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Novel fabrication of on-column capillary inlet frits through flame induced sintering of stainless steel particles

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ABSTRACT

A novel fritting technology was introduced for the fused-silica capillary. The technique involved sintering of stainless steel (SS) particles at the tip of capillary through flame heating. A simple butane gas based welding torch was used for sintering the SS particles. The new fritting technique, flame induced sintering of SS particles (FIS/SSP), was applied for making frits with different inlet diameters (75 μm, 100 μm, 250 μm and 530 μm). The changes in morphologies of SS particles during sintering process were identified by scanning electron microscopy (SEM). Frits with the length of 0.5–1 mm and capillaries with inner diameter about 50–100 μm were fabricated through suitable selection of experimental conditions (size of SS particles and heating mode). The frits prepared by FIS/SSP technique exhibited adequate separation properties and mechanical strength. Columns packed with C_{18} particles were stable with these frits in a few important chromatographic operations. Frits prepared by FIS/SSP technique was used in three typical separation modes namely, capillary electrochromatography (CEC), p-assisted CEC (p-CEC) and low pressure liquid chromatography (LPLC). Importantly, no bubble formation was noticed with the frit over a period of one week. A good peak symmetry and high efficiency for separation were obtained using pressure-assisted CEC, p-CEC and low pressure-driven separation modes.

1. Introduction

Miniaturization of liquid chromatography (LC) through the use of packed, open tubular, and monolithic capillary columns has opened up a number of possibilities for the rapid analysis of nanoliter and subnanoliter samples [1–4]. Because of the availability of numerous packing materials for conventional LC columns, the microsphere-packed columns are applied more popularly in the chromatographic separation fields as compared with monolithic columns and entrapped-microsphere columns [5–7]. Packed fused-silica capillaries with inner diameters in the range of 50 μm to 530 μm are typically used as columns in capillary electrochromatography (CEC), micro-high performance liquid chromatography (HPLC) and nano-HPLC [8]. The packing of micron sized particles into a capillary tubing and preparation of solid and stable column frits at the ends of the columns are the two important steps in the fabrication of capillary column. It has been noted that frit making is the most problematic step and challenging task to achieve good performances from the packed column. A 'good' frit should have the features such as high mechanical strength to sustain the high packing pressure, high permeability to have packing speed, short length to minimize the non-uniformity of packed bed, and good results for column-to-column separations. When many of the important features are inbuilt in a frit, it is necessary that the frit should be simple and fast to prepare so as to minimize the separation time. Generally, metal meshes as the frit of packing column result in a large dead volume for the chromatographic and EC separation. Also, the usage of in-line filters, to keep the stationary phase from leaving the column, limits the permeability and mechanical strength of the column along with band broadening during separation.

Over the past decades, many fritting strategies have been developed that include formation of hydrothermal frits for tapering the end of columns [9–13], coupled capillaries via a polytetrafluoroethylene sleeve or restrictors [11,14], silica-based sintered frits [15–17], and monolithic frits [18–21]. Knox and Grant made an inlet frit (without an outlet (retaining) frit) on quartz columns of 200 μm inner diameter (ID) by sintering a paste of native silica gel with wetted sodium silicate [22,23]. Frits made from mixtures of native silica and/or wetted with sodium silicate were mechanically more stable. Smaller frits (less than 1 mm) were made out of the packed bed itself by Lynen et al. [24], and further sintered by a perforated heating ribbon. Yamamoto et al. [25] obtained plate numbers >200,000 per meter for practically non-retained compounds (benzyl alcohol and benzaldehyde) on a packed bed with silica frits. Frits made directly from the packed bed did not cause much band broadening and were less prone to bubble formation [23,26,27].

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Maiolica et al. [28] reported the preparation of self-made frits for nanoscale columns by sol–gel technique. Wang et al. [29] inserted dry lichrosorb 50 μm Si 60A resins into a fused-silica capillary (about 0.5 mm), and then dipped it into a sol–gel solution for subsequent polycondensation. The on-column frit preparation and its utility for peptide separation in LC-MS/MS were described [29]. The columns with online frits could replace any capillary liquid transfer tubing without additional connectors. The performances of frits prepared with sol–gel, sintering, photopolymerization and modified sol–gel technologies were compared [30]. Sol–gel and photopolymerized frits were reported to have improved day-to-day and column-to-column reproducibility. Recently, a novel single-particle fritting technology has been developed to manufacture particulate-packed capillary columns with excellent robustness for CECs [30]. Superior performances relative to traditional sinter-fritted column were notified to a heat-free fritting process.

There are problems associated with each of the frit making strategies. Hydrothermal frit formation must be optimized for each packing material with respect to fritting temperature and duration of frit making. Optimization of the fritting parameters to provide a stable frit with consistent frit porosity is a time consuming process. The temperature and porosity of the frit are difficult to control in silica-based sintered frits. A partially intense heating in the sintering process could destroy the structure of silica particles, leading to deactivation of active sites on the surface of frits. This can cause fragility at the frit location and leads to bubble formation [31]. Few researchers have worked on 'frit making' by a sol–gel technique at room temperature [32]. Also, the sol–gel method requires extremely controlled experimental conditions as the sol–gel material dramatically shrink and results cavities within large pores in the capillary column [33]. Zhang and Huang [34] proposed a new methodology involving sol–gel technology. They prepared suspended silica solution in methylene chloride in the presence of trifluoro acetic acid as the solvent. A frit was fabricated by introducing a small amount of silica solution. Although the reaction could be completed by 30 min, the capillary required to be heated for an additional period of 6 h to form mechanically robust frit. Chen et al. [35] invented a photopolymerization method to prepare frits. Frits were fabricated in fused-silica capillary by UV-photopolymerization of trimethyl methacrylate and glycidyl methacrylate. This technique has been tried for frit making in CEC and not been investigated for programmed flow systems. The numerous problems, associated with frit formation and importance of the frits on the performances of separation, necessitate innovative approach for the fabrication of frits, especially with respect to speed of preparation without compromising the other important features of the frits.

In this paper, a simple, reliable, fast and robust frit making technique has been introduced. The fritting technology involved tapping of stainless steel (SS) particles at the capillary head and flame heating induced sintering of SS powder (FIS/SSP) (Fig. 1). In the new FIS/SSP fritting technology, SS particles were used for the preparation of frit to particulate filled capillary columns. The fabrication of inlet frit was completed within 20 s and frits had good porosity, permeability and were mechanically strong to hold the packing material. Two typical columns (column A and column B) with different capillary inner diameters (75 μm and 100 μm) were prepared. Frits prepared by FIS/SSP technique, were evaluated in three separation modes, capillary electro chromatography (CEC), pressure-assisted CEC (p-CEC) and low pressure driven liquid chromatography (LPLC). Baseline separation of the model analytes with high column efficiency and a good peak symmetry even for the last eluted compound of ethylbenzene were achieved.

2. Experimental

2.1. Instrumentation

CEC experiments were performed on an Agilent ^{3D}CE system (Agilent, Waldbronn, Germany) equipped with a diode array detector and the capability to apply up to 12 bar pressure to one or both ends of the capillary. Separation parameters were calculated using Agilent Chemstation Rev. A.10.02. A pneumatic pump (RPL-ZD10, Dalian Replete Scientific Instrument CO., Ltd, Dalian, China) and an ultrasonic bath (KQ-500E, Kunshan Ultrasonic Instrument CO., Ltd, Kunshan, China) were used to drive liquid and slurry into the capillary during column preparation. An FEI QUANTA 200 Scanning Electron Microscope (Philips-FEI, Holland) was used to study the morphologies of SS, the frits and packing columns. A capillary with a frit or packing bed was sectioned into 10 mm segments. These segments and SS powder were characterized without sputtering with gold prior to SEM analysis. Welding torch filled with butane gas (that could generate temperatures up to 1400 °C) was purchased from Liaocheng Kangda Grinding Tool Co., Ltd. (Shangdong, China).

2.2. Materials and chemicals

Fused-silica capillaries (50 μm, 75 μm, 100 μm and 250 μm as inner diameters with 375 μm as outer diameter; 530 μm inner diameter with 690 μm as outer diameter) were obtained from Yongnian Ruifeng Chromatography Ltd. (Yongnian, Hebei Province, China). 4 μm ODS silica gel (SP-120-4-ODS-BP) was purchased from Daiso Co. Ltd. (Osaka, Japan). SS powder (under 500-meshes) was purchased from Beijing Gelubo Alloy Material Limited Company. Tris(hydroxymethyl)aminomethane, acetonitrile (ACN) thiourea, benzene, toluene and ethylbenzene were reagent grade samples obtained from Beijing Chemical Reagent Company and/or Tianjing Chemical Reagent Company, China. Distilled water was obtained from a superpurification system (Danyangmen Corporation, Jiangsu, China). The mobile phase used in all experiments was ACN/Tris (90/10, v/v), pH = 8.5. The buffer was prepared with 10 mM Tris in H_2O. The pH was adjusted using a concentrated HCI solution, and then an appropriate amount of ACN was added in to obtain the mobile phase. The sample solutions were mixtures of benzene, methylbenzene and ethylbenzene with thiourea (as the dead time marker) dissolved in the mobile phase, with each component having a concentration in the range of 400–1000 mg/L. All solutions were degassed, ultra-sonicated and filtered through a membrane (0.22 μm) before use. The sample was introduced by electrokinetic injection at 10 kV for 5 s. The DAD wavelength range was set to 190–600 nm, and data was presented at 200 nm.

2.3. Frit preparation by FIS/SSP technique

The inlet frits were prepared in open fused-silica capillaries with different inner diameters, 75 μm, 100 μm, 250 μm and 530 μm. The procedure for frit formation (Appendix A: Part 1) is as follows. One end of the polyimide coated fused-silica capillary was tapped into 1.0 mL vial containing SS powder (<500 meshes). The tapping of SS particles into the end of capillary was monitored under microscope. The SS particles at the tip of capillary were sintered by periodic flame heating (~1400 °C, stepwise 10 s) using a commercial welding torch. Butane was used as the fuel that generates a high temperature of ~1400 °C. The flame heating was continued at the capillary head having the tapped SS particles. The SS particles became agglomerated at the tip of capillary. Few trials were needed to standardize the heating method.

2.4. Column packing with frits prepared by FIS/SSP technique

The capillary with a prepared frit was slurry packed with 4 μm ODS silica gel in a 90 mm × 1 mm (inner diameter) and SS column as slurry reservoir. The other capillary end was connected to the slurry reservoir by means of a screwed joint and a piece of 1/16 in. PEEK tubing as sleeve. The slurries (20% stationary phase in toluene/cyclohexanol, 1/1, v/v) were sonicated for 10 min and filled bubble-

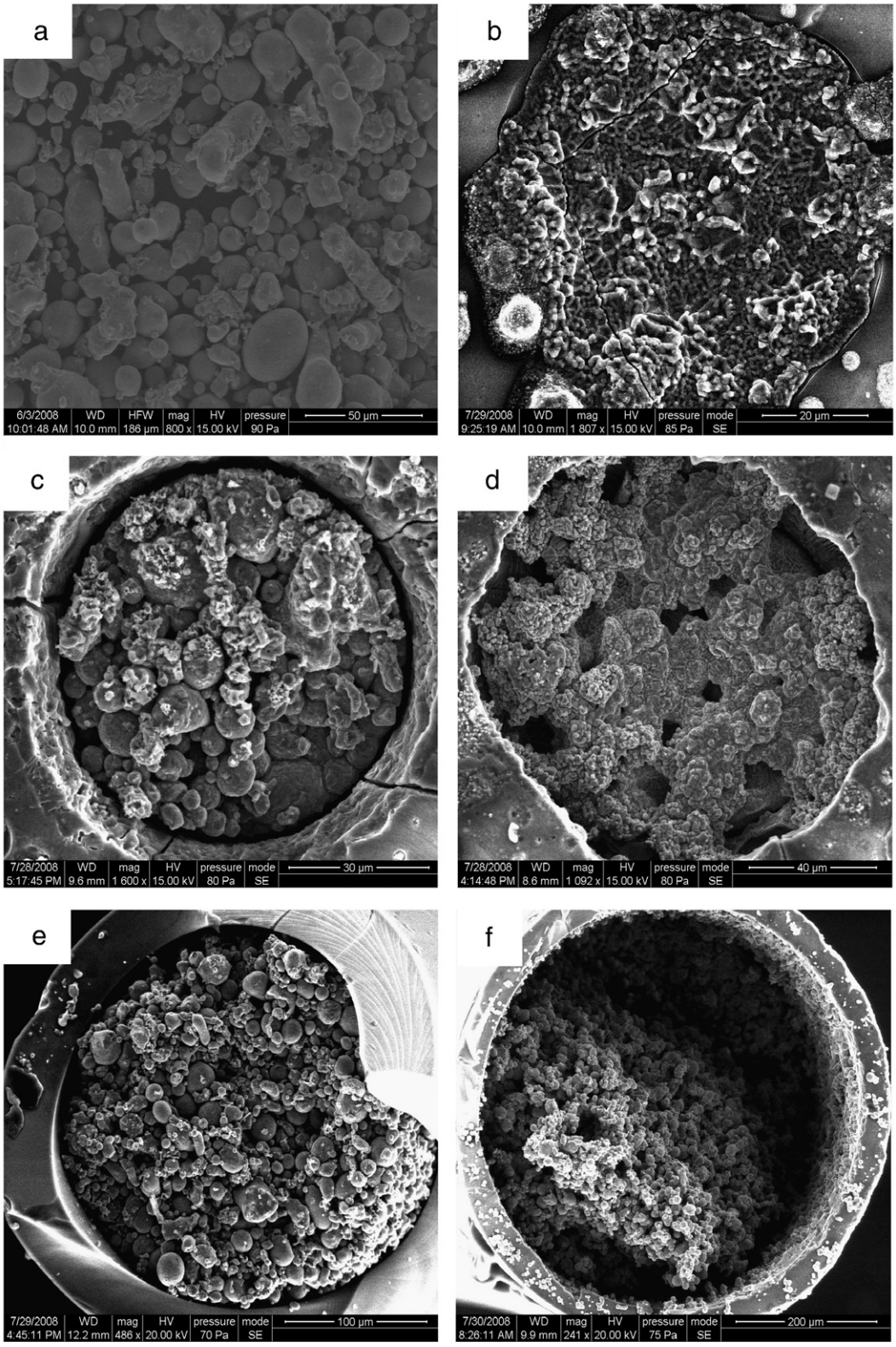

Fig. 1. SEM image of stainless steel particles and frits sintered about 20 s in different i.d. capillary tubes. a: tapped steel particles at the tip of the capillary, b–f: the sintered SS particles in the capillaries with inner diameters 50, 75, 100, 250 and 530 µm, respectively.

free into the slurry reservoir. The reservoir was rapidly closed and the capillary was immersed into an ultrasonic bath. The other end of the capillary was kept outside in a vertical position. Packing was carried out by the initial flushing of slurry liquid and followed by methanol for 1 h. After packing, the methanol in the capillary was flushed out with water and the frit was formed at the capillary end by heating the packing bed in the flame of welding torch. Sintering of frits was best done in water and this could prevent contamination of the column by the carbonaceous residues from methanol. Also carbonaceous impurities could interact with the analytes and affect the column

performances [36]. By using a combination of packing against gravity, using a balanced density solvent, and ultrasonic agitation to maximize the time, the slurry was kept in suspension so as to achieve reproducible results [37,38]. The packing process was monitored with the help of a microscope. A window was created by burning the polyimide coating of the capillary at the desired capillary position.

The packed capillary was installed in the electrochromatography system and equilibrated electrokinetically in the CE instrument by driving the buffer through the capillary at an applied voltage of 5 or 10 kV until a stable baseline was achieved.

3. Results and discussion

3.1. Frits fabrication and characteristics

Inlet frits of the fused-silica capillary were formed at the capillary head by FIA/SSP technique with inner diameters of 75 μm, 100 μm, 250 μm and 530 μm, respectively by burning butane at the tip of the capillary having tapped SS particles. A temperature as large as 1400 °C was subjected to sinter the SS particles. However, the heat could not penetrate into quartz part (melting point 1650 °C). The butane flame was applied for 10 s in a stepwise fashion. We employed FIA/SSP technique to prepare frits for fused-silica tubing of length in the range of 0.5–1.0 mm with inner diameters of 75, 100, 250 and 530 μm. The key controlling factors to produce robust frit were identified through few trials.

SEM image of SS particles and frits formed in different capillaries are presented (Fig. 1a–f). After heating for 10 s with butane flame, most of the SS particles became agglomerated in the capillaries. Robust frits were obtained when the butane flame heating was done with a stepwise heating of 10 s for two times. Few small holes or flaws were formed when the frit was refrigerated to room temperature. Morphologies of the successful frits (sintered about 20 s) formed in the capillaries with different diameters are shown (Fig. 1b–f). SEM images of the frit sintered in capillaries of different diameters at various times of sintering are presented in Appendix A (Part 2).

The frits sintered in the capillaries with inner diameters of 50 μm, 75 μm and 100 μm, could withstand up to a high pressure (1.2 MPa). However, the frits in the capillaries with inner diameters as 250 μm and 530 μm could not be retained in the capillary tubes once high pressure was turned on. This could be attributed to the probable weak interaction between the capillary wall and sintered SS particles for capillaries with larger diameters (>100 μm). With increase in capillary inner diameters or the decrease of fused-silica capillary wall thickness, physical interaction of sintered SS particles with silica surface could have been minimized. The frits prepared for capillary with inner diameters lesser than or equal to 100 μm, showed enough stability for the packed slurry. It is anticipated that SS particles could form a "steel-arch" structure after flame heating as the sintered SS particles wedged into the capillary wall [30]. The part of sintered SS particles and the "steel-arch" formed between capillary walls could hold the stationary phases in the capillary.

3.2. Performance of packed columns with frits prepared by FIS/SSP technique

Two typical columns (column A and column B) were prepared separately with inlet frits prepared the FIS/SSP for capillary with different inner diameters (75 μm and 100 μm respectively). Both columns were packed with 4 μm ODS silica gel. The description of the two columns is as follows. Column A: Capillary total length 33 cm (24.5 cm) 75 μm, packing length = 16.5 cm; Column B: Capillary total length 33 cm (24.5 cm) 100 μm, packing length = 16.5 cm. More details on the conditions used for the CEC experiment are given in Appendix A (Part 3).

The mechanical stability of the frits was tested in the modes of p-CEC, CEC and LPLC. No bleeding of particles was observed in all these experiments. Furthermore, the inlet frit was stable enough to hold packing material bed up to a pressure of 600 bar. The length of the frits could be controlled by the amount and size of SS particles tapped into the capillary head. Trial experiments revealed that the frit quality depended on the burning time and the tapping of SS powders into capillary end (Fig. 1). SS particles efficiently block the capillaries only if sintering was performed by stepwise heating (10 s each). Two successive heating for 10 s resulted in good 'frits' with a minimum failure (less than 30%) even during high pressure packing. The successful frit did not show any discernable deformation or shrinkage in the fused-silica capillaries. This was evident from microscopic observation. The micrographs of typical column with a sintered inlet frit and an outlet frit sintered by the packing stationary phase itself are presented in Fig. 2. Scanning electron microscopy (SEM) image of the frits (Fig. 2) showed that SS particles were steel-arched to each other and wedged to the inner wall.

3.3. Evaluation of chromatographic and electrochromatographic performances

Three typical separation modes such as CEC, p-CEC and LPLC were selected for the packed columns [39,40] with frits prepared by FIS/SSP technique. For the CEC mode, a pressure of 1.2 MPa was applied at both ends and a separation voltage was operated between the inlet and outlet. Pressurization between the sample inlet and outlet avoided the bubble formation. In the mode of p-CEC, pressure was only applied at the sample inlet and EOF was created by the high voltage, which were the driving forces for accelerating the separation. In the LPLC mode, a pressure of 1.2 MPa was applied at the inlet (the maximum limit of the instrument), and high voltage was not applied between the capillary inlet and outlet. Solution partitioning between the mobile and stationary phases is responsible for the retention of the model compounds.

A typical comparison of separation performance was made between the two columns, 75 (column A) and 100 μm (column B) i.d. independently with these three separation modes (Figs. 3 and 4). Experimental conditions were maintained almost the same in these experiments. The separation time was in the order of p-CEC > CEC > LPLC. Baseline separation of all model compounds was achieved for p-CEC, CEC and LPLC modes with a migration time of 3.20 min, 3.63 min and 14.66 min for the last eluted component, respectively on column A, whilst migration times were 2.76 min, 3.13 min and 13.66 min, respectively with column B. In LPLC mode, columns with the larger inner capillary diameters showed higher migration time than the capillary with smaller inner diameters. This could be attributed to the differences in the resistances. All analytes were eluted within 15 min from column A as well from column B. Thus, these columns showed good permeability for the analyte with the SS inlet frits. In the CEC mode, column with bigger inner diameters showed larger migration for the analyte than that with smaller inner diameter, due to differences in EOF.

Fig. 5 showed the van Deemter curves of column B in the modes of p-CEC, CEC and LPLC, which was obtained for ethylbenzene (the last eluted component). The van Deemter curves for p-CEC, CEC and LPLC had different trends over the full range of migration velocities. The curves were plotted using 12 points for each separation mode, with each point repeated at least three times, respectively. For p-CEC, when the inlet pressure was operated in the range of 0.2–1.2 MPa, with a stepwise increase of 0.1 MPa and at a constant operation voltage of 15 kV, a small change in migration velocity (range of 0.80–0.92 mm/s) was noticed. For CEC, when the voltage was operated in the range of 10–30 kV, with a stepwise change of 2 kV and at a nitrogen pressure of 1.2 MPa between the capillary ends, a large change in migration velocity in the range of 0.48 mm/s–1.80 mm/s was observed. It has

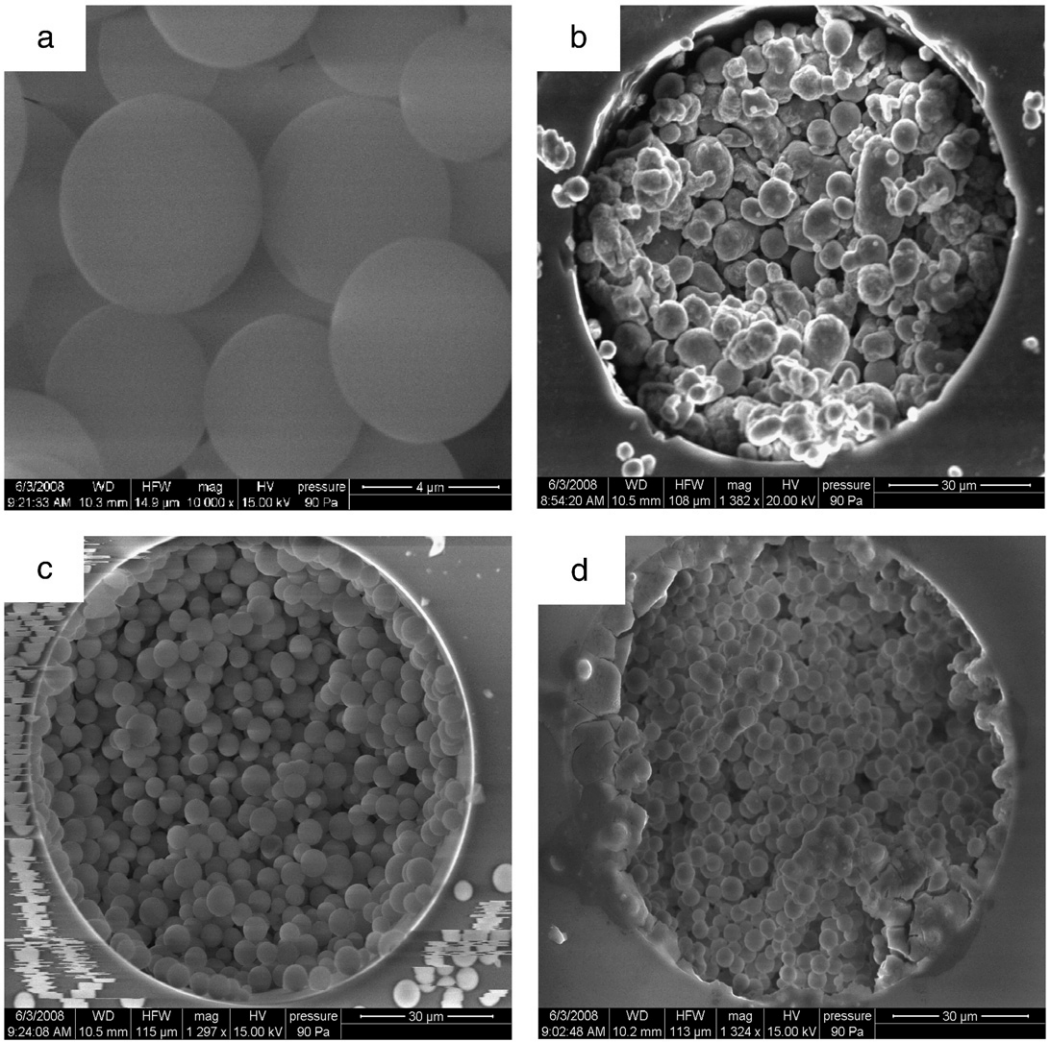

Fig. 2. SEM image of the stationary phase particles and the packed capillary column at different cross sections. a. the stationary phase particles, b. the cross section of sintered inlet frit, c. the cross section of stationary phase, and d. the cross section of outlet frit.

been noticed that the changes of separation voltage in the range of 10–30 kV led to more apparent effects to the migration of analytes, compared with that of inlet gas pressure. For LPLC, the pressure of nitrogen was varied at the capillary inlet from 0.2 MPa to 1.2 MPa with a step of 0.1 MPa. The migration velocity changed in the range of 0.08–0.46 mm/s. This suggested that the plate height difference in LPLC is dominated by the molecular diffusion in the limited pressure range of 0.2 MPa–1.2 MPa. Since this is dependent of velocity in the van Deemter equation, the curve displaced downward continually

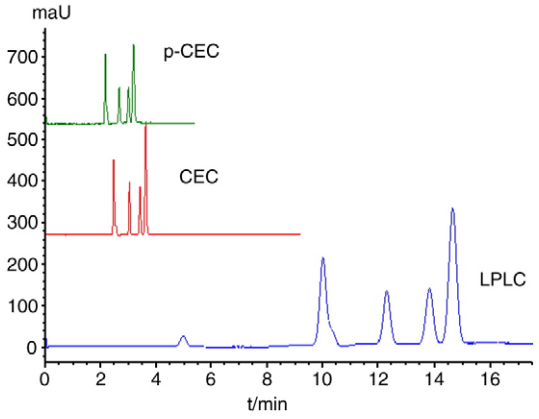

Fig. 3. Electropherograms of the four compounds using the prepared column in the modes of p-CEC, CEC and LPLC. Experimental condition: Capillary length 33 cm (24.5 cm) × 75 µm, packing length = 16.5 cm; injection: 10 kv × 5 s; 20 °C, 200 nm, buffer: ACN/10 mMTris = 9:1 (v/v); pH = 8.5. p-CEC 30 kV + 12 bar (inlet); CEC: 30 kV + 12 bar (both end); LPLC: 12 bar (inlet). Peak order: 1. thiourea, 2. benzene, 3. toluene, and 4. ethylbenzene.

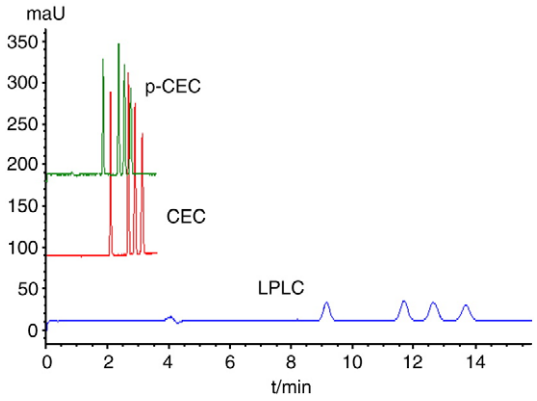

Fig. 4. Electropherograms of four compounds using the prepared column in the modes of p-CEC, CEC and LPLC. Experimental condition: Capillary length 33 cm (24.5 cm) × 100 µm, other experimental conditions and peak identification are the same as in Fig. 3.

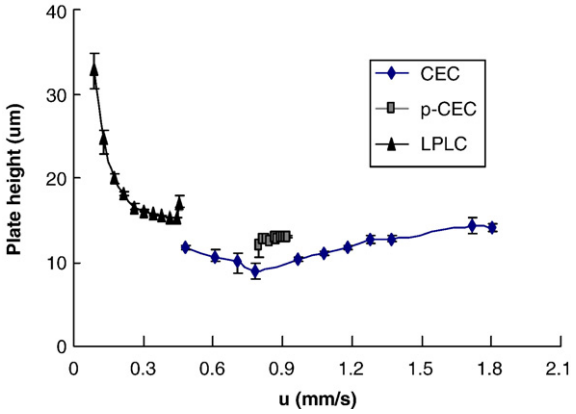

Fig. 5. Plate height of ethylbenzene as a function of migration velocity on column B in the modes of p-CEC, CEC and LPLC. Experimental conditions are the same as in Fig. 4.

with the increase of inlet gas pressure. If the analytes were separated in the same voltage, the fastest migration velocity would be obtained in the mode of p-CEC, whilst a highest efficiency would be achieved for the mode of CEC. The difference in EOF flow exhibited a different hydrodynamic profile than that generated by a pressure difference. Increased separation efficiency was achieved in CEC as compared to LPLC.

Symmetrical peaks were observed for the separated analytes with column B. Plots of symmetry factor vs linear velocity (mm s^{-1}) revealed similar trends (Fig. 6). The asymmetry of the bands arises due to adsorption of the hydrocarbon analytes onto the frit [28]. In our experiments, a satisfied symmetry factor was obtained in the range of 0.9–1.05 with these three separation modes. The results demonstrate that the frit is stable and practical. A good reproducibility of migration time for run to run ($n=10$ for column A, $n=6$ for column B) was noticed (Appendix A; Part 4) for column A and column B for the analytes (relative standard derivation (RSD): 1.94% (column A) and 1.48% (column B)).

4. Conclusions

Several frits making strategies have been evolved for capillary columns in the past decades. It is a challenging task for the separation scientists to investigate on new fritting technique that would resolve the tiresome and time consuming processes and thereby minimize the separation time. In this work, an easy and fast (within half a minute) frit making technique has been developed. Simple ingredients such as stainless steel powder and commercial butane gas based welding torch were used. Frits prepared by the new technique are mechanically strong (robust) and could be used in several separation modes. No apparent peak dispersion and bubbles were observed in the capillaries with 75 and 100 μm inner diameters over several hundreds of runs. Compared with other fritting methods, it can simplify the preparation of micro-columns, increase the preparation efficiency and will enlarge the practical application of CEC, micro-HPLC and nano-HPLC, particularly in the important area of hyphenation with mass spectrometry. This new frit making strategy could be extended for the packing capillary columns with a wide range of particulate materials such as porous graphitic carbon, polymer-based media and immobilized.

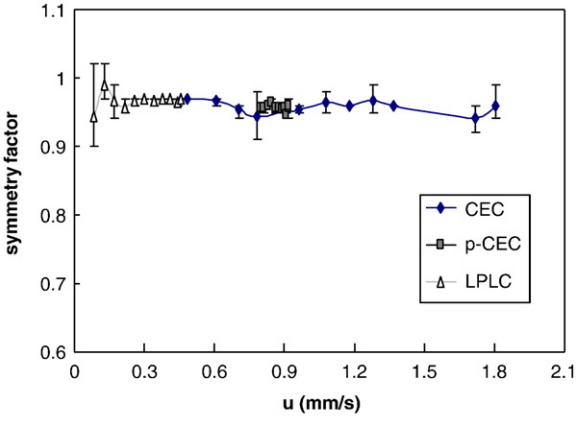

Fig. 6. Peak symmetry factor for ethylbenzene as a function of migration velocity.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.microc.2009.10.007.

References

[1] D. Janasek, J. Franzke, A. Manz, Scaling and the design of miniaturized chemical-analysis systems, Nature 442 (2006) 374–380.
[2] F. Svec, J.M.J. Frechet, New designs of macroporous polymers and supports: from separation to biocatalysis, Science 273 (1996) 205–211.
[3] R.J. Linhardt, T. Toida, Ultra-high resolution separation comes of age, Science 298 (2002) 1441–1442.
[4] J.P. Quirino, S. Terabe, Exceeding 5000-fold concentration of dilute analytes in micellar electrokinetic chromatography, Science 282 (1998) 465–468.
[5] L. Liu, P.S. Li, S.A. Asher, Entropic trapping of macromolecules by mesoscopic periodic voids in a polymer hydrogel, Nature 397 (1999) 141–144.
[6] F. Svec, C.G. Huber, Monolithic materials: promises, challenges, achievements, Anal. Chem. 78 (2006) 2101–2107.
[7] L. Ma, P.W. Carr, Loss of bonded phase in reversed-phase liquid chromatography in acidic eluents and practical ways to improve column stability, Anal. Chem. 79 (2007) 4681–4686.
[8] S. Ehlert, T. Rösler, U. Tallarek, Packing density of slurry-packed capillaries at low aspect ratios, J. Sep. Sci. 31 (2008) 1719–1728.
[9] C.J. Chen, C.H. Chang, G.R. Her, Use of a fritless dual tapered column and a low flow interface for capillary electrochromatography-mass spectrometry, J. Chromatogr. A 1159 (2007) 22–27.
[10] M. Mayer, E. Rapp, C.M. Marchk, G. Bruin, Fritless capillary electrochromatography, Electrophoresis 20 (1999) 43–49.
[11] G. Choudhary, C.S. Horváth, J.F. Banks, Capillary electrochromatography of biomolecules with on-line electrospray ionization and time-of-flight mass spectrometry, J. Chromatogr. A 828 (1998) 469–480.
[12] E. Rapp, E. Bayer, Improved column preparation and performance in capillary electrochromatography, J. Chromatogr. A 887 (2000) 367–368.
[13] K.D. Bartle, R.A. Carney, A. Cavazza, M.G. Cikalo, P. Myers, M.M. Robson, S.C.P. Roulin, K. Sealey, Capillary electrochromatography on silica columns: factors influencing performance, J. Chromatogr. A 892 (2000) 279–290.
[14] K. Tsukagoshi, S. Ishida, Y. Oda, K. Noda, B. Nakajima, Compact polytetrafluoroethylene assembly-type capillary electrophoresis with chemiluminescence detection, J. Chromatogr. A 1125 (2006) 144–146.
[15] R.T. Kennedy, J.W. Jorgenson, Preparation and evaluation of packed capillary liquid chromatography columns with inner diameters from 20 to 50 micrometers, Anal. Chem. 61 (1989) 1128–1135.
[16] N. Ishizuka, H. Minakuchi, K. Nakanishi, N. Soga, H. Nagayama, K. Hosoya, N. Tanaka, Performance of a monolithic silica column in a capillary under pressure-driven and electrodriven conditions, Anal. Chem. 72 (2000) 1275–1280.
[17] G.F. Wang, M. Lowry, Z.M. Zhong, L. Geng, Direct observation of frits and dynamic air bubble formation in capillary electrochromatography using confocal fluorescence microscopy, J. Chromatogr. A 1062 (2005) 275–283.
[18] A. Rocco, S. Fanali, Capillary electrochromatography without external pressure assistance: use of packed columns with a monolithic inlet frit, J. Chromatogr. A 1191 (2008) 263–267.
[19] J.R. Chen, R.N. Zare, F. Svec, E. Peters, Macroporous photopolymer frits for capillary electrochromatography, Anal. Chem. 72 (2000) 1224–1227.

[20] C. Legido-Quigley, N.W. Smith, Study of short polystyrene monolith-fritted micro-liquid chromatography columns for analysis of neutral and basic compounds, J. Chromatogr. A 1042 (2004) 61–68.
[21] C. Legido-Quigley, N.W. Smith, C. Legido-Quigley, N.W. Smith, Short polystyrene monolith-fritted micro-liquid chromatography columns for rapid isocratic analysis of pharmaceuticals direct from plasma, Anal. Bioanal. Chem. 385 (2006) 686–691.
[22] J.H. Knox, I.H. Grant, Miniaturisation in pressure and electroendosmotically driven liquid chromatography: some theoretical considerations, Chromatographia 24 (1987) 135–143.
[23] J.H. Knox, I.H. Grant, Electrochromatography in packed tubes using 1.5 to 50 μm silica gels and ODS bonded silica gels, Chromatographia 32 (1991) 317–328.
[24] F. Lynen, A. Buica, A. Villiers, A. Crouch, P. Sandra, An efficient slurry packing procedure for the preparation of columns applicable in capillary electrochromatography and capillary electrochromatography-electrospray-mass spectrometry, J. Sep. Sci. 28 (2005) 1539–1549.
[25] H. Yamamoto, J. Bauman, F. Erni, Electrokinetic reversed-phase chromatography with packed capillaries, J. Chromatogr. A 593 (1992) 313–319.
[26] H. Rebscher, U. Pyell, A method for the experimental determination of contributions to band broadening in electrochromatography with packed capillaries, Chromatographia 38 (1994) 737–743.
[27] R.T. Kennedy, J.W. Jorgenson, Quantitative analysis of individual neurons by open tubular liquid chromatography with voltammetric detection, Anal. Chem. 61 (1989) 436–441.
[28] A. Maiolica, D. Borsotti, J. Rappsilber, Self-made frits for nanoscale columns in proteomics, Proteomics 5 (2005) 3847–3850.
[29] L.C. Wang, C.Y. Okitsu, H. Kochounian, A. Rodriguez, C.L. Hsieh, E. Zandi, A simple and inexpensive on-column frit fabrication method for fused-silica capillaries for increased capacity and versatility in LC-MS/MS applications, Proteomics 8 (2008) 1758–1761.
[30] B. Zhang, E.T. Bergstrom, D.M. Goodall, P. Myers, Single-particle fritting technology for capillary electrochromatography, Anal. Chem. 79 (2007) 9229–9233.
[31] F. Svec, E.C. Peters, D. Sýkora, C. Yu, J.M.J. Fréchet, Monolithic stationary phases for capillary electrochromatography based on synthetic polymers: designs and applications, J. High. Resolut. Chrom. 23 (2000) 3–18.
[32] T. Hyotylainen, M.L. Riekkola, Solid-phase extraction or liquid chromatography coupled on-line with gas chromatography in the analysis of biological samples, J. Chromatogr. B 817 (2005) 13–21.
[33] J.R. Chen, R.N. ZareEric, C. Peters, F. Svec, J.J. Frechet, Semipreparative capillary electrochromatography, Anal. Chem. 73 (2001) 1987–1992.
[34] X. Zhang, S. Huang, Single step on-column frit making for capillary high-performance liquid chromatography using sol–gel technology, J. Chromatogr. A 910 (2001) 13–18.
[35] J.R. Chen, M.T. Dulay, R.N.Z. Svec, E. Peters, Macroporous photopolymer frits for capillary electrochromatography, Anal. Chem. 72 (2000) 1224–1227.
[36] H. Rebscher, U. Pyell, In-column versus on-column fluorescence detection in capillary electrochromatography, J. Chromatogr. A 737 (1996) 171–180.
[37] B. Lumley, T.M. Khong, D. Perrett, An improved procedure for packing capillaries for capillary electrochromatography, Chromatographia 54 (2001) 625–628.
[38] T.M. Zimina, R.M. Smith, P. Myers, Comparison of ODS-modified silica gels as stationary phases for electrochromatography in packed capillaries, J. Chromatogr. A 758 (1997) 191–197.
[39] Y.P. Zhang, L.Q. Fan, K.P. Lee, Y.J. Zhang, S.H. Choi, W.J. Gong, Preparation of monolithic capillary columns for capillary electrochromatography by γ-ray irradiation, Microchim. Acta 158 (2007) 353–360.
[40] Y.P. Zhang, X.W. Ye, L.B. Qu, S.H. Choi, A.I. Gopalan, K.P. Lee, Novel method to prepare polystyrene-based monolithic columns for chromatographic and electrophoretic separations by microwave irradiation, J. Chromatogr. A 1188 (2008) 43–49.