Electrochromatography and micro high-performance liquid chromatography with 320 µm I.D. packed columns

Chao Yan☆, Daniel Schaufelberger, Fritz Erni*

Analytical Research and Development, Sandoz Pharma Ltd., CH-4002 Basle, Switzerland

Abstract

Electrochromatography is a chromatographic method in which the mobile phase (liquid or supercritical fluid) is "pumped" through a stationary phase in a microbore or capillary column by electroosmosis using an electric field. The technique permits separation of charged and uncharged compounds with higher resolution and superior efficiency when compared with micro-HPLC with an identical column. It is desirable to work with packed capillary columns with wide diameter in electrochromatography in order to improve detectability and column loadability. This study shows that we have moved a step forward towards this goal in spite of problems and difficulties, due to Joule heating, frit making and column packing in using wide-diameter columns. The paper demonstrates that the pressure pump of micro-HPLC with a commercially available 320 µm I.D. column can be replaced by the electroosmotic "pump" of capillary zone electrophoresis. Experiments were carried out in a chromatographic system under both electroosmosis and pressure-driven flow with 320 and 50 µm I.D. columns packed with 3- and 5-µm ODS. The advantage of electrochromatography over conventional micro-HPLC is shown.

1. Introduction

Electrochromatography (EC) is a developing analytical technique in which the mobile phase fluid is driven through a stationary phase in a microbore or capillary column by electroosmosis using an electric field. Retention in EC is governed both by the electrophoretic mobility of the solutes and their partitioning between the stationary and mobile phases. Micellar electrokinetic capillary chromatography (MECC) with pseudostationary phase also depends on electroosmosis as the driving force and it has been used successfully for separation and analysis [1]. However, we prefer to exclude MECC from EC since it may have its own definition.

Microbore and capillary column high-performance liquid chromatography (micro-HPLC) have undergone moderate development since the pioneering work of Scott and Kucera [2]. This technique provides good selectivity in a wide range of applications [3–6]. The mobile phase in micro-HPLC is driven through the column by applying high pressure and this induces laminar flow which causes a parabolic velocity profile of the mobile phase and thus reduces the plate number.

In contrast to micro-HPLC, capillary zone electrophoresis (CZE) [7] provides excellent efficiency in addition to its characteristics of

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* Corresponding author.
☆ Present address: Department of Chemistry, Stanford University, Stanford, CA 94305-5080, USA.

relatively simple instrumentation. Although this powerful technique enjoys the reputation of high resolution and superior efficiency in separation of charged molecules, its power diminishes in case of uncharged molecules and charged molecules with identical electrophoretic mobility.

EC has the potential to serve as an ideal bridge connecting micro-HPLC and CZE and, therefore, to allow the combination of the high efficiency in CZE with the wide range of application and selectivity in micro-HPLC.

As early as in 1952, Mould and Synge [8] applied electroosmotic flow (EOF) in thin-layer chromatography for the separation of substances according to their molecular masses. Twelve year later, Pretorius et al. [9] demonstrated, with unretained solute, the improvement of efficiency by using electroosmosis in both thin-layer and column chromatographic systems. An electroosmotic pump for isotachophoresis was described by Ryšlavý et al. [10]. In 1981 [7], Jorgenson and Lukacs reported the results of the performance of electroosmotic pumping in a chromatographic system. Tsuda [11,12] separated compounds with similar capacity factors but different charges using combined pressure and voltage. A systematic study on EC with packed columns was reported by Knox and Grant [13]. Their results of higher plate efficiency in EC over HPLC are quite convincing. However, they pointed out that the big obstacle lies in the effect of Joule (self) heating which limits the useful column inner diameter to 75 μm. The possibility and limitation of applying electroosmosis in a capillary packed with reversed phase were also investigated by Yamamoto et al. [14] and others [15,16]. Packed capillary columns with 50, 75 and 100 μm I.D. were successfully used in EC.

One of the major challenges to the future development of EC, as in micro-HPLC and CZE, is the detector sensitivity. With the use of small-I.D. columns, the sensitivity of on-column detection is restricted by the limited optical path length although several approaches have been developed to overcome this obstacle. The main objective of this investigation is to explore the possibility and limitation of using wide-diameter columns, such as 320 μm I.D. columns which are routinely used in micro-HPLC, in order to ex-

tend the applicable range of EC by improving the detectability and column loadability.

In this article, we report the results of our investigations on EC with 320 μm I.D. columns packed with 3-μm ODS. In addition, a new concept of column arrangement with a 50 μm I.D. column packed with 3-μm ODS in EC will be described.

2. Experimental

2.1. Apparatus

The outline of the chromatographic system used for both EC and micro-HPLC in this study was described previously [14]. A schematic diagram is shown in Fig. 1. The stainless-steel six-port rotary valve including an injection port (Model 7010; Rheodyne, Cotati, CA, USA) serves as a injection manifold. A high-pressure pump (Model 100 solvent metering system; Altex, Berkeley, CA, USA) connected to the injection manifold for HPLC operation was also used for filling the column with mobile phase and eliminating gas bubbles from the column for EC. A carbon electrode connected to the power supply (Alpha MKII, Model 2907 P, 0–60 kV; Brandenberg, Surry, UK) was dipped into the anode chamber filled with mobile phase. The chamber was connected to the injection manifold by a stainless-steel tube. So, the high voltage was
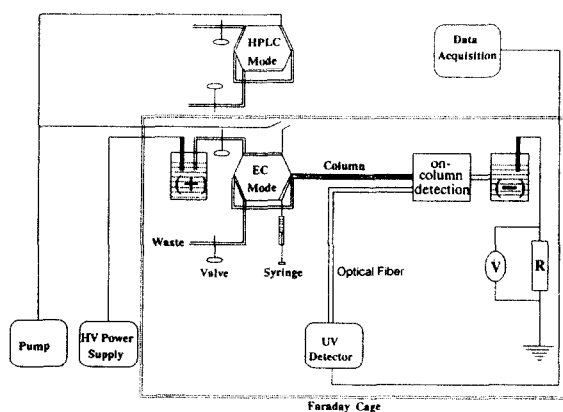

Fig. 1. A schematic diagram of the equipment used for both electrochromatography and micro-HPLC. HV = High-voltage.

applied to the stainless-steel six-port valve through the anode chamber (see Fig. 1). The outlet of the column was inserted into a capped cathode vial containing an electrode led to earth via an electrical resistor which was connected to an electrometer (168 Autorang DMM; Keithley Instruments, Cleveland, OH, USA) to measure the current. On-column detection was carried out with a modified UV detector (Model 783A; Applied Biosystems, Foster City, CA, USA) connected to a pair of optical fibres (about 60 cm long, 600 μm I.D., 1 mm O.D.; Laaber, Rüsselsheim, Germany). The equipment was partially housed within a Faraday cage which ensured the automatic disconnection of the high-voltage power supply whenever the cage was opened.

2.2. Columns

The 320 μm I.D. (450 μm O.D.) column packed with 5-μm Spherisorb ODS was obtained from LC Packings (Amsterdam, Netherlands). The original frit (a piece of PTFE filter) had to be removed after several runs because leaking was observed in the joint (glued) area between the column end and a piece of empty capillary. The new outlet frit was made as follows. First, a frit was sintered [14] at the end of a piece of empty capillary (*ca.* 15 cm × 50 μm I.D. × 280 μm O.D.) on which a detection window was created by burning off about 3 mm of the polyimide coating. The capillary with the sintered frit was then inserted into the 320 μm I.D. column and glued. The inlet frit was made in the same way but without detection window (see Fig. 2a).

The 50 μm I.D. (280 μm O.D.) column was packed with 3-μm Hypersil ODS (Shandon Southern Products, Runcorn, UK) similarly to the way described by Yamamoto *et al.* [14], but with the following simplification: the outlet of the packed column with a sintered frit was connected to a piece of empty capillary (on which a window was created) by means of a PTFE sleeve connector (250 μm I.D., LC Packings) (see Fig. 2b). In doing so, we were able to avoid the tedious and time-consuming procedure of packing and depacking the column with silica gel prior to packing with reversed phase [14].
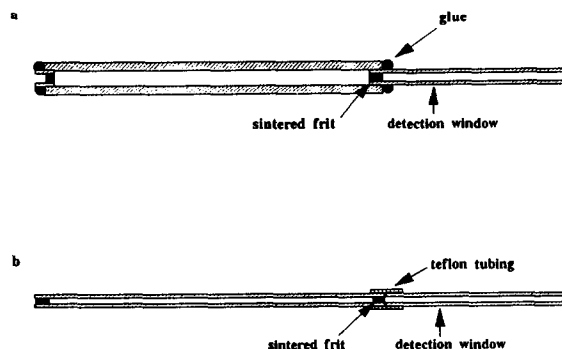

Fig. 2. Schematic presentation of the cross-section of the columns: (a) 320 μm I.D. packed column; (b) 50 μm I.D. packed column.

2.3. Chemicals

Sodium tetraborate, acetonitrile, thiourea, benzylalcohol, benzaldehyde, benzene and naphthalene were obtained from Merck (Darmstadt, Germany). The mobile phase was prepared by mixing 4 mM sodium tetraborate buffer (pH 9.1) with acetonitrile followed by filtration through a nylon-66 membrane (0.22 μm pore size) and by degassing (ultrasonic bath and helium).

2.4. Procedures

EC experiments were carried out as follows. First the column was connected to the injection manifold (set at HPLC mode, see Fig. 1) and flushed with pure buffer. The pump was switched off but not disconnected until the pressure reading dropped to zero. Then the above procedure was repeated, but this time using selected mobile phase instead of pure buffer. The column was then mounted as shown in Fig. 1. The outlet was inserted into the capped cathode chamber filled with mobile phase. After the pressure reading dropped to zero, the pump was disconnected and the injection manifold was switched to EC mode. Electrokinetic injection was carried out by filling the injection manifold with sample solution and then applying a low voltage (5 kV) across the column for about 5 s. The sample solution in the injection manifold was then thoroughly washed out with the mobile phase and the outlet valve

was closed. Finally, high voltage was applied and elution proceeded to start EC.

Micro-HPLC was carried out using the same chromatographic system with both hydrodynamic and electrokinetic injections. The hydrodynamic injection was carried out as follows. The injection manifold in the EC mode was filled with the sample solution. It was then switched to HPLC mode and low pressure (*ca.* 10 bar) was applied for *ca.* 3 s. The injection manifold was switched to the EC mode and flushed with mobile phase and the outlet valve was closed. The injection valve was switched back to HPLC mode and pressure was applied and elution proceeded.

The UV detector output was collected and analyzed by means of a chromatography laboratory automation system (CLAS; Perkin-Elmer, Norwalk, CT, USA). The experimental system was not thermostated or cooled except (when the ventilation system was off) a fan was used to blow air onto the column to encourage heat dissipation. Sample concentration of test mixture was estimated to be in the range of 0.5 to 1.0 mg/ml.

3. Results and discussion

3.1. EC with 320 μm I.D. column

Fig. 3 shows the EC separation of a test mixture of four neutral compounds on a commercially available 490 mm × 320 μm column packed with 5-μm Spherisorb ODS. The elution was carried out with acetonitrile–4 mM sodium tetraborate buffer (pH 9.1) (90:10, v/v) at an applied voltage of 50 kV. Taking thiourea as an "unretained" solute marker, an electroosmotic velocity of 0.35 mm/s was calculated. Reduced plate heights were 3.1 for thiourea and 3.2 for benzaldehyde. In another experiment, using a mobile phase with 80% organic modifier, the influence of applied voltage on column efficiency was studied. EC parameters for benzaldehyde are listed in Table 1. Note that the plate height decreases with increasing applied voltage, indicating that a better column efficiency is attain-
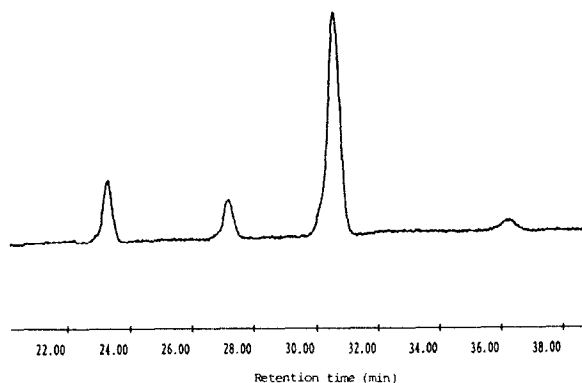

Fig. 3. Electrochromatographic separation of test mixture with 490 mm × 320 μm I.D. column packed with 5-μm Spherisorb ODS. Mobile phase: CH_3CN–4 mM sodium tetraborate (pH 9.1) (90:10, v/v). Solutes in order of elution: thiourea, benzylalcohol, benzaldehyde, benzene.

able at even higher voltage (higher electroosmotic velocity). However, plots of applied voltage *vs.* measured current (Fig. 4) indicate, at least for 80% acetonitrile, a non-linear relationship, suggesting that Joule heating occurs within the column. In fact, attempts to increase the EOF in this particular column by applying higher voltages (56 kV) did not succeed because of the formation of bubbles inside the column.

In addition, the original frit of the 490 mm × 320 μm I.D. column did not withstand repeated operation and we therefore shortened the column and inserted, at both ends, empty 50 μm I.D. capillaries, carrying each a sintered frit (see Fig. 2a). The following experiments were all carried out with columns modified in this way.

A plot of EOF *vs.* applied voltage (V) and

Table 1
Electrochromatographic parameters for benzaldehyde on the 490 mm × 320 μm I.D. packed column

V (kV)	t_R (min)	N	H (μm)	h
30	54	20 000	25	5.0
35	41	26 000	19	3.8
40	36	35 000	14	2.8
45	32	41 000	12	2.4
50	29	46 000	11	2.2

Mobile phase: CH_3CN–4 mM sodium tetraborate (pH 9.1) (80:20, v/v).

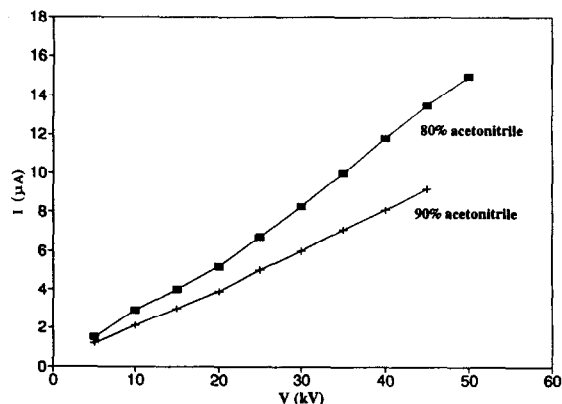Fig. 4. Ohm's law plots for the 490 mm × 320 μm column.

current (I) from data obtained with a 250 mm × 320 μm I.D column is shown in Fig. 5. The relationship between EOF and I is linear, as commonly observed in CZE [7] and MECC [17], and suggests that there was no significant effect of Joule heating in the column under these experimental conditions. However, this could be misleading since we were working in a relatively narrow range of voltage (see also Fig. 4).

By applying 40 kV voltage across the column, we were able to generate a EOF of 0.43 mm/s, which was still lower than one would like to have. The dependence of linear velocity on the concentration of organic modifier in the mobile phase is shown in Fig. 6. Results indicate that, at
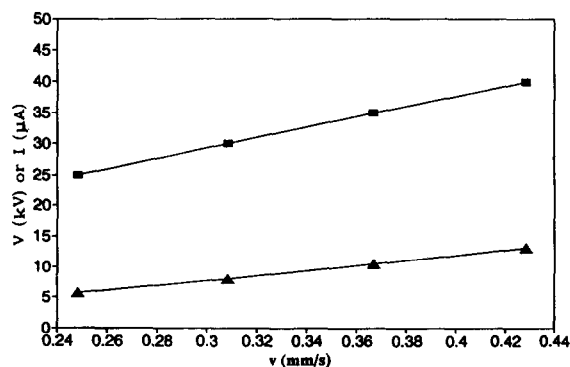

Fig. 6. Effect of organic modifier (CH_3CN) on electroosmotic velocity for 150 mm × 320 μm I.D. packed column at constant voltage of 25 kV.

concentrations of organic modifier higher than 40%, the EOF significantly decreases.

In order to compare the performance of EC with that of micro-HPLC, separations were carried out on the same column with both electroosmosis- and pressure-driven flow under otherwise the same conditions. Fig. 7 shows the plots of reduced plate height vs. linear velocities (Van Deemter plot) generated with both electroosmosis- and pressure-driven flow. The direct comparison shows that, in the range of 0.2 to 0.5 mm/s linear velocity, EC is more efficient than micro-HPLC. Higher velocities could not be achieved by EOF (see above) but one would

Fig. 5. Dependence of electroosmotic velocity (v) on applied voltage (V, ■) and current (I, ▲) for 250 mm × 320 μm I.D. column packed with 5-μm Spherisorb ODS. Mobile phase: CH_3CN–4 mM sodium tetraborate (pH 9.1) (80:20, v/v). Thiourea was used as unretained solute marker.
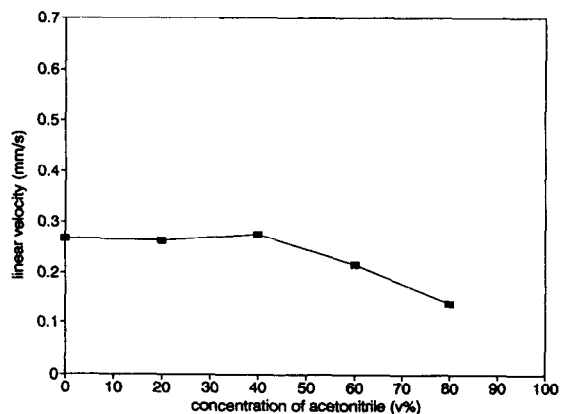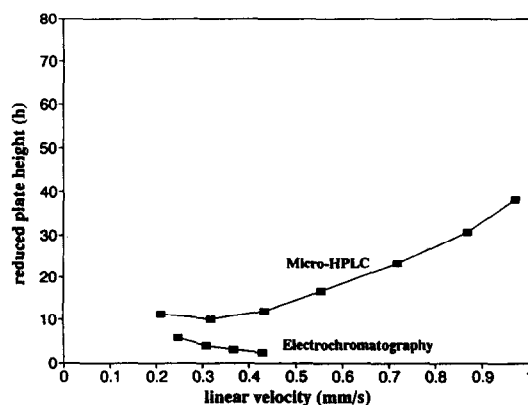

Fig. 7. Dependence of reduced plate height on mobile phase velocity obtained from both electroosmosis- and pressure-driven flow. Conditions as in Fig. 5.

predict that the efficiency might even be improved with flows higher than those achieved under the present experimental conditions. Micro-HPLC, on the other hand, is characterized by a decrease in efficiency at higher linear velocities.

The higher efficiency of EC is convincingly demonstrated in Fig. 8 showing the expanded peaks for thiourea on the same column using, respectively, electroosmosis-driven and pressure-driven flow under otherwise identical conditions.

3.2. EC with 50 μm I.D. column

At the current state of the development of EC, the best results are obtained with 50 μm I.D. capillaries. Similar to the concept introduced recently by Van Soest et al. [15], we used a new method for column arrangement: a packed capillary column with sintered frits at both ends was connected to a piece of empty capillary carrying a window for on-column detection. The connection was made by a PTFE sleeve with an inner diameter barely smaller than the outer diameter of the capillary. A schematic drawing of this two-stage column is given in Fig. 2b. Note that, instead of using a piece of filter as an outlet frit [15], we sintered the frit by gentle heating of the column end (ca. 2 mm) filled with 4-μm silica
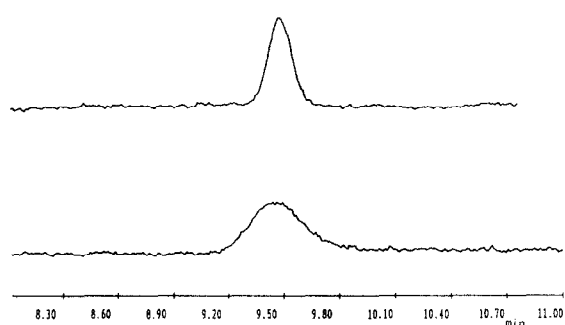
wetted with sodium silicate solution [14]. With the sintered frit we were able to make a firm and direct column–capillary connection (without using any filter in between). In our experience, this arrangement significantly reduces the chance of bubble formation in the joint area. Following this design, we packed a 230 mm × 50 μm I.D. column with 3-μm Hypersil ODS and used it for the separations of a test mixture in both EC and micro-HPLC. For comparison purposes, the applied voltage in EC was adjusted to yield the same linear flow (0.8 mm/s) as obtained with micro-HPLC. The chromatograms from the two non-optimized separations are shown in Fig. 9 and prove that the efficiency of electroosmosis-driven chromatography is higher than the efficiency of pressure-driven chromatography. The columns of the type described in Fig. 2b are particularly convenient and their preparation is less time-consuming compared with the previously used method with the outlet frit located several centimetres from the end of the capillary [14]. Another advantage of the column arrangement is that the empty capillary carrying the detection window can be retained so that there is no need to replace it if a new packed column is mounted.

Fig. 8. Comparison of peak broadening between EC (upper; h = 4.4, applied voltage 40 kV) and HPLC (lower; h = 19.1, pressure 20 bar) on a 250 mm × 320 μm I.D. packed column. Conditions as in Fig. 5 except for applied voltage and pressure.
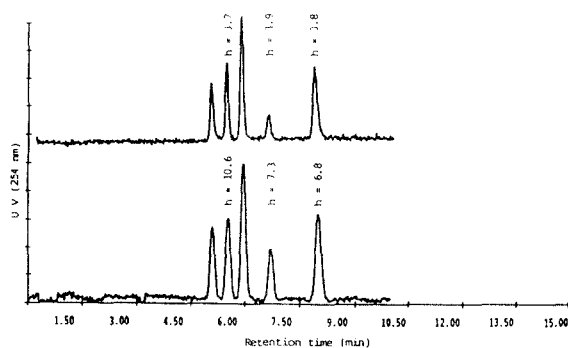

Fig. 9. Electrochromatographic separation of test mixture on a 230 mm × 50 μm I.D. column packed with 3-μm Hypersil ODS. Mobile phase: CH_3CN–4 mM sodium tetraborate (pH 9.1) (80:20, v/v). Solutes in the order of elution: thiourea, benzylalcohol, benzaldehyde, benzene, naphthalene. (Top) EC, applied voltage 23 kV; (bottom) micro-HPLC, pressure 130 bar.

3.3. Major problems and difficulties in EC

Joule heating

Although it is believed that EC is indeed a promising separation technique, there are practical problems and difficulties, especially when wide-diameter columns are used. The major obstacle is Joule heating [13,18]. Joule heating of an EC system results in a temperature gradient in the column which consequently causes band broadening. What makes things worse is that the temperature increase in the packed column, where the particles and frits act as "boiling chips", can assist in the formation of bubbles which generate baseline noises and dried-out sections in the column and eventually break the current and thus stop the EOF. Shown in Fig. 4 are Omh's law plots for the 490 mm × 320 μm I.D. column packed with 5-μm Spherisorb ODS, where the Joule heating is clearly indicated by the deviations of the plots from linearity. According to Knox and Grant [18] and Poppe and co-workers [19,20], the temperature difference between the centre of a cylinder and its wall is given by

$$\Delta T = \frac{Qd^2}{16K} = \frac{EI}{4\pi K} \qquad (1)$$

where Q is the rate of heat generation per unit volume within the cylinder, d is the column diameter, K is the thermal conductivity of the medium, E is the field strength and I is the current. The self heating is much greater in a wider-bore column since it is proportional to the square of the column diameter.

While the theoretical calculations led to the speculations that the use of a packed capillary column in EC may be limited to columns with I.D. < 100 μm, this experimental investigation shows that 320 μm I.D. columns can be used in EC in practice. This is probably due to the following reasons. (a) The amount of heat generated in EC with a packed column, as compared to CZE with an empty tube with the same diameter, is severely reduced because of the relatively low electric current (0–20 μA, see Fig.

4) due to the high resistance of the mobile phase (e.g., with 80% acetonitrile) plus the packing material. (b) The Joule heating effect on peak dispersion in EC is mainly caused by the changes of partition of the solutes (usually neutral) due to the temperature gradient in the column, in contrast to CZE where, in addition to the migration changes caused by the parabolic flow profile of the mobile phase due to the temperature gradient, the electrophoretic mobility of the solutes (usually charged) depends on the mobile phase viscosity. (c) The parabolic flow profile generated by the self-heating may be counterbalanced to some extend by the opposite parabolic profile caused by the relatively lower EOF in the centre of the column due to the partial coverage of the silica surface of the packing particles (ODS). To summarize, the influence of inhomogeneity of temperature on peak broadening due to Joule heating is much less in EC than in CZE.

Nevertheless, the formation of bubbles in the column is still a major problem in EC performance. Therefore, thorough degassing of the mobile phase is extremely important and efficient cooling system for further development of EC with wide-diameter columns may be necessary.

Frit

Frit is the key to the success of using electroosmotic pumping in a chromatographic system. If fluorescence detector (on-column detection) is used, a window can be created within the packed section of the column [13] and this makes the outlet frit-making easier and also preserves the integrity of the migrating zone of the solutes. This sensitive device, however, is limited to detect only fluorescent solutes and there are many cases where other type of detector are desirable. The more commonly utilized UV detector requires a window created on a piece of empty capillary after the frit. While sintering a frit on a narrow-diameter column has been done without problems [7,13,14, and this paper], it becomes increasingly difficult when dealing with columns with wider diameter (say, 320 μm or up). Therefore, some sort of arrangement has to

be made (see, e.g., Fig. 2) to construct a frit and create a window. A good frit should possess at least the following characteristics: (a) it must be sufficiently mechanically strong to stand high pressure in the process of column packing; (b) it should be sufficiently chemically inert to bear the environment formed by the organic modifier and running buffer with certain range of pH value; (c) it must be sufficiently porous to allow the mobile phase and the solutes pass through (without significant back pressure) but, at the same time, not too porous to prevent the stationary phase bleeding; (d) it should not introduce significant dead volume to cause extra peak broadening.

Column packing

Column packing is crucial for the improvement of the performance of EC, as well as for the future commercialization of this technique. Knox and Grant [18] have predicted theoretically and proven experimentally [13] that the EOF is essentially unaffected by particle size at least down to 1.5 μm. As the particle size is reduced below 1 μm the plate height will be dominated by the B term (axial diffusion) in the Van Deemter equation ($H = A + B/u + Cu$). Should this theoretical prediction be substantiated, an efficiency of a million theoretical plates in less than 30 min could be achievable in EC. However, to pack such a column efficiently with fine particles will be a real challenge since, conventionally, extremely high pressure is required. Especially when coming to wide-diameter columns, application of such high pressure could be disastrous. Therefore, alternative ways have to be found out to pack micron and submicron particles in stable and uniform beds in order to take the full advantages of EC. Further investigation in this direction is underway.

4. Conclusions

EC is an unique separation technique which combines the high efficiency of CZE and the good selectivity of micro-HPLC. It can be used to separate both neutral and ionized molecules because retention is governed by the electrophoretic mobility of the solutes, as in CZE, as well as by their partitioning equilibrium, as in HPLC. The use of commercially available 320 μm I.D. packed columns in EC has been demonstrated and the advantages of EC compared to micro-HPLC are obvious. It is anticipated that even wider-bore columns could be used and that this would further improve the detectability and column loadability.

The new arrangement for EC proposed by Van Soest et al. [15] represents an important step towards a wider use of EC and together with the method presented here, further emphasizes its potential. As a matter of fact, packed columns with narrow diameter (such as 50 μm I.D.) might be routinely used in commercially available CZE instruments. Further investigation in this direction is already in progress.

5. References

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