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Electrochromatography with a 2.7 mm inner diameter monolithic column

Qi-shu Qu, You-zhao He*, Wu-er Gan, Ning Deng, Xiang-qin Lin

Department of Chemistry, University of Science and Technology of China, Hefei, Anhui 230026, PR China

Abstract

Monolithic columns of 2.7 mm I.D. have been prepared and used in electrochromatography (EC) separation. Although capillary electrochromatography (CEC) has higher separation efficiency, it displays some shortcomings, such as limited sample loadability and restricted concentration detectability etc. In this paper, we investigate the feasibility of EC separation with millimeter diameter monolithic columns. By using a designed preparation method of monolithic column packed with about 150 μm quartz sand, the effect of Joule heating can be reduced, and the processes of frit making and column packing can be avoided. The concentration detectability of the EC is improved comparing with that of CEC. Moreover, the separation efficiency of 52 000 plates/m was achieved with a 70 mm length and 2.7 mm I.D. monolithic column.
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1. Introduction

Capillary electrochromatography (CEC) is a developing analytical technique, which combines the advantages of high selectivity of high-performance liquid chromatography (HPLC) and high separation efficiency of capillary electrophoresis (CE). CEC was introduced by Pretorius et al. in 1974 [1] and demonstrated as a powerful separation technique by Jorgenson and Luckacs in 1981 [2]. In current CEC practices, two main types of capillary columns have been adopted, viz. packed and open tube columns. Because the former displays higher sample capacity and better retention capability than those of the latter, it has become the predominant style for CEC separation.

However, column frits should be prepared in a packed capillary column in order to prevent loss of stationary phase. There are several ways to prepare column frits. For instance, the frits were prepared by the polymerization of potassium silicate and formamide, by sintering spherical silica gel wetted with potassium silicate solution and by sintering of pure spherical silica gel etc. [3]. All the frits showed their limitations in separation velocity and column-to-column reproducibility. In addition, bubble formation in frits is another shortcoming during the CEC run. Because of the differences in the resistance and permeability between frits and the packed part, Joule heating can make bubble formation easier in the frits than in packed part. Once small bubbles are pro-

*Corresponding author. Fax: +86-551-363-1760.
E-mail address: yzhe@ustc.edu.cn (Y.-z. He).

duced in a column, electric current of CEC can be reduced or even cut off.

In order to overcome the frit shortcomings, monolithic capillary columns have been developed in the CEC technique. The preparation methods of monolithic capillary columns include organic polymer-based polymerization [4–11], bonded silica stationary phase immobilized through the use of sol–gel techniques [12–22] and sintered octadecylsilica [23]. With the monolithic capillary columns, the CEC can eliminate the disadvantages caused by frits and packed capillaries. So the separation method has been paid more attention by analytical chemists.

However, both the packed and monolithic capillary columns have their disadvantages, such as limited sample loadability and restricted concentration detectability etc. In fact, CEC columns with I.D. greater than 150 μm have been successfully employed by several groups. Yamamoto et al. [24] demonstrated the possibility of using a packed column with I.D. greater than 200 μm. Pusecker et al. [25] used a packed column of 250 μm I.D. coupled with a NMR spectrometer using a relatively low working voltage, which can reduce the effect of Joule heating. Yan et al. [26] showed that 320 μm I.D. packed columns could be used in CEC separation, although slightly positive deviations from the linearity of its Ohm plots were observed. Recently a semi-preparative separation, which was performed with a 550 μm I.D. packed column and low separation voltage (<5 kV), was reported by Chen et al. [27] and 60 000 plates/m was achieved. In fact, Pretorius et al. [1] were the first group to introduce a 1 mm I.D. packed column in EC separation in 1974. The separation method with a column diameter greater than 500 μm can be called electrochromatography (EC) separation. Till now, monolithic columns with an inner diameter greater than 1 mm have not been used in EC separation. But they have already been employed in HPLC separation successfully [17–19].

Based on the works mentioned above, large diameter monolithic columns may be used in EC separation if their Joule heating can be limited. In this paper, an EC separation with a 2.7 mm I.D. monolithic column and separation voltage lower than 1000 V was presented, and characteristics of the column were investigated. This type of EC separation may

be developed as a complementary method for CEC to improve the sample capacity and concentration detectability, may carry out preparative separation, and may become an effective separation technique for electrokinetic flow analysis [28,29].

2. Experimental

2.1. Chemicals and materials

Fused silica tubes of 2.7 mm, 4 mm, 5 mm and 6 mm I.Ds. were purchased from Glass Instrument (Shanghai, China). Deionized water was obtained from Kesheng (Hefei, China). Chromatographic grade acetonitrile was purchased from Tedia (Fairfield, USA). Chromatographic grade methanol was obtained from Hanbang Science & Technology (Jiangsu, China). Analytical grade of quartz sand with 120~180 μm granularity (called "fine quartz sand" in this paper), glacial acetic acid, sodium acetate, hydrochloric acid, potassium hydroxide, tris (hydroxymethyl) aminomethane (Tris), thiourea, benzal chloride, benzyl chloride, benzaldehyde and formamide were purchased from Chemical Reagent (Shanghai, China).

2.2. Formation of submicron silica

Submicron silica was prepared before the synthesis of monolithic columns. Its preparation method refers to Stöber's work [30], which was based on hydrolysis and subsequent condensation of alkoxysilane in a short chain alcohol solution with ammonia as the catalyst. The particle size of the submicron silica prepared by the Stöber method was around 500 nm.

2.3. Preparation of monolithic EC columns

Fused-silica tubes with different inner diameter were pretreated before use. The tubes were immersed into 2 M KOH solution for 2 h and then washed with deionized water. After being dried at 150 °C for 1 h and cooled to room temperature, the tubes were clamped vertically, added with the fine quartz sand slowly and vibrated till the required height. The monolithic columns were fabricated by the method

similar to that used to cast column frits in the fused-silica tube for the packed column of HPLC [12]. Submicron silica (0.4 g) was dissolved in 1.4 ml 2.5 M KOH solution and formed a potassium silicate solution. Formamide was added into the potassium silicate solution to a weight ratio of 6% drop by drop and stirred thoroughly until formamide was completely dispersed. Hydrolysis of formamide in the basic potassium silicate solution led to the formation of ammonium by the reaction expressed as:

$$HCONH_2 + KOH + H_2O \rightarrow HCOOK + NH_4OH$$
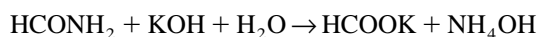
The produced ammonium hydroxide gradually reduced the pH of the solution from ca. 11.8 to 10.8, and initiated the polycondensation of silica [31]. When formamide has completely dispersed in the potassium silicate solution, the mixture solution was added slowly into the fused-silica tube packed with fine quartz sand. When the whole column was filled with the solution, it was sealed up at the bottom end, placed vertically in a drying oven and kept at 60 °C for 1 h. Then the oven temperature was raised up to 150 °C and kept for 5 h. After polymerization, the excessive tube of the column was cut off. Before EC separation, the column was flushed with buffer solution for 60 min.

2.4. SEM characterization of monolithic columns

Scanning electron microscopy (SEM) was used for the characterization of the monolithic columns. All electron microscopy images were obtained by a Hitachi X-650 scanning electron microscope. The SEM images of the column cross-section were obtained after the monolithic columns were cut transversely and smoothly.

2.5. Electrochromatography

A schematic diagram of the home-made EC system is shown in Fig. 1. All the EC separations were performed with the system equipped with an UV absorption cell and detected at 254 nm with a spectrophotometer (WFX-1B. Second Optical Instrument Factory, Beijing, China). Neither pressurizing nor cooling steps were applied to the EC separation.
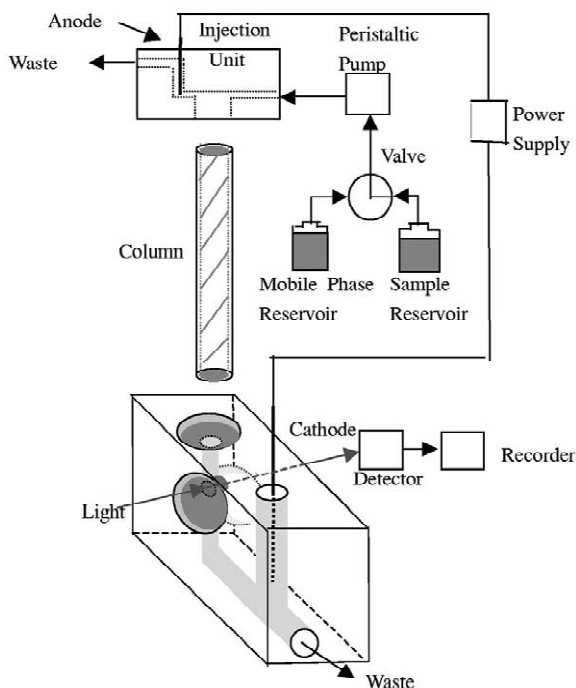

Fig. 1. Schematic diagram of the EC separation system.

Sample solution was injected into the monolithic column electrokinetically by an injection system, including a home-made injection unit, a peristaltic pump (Guokang Instrument, Xinchang, Zhejiang, China) and a three-way valve (Zhaofa Automatic Analysis Institute, Shenyang, China). During injection, approximate 10 µl sample solution was introduced into the injection unit by the peristaltic pump. Then the valve turned back and the buffer solution was introduced into the injection unit. Tris–HCl (50 mM) buffer stock solution was prepared by dissolving 1.211 g Tris in 100 ml deionized water, adjusted to pH 8.0 with 0.1 M HCl solution by a pH meter (PHB-1, Sanxin Instrument Factory, Shanghai, China), and then diluted to 200 ml with deionized water. A 2 mM Tris–HCl buffer solution, viz. mobile phase, was prepared with CH_3CN and pH 8.0, 50 mM Tris–HCl in a volume ratio of 96:4. The sample solution was electrokinetically injected into the column at 1000 V provided by an electrophoretic power supply (DYY-III-4, Liuyi Instrument Factory, Beijing, China) when it passed through the top of the column. The injection volume can be controlled by

the flow-rate of the peristaltic pump and the injection time of the valve. By using this injection system, the redundant sample solution can be flushed out to a waste vial and the fresh buffer solution can be supplied before running the EC. Thus, the sample contamination can be avoided and the reproducibility of sample injection can be improved.

3. Results and discussion

3.1. Column preparation

A simple preparation method for the monolithic columns was developed by immobilizing the fine quartz sand with silica xerogel in this paper. This technique provides a stationary phase with low thermal effect, which can reduce Joule heating and improve reproducibility during EC running.

In the processes to fabricate the monolithic columns, filling the column with fine quartz sand is an important step. By packing the fine quartz sand in the columns, slight shrinkage during the column formation will be avoided and the silica xerogel can be attached tightly on to the inner surface of the fused-silica tube. By packing the fine quartz sand in the columns, the porosity of the monolithic columns can be decreased. As a result, relatively low current can be achieved and the produced heat can be dispersed by fine quartz sand. Thus, Joule heating can be limited, as observed in Fig. 6 by comparing the 2.7 mm I.D. column with fine quartz sand to the 3.0 mm I.D. one without quartz sand. And by packing the fine quartz sand in the columns, it can also be beneficial to enhance and stabilize the electro-osmotic flow (EOF) on the surface of the silica xerogel.

3.2. SEM characterization of the monolithic column

The SEM micrographs of the monolithic columns are illustrated in Figs. 2–5.

Fig. 2 shows the cross-section micrograph of a monolithic column at a magnification of ×30. This micrograph reveals that the entire cross-section of the fused-silica tube filled with the fine quartz sand was immobilized by silica xerogel. The micrograph
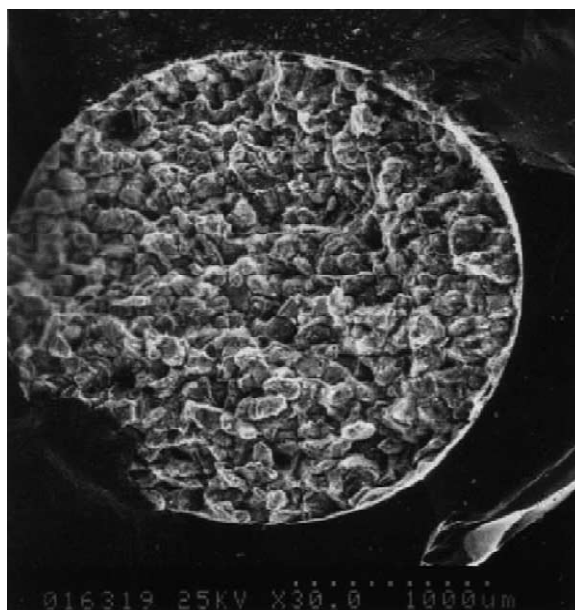

Fig. 2. Scanning electron micrograph of the monolithic column prepared in 2.7 mm I.D. fused-silica tube. Magnification, ×30.
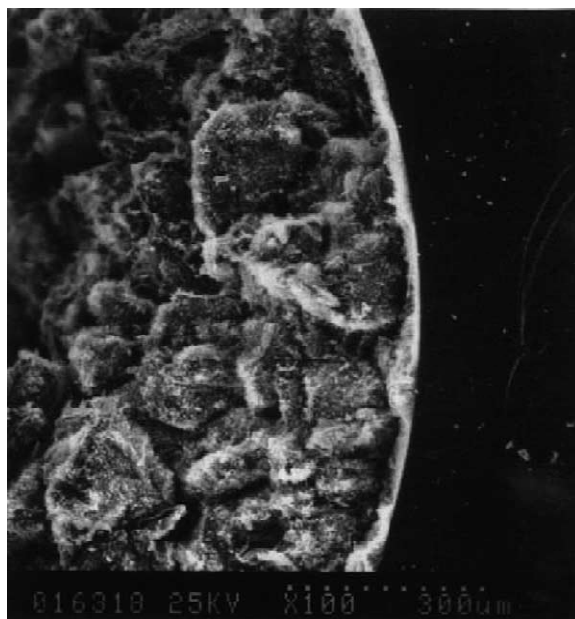

Fig. 3. Scanning electron micrograph of a monolithic column at the interface of the monolithic rod and inner wall of fused-silica tube. Magnification, ×100.
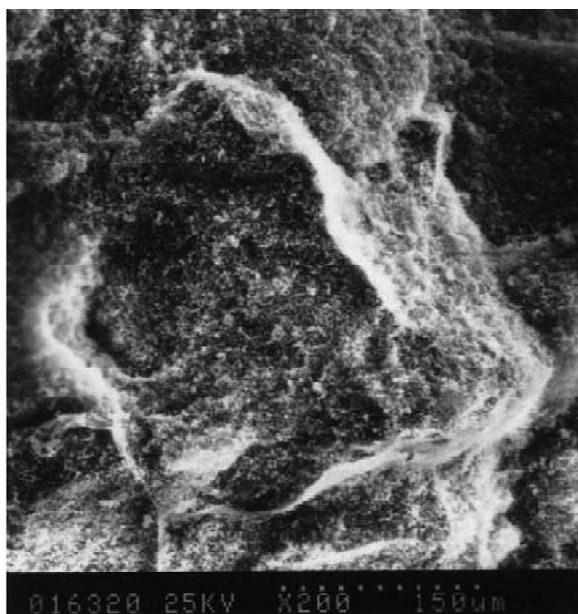
Fig. 4. Scanning electron micrograph of quartz sand immobilized by silica xerogel. Magnification, ×200.

Fig. 5. Scanning electron micrograph of stationary phase. Magnification, ×10 000.

of ×100 in Fig. 3, viz. an enlarged one of Fig. 2, shows clearly that the fine quartz sand immobilized by silica xerogel is attached tightly on to the inner wall of fused-silica tube. Fig. 4 shows that the fine quartz sand particles were attached to each other tightly by silica xerogel. Additionally, in Fig. 5, a partial view of the surface of the stationary phase at a magnification of ×10 000, reveals the surface of the silica xerogel and its morphological characterization, and it does have the pore canals ranged from 0.3 μm to 2 μm. EOF can be generated in the canals, but its velocity will not be depressed within the canal ranges [32–34].

Among the monolithic columns with different diameters, their configuration appeared very similar. Fig. 6 summarizes the Ohm plots of various I.D. columns. It shows that even if the I.D. of the columns with fine quartz sand rises up to 6 mm and the working current increases to 1500 μA, the deviation from the linearity between electric field strength (Fig. 6) and current can not be observed except for the 3 mm I.D. one without fine quartz sand. According to Ohm's law, the relationship between electric field strength and current can be expressed as Eq. (1):

$$I = \pi r^2 \lambda \phi c E \qquad (1)$$

where I is working current, r is column radius, λ is molar conductivity of the buffer solution, ϕ is
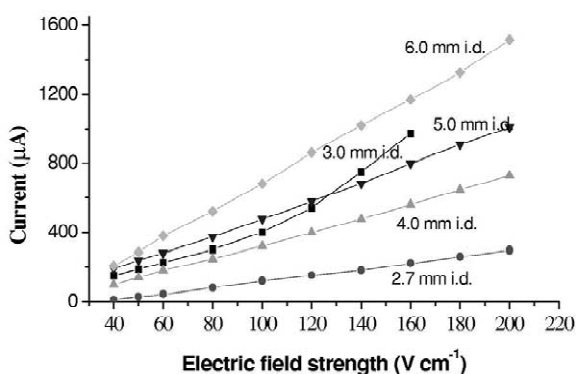

Fig. 6. Effect of electric field strength (E) on current for monolithic columns with various inner diameters. The monolithic columns are filled with 120–180 μm fine quartz sand except for the 3 mm I.D. one. Buffer solution: pH=4.0, 1 mM HAC–NaAC. UV detection is at 254 nm.

porosity of the column, c is buffer concentration and E is electric field strength. If the excessive Joule heating occurs, the Ohm plots will show positive deviation from its linearity since the molar conductivity of buffer solution and the diffusion coefficient of the buffer ions will be affected by temperature with positive correlation. Our experimental data indicate that there is no excessive Joule heating occuring within the column ranges from 2.7 mm to 6.0 mm I.D., except for the 3 mm I.D. column, in which fine quartz sand is not used. It suggests that these large diameter columns can be adapted for the EC separation. In addition, by comparing the 2.7 mm I.D. monolithic column with fine quartz sand to the 3 mm I.D. one without quartz sand in Fig. 6, we can find clearly that the current decreased a lot by filling with quartz sand. The experimental results indicate that the heat generated in the column can be reduced by adding quartz sand.

3.3. Electro-osmotic flow in the monolithic column

EOF plays an important role in EC separation for it can provide the driving flow and its flat flow profile can reduce the axial dispersion of the separated zones [10,35]. In this paper, fine quartz sand is used as the stationary phase bed and provides negative surface charges under the EC conditions. However, silica xerogel prepared from formamide can offer positive charges. Our experimental results showed that the EOF direction could be reversed by increasing the amount of formamide added into the potassium silicate solution during the polymerization of the monolithic column. The electro-osmotic flow velocity ν_{eo} can be expressed as:

$$\nu_{eo} = \frac{\epsilon_0 \, \epsilon_r \, \zeta \, E}{\eta} \tag{2}$$

where ν_{eo} is electro-osmotic velocity, ϵ_o is permittivity of vacuum, ϵ_r is relative dielectric constant, ζ is zeta potential, η is viscosity of buffer solution. Fig. 7 shows the effect of electric field strength on EOF velocity. As shown in the figure, the EOF velocity increases linearly with the increase of the electric field strength. It indicates that the influence of Joule heating in the 2.7 mm column will not be excessive within the range of electric field strength.
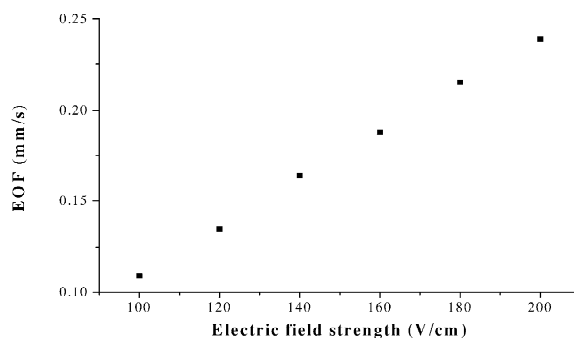

Fig. 7. Effect of electric field strength (E) on electroosmotic velocity (EOF) with a 40×2.7 mm I.D. monolithic column. Buffer solution: a volume ratio of 4% 50 m*M* Tris–HCl (pH= 8.0) in acetonitrile. UV detection is at 254 nm.

Otherwise, the EOF velocities will departure from linearity because of the temperature effect on η in Eq. (2). It proves again that the EC separation with the large diameter monolithic columns is possible.

3.4. Chromatographic characterization of monolithic columns

One of the main influences on separation efficiency is Joule heating during the EC run. The effect of Joule heating can result in a temperature gradient in the radial monolithic column and can consequently cause the sample zone broadening. Knox and Grant [31] analyzed this phenomenon. The temperature difference between the center and inner wall of a column can be expressed by Eq. (3):

$$\Delta T = \frac{Q d_c^2}{16 \, K} = \frac{E^2 \lambda \, c \, \phi \, d_c^2}{16 \, K} \tag{3}$$

where Q is the rate of heat generation in per unit volume of the column, d_c is the I.D. of the column and K is thermal conductivity. From Eq (3), it seems that it is impossible to use a large-diameter column for EC because the self-heating generated in a column is proportional to the square of column diameter. Knox and Grant substituted typical values of CEC into Eq. (3), such as $E=50\,000$ V m^{-1}, $\lambda=0.015$ m^2 mol^{-1} Ω^{-1}, $c=10$ mol m^{-3}, $\phi=0.75$, $d_c=100$ μm and $K=0.4$ W m^{-1} K^{-1}. The results can be $Q=280$ W cm^{-3}. Generally, the range of electric field strength can be 30 000–90 000 V m^{-1} and that of buffer concentration can be 1–10 mol

m^{-3} in CEC. In contrast to CEC, the heat generated in per unit volume of a column in HPLC is 0.2 W cm^{-3} only [32]. So they came to the conclusion that the heat generated in per unit volume of CEC is about 1500 times larger than that of HPLC under typical operating conditions. In accordance with our EC conditions, we substitute the same values of λ and K and those of E, c, ϕ and d_c, which are 20 000 V m^{-1}, 2 mol m^{-3}, 0.4 [36] and 2.7 mm, respectively, into Eq. (3). It gives $Q = 4.8$ W cm^{-3} and
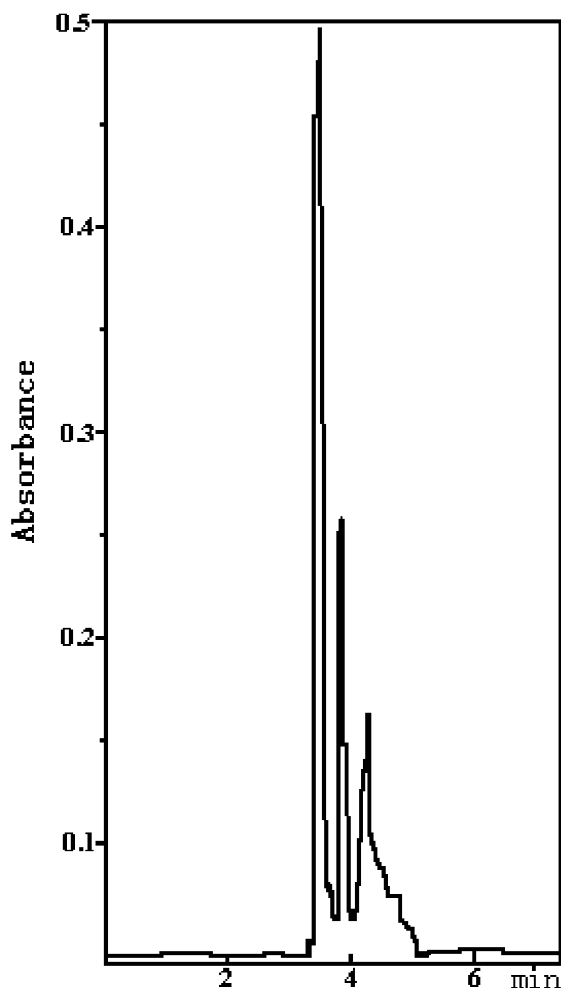

Fig. 8. Electrochromatogram of a mixed sample with a 70×2.7 mm I.D. monolithic column. Buffer solution: a volume ratio of 4% 50 m*M* Tris–HCl (pH=8.0) in acetonitrile; injection voltage and time is 1000 V and 2 s, and electrophoresis voltage is 1000 V; detection wavelength is 254 nm; Solutes: 1, 0.5 m*M* benzaldehyde; 2, 0.2 m*M* benzyl chloride; 3, 0.2 m*M* benzal chloride.

indicates that the heat generated in per unit volume of EC is about 24 times larger than that of HPLC and the experimental data aforementioned show clearly that the heat generated in the 2.7 mm I.D. columns is also accordant with the theoretical calculation. Further work will be carried out to decrease the center-to-wall temperature difference.

As an example, Fig. 8 shows EC separation of a simulated sample with a home-made 70 mm length, 2.7 mm I.D. monolithic column. The separation model was a typical normal-phase EC as expected. The separation efficiency achieved for benzaldehyde was about 52 000 theoretical plates per meter. In the figure, we can find clearly that the determination signal with 2.7 mm I.D. columns is much higher than that with normal CEC. The calculated detection limit of benzaldehyde is 0.3 μg/ml in the 2.7 mm I.D. columns. Scherer and Steiner showed that the detection limit of propyl benzoate was 11.5 μg/ml with a 100 μm I.D. capillary column [37]. In addition, a commercial detector can be employed for the proposed separation method, such as a simple spectrophotometer, for this work. So the trace concentration analysis that cannot be achieved in CEC, can be truly done with the proposed EC separation. Typical daily electrochromatograms for thiourea obtained by using a 40 mm length, 2.7 mm I.D. monolithic column showed consistent migration time and peak height. It indicated that the reproducibility of the column is good. The RSD of three individual separations for the migration time and peak height is 5.4% and 6.8%, respectively.

4. Conclusion

In sum, the EC of 2.7 mm I.D. monolithic columns filled with 120–180 μm quartz sand can be chosen as an effective separation technique, which can be a complementary separation method to CEC. It has satisfactory separation efficiency and repeatability. Several limitations of CEC, such as concentration detectability and sample loadability etc. can be improved by using the proposed separation technique. The EC technique can be developed as a complementary separation method for CEC, as a preparative separation method, and as a separation technique for electrokinetic flow analysis. The im-

provement of column preparation reproducibility, separation efficiency and the development of a reversed-phase model are our planned research topics.

Acknowledgements

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