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Exact electric field strength over the packed capillary electrochromatography column bed, theoretical considerations and practical determination

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Abstract

On the basis of the experiments, it was proved that Ohm's law holds true in a capillary electrochromatography (CEC) column. By using the additivity of the potential drop over the packed and unpacked sections, the exact values of the electric field strength over the packed CEC column bed were determined experimentally. The ratio of the resistance of packed CEC column sections to that of an open capillary of the same length ($\bar{R}_p/R_{o,L_p}$) were calculated. Some theoretical calculations were made to show how some structural parameters of particle lattice affect the resulting $\bar{R}_p/R_{o,L_p}$ value of the column bed formed accordingly. It is suggested that to correctly elucidate the EOF phenomenon in a CEC column, the potential drop over the packed and unpacked column sections, E_p and E_o, should be accounted for respectively, rather than using the average field strength over the total column length.

Keywords: Capillary electrochromatography; Packed column bed; Electric field strength; Resistance of CEC column; Resistivity of the mobile phase

1. Introduction

Capillary electrochromatography is an emerging separation technique that combines the separation power of high performance liquid chromatography (HPLC) with the high efficiency of capillary zone electrophoresis (CZE) [1–10]. Since Knox and Grant [2,3] laid the theoretical foundation of this technique, a number of papers have been published by Choudhary and Horváth [11], Rathore and Horváth [12,13] and Wan [14–18], and Cikalo et al. [19], studying theoretically the parameters, such as the electric field strength E, influencing CEC column performance. Wan [15] described a method for characterization of the columns used in CEC that is based on measurements of relative electric conductivity of the packed and open segments of the column.

In practical CEC works, E values used to be calculated over the total length of the column, L, i.e., by dividing the running voltage by L, although Choudhary and Horváth [11] measured the conductance ratio of the packed segment and that of empty capillary, ratios ranging from 0.30 to 0.42 were obtained. Indeed, the difference between the conductance of packed and open sections of the column have been noticed by authors. As a result, the real electric field strength over the packed column bed (E_p) cannot be expected to be equal to the average field strength [12] calculated as mentioned above.

In this paper the exact values of E_p in a CEC column were determined experimentally using columns packed with polystyrene-divinylbenzene encapsulated silica stationary phases (PS-DES) with diameters of 0.35 μm as well as 5 μm, respectively. Some theoretical consideration was put forward. The results showed that to correctly elucidate the EOF phenomenon in a CEC column, the potential drop over the unpacked column sections, E_p and E_o, should be accounted for respectively.

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2. Experimental

2.1. Instruments and reagents

2.1.1. Instruments

To measure the current through CEC columns under a certain applied voltage, a CS2671A type electric power source (0–10 kV, Changsheng Instrument Co. Ltd., Nanjing, China) was used. A UT58A type digital amperometer (Youlide Technology Co. Ltd., Shenzhen, China) was used to measure the current. The capillaries (250 μm i.d.) were from Yongnian Optical Fiber Factory (Hebei, China).

2.1.2. Reagents

HPLC-grade solvents for chromatographic separations were obtained from Fisher Chemicals, USA. Tri(hydroxymethyl)-amino-methane (Tris) was from Sigma Chemical Co. (St. Louis, MO, USA). Other reagents employed for the synthesis of the stationary phases as well as for the preparation of the mobile phases were all of analytical reagent grade, all obtained from Beijing Chemical Reagent Factory.

2.2. Preparation and test of the packed columns

The columns tested were packed with PS-DES particles of 5 and 0.35 μm diameters. The synthesis [20] as well as the preparation of the columns [21] has been reported in the literature.

During the whole experiment a voltage of 8 kV was applied on the column. Before use, the mobile phases were filtered through 0.45 μm filtering membrane and ultrasonicated for 5 min. The columns tested were equilibrated with the mobile phase before testing, subsequently running at a low voltage of 1 kV until the current was stable. All the experiments were done at room temperature. When the column is cut too short, leading to high electric strength, it was necessary to apply a gas pressure of 0.2 MPa on both electrolyte vials to avoid bubble formation. Otherwise, the inlet and outlet were all kept at atmospheric pressure.

3. Theoretical considerations

3.1. Determination of resistance values of the packed and open column segments

For metallic conductors, the resistance can be calculated according to Ohm's law. However, it has long known that there are two effects of the passage of a direct current through an electrolyte solution which make the measurement of electrolytic resistance by using direct currents problematic. They are caused by polarization at the surface of the electrodes, which set up an opposing electromotive force; as well as by the unequal dilution of the solution which is causative of a subsidiary electromotive force either in the same direction as that applied or in the reverse, depending on which solution in one of the electrolytic cells becomes the more dilute. Both effects would normally deter the application of Ohm's law from the electrolytic cell as a whole. To eliminate both these effects, alternative current is used to measure the resistance. Electrophoresis is typically a phenomenon of conduction of a direct current through electrolyte solution. For people who study electrophoresis, the problem they would be facing is that the above-mentioned effects prevent them from using the easily obtained experimental data into the study of potential drops inside a column. In this paper we tried to test the possibility to measure the resistance of the capillary by using direct current through it. If under certain circumstances the Ohm's law still holds true, then it is much easier to deal with parameters such as the potential drops in the column.

In this paper, we experimentally proved that when the current through the column is not too strong, Ohm's law still kept true during an electrophoretic process, as a result potential drops over the two segments in the column followed rule of additivity. On the basis of the discovery, exact electric field strengths over packed and open segments of the column were determined.

Suppose the inner cross-section of a CEC column is A_o and in its packed segment a part of its cross-section remaining to be occupied by the mobile phase, A_p. According to Ohm's Law, under a potential U the current I through the whole column can be described as:

$$I = \frac{U}{R} = \frac{U}{(L_o\rho/A_o) + (L_p\rho/A_p)} \tag{1}$$

where

$$R = R_o + R_p \tag{2}$$

$$R_o = \frac{L_o\rho}{A_o} \tag{3}$$

$$R_p = \frac{L_p\rho}{A_p} \tag{4}$$

in which R as well as L are the resistance values and the lengths of the column, with subscripts o and p denote those of the open or packed segments, respectively; A_p is the cross-sectional area of a capillary with equivalent resistance to the packed column bed; ρ is the resistivity of the mobile phase.

The potential drop over the open or packed segment of the column can be calculated according to following equations:

$$U_o = IL_o\frac{\rho}{A_o} \tag{5}$$

$$U_p = IL_p\frac{\rho}{A_p} \tag{6}$$

Eq. (5) can be rearranged into:

$$\frac{\rho}{A_o} = \frac{U_o}{IL_o} \tag{7}$$

This measured ρ/A_o can be used to calculate the potential drop over the packed bed of a CEC column with known L_o

and L_p values: if A_o, ρ in the following measurement are held constant, with the current I through the column measured, the potential drop over the open segment of the column, U_o, can be calculated. U_p can subsequently be calculated as follows:

$$U_p = U - U_o \tag{8}$$

3.2. Determination of $\bar{A}_p/A_o$

Some authors have already pointed out that, due to the different $\bar{A}_p$ and A_o values, the potential drop over the same lengths of the open and packed segments are different [12].

After rearranging Eqs. (5) and (6), it follows:

$$I = \frac{U_o A_o}{L_o \rho} = \frac{U_p \bar{A}_p}{L_p \rho} \tag{9}$$

$$\frac{\bar{A}_p}{A_o} = \frac{L_p}{L_o}\frac{U_o}{U_p} \tag{10}$$

As the stationary phase particles are randomly packed into column bed, it is impossible to calculate $\bar{A}_p/A_o$ from structural parameters of the column bed such as particle size and so on. However, suppose the column bed is formed following the close packing models with stationary phase particles of uniform diameter, the $\bar{A}_p/A_o$ values can be theoretically calculated.

Fig. 1 showed a hexagonal portion of a close packing mode column bed. When the direction of electric current is horizontal as shown in the figure (it will be shown later that sometimes this is very important), on the cross-sections A–E (see Fig. 2), the intersections of the particles as the intersecting

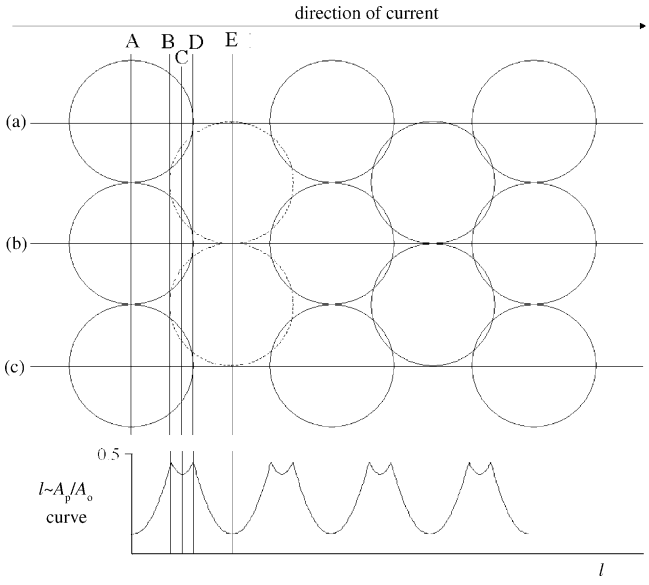

Fig. 1. Schematic of a close packing [22] column bed, and the local A_p/A_o as function of l. For the definitions of A_p and A_o, see text.

plane moves successively from A on (A is chosen as an original point because from there on, the periodical change of A_p/A_o can be seen as function of l, l is the distance from the origin. If the column boundary effect on the cross-sectional open area can be neglected, it can be seen in Fig. 2 that in intersection A, the ratio A_p/A_o is equal to the ratio of the a pocket intersection area with the area of the Δabo. The latter ratio can be shown to be equal to $1 - \left(\pi/2\sqrt{3}\right)$, which is the ratio of the area of an equilateral triangle as the unit, to the

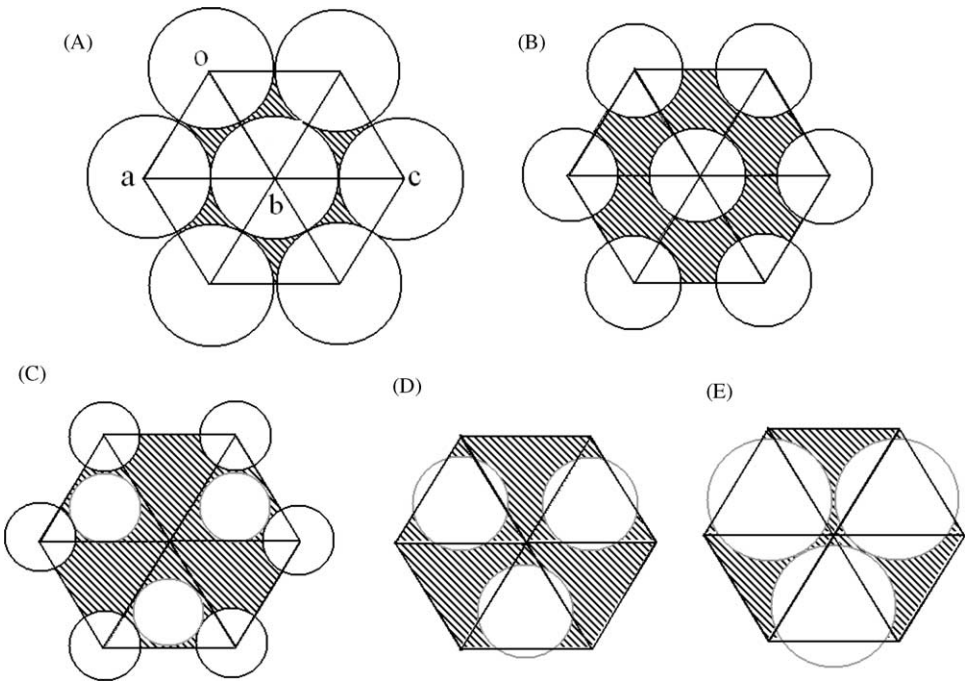

Fig. 2. Schematic of the cross-sections A–E.

difference between the triangle and half the area of a circle. The areas of the circles changes when the intersectional plane moves to the direction of the current. In some cases the intersections of next layer particles appear in the cross-sectional plane (e.g. C and D in Fig. 2, plane B is just tangent to next layer particles). Thus, the pocket intersection areas change with l. It can be observed in Fig. 1 that in between A and B the ratio A_p/A_o equals to the difference of a triangular area with half the area of the circle, in between B and C, to the difference with half the sum of two circle areas, with the area of the triangle as the unit. Moving on to D and E, although there is a displacement of the circles in relation to the hexagon, the ratio of their areas keeps the same, so in between C and D as well as in between D and E the ratio A_p/A_o equals to the value in between B and C as well as A and B.

From Eqs. (3) and (4) it can be derived that in a packed column bed whose length is L_p, the ratio of its resistance to that of an unpacked capillary of the same length, $\bar{R}_p/R_{o,L_p}$, can be calculated as:

$$\frac{\bar{R}_p}{R_{o,L_p}} = \frac{\rho\int_0^{L_p} dl/A_p}{\rho\int_0^{L_p} dl/A_o} \tag{11}$$

A_o is constant; therefore it follows:

$$\frac{\bar{R}_p}{R_{o,L_p}} = \frac{\int_0^{L_p} dl/(A_p/A_o)}{L_p} \tag{12}$$

Eq. (12) compares the resistance of the column bed with that of an open capillary of the same length. On the same basis, from Eqs. (3) and (4),

$$\frac{\bar{R}_p}{R_{o,L_p}} = \frac{A_o}{A_p} \tag{13}$$

can be derived.

4. Results and discussion

4.1. Experimentally determined $\bar{R}_p/R_{o,L_p}$ values from CEC columns

Two CEC columns of different lengths packed with stationary phases of different particle diameters, using mobile phases of very different conductivities, were tested for their $\bar{R}_p/R_{o,L_p}$ values. The current through the columns were measured when the readings were stable. After the initial currents were measured, the open sections of the columns were cut piece by piece, leading to different L_o values. Every time a piece of the capillary was cut, the current was measured after stabilization of the reading. The results are shown in Fig. 3. From the figure it can be seen that although the particle diameters of the phases and the mobile phases were all different, the measured resistance values were far apart, the L–R relationships were all linear.

If Ohm's law is valid in an electrophoretic column, when ρ, A_o and R keep constant, R should be a linear function of L_o

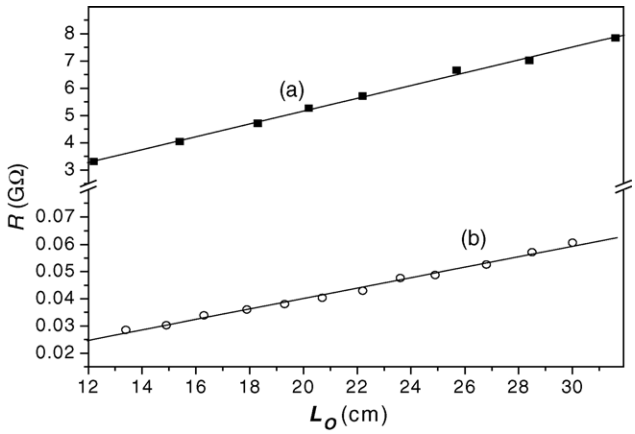

Fig. 3. Plot of the L_o vs. R. For experimental conditions see text.

(Eqs. (2) and (3)). The experimental results indeed showed a linear relationship between R and L_o using a CEC column having a packed length L_p and different L_o, filled with the same mobile phase and under the same potential drop through the whole column.

There is no doubt that the two effects of the passage of a direct current through an electrolyte solution normally cause problems in measuring resistance of an electrolyte solution. However, in our case probably the opposing electromotive force caused by polarization at the surface of the electrodes is too weak in comparison to the high voltage applied, and the time for the fulfillment of the experiment does not suffice the current to cause significant changes in the composition of the solution in the cell, as a result Ohm's law appeared to be well applicable.

From the experimental results, it can be calculated that the $\bar{R}_p/R_{o,L_p}$ values were 2.88 and 2.57, respectively for the 5 and 0.35 μm packing columns, respectively. These results coincide rather well with the figure obtained by Choudhary and Horváth 2.85 [11].

4.2. Calculation of $\bar{R}_p/R_{o,L_p}$ values of columns with different packing beds

As mentioned above, $\bar{R}_p/R_{o,L_p}$ can be calculated from Eq. (13) if A_p/A_o can be derived. In the case of the close packing mode column bed, this can be realized: when l is between A and B in Fig. 1, A_p/A_o is equal to:

$$F_1(l) = \frac{(\sqrt{3}/4) - (\pi/2)\cdot[(1/2)^2 - l^2]}{\sqrt{3}/4} \tag{14}$$

in which the first term is the area of an equilateral triangle, the second is half the area of the circle, with the diameter of the circle in plane A being unit. When l is between B and C, A_p/A_o is equal to:

$$F_2(l)=\frac{\sqrt{3}/4-(\pi/2)[(1/2)^2-l^2]-(\pi/2)\left[(1/2)^2-(\sqrt{6}/3-l)^2\right]}{\sqrt{3}/4} \quad (15)$$

the last term is the area of the next layer particle intersection.

With the expressions known, $\bar{R}_p/R_{o,L_p}$ can be calculated using Eq. (12):

$$\frac{\bar{R}_p}{R_{o,L_p}}=\frac{\int_0^a dl/F_1(l)+\int_a^b dl/F_2(l)}{L} \quad (16)$$

$\bar{R}_p/R_{o,L_p}$ is the average value over a length of the column. As mentioned above, A_p/A_o is a periodical function of l, thus this ratio over a length of the packed column bed should equal to the ratio calculated over a section of the column with a length of a period of the function. The resistance of the whole column can be calculated by adding those of many segments A–C and C–E, it can be shown that the resistance values of these two segments are the same. The rest of the column, the first and last half-particle segments of the column, have different resistance values, however if the length of the column is much longer than the diameter of the particle, $\bar{R}_p/R_{o,L_p}$ calculated within a range between A and C can be used to stand for the value over the whole packed bed.

In doing so and assume the particle diameter being unit, L is equal to $\sqrt{6}/6$, half the distance between the particle layers, a, b can be shown to be equal to $\sqrt{6}/3-0.5$ and $\sqrt{6}/6$, respectively. The result of the calculation is $\bar{R}_p/R_o=5.187$.

Some interesting results were obtained from column bed of various packing modes. First, for column bed packed after the two close packing models, hexagonal and cubic, there is no different with respect to the $\bar{R}_p/R_{o,L_p}$ value, as long as the direction of the current is perpendicular to the layers. This can be explained using Fig. 2: as long as the third layer of particles are positioned on the pockets formed by the second layer to form close packing, A_p/A_o values are independent on the way the further layers of particles are packed on the layers already packed. The same $\bar{R}_p/R_{o,L_p}$ value can be expected.

However, when the lattice of the particles through which the current flows turns a certain angle (54.74°) so that the column bed is of the surface center cubic lattice, although the density of the particle bed remains the same, the calculated $\bar{R}_p/R_{o,L_p}$ value changes drastically to 3.451. This discrepancy can be attributed to the different ways of change of the open tunnel width in the column bed. When a current flows through the hexagonal lattice, in some cases the tunnel for the current are very narrow (can be as narrow as 9.31% of the original capillary cross-section, Fig. 2A). This results in high resistance of the hexagonal close packing column bed. When the current flows through a surface center cubic lattice column bed, the cross-section of the tunnel widths are more even, as a result a column of the latter model will result in lower resistance.

Although all the above-mentioned results are obtained from an idealistic model, it does set a limit on the highest possible resistance that can be realized by packing uniform diameter particles into a CEC column. In reality, $\bar{R}_p/R_{o,L_p}$ values of 2–3 are measured, as seen from our results and from Choudhary and Horváth [11]. These low values are probably caused by the intervals between the particles or by smaller particles in the lattice (wider tunnels). With the same principle these effects can be calculated: if a column bed is constructed by a hexagonal close packing lattice, yet in the lattice there is a same interval between every particle, it is surprise to find that it needs only a clearance as small as ca. 8% of the particle diameter to decrease the calculated $\bar{R}_p/R_{o,L_p}$ values from 5.19 to 2.85. In other words, in a evenly packed column bed by the particles having a diameter of 5 μm which are ca. 0.4 μm apart, the resistance would be nearly half that of a same column when the particles are really closely packed.

On the other hand, however, if in every two layers of the particles among every hexagonal arranged particles of the same diameter, there is in the center a small diameter particle, say of 80% of the other's diameter, such a column bed would result in a $\bar{R}_p/R_{o,L_p}$ value of 4.11. The above-mentioned model actually means a particle distribution of 87.5% normal-sized particles and 12.5% small diameter particles. Although it still demands an idealistically even distribution of particles, it does somehow approach to the real situation.

4.3. Meaning of $\bar{R}_p/R_{o,L_p}$ values

In this paper $\bar{R}_p/R_{o,L_p}$ is measured experimentally as well as calculated for some kinds of column beds. The calculated $\bar{R}_p/R_{o,L_p}$ value for close packing mode of the column bed probably sets a limitation of the resistance of CEC packed in all ways; this ratio may be used to control the closeness of the column bed. Besides, by calculating the $\bar{R}_p/R_{o,L_p}$ values for column beds packed after different models, it might uncover some clue to the relationship between the column bed quality and some structural parameters of the particle lattice, such as the evenness of the particle diameters, the distribution of the pockets of various sizes etc.

The experimentally measured $\bar{R}_p/R_{o,L_p}$ value, on the other hand, can be used to determine the exact field strength over the packed and unpacked portion of the CEC column, E_p and E_o, respectively. A literature search showed that many authors did not explicitly report the exact field strength over the column bed. It is very probable that the field strength they reported is neither E_p nor E_o, rather, it was an average field strength calculated by dividing the total applied voltage by the total column length, see Fig. 4.

From theoretical point of view, failure to clarify the field strength over the different sections of CEC column might be more problematic than it is viewed at the first glance: EOF in a CEC column might be a result of a synchronized effect of the electroosmotic forces, both in the packed section of the column and in the unpacked. How the two forces work together to cause the final EOF is apparently a somehow complicated problem: the zeta potentials in the packed section of the CEC column is likely to differ from that in the

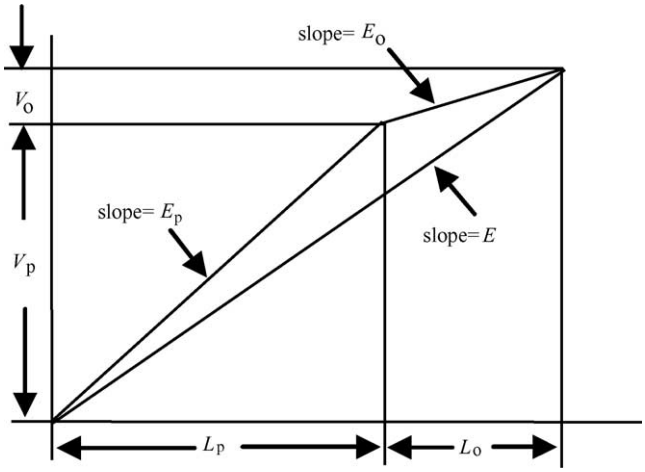

Fig. 4. Plots of the actual E_p and E_o, and average field strength E in the packed and the open segments.

open section, whereas due to the difference in total tunnel cross-section in the packed bed and that in the unpacked, the linear flow rate through the stationary phase is different in the former from that through the latter. How should these considerations be put together to give a better elucidation of the EOF as a whole in a CEC column might still be a problem to be studied. It is our point of view that, at any rate, E_p and E_o should be accounted for respectively.

5. Conclusions

On the basis of the experiments it was proved that Ohm's law holds true in a CEC column, as a result by applying the resulting additivity of the potential drop over the packed and unpacked sections, the potential drop over the column packed bed can be measured by using Eqs. (7) and (8). The experimentally measured $\bar{R}_p/R_{o,L_p}$ values are compared with theoretical values obtained by calculating using a current through a close packing particle lattice as a model. Further calculations showed how the structural parameters of the lattice such as the distance between the particles and the evenness of the particle size affected the $\bar{R}_p/R_{o,L_p}$ value. It is probably necessary to account for E_p and E_o by using the method suggested in this paper or any other way, for the correct elucidation of EOF in a CEC column.

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