ELSEVIER

Journal of Chromatography A, 737 (1996) 243–253

JOURNAL OF CHROMATOGRAPHY A

Rectangular capillary electrophoresis: study of some dispersive effects

A. Cifuentes*, M.A. Rodríguez, F.J. García-Montelongo

Departamento de Química Analítica, Bromatología y Toxicología, Facultad de Químicas, Universidad de La Laguna, Avenida Trinidad s/n, 38204 La Laguna (Tenerife), Spain

First received 10 October 1995; revised manuscript received 18 December 1995; accepted 29 December 1995

Abstract

Different phenomena occurring during capillary electrophoretic (CE) separations (i.e., heat generation, spontaneous injection, siphoning effect and adsorption of the solute on the capillary wall) were investigated in cylindrical and rectangular capillaries of large cross-sectional area. The equation that relates the plate height to the extent of adsorption in a rectangular duct is shown and compared with that for cylindrical capillaries. Theoretical and experimental results obtained with capillaries of both geometries are compared. The advantages and drawbacks of using rectangular capillaries in micropreparative CE are discussed. A simple modification to the commercial CE instrument used in this work allowed the easy installation of rectangular capillaries within the apparatus.

Keywords: Heat generation; Adsorption; Siphoning effect; Rectangular capillaries; Dispersive effects

1. Introduction

Tiselius [1] suggested in 1937 the use of rectangular geometries in electrophoresis, but only recently [2–5] has the employment of rectangular geometries in capillary electrophoresis (CE) been addressed. Briefly, the latter works have shown that: (a) better heat dissipation can be obtained by using rectangular columns [1–3] instead of circular columns, allowing faster separations [5]; (b) the width of the rectangular capillaries can be increased without altering their heat dissipation, increasing sample capacity [3,4]; and (c) rectangular ducts show better sensitivity for path-length-dependent detection systems (UV absorption, fluorescence, etc.) [4]. Also, it has been theoretically discussed that the so-called siphoning effect and spontaneous injection should be less critical using rectangular columns [5]. Mostly, the advantages of using rectangular ducts arise from their narrower characteristic dimension, i.e., height, compared with that of circular capillaries, i.e., diameter, for tubing of equal cross-sectional area [3].

CE performed in circular capillaries coupled to electrophoresis using rectangular cross-sections has recently been described for continuous electrophoretic separations [6]. Also, the use of micromachined channels (typically from 10×30 μm up to 10×100 μm) on a glass chip has

* Corresponding author. Present address: Instituto de Química Orgánica (CSIC), Juan de la Cierva 3, 28006 Madrid, Spain.

0021-9673/96/$15.00
PII S0021-9673(96)00002-7

shown interesting possibilities for obtaining fast separations in CE, using very high electric fields in miniaturized systems [7–9]. In two recent studies [5,10], the use of rectangular capillaries in CE has been shown to be a very promising tool for the separation of substances on both analytical and micropreparative scales. Experimental evidence was given about the possibility of obtaining faster separations with rectangular ducts than with cylindrical tubing while maintaining efficiency. Also, it was shown theoretically that higher production ratios in micropreparative scale should be accessible by using rectangular capillaries [10].

In two different studies [5,11], the effect of the pressure-induced flow arising from the difference between the buffer levels in the vials at the capillary ends was discussed. This pressure-induced flow, known as hydrostatic flow or the siphoning effect, was shown to have a negative effect on the efficiency as a result of its parabolic velocity profile. Moreover, it was predicted theoretically that the larger the capillary radius and the greater the difference between the buffer levels, the poorer is the efficiency obtained. However, it has been predicted theoretically that this negative effect on the efficiency can be dramatically reduced by using rectangular capillaries of equal cross-sectional area to that of a circular capillary [5].

On the other hand, the electrostatic adsorption phenomenon occurring in CE between the fused-silica capillary wall, negatively charged, and analytes bearing positive charge seems to be the main cause of the decrease in efficiency observed for this type of compounds [12]. Thus, for the same separation conditions, the higher the number of positive charges on the analyte the poorer is the efficiency obtained, as usually observed for basic proteins [13,14]. Furthermore, analyte adsorption on the internal surface of the capillary can cause poor reproducibility of migration times and low analyte recoveries.

Many methods have been developed in CE to reduce the interaction between analytes and the silica surface (see Refs. [15–17] for up-to-date reviews). However, little theoretical work has been done in CE on the adsorption phenomena occurring between analytes and the capillary wall [18–20]. Moreover, to our knowledge, none of these studies, either theoretical or experimental, has dealt with the influence of the capillary geometry on the adsorption phenomenon occurring in CE.

The aim of this work was to provide more insight into the advantages and drawbacks arising from the use of rectangular geometries compared with those using cylindrical ones. Thus, circular and rectangular geometries were compared in terms of heat dissipation, spontaneous injection and siphoning effect. Also, a comparative study on the adsorption process occurring in rectangular and circular capillaries is shown. The equations describing the band broadening associated with both the adsorption and the siphoning effect in capillaries of both geometries are shown.

2. Experimental

2.1. Instrumentation

Two different CE instruments were employed to carry out the experiments. A laboratory-made instrument as described previously [21] was used for measuring the heat generation using forced air as cooling fluid. A P/ACE 2000 HPCE electrophoresis apparatus (Beckman, Fullerton, CA, USA) controlled by a PS/2 486 computer was used for the rest of the experiments. The P/ACE cartridge cover where the capillary is placed had to be slightly modified in order to allow the installation of the rectangular column. The modification is described below.

The fused-silica capillaries (Composite Metal Services, Worcester, UK) were circular with 180 μm I.D. and 360 μm O.D. (90 μm wall thickness) and rectangular with 500 μm width and 50 μm height with wall thickness ca. 100 μm, both with a polyimide external coating and similar cross-sectional areas. Capillaries with 50 cm total length and 25 cm effective length were used in the laboratory-made apparatus and capillaries with 27 cm total length and 20 cm effective length in the Beckman system (note that in this

instrument 4 cm of the effective length of the capillary is not cooled by the liquid). All the injections were carried out at the anodic side using controlled time and voltage. Rectangular columns were positioned so that detection was across the 500-μm axis of the capillary. Detection took place at 214 nm. All the data were collected and analysed using System Gold software from Beckman.

2.2. Samples and chemicals

Horse and sperm whale myoglobins, bovine α-lactalbumin, substance P fragment 1–7 (all from Sigma, St. Louis MO, USA) and 2-methylpyridine (from Merck, Darmstadt, Germany) were used as samples. The compounds were dissolved at the concentrations indicated in each case in water purified with a Milli-Q water system (Millipore, Bedford, MA, USA). Samples were stored at −5°C and heated to room temperature before use. Formic acid, acetic acid (both from Merck) and 2-(N-cyclohexylamino)ethanesulfonic acid (CHES) (from Sigma) were used as received in the different running buffers. The pH of these solutions was adjusted using 1 mol/l sodium hydroxide solution.

2.3. Methods

The cartridge of the P/ACE system where the capillaries are held, was slightly modified in order to allow the installation of the rectangular geometry. Originally the cartridge cover showed a prominence which presses part of the capillary when the cartridge is closed. The role of this prominence seems to be to improve the cooling process by reducing the dead volume around the capillary. However, when the cartridge is being closed when holding a rectangular column inside, the prominence breaks the column. The modification consisted in removing this prominence by making new cartridge covers. The rest of the characteristics and dimensions of the cover were as provided originally by the manufacturer. All the experiments performed using the P/ACE 2000 system were carried out with the modified cover.

In order to condition both types of capillary in a similar way, the columns were initially rinsed with 0.1 mol/l sodium hydroxide solution for 30 min. Each time a different buffer was used it was flushed through each capillary for 15 min.

To carry out the study of spontaneous injection, the P/ACE instrument was used under manual control so that both types of capillary were in contact with the sample for 2 s. The time between transferring the capillary inlet from the sample solution and reinserting it in the inlet vial was 15 s. In order to prevent hydrodynamic injection, the meniscus in the outlet vial (vial 1) was controlled to be at the same height as the meniscus of the sample solution (vial 2). To keep a constant level of liquid in these two vials, an outlet vial (vial 3) different from that used before was used during the separation. Once the experiment had been performed with the rectangular column, the cartridge was exchanged for another one with a circular capillary inside and the experiment was repeated under identical conditions. This operation was repeated three times for each capillary.

3. Results and discussion

3.1. Heat generation

It has been discussed theoretically [2,3] and reported experimentally [5] that by using rectangular capillaries better heat dissipation compared with that from circular capillaries can be obtained. In a previous study [5], although sufficient evidence was obtained on the better heat dissipation arising from the use of rectangular ducts, the comparison between both types of geometry was made using a circular capillary with a cross-sectional area 25% larger than that of the rectangular capillary. This had to be done owing to the difficulty of finding rectangular and circular columns with the same cross-sectional area.

In Fig. 1 a comparison is made using a circular (180 μm I.D.) and a rectangular column (500 × 50 μm) of similar cross-sectional area. Two different cooling procedures are also compared, i.e. forced air (dashed lines) and liquid (solid

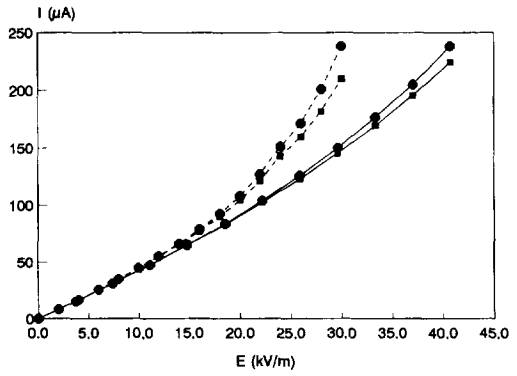

Fig. 1. Electric current versus electric field for (●) a circular and (■) a rectangular capillary. The cooling fluids were forced air (dashed lines) and liquid (solid lines). Buffer, 25 m*M* CHES (pH 10). All conditions as indicated in Experimental.

lines). As can be seen, for the same cooling system a lower electrical current is obtained with the rectangular than with the circular column at electric fields higher than 20 kV/m. Further, the higher is the electric field and the less effective is the cooling procedure employed, the larger are the differences between the two columns.

This can be seen in Fig. 2, where a comparison between the two geometries and for the two different cooling fluids is made in terms of differences in electric current as a percentage, i.e., $100[(I_c - I_r)/I_r]$, where I_c and I_r are the electrical currents in the circular and rectangular

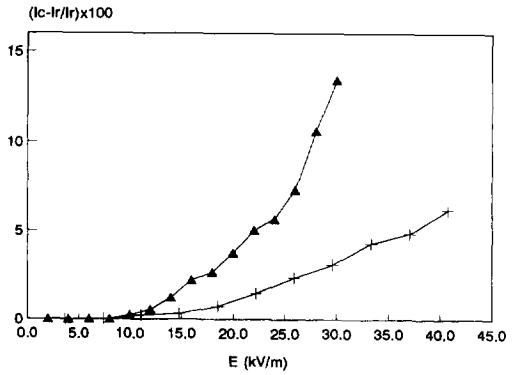

Fig. 2. Electric current differences between a circular and a rectangular column, calculated as 100 $[(I_c - I_r)/I_r]$ versus electric field for two different cooling fluids: (▲) forced air and (+) liquid.

capillary, respectively. As can be seen, there was a ca. 15% of difference in electric current when forced air was used for cooling and an electric field of 30 kV/m was applied. The differences observed between the two capillaries were smaller (up to 6%) when a liquid was used as a cooling fluid.

3.2. Spontaneous injection

Prior to carrying out the study on spontaneous injection into both types of columns the differences in sensitivity arising from the different optical path lengths used for the two capillaries were investigated.

To carry out this study, the same 2% solution of acetone in water was flushed through both capillaries. Surprisingly, without optimization of the position of the rectangular capillary (500 μm optical path length) at the detection point, no gain in signal was observed compared with that from the circular capillary (180 μm), giving both columns similar absorbance values, i.e., 0.0501 and 0.0496 for the rectangular and circular columns, respectively. When the position of the rectangular column at the detection point was optimized by using a microscope, a 1.6 times higher signal compared with that from the circular capillary was obtained. This increase is smaller than that expected theoretically, i.e., 2.8-fold according to the Lambert–Beer law. Two possible reasons can explain this unexpected behaviour: (1) a non-observed inclination of the capillary at the detection point which would reduce the effective optical path length; and/or (2) a smaller number of photons passing through the rectangular column as a result of both the smaller width (50 μm) of the rectangular capillary compared with that of the circular capillary (180 μm I.D.) and the dimensions of the slide (100 μm) provided by the manufacturer with the cartridge prior to the detection window. Thus, under these conditions part of the incident light would not pass through the rectangular column, thus giving a lower signal than expected.

This behaviour has been reported previously [4] by comparing the sensitivity obtained with a

50×1000 μm rectangular column positioned in two different ways, so that detection was across the 50-μm axis or the 1000-μm axis of the rectangular duct. Comparison showed that a 15-fold increase in signal was obtained by using the longer axis, instead of the 20-fold increase expected according to the Lambert–Beer law.

For convenience, we decided to use conditions such that, for the same amount of sample injected, a similar absorbance value was obtained for both types of geometries for all the experiments. This was easily controlled by injecting the acetone solution as indicated above. In this way, comparisons between the two columns in terms of spontaneous injection, siphoning effect and adsorption can be made directly from the electropherograms without further calculations.

It has been reported that spontaneous or ubiquitous injection [22,23] as occurs in CE seems to lead to errors in calibration and quantification for which compensation is difficult even when internal standards are employed. In addition to spoiling quantification, this phenomenon can diminish the efficiency of the separation [23].

On the other hand, it has been discussed theoretically [5] that by using rectangular capillaries the amount introduced within the capillary by spontaneous injection would be greatly reduced. Fig. 3 shows an experimental example of this phenomenon. As can be seen, under identical experimental conditions for both geometries the spontaneous injection occurring in a rectangular capillary (Fig. 3B) is 4–5 times smaller than that in the circular capillary (Fig. 3A) (note that the absorbance axes differ in Fig. 3A and B). In Fig. 3B it is shown that under these conditions the protein with the lowest concentration, i.e., sperm whale myoglobin, was not distinguished from the noise in the rectangular column, and therefore no injection of this protein can be considered.

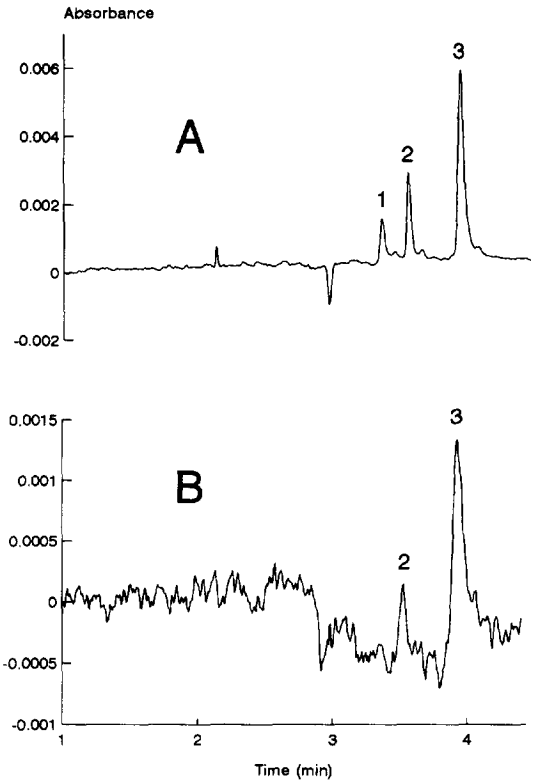

Fig. 3. Electropherograms showing the different amounts introduced into the capillary by spontaneous injection into (A) a circular and (B) a rectangular column. Buffer, 25 m*M* CHES (pH 10); both capillaries, circular (180 μm I.D.) and rectangular (500×50 μm), with L_t 27 cm and L_c 20 cm; sample, 1 = sperm whale myoglobin (0.25 mg/ml), 2 = horse myoglobin (0.45 mg/ml) and 3 = bovine α-lactalbumin (0.73 mg/ml); run voltage, 4.5 kV; detection at 214 nm.

3.3. Siphoning effect

The length-based peak variance induced by the different heights of the buffer levels in a circular capillary is given by [5]

$$\sigma_c^2 = 6.51 \cdot 10^{-4} \cdot \frac{h^2 \rho^2 g^2 r^6 t}{\eta^2 L^2 D_m} \quad (1)$$

and for a rectangular column

$$\sigma_r^2 = 3.36 \cdot 10^{-2} \cdot \frac{h^2 \rho^2 g^2 a^6 t}{\eta^2 L^2 D_m} \quad (2)$$

where r is the radius of the circular capillary (90 μm), a the half rectangular column height (25 μm), t the analysis time, ρ the buffer density, η the buffer viscosity, g the acceleration due to gravity and h the difference between the levels of buffer.

For example, employing identical separation

conditions for a circular capillary of 180 μm I.D. and a rectangular column of 500 × 50 μm, with a total length $L = 0.27$ m and for an analysis time of 3 min, using a solute whose diffusion coefficient is $D_m = 10^{-10}$ m^2/s and the density and viscosity of water at room temperature, and for a very small difference in heights between the buffer levels such as $h = 5$ mm, Eqs. 1 and 2 predict a volume variance in the circular column of $\sigma_c^2 = 2.05 \cdot 10^{-5}$ m^2 and in the rectangular column $\sigma_r^2 = 4.86 \cdot 10^{-7}$ m^2. Hence under these conditions the influence of this effect on the efficiency is 42 times smaller using the rectangular column.

From these results, it also can be deduced that rectangular capillaries would be well suited for micropreparative isoelectric focusing, since the moving of focused zones by pressure could be done with a smaller decrease in efficiency than that normally observed with circular geometry. Moreover, the recently described flow counterbalanced capillary electrophoresis [24] can also greatly benefit from this advantageous characteristic of rectangular columns, since the pressure used for moving the analytes back and forth across the detection window will induce a much lower peak dispersion.

As experimental evidence of this different behaviour, Figs. 4 and 5 show the influence of h, measured as inlet buffer level minus outlet buffer level, on the separation of three proteins in a circular and a rectangular capillary, respectively, under the same conditions. As can be seen, in the circular column the efficiency, and with it the resolution, are ruined when h increases, whereas in the rectangular column no variation is observed. Further, in the circular column a progressive decrease in the analysis times is also observed.

Fig. 6 shows the analysis time variation obtained when seven consecutive runs were performed in a circular and in a rectangular column, leaving the P/ACE apparatus working in the automatic mode without changing buffer vials between injections. In this case, progressively longer analysis times were obtained in the circular capillary. This effect is produced by the

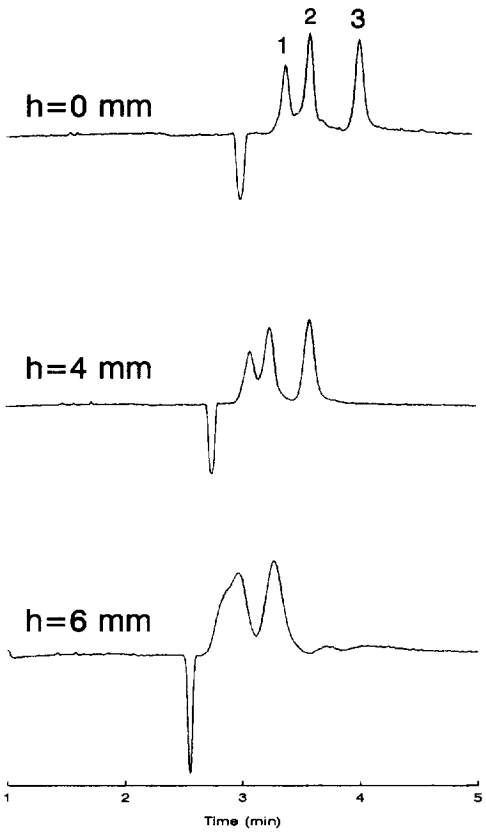

Fig. 4. Influence of the siphoning effect on the separation in a circular capillary for differences between the buffer levels (inlet – outlet) of (upper) 0, (middle) 4 and (lower) 6 mm. Injection, 2 kV for 2.5 s. All conditions as in Fig. 3.

decrease in the buffer level in the inlet vial and the corresponding increase in the level in the outlet vial as a result of the cathodic electroosmotic flow. Under identical conditions, no differences were observed using the rectangular capillary.

Although these results are specific for our separation conditions (length of capillary, run voltage, electroosmotic flow, type of vials, etc.), the implications are straightforward since these types of capillaries are normally used for micropreparative purposes, where repeated separations and collections are normally done, and exquisite analysis time control is mandatory.

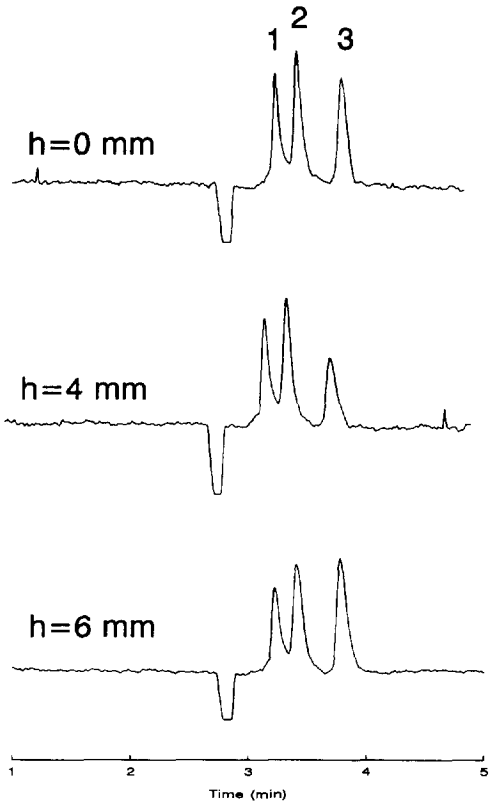

Fig. 5. Influence of the siphoning effect on the separation in a rectangular capillary for differences between the buffer levels (inlet − outlet) of (upper) 0, (middle) 4 and (lower) 6 mm. All conditions as in Fig. 4.

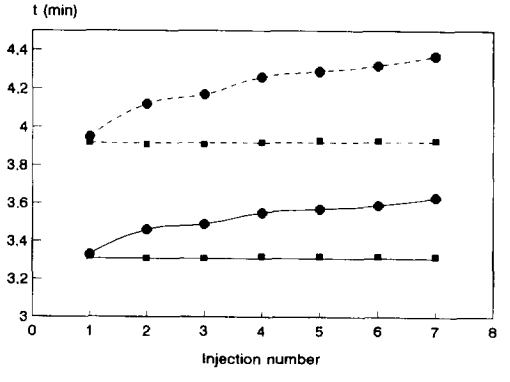

Fig. 6. Reproducibility of analysis time in (●) a circular and (■) a rectangular capillary. Dashed lines, α-lactalbumin; solid lines, whale myoglobin. All conditions as in Fig. 4.

3.4. Adsorption phenomena

In a first approximation, we can carry out a simple theoretical comparison of the extent of adsorption expected from circular and rectangular capillaries. To do this, we can make an initial estimation of the capacity factors k' that both geometries would provide. We can use the relationship [25,26] in which the capacity factor is related to the equilibrium distribution coefficient K and to the ratio of the surface area of stationary phase A_s to the volume of buffer V_b in the column:

$$k' = \frac{A_s}{V_b} \cdot K \tag{3}$$

In CE, A_s can be considered equal to the internal surface area of the capillary wall and the distribution coefficient K, here in units of length, is given by [25]

$$K = \left(\frac{c_s}{c_b}\right)_{eq} \tag{4}$$

where c_s is the solute concentration per unit area in the stationary phase relative to that per unit volume of buffer c_b, both at equilibrium. Next, we can assume that for the same column material (i.e., fused silica) and under identical separation conditions, the K value should be similar for both types of geometries. An initial comparison can be done for the case that the I.D. of the circular capillary, e.g., 50 μm, has the same value as the height of the rectangular capillary, e.g., 500 × 50 μm. Under these conditions, a k' two times smaller is obtained by using the rectangular duct. However, the thermal deformation will be lower with the circular tube, while the detection will be less sensitive with this column.

For the case in which we are mostly interested, i.e., micropreparative use of CE, a comparison between columns of similar cross-sectional areas is in order. In our case we compare a 180 μm I.D. circular column with a 500 × 50 μm rectangular capillary. A simple calculation shows that the 50 × 500 μm rectangular capillary provides a k' value 2.1 times higher than that from the 180 μm I.D. circular column, as a result of the larger

internal surface area of the rectangular geometry.

At first sight it would seem that the rectangular columns are even less suited than the circular columns for micropreparative CE when some adsorption phenomenon is taking place. However, let us consider the effect of k' on the efficiency of the separation.

The partition phenomenon of the analyte taking place between the buffer and the capillary wall during CE separations has been addressed by using different mathematical models, e.g., derived from plate height theory [18,19,27–29], the Lapidus–Amundson kinetic model [19,30] or stochastic simulation methods [19,31].

Giddings [27] described in 1961 the adsorption phenomenon for chromatographic separations where a stationary film of uniform thickness is employed and the mobile phase moves with uniform velocity (i.e., plug flow profile) within a cylindrical column. For this study he used the plate height theory developed by himself. This case is a more than acceptable approximation of the adsorption phenomena occurring in CE, in which the silica capillary wall acts as stationary phase and where the buffer moves with a more or less plug flow profile. The results of Giddings were extended by Martin and Guiochon [18] to CE using the electroosmotic flow as driving force and a partly flat, partly parabolic flow profile, which can better represent the electroosmotic velocity profile. For the simplest case (i.e., perfect plug flow profile), Giddings [27] and Martin and Guiochon [18] arrived at the same equation:

$$H_{C,ads} = \frac{(1-R)^2}{4} \cdot \frac{vr^2}{D_m} \tag{5}$$

where $H_{C,ads}$ is the plate height related to the adsorption process in a circular capillary, R is the retention factor and v is the average velocity (i.e., including electroosmotic flow) of the analyte. Eq. 5 can be written in terms of the capacity factor k', which is related to R by

$$R = \frac{1}{1+k'} \tag{6}$$

and with $v = \mu E$ one has

$$H_{C,ads} = \frac{1}{4}\left(\frac{k'}{1+k'}\right)^2 \frac{\mu E r^2}{D_m} \tag{7}$$

where μ is the apparent electrophoretic mobility of the analyte and E the electric field strength. This equation, as stated above, only applies to those cases in which a perfect plug flow profile is taking place, it neglects any deformation of the flow close to the capillary wall and it assumes that the analyte moves through the capillary as a Gaussian zone. The last aspect implies that the distribution towards the wall is linear, i.e., the concentrations and/or distribution coefficients are so low that the surface adsorbent is not saturated. Although in electrically driven systems some deformation of the flow profile close to the capillary wall is expected to occur [32], this equation can be used as a good approximation for describing the influence of the adsorption process on the plate height in CE.

In a similar way as shown in Eq. 5, Giddings [27] described the plate eight for a chromatographic separation where a stationary film of uniform thickness is employed and the mobile phase moves as a perfect plug flow flow profile between parallel faces of infinite width. Using the same steps as used above, we obtain

$$H_{R,ads} = \frac{2}{3}\left(\frac{k'}{1+k'}\right)^2 \frac{\mu E a^2}{D_m} \tag{8}$$

where $H_{R,ads}$ is the plate height related to the adsorption process in a rectangular geometry and a is the half distance between the faces. Eq. 8 only applies assuming the same limitations as explained above (e.g., perfect plug flow profile and analyte moving as a Gaussian zone). Also, this equation assumes the no occurrence of any "end effect", i.e., the separation process is considered to take place between parallel faces of infinite width. However, for our theoretical study we assume that Eq. 8 can be used as a good approximation in rectangular capillaries provided that a large width-to-height ratio ϕ is used (for the rectangular column used in this work $\phi = 10$). The inclusion of the "end effect" would require too detailed mathematical work, as was shown before [3], which is not the purpose of this paper.

As can be seen, there is a clear similarity between Eqs. 7 and 8. In both equations the plate height is related to the capacity factor, the analyte velocity, its diffusion coefficient and the capillary geometry, via the column dimensions r and a, and the quantities 1/4 and 2/3, which in chromatography are configuration factors dependent on the geometry of the stationary phase [25], and in CE we can consider them to be associated with the capillary shape. By inserting Eq. 3 into Eq. 7 and 8, we obtain for circular capillaries

$$H_{C,ads} = \frac{1}{4}\left(\frac{K}{\frac{r}{2}+K}\right)^2 \frac{\mu E r^2}{D_m} \quad (9)$$

and for rectangular capillaries

$$H_{R,ads} = \frac{2}{3}\left(\frac{K}{\frac{a\phi}{1+\phi}+K}\right)^2 \frac{\mu E a^2}{D_m} \quad (10)$$

Now we can carry out a more precise comparison between the two different geometries in terms of the adsorption phenomenon and its influence on the efficiency of the separations.

Under the same conditions, as stated above, it is possible to assume that, independent of the geometry, very similar K values will be obtained for both columns, provided that the rest of the conditions are the same. We have plotted in Fig. 7 the plate height that would be obtained as a function of different K values. A circular capillary of 180 μm I.D. and a rectangular capillary of width-to-height ratio $\phi = 10$ were used, both capillaries having similar cross-sectional areas. As can be seen, some differences occur between these capillaries. For instance, by using the rectangular capillary the theoretical plate due to adsorption for $K = 1.35 \cdot 10^{-6}$ m (which would correspond approximately to $k = 0.06$ in this column and $k' = 0.03$ in the cylindrical column) is 20% smaller than that obtained with the circular capillary for the same K value. This difference is explained through the dependence of the plate height on the capillary geometry, via the parameters a, ϕ and r, as can be observed in Eqs. 9 and 10.

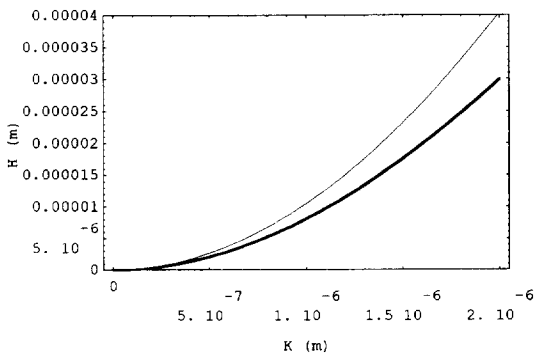

Fig. 7. Theoretical plate height H vs. equilibrium distribution coefficient K for three capillaries of different geometries and similar cross-sectional areas according to Eqs. 9 and 10. Conditions: (thin line) circular capillary with 180 μm I.D.; (thick line) rectangular capillary with 500×50 μm ($\phi = 10$). Electric field strength $E = 17\,000$ V/m; diffusion coefficient $D_m = 10^{-10}$ m^2/s; electrophoretic mobility of analyte = $66 \cdot 10^{-9}$ m^2/s·V.

In Fig. 8 we have plotted the theoretical plate height versus the separation electric field for both columns. A K value of 10^{-6} m was used for both columns, which provides different k' values as indicated above. As can be seen, the higher the electric field applied, the larger is the plate height obtained. Moreover, smaller plate heights, i.e., ca. 20–30% smaller, would be expected to occur by using a rectangular column over all the range of electric fields.

From these theoretical results, it seems that,

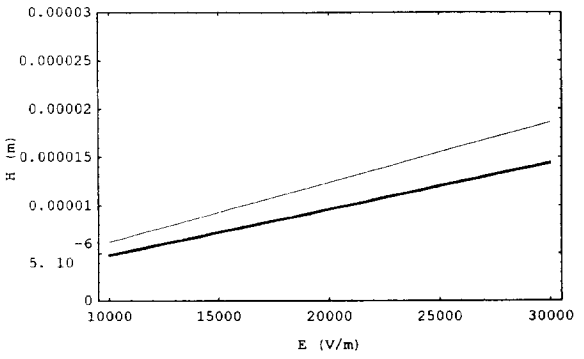

Fig. 8. Theoretical plate height H vs. electric field for two capillaries of different geometries and similar cross-sectional areas according to Eqs. 9 and 10. The same equilibrium distribution coefficient, $K = 10^{-6}$ m, was used for both columns. All conditions as in Fig. 7.

contrary to what was initially expected, the adsorption occurring in a rectangular capillaries does not bring about a more negative effect on the separation than that obtained with a circular column. Further, the use of rectangular ducts seems to provide slightly better efficiencies than those with circular columns. In order to probe these results, we carried out different experiments by using the 180 μm I.D. circular and the 500×50 μm rectangular capillaries.

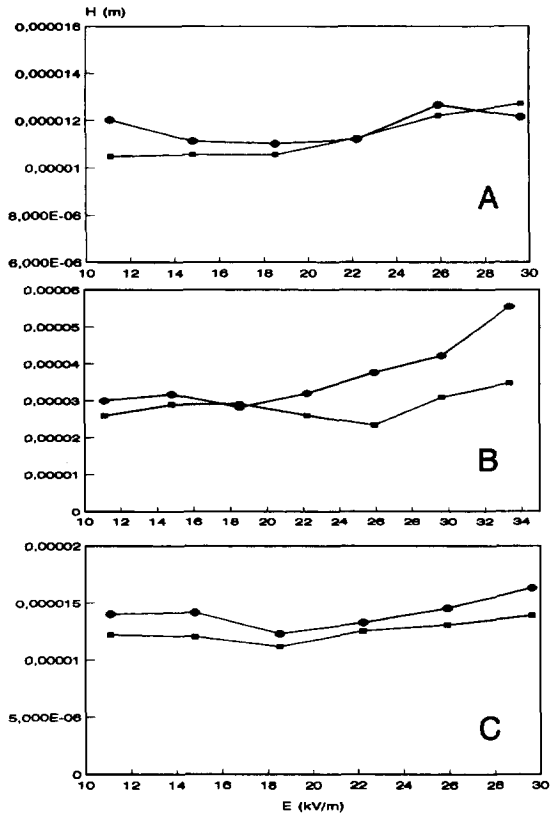

Fig. 9. Experimental values of plate height (H) versus electric field (E) for three different compounds in (●) a circular and (■) a rectangular capillary. (A) Buffer, 0.15 mol/l acetic acid–0.15 mol/l formic acid (pH 2.6); injections, 1.5 kV for 2.5 s of 0.2 mg/ml substance P 1–7 fragment. (B) Buffer, 25 mM acetic acid (pH 5.5); injection, 2 kV for 2.5 s of 1% 2-methylpyridine in water. (C) Buffer, 25 mM CHES (pH 10); injection, 2 kV for 2.5 s of 1.1 mg/ml sperm whale myoglobin. Other conditions as in Fig. 3.

Three different compounds were used to carry out these experiments, namely sperm whale myoglobin, 2-methylpyridine and substance P fragment 1–7 (a highly basic peptide) at pH 10, 5.5 and 2.6, respectively. Under these conditions, some adsorption can be expected to occur between these substances and the capillary wall, since the three compounds will bear some positive charge and, at their respective separation pH values, the capillary wall will carry a negative charge.

The results obtained are shown in Fig. 9. As can be seen, in all instances very similar efficiencies were obtained with both type of geometries, slightly better efficiencies being observed when using the rectangular column. Although the lack of knowledge of the absolute values of k' precludes a direct comparison between the experimental and theoretical values, it can be seen that similar tendencies are observed with both kinds of values. However, in general, the experimental differences observed between the two columns were not as high as those predicted by theory. Several factors could explain this disagreement, e.g., the high R.S.D. obtained for the efficiency values (i.e., up to 10%, $n = 3$), the lack of inclusion of other dispersive effects such as axial molecular diffusion, thermal effects and injection, in the theoretical calculations and the assumption that no "end effect" in the rectangular capillary occurs.

4. Conclusions

It has been demonstrated that the use of rectangular capillaries diminishes the adverse influence of some undesirable effects such as heat generation, spontaneous injection and siphoning effects on CE separations. Moreover, it has been shown theoretically and experimentally that the effects of adsorption phenomena on the separation efficiency are comparable, or slightly less critical, when rectangular capillaries are used instead of cylindrical capillaries.

Acknowledgements

The authors thank Professor H. Poppe, Dr. J.C. Diez-Masa and Dr. M. de Frutos for stimulating discussions and J.M. Campos for the modification of the cartridge cover. Financial support of this work by CICYT (Spain), grant AMB92-0515, and the Local Government of the Canary Islands is acknowledged.

References

[1] A. Tiselius, Trans. Faraday Soc., 33 (1937) 524.
[2] M. Janson, A. Emmer and J. Roerade, J. High Resolut. Chromatogr., 12 (1989) 797.
[3] A. Cifuentes and H. Poppe, Chromatographia, 39 (1994) 391.
[4] T. Tsuda, J.V. Sweedler and N. Zare, Anal. Chem., 62 (1990) 2149.
[5] A. Cifuentes and H. Poppe, Electrophoresis, 16 (1995) 2051.
[6] J.M. Mesaros, G. Luo, J. Roerade and A.G. Ewing, Anal. Chem., 65 (1993) 3313.
[7] D.J. Harrison, K. Fluri, K. Seiler, Z. Fan, C.S. Effenhauser and A. Manz, Science, 261 (1993) 895.
[8] D.J. Harrison, A. Manz, Z. Fan, H. Ludi and H.M. Widmer, Anal. Chem., 64 (1992) 1926.
[9] S.C. Jacobson, R. Hergenroder, L.B. Koutny, R.J. Warmack and J.M. Ramsey, Anal. Chem., 66 (1994) 1107.
[10] A. Cifuentes, X. Xu, W.Th. Kok and H. Poppe, J. Chromatogr. A, 716 (1995) 141.
[11] E. Grushka, J. Chromatogr., 559 (1991) 81.
[12] J.W. Jorgenson and K.D. Lukacs, Science, 222 (1983) 266.
[13] G.J.M. Bruin, J.P. Chang, R.H. Kulman, K. Zegers, J.C. Kraak and H. Poppe, J. Chromatogr., 471 (1989) 429.
[14] H.H. Lauer and D. McManigill, Anal. Chem., 58 (1986) 166.
[15] W.G. Kuhr, Anal. Chem., 62 (1990) 403R.
[16] W.G. Kuhr and C.A. Monning, Anal. Chem., 64 (1992) 389R.
[17] C.A. Monning and R.T. Kennedy, Anal. Chem., 66 (1994) 280R.
[18] M. Martin and G.A. Guiochon, Anal. Chem., 56 (1984) 614.
[19] M.R. Schure and A.M. Lenhoff, Anal. Chem., 65 (1993) 3024.
[20] V.P. Adreev and E.E. Lisin, Chromatographia, 37 (1993) 202.
[21] A. Cifuentes, M. de Frutos, J.M. Santos, and J.C. Diez-Masa, J. Chromatogr. A, 655 (1993) 63.
[22] H.A. Fishman, N.M. Amudi, T.T. Lee, R.H. Scheller and R.N. Zare, Anal. Chem., 66 (1994) 2318.
[23] E.M. Grushka and R.M. McCormick, J. Chromatogr., 471 (1989) 421.
[24] C.T. Culbertson and J.W. Jorgenson, Anal. Chem., 66 (1994) 955.
[25] J.C. Giddings, Unified Separation Science, Wiley, New York, 1991.
[26] H. Poppe, J. Chromatogr. A, 656 (1993) 19.
[27] J.C. Giddings, J. Chromatogr., 5 (1961) 46.
[28] R.J. Wieme, in E. Heftmann (Editor), Chromatography, a Laboratory Handbook of Chromatographic and Electrophoretic Methods, Van Nostrand Reinhold, New York, 1975.
[29] S. Hjerten, Electrophoresis, 11 (1990) 665.
[30] L. Lapidus and N.R. Amundson, J. Phys. Chem., 56 (1952) 984.
[31] M.R. Schure, Anal. Chem., 60 (1988) 1109.
[32] J.A. Taylor and E.S. Yeung, Anal. Chem., 65 (1993) 2928.