

3. Results and discussion

3.1. Dependence of the EOF on the average particle diameter of the packing material

The velocity of the EOF, u_{EOF} , is given by the Smoluchowski equation:

$$u_{\text{EOF}} = \frac{\epsilon_0 \epsilon_r \zeta E}{\eta} \quad (1)$$

where ϵ_0 is the dielectric constant of the vacuum, ϵ_r is the dielectric constant of the mobile phase, ζ is the zeta potential, E is the applied electrical field strength and η is the dynamic viscosity of the mobile phase. The velocity u_{EOF} is independent of the average particle diameter, d_p , of the packing material as long as d_p is greater than 40-times the thickness of the electrical double layer δ . The thickness of the electrical double layer δ is defined by Eq. (2):

$$\delta = \left(\frac{\epsilon_0 \epsilon_r RT}{2cF} \right)^{1/2} \quad (2)$$

where c is the concentration of the buffer solution and F is the Faraday constant. It is obvious that δ is affected by the concentration of the electrolyte which usually varies between 1 and 100 mM. Therefore, the minimum particle diameter that can be used is always related with the buffer concentration. Table 1 lists the thickness of the electrical double layer δ and the resulting minimum particle diameter at various buffer concentrations. According to Knox and Grant [6] the minimum particle size is assumed to be equal to about 40 δ .

In a previous paper we already reported on the

Table 1

Influence of the buffer concentration on the thickness of the electrical double layer and on the minimum particle size of the packing material

c (mol l ⁻¹)	δ (nm)	$d_{p,\min}$ (μm)
0.001	10	0.4
0.01	3	0.12
0.025	2	0.08
0.1	1	0.04

Table 2

Characteristic data of the native silicas determined by nitrogen-sorption measurements at 77 K^a

d_p (μm)	a_s (BET) (m ² g ⁻¹)	V_p (G) (ml g ⁻¹)	p_d (BJH) (\AA)
3	336	1.21	155
1	665	0.61	28
0.5	546	0.53	36
0.2	750	0.65	33

^aThe specific surface area a_s (BET) has been determined according to BET, the specific pore volume V_p (G) according to the Gurwitsch rule and the average pore diameter has been determined from the desorption branch of the nitrogen isotherm using the Barrett, Joyner and Halenda method. The values of p_d vary only slightly for silanized materials. The difference is about 2 \AA .

application of submicron size reversed-phase silica packings with a diameter of 0.2, 0.5 and 1 μm without compromising the EOF [7]. Since these materials were porous the question arises whether the EOF is exclusively generated at the outer surface of the particles or also at the inner surface of the silica beads. The average pore diameter of the reversed-phase packing is in the low mesoporous range (see Table 2) and is equivalent to the thickness of the double layer δ at a buffer concentration of 25 mM. According to Knox and Grant [6] a significant intraparticle flow can be excluded under these conditions. Contrasting with their opinion, other authors discussed the presence of an interparticle flow even at small pore diameters. Lobert and Engelhardt [8] studied the effect of the average pore diameter of silica packings on the EOF. They noticed an increase of the EOF with increasing pore diameter under otherwise constant conditions.

Facing these results, the question arises whether the EOF will be affected when nonporous silicas instead of porous packings are applied. Fig. 1 clearly indicates that nonporous *n*-octadecyl bonded packings generate an EOF as high as mesoporous packings. The absence of a porous structure does not influence the EOF velocity. This implies that the

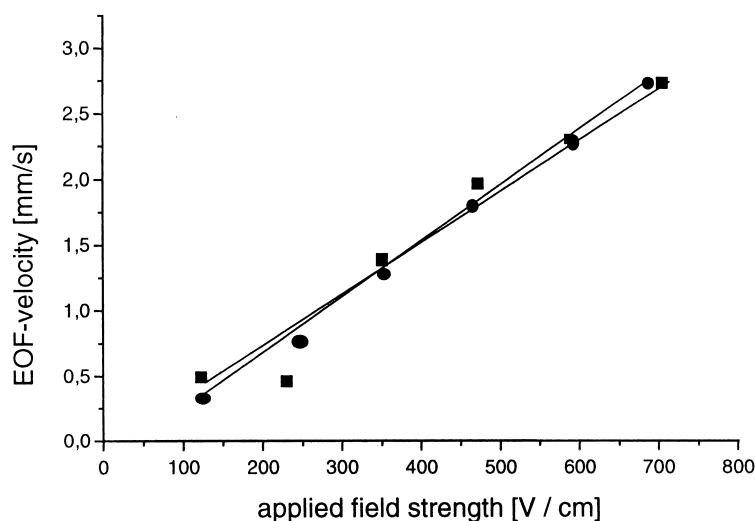


Fig. 1. Influence of the particle diameter of nonporous *n*-octadecyl bonded silica packings on the EOF. Mobile phase: ACN–5 mM Tris–HCl, pH 8 (80:20); capillary 1: 31.5 cm (effective length 23.5 cm) \times 100 μm , packed with MICRA NPS polymeric ODS 3 μm (square), capillary 2: 34 cm (effective length 26 cm) \times 100 μm , packed with MICRA NPS polymeric ODS 1.5 μm (circle).

EOF of a porous packing is not only generated between the particles but also within the pores of the silica. To fully answer that question further studies are required.

3.2. Influence of the zeta potential of the reversed-phase silica packing on the EOF

In contrast to CE, the EOF in a packed fused-silica capillary is more or less exclusively generated by the packing material, i.e., by the residual silanol groups at the surface of the silica particles [9]. Since the amount of these silanol groups is strongly dependent on the pH of the mobile phase the EOF is also affected by the pH. At high pH values the silanol groups are deprotonated leading to a high EOF. At a pH between 2 and 3, which is the pH of zero charge of the silica beads, the EOF is only about 0.5 mm s^{-1} . This is in agreement with independent measurements of the zeta potential of reversed-phase silicas as a function of pH. In these experiments the zeta potential has been determined by measuring the

electrophoretic mobility of the silica with laser doppler anemometry.

Fig. 2 clearly indicates that there is nearly no hysteresis between the descending and the ascending pH curves. Measuring the same dependency on unpacked fused-silica capillaries a pronounced hysteresis is observed [10]. Experiments by Schwer and Kenndler [11] have shown that the zeta potential of fused-silica capillaries reaches a value of about -120 mV at pH 9 whereas the zeta potential of the *n*-octyl bonded silica is only about -70 mV . This corresponds to the observed zeta potential for coated capillaries [11]. Besides the pH of the buffer solution the zeta potential of an unpacked fused-silica capillary as well as the zeta potential of the *n*-octyl bonded silica is also affected by the acetonitrile content of the mobile phase. Since the increase of the acetonitrile concentration decreases the zeta potential of a bare fused-silica as well as the one of a coated capillary [12] the increase in EOF with increasing acetonitrile content in a packed capillary should be attributed to the decrease of the hydronium ion

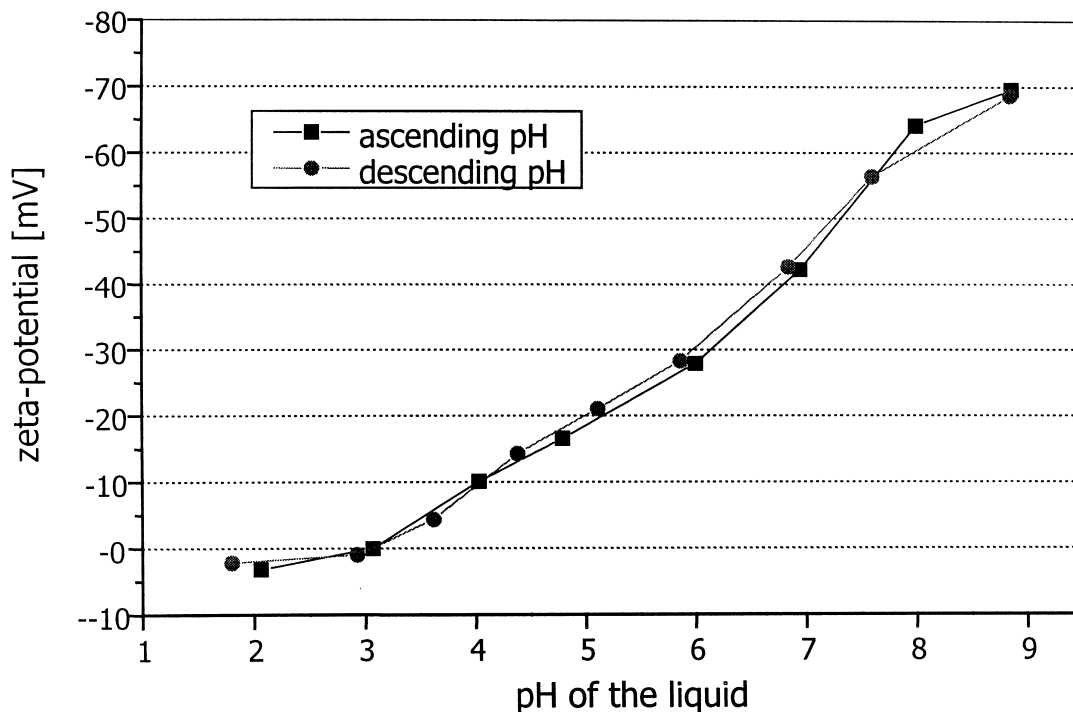


Fig. 2. Influence of the pH value of the liquid on the zeta potential of *n*-octyl bonded silica. Silica: $0.5 \mu\text{m C}_8$ suspended in ACN–25 mM Tris–HCl (80:20, v/v).

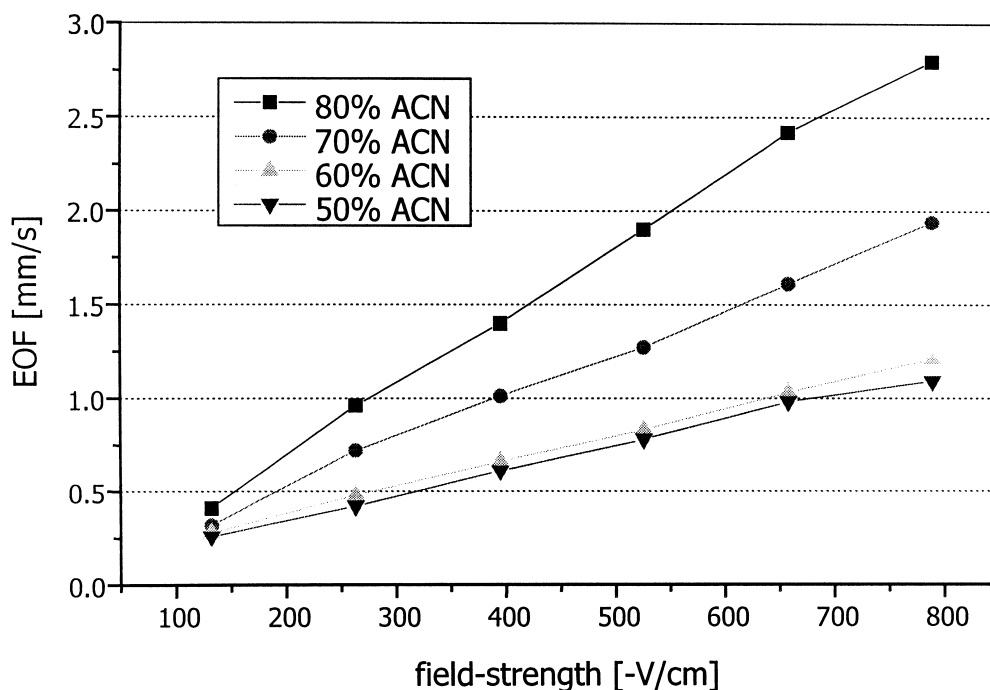


Fig. 3. Influence of the acetonitrile content of the mobile phase on the EOF of a packed fused-silica capillary. Capillary: 38 cm (effective length 8.5 cm) \times 100 μ m, packed with 0.5 μ m C_8 ; mobile phase: $x\%$ ACN–(100– $x\%$) 25 mM Tris–HCl, pH 8, 20°C, analyte: thiourea.

concentration with increasing acetonitrile content (Fig. 3).

3.3. Efficiency of CEC capillaries packed with *n*-octyl bonded silicas with an average particle diameter between 0.2 and 3 μ m

The plate height concepts developed for HPLC have simply been transferred to CEC neglecting the effect of the electrical field on mass-transfer phenomena. Dittmann et al. [13] discussed the individual contributions to total plate height in CEC based on the model of Horvath and Lin [14]. Knox [15] has described the effect of Joule heating on the column performance in CE and CEC.

Experimental data have shown that the minimum plate height in CEC amounts to about $H \cong 1\text{--}2 d_p$ whereas in HPLC the minimum value is $2 < d_p < 5$. Typically, for capillaries packed with 3 μ m Hypersil ODS material plate numbers of about 200 000 per meter column length were achieved [16–19]. The higher separation efficiencies in CEC can be attributed to the Eddy-diffusion term which is much smaller

than in pressure-driven systems. The plate height in CEC can be calculated by an equation proposed by Horvath and Lin [14]:

$$H = 2\lambda d_p + \frac{2\gamma D_m}{u} + 55 \cdot 10^{-3} d_p^2 \quad (3)$$

where d_p is the average particle diameter of the packing material in μ m, λ is the structural parameter, γ is the tortuosity and D_m is the diffusion coefficient of the analyte in the mobile phase. From this equation it is evident that the Eddy-diffusion term, A , and the mass transfer term, C , decrease with decreasing particle size whereas the axial diffusion term, B , is independent of the particle diameter. According to Eq. (3), the plate height H for a capillary packed with 0.5 μ m silica beads is about 0.6 μ m. The experimentally obtained data for the plate height, H , of such a capillary are three-times higher indicating the presence of dispersive effects like Joule heating or inequalities in the packing. Extra column contributions may also arise from the injection and detection system. It is well known that current UV on-column detection systems lack sen-

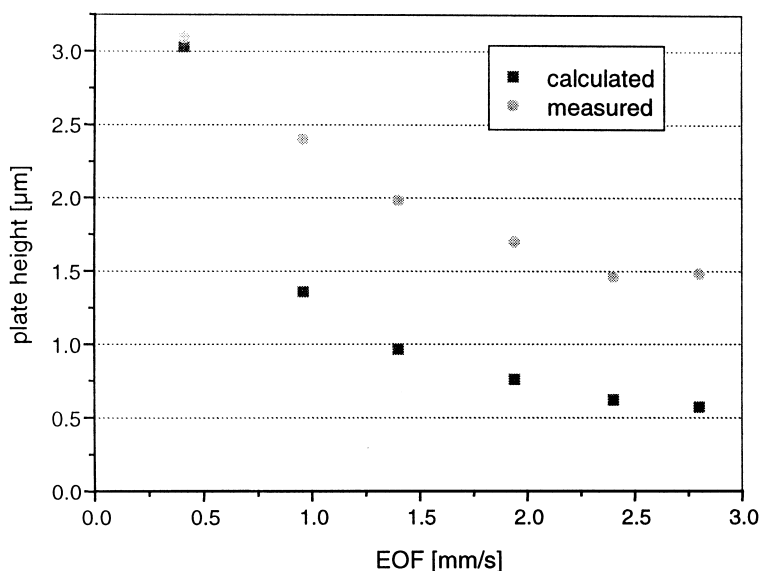


Fig. 4. Calculated and measured $H(u)$ curves for *n*-octyl bonded $0.5 \mu\text{m}$ silica. Calculated curve: according to Eq. (3), parameters used according to Table 4; measured curve: capillary: 38 cm (effective length 8.5 cm) \times $100 \mu\text{m}$, packed with $0.5 \mu\text{m}$ C_8 ; mobile phase: ACN–25 mM Tris–HCl, pH 8 (80:20), 20°C , analyte: thiourea.

sitivity and may decrease column efficiency. From Fig. 4 it is also evident that the $H(u)$ curve for the $0.5 \mu\text{m}$ silica is still descending at EOF velocities usually generated in CEC. This holds for both the measured and the calculated curve. To achieve the minimum plate height higher EOF velocities in the order of $4\text{--}5 \text{ mm s}^{-1}$ must be attained.

The values listed in Table 3 clearly indicate that at a moderate EOF of about 3 mm s^{-1} (which has been generated with the materials listed in Table 3) the plate height in CEC is mainly determined by the axial diffusion especially when submicron size silicas are applied. The contribution of the A and the C terms to the total plate height can be neglected at particle diameters $\leq 1 \mu\text{m}$. Since the axial diffusion is independent of the particle diameter of the packing a further decrease in particle size down to $0.5 \mu\text{m}$

will hardly affect the plate height, H . It must be emphasized that this statement holds only at moderate EOF velocities of about 3 mm s^{-1} . As soon as higher EOF velocities are generated the dominant effect of the axial diffusion diminishes due to the increase of the A and C terms. At EOF velocities of about 4 mm s^{-1} the influence of the particle size of the packing material on the plate height becomes more pronounced leading to improved column performance when submicron size silicas are applied. Unfortunately, we were not able to generate such high velocities due to the limitation in the minimum column length. To fully exploit the potential of submicron size silica packings it is mandatory to achieve these high velocities by reducing the total capillary length and/or by the application of higher voltages.

Table 3

Contributions of the A , B and C terms to plate height at a particle size of (a) $3 \mu\text{m}$, (b) $1 \mu\text{m}$, (c) $0.5 \mu\text{m}$ ^a

d_p (μm)	u (mm s^{-1})	H (μm)	A (μm)	B/u (μm)	Cu (μm)
3	3.0	2.76	0.87	0.40	1.49
1	3.0	0.86	0.29	0.40	0.165
0.5	2.8	0.61	0.14	0.43	0.036

^a The constants used are: $\gamma=0.6$, $\lambda=0.1$ and $D_m=10^{-9} \text{ m}^2 \text{ s}^{-1}$.

The efficiency of a CEC separation is not only affected by the dispersion effects described by the van Deemter equation but also by additional contributions like Joule heating. The heat that is generated in a packed column in CEC is about 1500-times higher than in HPLC (depending on the conductivity of the mobile phase) due to the high voltages that are applied at the capillary. The temperature in the center of the capillary is higher than at the capillary walls leading to a parabolic flow distribution which is superimposed on the actual flat flow profile. The dispersive effect of the flow profile disturbance on the efficiency of a packed CEC capillary is described by the following equation [14]:

$$\Delta H = \frac{7 \cdot 10^{-9} u_{\text{EOF}} \lambda^2 \epsilon^2 d_c^6 E^4 c^2}{D_m \kappa^2} \quad (4)$$

where ϵ_r is the dielectric constant of the mobile phase, ϵ_0 is the dielectric constant of the vacuum, ϵ is the total porosity of the packed capillary, ζ is the zeta potential, d_c is the inner diameter of the capillary, E is the applied electrical field strength, c is the concentration of the electrolyte, D_m is the diffusion coefficient of the analyte in the mobile phase, η is the viscosity of the mobile phase and κ is the thermal conductivity.

The main parameters that influence the degree of dispersion are the inner diameter of the capillary, the concentration of the electrolyte and the applied field strength. Table 4 lists the calculated thermal contribution, H_T , and the plate height that should be generated at EOF velocities between 0.4 and 2.8 mm s⁻¹. The value of H_T drastically increases at an electrical field strength $E > 400$ V cm⁻¹. At a field

strength, E , of 790 V cm⁻¹ the contribution of H_T is about one third of the overall plate height (Table 4). Despite the fact that this effect is independent of the particle size of the packing material it becomes much more pronounced at small particle sizes because plate height itself decreases with decreasing particle size. To obtain maximum column performance it is mandatory that the thermal effects are controlled and reduced. This can be achieved by optimizing the inner diameter of the capillary and the buffer concentration. Cooling might also be a solution but one has to be aware that it does not help against radial temperature differences.

3.4. Optimization of a CEC separation with respect to column efficiency and analysis time

As in HPLC, the efficiency and the analysis time of a separation not only depends on the type of mobile and stationary phase but also on the instrumental set-up. To achieve high plate numbers and short analysis times high electrical field strengths are required:

$$N = \frac{L}{H} = \frac{L \epsilon_0 \epsilon_r \zeta E}{2 D_m \eta} \quad (5)$$

where ϵ_0 is the dielectric constant of the vacuum, ϵ is the dielectric constant of the mobile phase, ζ is the zeta potential, E is the applied electric field strength, D_m is the diffusion coefficient of the analyte in the mobile phase, η is the viscosity of the mobile phase and L is the length of the capillary [15].

The electrical field strength is determined by the maximum voltage the power supply of the instrument can deliver and the total length of the capillary. Most of the commercially available CEC instruments are modified CE instruments. Those instruments usually requires a minimum total capillary length of about 25 cm which is far too long for the generation of high-speed and high-performance CEC separation. Some authors [20–22] have already successfully demonstrated that it is possible to perform CEC separations with capillaries having a packed bed < 10 cm. Therefore from the users point of view the total length of the capillaries can easily be reduced to < 10 cm. From the engineers point of view this gives rise to some problems because a decrease in column

Table 4
Thermal contribution to plate height at various field strengths^a

E (V cm ⁻¹)	H_T (μm)	H (μm)	H_T/H
132	$5 \cdot 10^{-6}$	3.00	$1.6 \cdot 10^{-6}$
263	0.0002	1.36	$1.5 \cdot 10^{-4}$
395	0.004	0.98	$4.1 \cdot 10^{-3}$
526	0.02	0.78	0.03
658	0.075	0.65	0.12
789	0.225	0.61	0.37

^a The constants used are: $\epsilon_r = 41.07$, $\epsilon_0 = 8.85 \cdot 10^{-12}$ C² J⁻¹ m⁻¹, $\zeta = 50$ mV, $\lambda = 0.015$ m² mol⁻¹ O⁻¹, $\epsilon = 0.75$, $d_c = 100$ μm, $c = 25$ mM.

length will lead to an increase in field strength and therefore to higher Joule heating. To implement CEC as a routine method in chemical and pharmaceutical industry it is imperative to overcome these problems and to develop a conveniently designed CEC systems which is robust enough for routine use.

4. Conclusions

The application of submicron size silica beads as packing seems to be a promising tool to increase the efficiency and the velocity of a CEC separation. So far, the use of these particles is restricted due to instrumental limitations. To fully exploit the potential of the submicron size materials it is mandatory to develop a miniaturised CEC system that allows the use of capillaries with a total length much shorter than the ones commonly used and that offers improved injection and detection units.

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