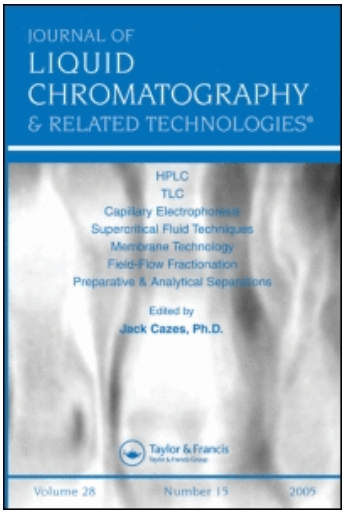

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Rapid Preparation of Monolithic Silica Column for Electrophoretic and Chromatographic Separation by Microwave Irradiation

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Abstract: A porous monolithic sol-gel column with the solution of methacryloxypropyltrimethoxysilane in toluene with an acid catalyst was quickly prepared using microwave irradiation. Three typical silica based capillary monolithic columns prepared were used to evaluate the separation of some typical model compounds in the modes of CEC, pressure assisted CEC, and low pressure driven separation. Baseline separation of the analytes including thiourea, benzene, toluene, ethyl benzene, biphenyl, and naphthalene could be obtained by the polymer with a length of 10 cm. A scanning electron micrograph of a cross section of the capillary column showed that the gel took the form of a spherical particle aggregate and adhered to the column inner wall.

Keywords: Electrochromatography, *In-situ* polymerization, Microwave irradiation, Monolithic column

INTRODUCTION

As a hybrid of high performance liquid chromatography (HPLC) and capillary electrophoresis (CE), capillary electrochromatography (CEC)

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has attracted numerous groups to this rapidly growing research area over the past decade.[1–3] Monolithic capillary columns, based on a continuous and porous media produced *in situ*, are one of the most competitive chromatographic column technologies because of their unique properties like fast separation, high lineal flux velocities, and nonrequirement of frits.[4–6]

The preparation of most monolithic columns such as silica based and organic polymer based monolithic columns for HPLC, micro-HPLC, and CEC by in situ polymerization are generally UV and thermally initiated.[7,8] In a general sense, photopolymerization refers to the use of electromagnetic irradiation as the energy source for the polymerization of monomers, oligomers, and polymers. Although ultraviolet visible light and conventional heat are mainly used for this purpose, photopolymerization can also be induced by ionizing radiation (e.g., electron beam, γ, and x-ray), infrared, microwave, or even ultrasound.[6,9] Methacryloxypropyltrimethoxysilane (MPTMS), which contains both methacrylate and alkoxysilane groups, was often used to prepare a photopolymerized or thermal polymerized sol-gel in a single step reaction.

The Zare group have used it to fabricate porous CEC monolithic columns and sol-gel frits using Irgacure 1800 as photoinitator, but the capillary generally had not enough mechanical stability after the removal of a stripe of polyimide coating.[10–13] Moreover, Zheng, et al. prepared the similar monolith in a 100 μm I.D. UV transparent capillary using MPTMS as the monomer.[14] The feasibility of applying this column for the CEC-MS separation and detection of 16 polycyclic aromatic hydrocarbons (PAH), and 11 alkyl phenyl ketones was investigated. A porous polymerized sol-gel (PSG) monolith was also synthesized in the separation channel of a borosilicate glass chip via UV irradiation of a mixture of MPTMS, an acid catalyst, a porogen, and a photoinitiator.[15] Toyo'oka and coworkers performed capillary pretreatment using MPTMS to form an anchor onto the silicate matrix and prevent the gel from being leached out of the capillary.[16,17] The polymerization process could also be initiated in principle by thermal initiation, but capillaries filled with the similar reactant solutions must be incubated at 60°C for 24 h.[18]

Initiation by conventional heating presents the disadvantage of long reaction time due to the slow heat convection, while photopolymerization necessitated use of expensive capillaries with UV transparent outer coatings. Compared with traditional methods, microwave irradiation has the advantages of being volumetric, direct, selective, and instantaneously controllable. Microwaves can penetrate the material placed inside its fields. All the molecules of material are subject to the electromagnetic field, although the field strength decreases as it gets deeper into the material. Here, to the best of our knowledge, it was first attempted to prepare the monolithic capillary column using microwave irradiation instead of

the traditional methods of UV light and thermal initiation. The chromatographic and electrophoretic behaviors of the studied monolithic columns have been comparatively evaluated in the modes of CEC, pressure assisted CEC, and low pressure driven separation in this work. Various different operational parameters, such as column temperature, separation voltage, acetonitrile content, and buffer pH, were varied to assess their influence on column performance. Baseline separation of some typical neutral compounds could be obtained under the optimal condition, such as thiourea, benzene, toluene, ethyl benzene, biphenyl, and naphthalene. It provided a viable alternative to either thermally initiated or photopolymerization for the novel preparation of monolithic columns.

EXPERIMENTAL

Instrumentation

All CEC experiments were performed on a Agilent ^{3D} CE system (Agilent Technologies, Inc., Walbronn, 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. The rinse of all prepared monolithic columns was carried out using a HP1100 Series HPLC system (Agilent Technologies, Inc., Walbronn, Germany) equipped with a quaternary pump. The irradiation step was carried out in a home microwave oven (Midy Co.Ltd., Guangdong, China) with the largest output power of 700 W and a frequency of 2450 Hz. An FEI QUANTA 200 Scanning Electron Microscope (Philips-FEI, Holland) was used to study the morphology of the monolith. A capillary with the monolith was sectioned into 10 mm segments without sputtering with gold.

Materials and Chemicals

Fused silica capillaries (75 μm inside diameter, 375 μm outside diameter) were purchased from Yongnian Ruipu Optic Fiber Plant (Yongnian, Hebei Province, China). MPTMS, AIBN, Sodium dodecyl sulfate (SDS), acetonitrile (ACN), ammonium acetate, thiourea, benzene, toluene, ethyl benzene, biphenyl, naphthalene, were purchased from Beijing Chemical Reagent Company and Tianjing Chemical Reagent Company, China. Distilled water was obtained from a superpurification system (Danyangmen Corporation, Jiangsou, China). The buffers used in the experiments were prepared with various volume ratios of 50 mM ammonium acetate, water, and CAN; all solutions were degassed with ultrasonication and filtered through a membrane (0.45 μm) before use. In a typical

chromatographic and electrophoretic experiment, aromatic hydrocarbons were dissolved in methanol and injected for peak identification.

Preparation of the Sol-Gel Columns

Prior to filling the reactants into the capillary, it was pretreated with the following procedure.[3,18] First, the capillary column with a length of 40 cm was rinsed with 1 M NaOH for 30 min and then with 0.1 M HCI for 30 min. After subsequent flushing with H_2O for about 30 min, it was dried by the passage of nitrogen gas. The purpose of capillary pretreatment is to increase the concentration of surface silanol groups. Since silanol groups on the capillary surface represent the principal binding sites for in situ created sol-gel stationary phases, higher concentration of these binding sites on the capillary surface would facilitate the formation of highly secured sol-gel stationary phases through chemical bonding with the capillary inner walls. In general, the sol-gel reaction in our experiment involved the following steps:[19,20] (1) the hydrolysis of MPTMS; (2) as the sol-gel reactions proceed, the products of hydrolysis undergo polycondensation reactions in a variety of ways: the hydrolyzed product of MPTMS; its product and the silanol or alkoxy group on the growing sol-gel network; the silanol groups on the inner capillary surface and sol-gel reaction products in their vicinity; (3) the condensed products can then undergo further condensation reactions to create a three-dimensional sol-gel work. The sol-gel network growing in the vicinity of the capillary walls may eventually become anchored to the inner capillary surface through chemical bonding with the silanol moieties residing along the inner fused silica capillary surface. In addition, the break up of carbon-carbon double bonds (C = C bonds) of molecules in the monomer due to microwave irradiation could lead to polymerization quickly. By this process of destroying carbon-carbon double bonds, the molecule itself becomes highly reactive and links itself to another highly reactive molecule. By this forming of very long macromolecules the liquid monomer changes to a solid polymer that can have totally different properties from the liquid monomer.

In the sol-gel reaction, the HCl catalyst plays an important role in the hydrolysis and condensation reactions.[10–13] Polymerization having morphologies with different permeabilities and surface areas were prepared by varying the ratio of the monomer stock solution to toluene (porogen). The porogen acts as a through pore template and solubilizer of the silane reagent during the reaction. It is noted that alkoxysilane based sol-gel precursors usually show poor solubility in water. Therefore, we choose SDS as one of the sol solution components to achieve a homogeneous system with all the sol-gel ingredients effectively dissolved

together.[18] Here, some columns were prepared with some changes, such as the acid degree, mixture composition, irradiation power, irradiation time, and three typical columns were chosen for the comparative evaluation.[21,22] Briefly, a monomer stock solution, a mixture of 225 μL MPTMS and 75 μL 0.1 M hydrochloric acid, was stirred for 30 min. at room temperature, then 1700 μL toluene and 89 mg AIBN were added to the monomer stock solution in the absence of SDS (Column A). In the comparative experiment, a reactant was respectively prepared with the above solutions, subsequently, 100 mg SDS was additionally added to the monomer stock solution for obtaining the homogenous phase and stirred for another 30 min at room temperature (Columns B and C). After the pretreated capillary was partially filled with the mixture to a set position, the capillary was sealed at both ends with glue and rubber stoppers. The partially filled capillaries were irradiated in a home microwave oven using an output power of 350 W.

After irradiation, the capillaries were washed with methanol using a HPLC pump to remove any unreacted reagents and cut to 33 cm for the next use. A window about 1 cm length was created immediately by scraping the polyimide coating of the capillary after the column bed. Once fabricated, the capillary was installed in an Agilent CE cartridge. The monolithic capillary was conditioned with the separation solution for approximately 5 min using a syringe and a hand held vise. The column was further conditioned electrokinetically in the CE instrument by driving the mobile phase through the capillary at an applied voltage of 5 or 10 kV until a stable baseline was achieved.

RESULTS AND DISCUSSION

Chromatographic and Electrophoretic Performances on the Typical Columns

The effects of irradiation time were varied to investigate the polymer effects from 5 min to 100 min at any output power of the microwave oven from 700 W to 70 W; the monoliths were formed easily, which showed that microwaves can penetrate the fused silica capillary inside the electromagnetic fields, even using the lowest power (70 W). After columns A, B, and C were prepared by microwave irradiation with an output power of 350 W, a reversed phase mechanism was observed in three modes, including CEC, pressure assisted CEC, and low pressure driven separation. Solution partitioning between the mobile and stationary phases is the main mechanism responsible for retention of the neutral analytes. The elution order of the column is similar to that of reversed phase chromatography, the analytes with larger molecular weight or more hydrophobic

analytes were eluted later than the analytes with smaller molecular weight or more hydrophilic analytes. Elution of the analytes was obtained for the mixture using columns A, B, and C in the above three modes (see Figure 1). For the CEC mode, a pressure of 12 bar was applied at both ends and a separation voltage was operated between the inlet and outlet. Pressurization between the sample inlet and outlet could avoid the bulb forming. In the mode of pressure assisted CEC (p-CEC), pressure only added at the sample inlet and EOF was created by the high voltage, which was the driving force for accelerating the separation. The versatility of columns A, B, and C was also investigated in the mode of low pressure driven separation, the 12 bar pressure was only applied at the inlet (the maximum limit of the instrument). At this time, no high voltage was applied between the capillary inlet and outlet, but all analytes still eluted showing a good permeability for columns A, B, and C. The chromatographic and electrophoretic separation of the typical model compounds were comparatively shown for column A, B, and C (see Figure 2).

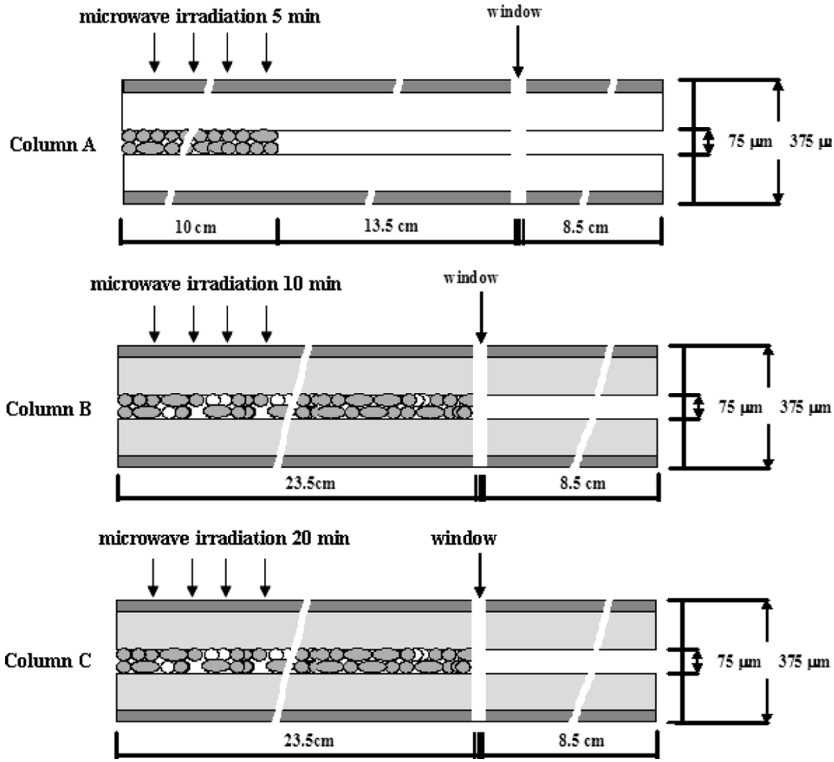

Figure 1. Schematic diagram of preparation of monolithic columns by microwave irradiation.

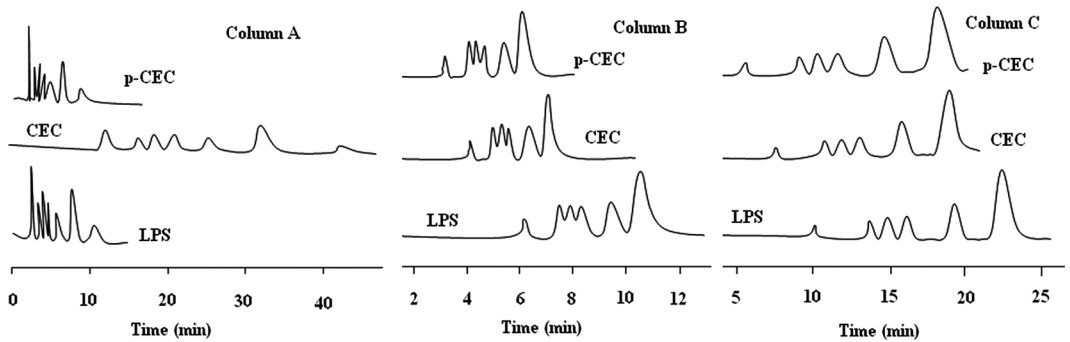

Figure 2. Electrochromatograms of the separation of the analytes in the modes of p-CEC, CEC, and low pressure driven separation for monolithic columns A, B, and C. Experimental condition: Capillary length 33 cm (24.5 cm) × 75 μm; injection: 10 kV*5s; 20°C; DAD detector. A: pressure driven and operating voltage = 12 bar (inlet) +20 kV; B: pressure driven and operating voltage = 12 bar (both) +20 kV; C: pressure-driven = 12 bar (inlet). Column A: 50 mM ammonium acetate: water: acetonitrile = 1:5:4 (*v*/*v*/*v*); Column B: 50 mM ammonium acetate, water, and acetonitrile = 1:4:5 (*v*/*v*/*v*); Column C: 50 mM ammonium acetate: water: acetonitrile = 1:4:5 (*v*/*v*/*v*); pH = 7.16. Peak identification: 1. thiourea, 2. benzene, 3. toluene, 4. ethyl benzene, 5. biphenyl, 6. naphthalene, 7. phenanthrene.

Apparently, it is easily understood that the separation time becomes shorter and shorter in the order of pressure assisted CEC, CEC and low pressure mode, respectively.

The effect of sol-gel polymer length in the capillary on analyte elution times was studied for lengths of 0, 10, 23.5 cm from the inlet to another end (see Figure 1). As the length of the segment increased, the resolution increased while each component eluted later. Baseline separation of the test mixture was still achieved for monolith length equal to 10 cm; it was better than the results from the previous reports, which the sol-gel polymer was prepared by UV-photopolymerization.[10–13] Furthermore, the resolution and retention time of model analytes also increased with the prolonged irradiation time from 10 min to 20 min for columns B and C. At high field strengths, it is expected that peak broadening will occur as a result of Joule heating. Nevertheless, we observe that peaks are sharpened further at higher field strengths. It appears that efficient dissipation of the Joule heat generated at high field strengths occurs with the monolith. In our case, there was only one exception that peak broadening of column A was apparent in the mode of CEC, it was probably attributed to the long blank column after the monolith or the absence of SDS in the monomer mixture.[18]

It is worth noting that three columns withstand pressures of up to 30 MPa with no detectable damage. The network is achieved via the hydrolysis of an alkoxy silicate followed by condensation then polymerization. Denser monolithic beds are less permeable and higher pressures are needed to drive liquid flow through. Superficially, column permeability may seem irrelevant in CEC separation since EOF is the driving force, in a CEC, to propel the mobile phase through the column without requiring mechanical pressure.[21,22] However, columns with high permeability provide some significant advantages, especially in p-CEC operation, pressure driven operation, and sample injection or quick flushing of the capillary during column regeneration or equilibration.[25,26] The macroporous monolithic structures facilitate the mobile phase flow through the pores, and thereby, promotes effective solute/stationary phase interaction by bringing them together. Effective solute transport mechanism operating within this monolithic structure due to mobile phase flow through the macropores, together with the flat flow profile of EOF, leads to high speed and separation efficiency in CEC.

Effects of Temperature and Operation Voltage on Column A

Temperature is a controllable parameter that can be used to further optimize CEC, p-CEC, and low pressure driven separation. It is apparent that the relatively small changes in temperature ($\pm 10^{\circ}C$) cannot be

neglected since they correspond to significant changes in migration time. Figure 3 illustrates that when the column A temperature was increased from 10°C to 40°C, the theoretical plate numbers decreased, and the migration times of the analytes shortened as the electroosmotic flow velocity increased in the mode of p-CEC. This change in the electroosmotic flow velocity in the prepared column is ascribed only to changes in the buffer viscosity and not to structural changes in the monolith structure as a function of temperature. The same trend of increasing migration time with decreasing temperature is observed for pressure driven (12 bar) separation and CEC mode (not shown).

Joule heat produced in the column is an important factor influencing the separation, which is a result of the heat generated by the passage of an electrical current through the capillary. In our work, the effect of operating voltage for columns A in the mode of p-CEC mode was also investigated in Figure 4. As the voltage increases from 10 kV to 25 kV, the migration time of the last eluted peak gradually decreased for column A. No bulb was formed in our experiment, which

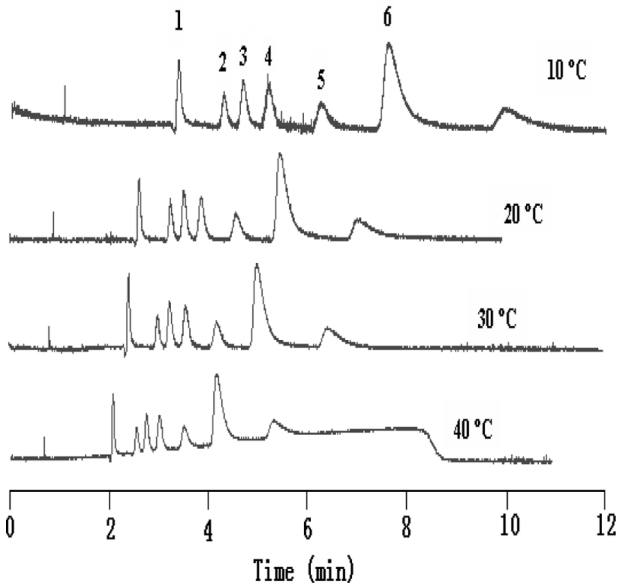

Figure 3. Effects of temperature on the migration time and theoretical plate number of seven analytes on column A in the mode of pressure assisted CEC. Experimental condition: 12 bar (inlet) +20 kV; buffer 1:5:4, injection:10 KV*5s; pH = 7.16; Temperature: 10°C ~ 40°C. Other condition and peak identification is the same as Figure 2.

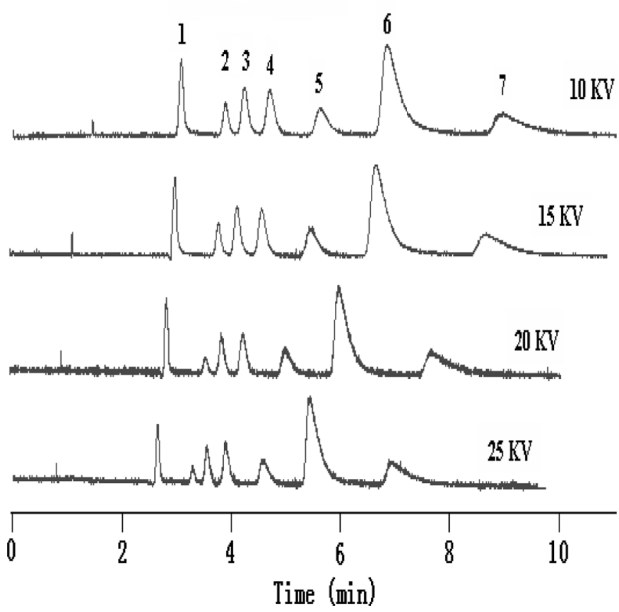

Figure 4. Effects of voltage on the migration times on column A in the mode of p-CEC. Experimental condition: 12 bar (inlet) +10 ~ 25kV; buffer 1:5:4, injection:10 KV*5s; pH = 7.16. Other condition and peak identification is the same as Figure 2.

showed that the Joule heating effect with small inner diameters (75 μm) were not very serious.

Effects of Acetonitrile Content and pH on Column A

Figure 5 illustrates the dependence of the migration times of all analytes on acetonitrile concentration in the buffer on the monolithic column A. The acetonitrile composition of the mobile phase or buffer is in the range of 40% to 70% (v/v). Only one peak is observed for the analytes when acetonitrile concentration equals 70%. At 40% (v/v) acetonitrile, baseline separation of seven compounds is achieved during 10 min, with peak shapes being symmetrical. Partitioning of the analytes from the mobile phase to the monolith phase, which is hydrophobic, is affected by the amount of acetonitrile present in the mobile phase. At low volumes of acetonitrile (i.e., higher volumes of water), partitioning is altered in favor of the analytes, therefore, the analytes interact longer with the monolith surface. The change in the elution times of the analytes with acetonitrile concentration are expected for a reversed phase mechanism. In typical

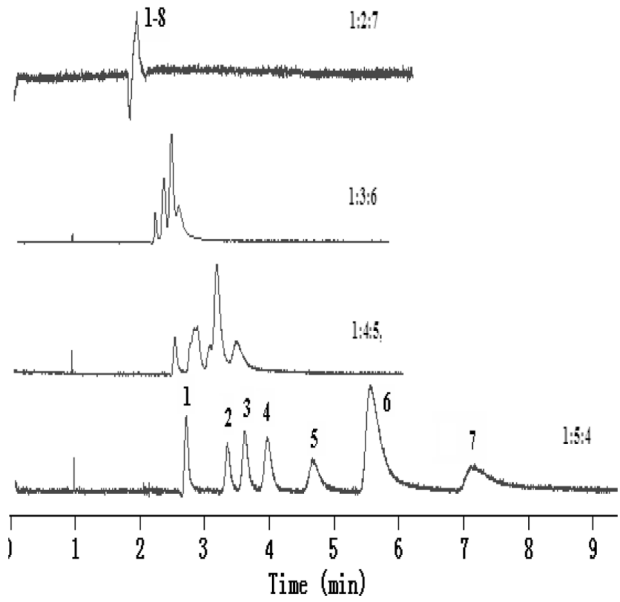

Figure 5. Effects of acetonitrile content on the electrochromatographic separation of seven analytes on column A in the mode of p-CEC. Experimental condition: 12 bar (inlet) +20 kV; temperature = 20°C; injection 10KV*5s; pH = 7.16. Buffer: 50 mM ammonium acetate: water: acetonitrile (*v*/*v*/*v*); 1:2:7 (a); 1:3:6 (b); 1:4:5 (c); 1:5:4 (d). Other condition and peak identification is the same as Figure 2.

reversed phase chromatography, the elution times decrease with an increase in organic solvent concentration in the mobile phase. The pH effect for the separation of all analytes is also investigated, electroosmotic flow velocities do not change, apparently due to the relative stability of total surface charge in the range of $4.02 \sim 7.16$ (not shown).

In addition, a system of study of the run-to run reproducibility of column A ($n = 6$) was carried out with a reproducibility of retention time and retention factor ($k = (t_r - t_0)/t_0$) in the range of $1.63\% \sim 1.88\%$ and $0.29\% \sim 0.43\%$, respectively, whilst the reproducibility for another three columns was in the range of $28.74\% \sim 29.45\%$ and $1.33\% \sim 2.61\%$. The experiments were carried out in the mode of p-CEC with an operating voltage of 30 kV and a buffer solution of 50 mM ammonium acetate/water/acetonitrile (1:5:4, *v*/*v*/*v*). The chromatographic parameters were shown in Table 1. Furthermore, for every single capillary monolithic column (A, B, and C), no apparent decrease of theoretic plates was observed after the use of over 200 runs in three weeks, and baseline separation for all test compounds can still be obtained, which shows the silica based

Table 1. Reproducibility of run to run, and column to column for column A

Reproducibility		Retention time (t^*, min)				Retention factor		
		t_0	t_1	t_2	t_3	k_1	k_2	k_3
Run to run	1	3.02	4.22	5.54	6.93	/	/	/
	2	2.99	4.16	5.46	6.83	0.40	0.83	1.30
	3	2.97	4.15	5.45	6.83	0.39	0.83	1.29
	4	2.94	4.11	5.39	6.74	0.40	0.83	1.30
	5	2.92	4.07	5.34	6.70	0.40	0.83	1.29
	6	2.89	4.07	5.30	6.64	0.40	0.83	1.30
RSD (%)		1.87	1.63	1.88	1.79	0.43	0.29	0.36
Column to column	1	3.32	4.20	5.11	5.93	0.27	0.54	0.79
	2	1.95	2.47	3.00	3.50	0.26	0.53	0.79
	3	2.18	2.77	3.39	3.98	0.27	0.55	0.83
RSD (%)		29.45	29.43	29.30	28.74	1.33	1.93	2.61

*t_0-thiourea, t_1-benzene, t_2-toluene, t_3-ethyl benzene.

capillary monolithic columns prepared by microwave irradiation have a good mechanical strength and permeability.

Comparative Theoretic Plates and Retention Factors on Columns A, B, and C

Figure 6 illustrates the comparative results of theoretic plates per column (N/column) and retention factors for the columns A, B, and C in the mode of p-CEC mode. With the shortest monolith length of 10 cm, the least polymer is formed in a column with which the analytes can interact. With the increase of monolith length, the k value of each analyte increases, which is indicative of stronger retention of the analytes, but the theoretic plates decrease, which is probably attributed to the effect of eddy diffusion. With the increase of irradiation time, the monoliths seemed to have formed more uneven pores. Clearly, the choice of irradiation time and intensity is the important factor to control the morphology of the PSG for the successful separation of the sample mixtures. These experiments are carried out using a mobile phase or buffer with a 50 mM ammonium acetate, water, and acetonitrile = 1:4:5 ($v/v/v$). It should be noted, that baseline separation of the mixture can be obtained for column A with the largest theoretic plates and fastest separation time when the ACN decrease to 40%. For the monolithic columns A, B, and C, the efficiencies up to 10427 plates/column, 4993 plates/column, and 1910 plates/column, respectively, were achieved for thiourea, a less retained compound.

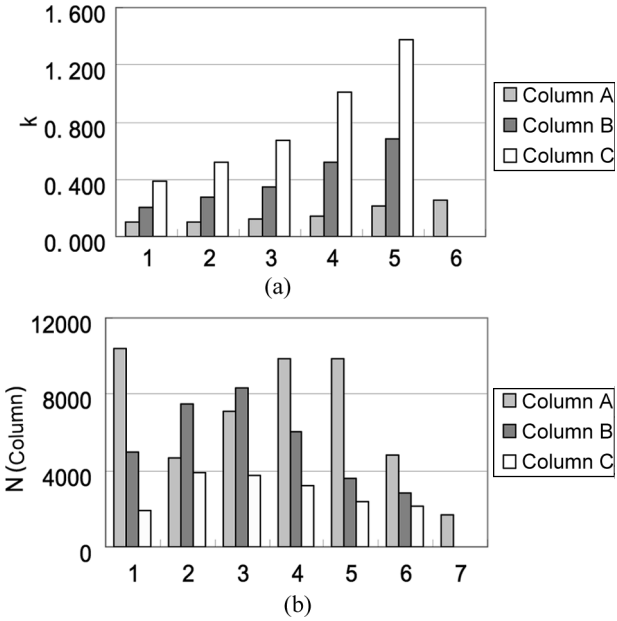

Figure 6. Comparative theoretic plates and retention factors for columns A, B, and C in the mode of p-CEC. Experimental condition: 50 mM ammonium acetate, water, and acetonitrile = 1:4:5 ($v/v/v$); pH = 7.16; injection: 10 kV*5s; 20°C; DAD detector. Peak identification: 1. thiourea, 2. benzene, 3. toluene, 4. ethyl benzene, 5. biphenyl, 6. naphthalene, 7. phenanthrene.

Characterization of the Prepared Columns

Strict control of the morphology of the monolithic stationary phase is important to obtain a generic porous monolithic material that provides a good separation efficiency and a low resistance to flow. [6,7,27] The latter is of prime importance, since it enables easy flushing of the column with liquids that are used in the subsequent separation in the modes of p-CEC, CEC, and low pressure driven separation. Figure 7 illustrated columns, A, B, and C in which the well developed porous structure is visible and no gap is seen between the monolith and the capillary walls confirming the efficiency of attachment. The network is achieved via the hydrolysis of an alkoxy silicate followed by condensation then polymerization by microwave irradiation. The pore and channel size of monoliths for column B and C seemed have formed bigger particle size and pores with increasing polymerization reaction time. The SEM micrographs of each column shows a structure comprised of an interconnecting network less than 1.5 μm spherical structures, throughout which micrometer sized

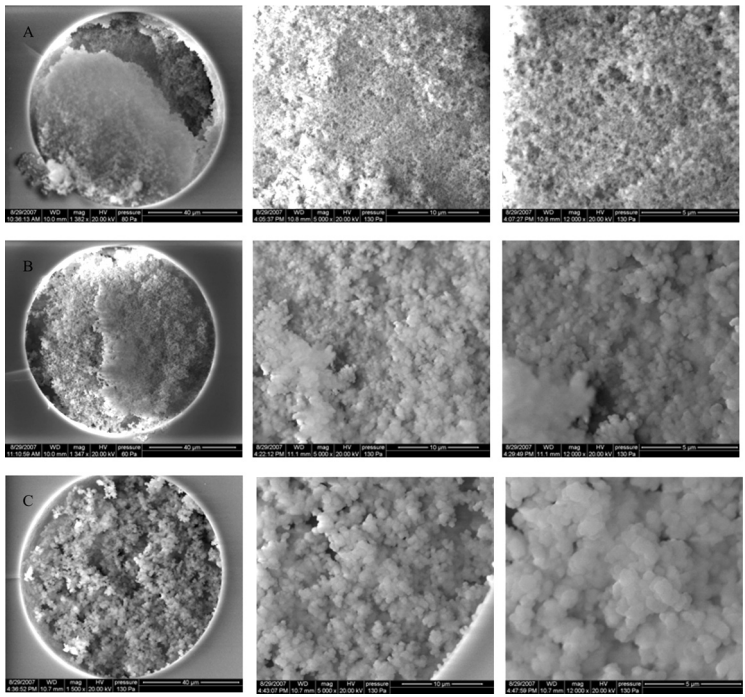

Figure 7. Scanning electron micrograph of monolithic columns A, B, and C.

through pores are interspersed. Although the density of the microwave initiation sol-gel skeleton seemed less than those previously reported,[7–9] better permeabilities were obtained, which ensured the quick separation in three modes. This was probably attributed to the different initiation method for the polymerization process.

CONCLUSIONS

A simple and fast procedure for the in situ preparation of a sol-gel monolith in a capillary was first attempted by microwave irradiation. The main advantage of microwave irradiation as an energy source is its short reaction time and a required lower expense, which possesses significant advantages over conventional heating. On the other hand, use of capillaries with UV transparent outer coatings in the photopolymerization was avoided, which tend to be more expensive than conventional polyimide coated capillaries. Here, our first attempts showed that microwave

radiation synthesis of porous sol-gel supports within the capillary are a viable alternative to either thermally initiated or photo polymerization. To better understand and control the microwave irradiation accurately, how electromagnetic fields are established inside the capillary and materials, and how they interact with the material at the molecular level, further research studies should be carried out.

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REFERENCES

1. Kavita, M.; Nelu, G. Application of monolithic columns in high performance liquid chromatography. J. Liq. Chromatogr. & Rel. Technol. **2005**, *28*, 1055–1074.
2. Zhang, Y.P.; Lee, K.P.; Choi, S.H.; Gopalan, A. Comparative studies of phemyl alcohols using HPLC and HPCE. Electrophoresis **2004**, *25*, 2711–2719.
3. Zhang, Y.P.; Ye, X.W.; Tian, M.K.; Qu, L.B.; Lee, K.P. Novel method to prepare polystyrene-based monolithic columns for chromatographic and electrophoretic separations by microwave irradiation. J. Chromatogr. A **2008**, *1188*, 43–49.
4. Chankvetadze, B.; Yamamoto, C.; Tanaka, N.; Nakanishi, K.; Okamoto, Y. High-performance liquid chromatographic enantioseparations on capillary columns containing monolithic silica modified with cellulose tris(3,5-dimethylphenylcarbamate). J. Sep. Sci. **2004**, *27*, 905–911.
5. Shi, Z.G.; Feng, Y.Q.; Da, S.L. Study of the preparation conditions of silica monoliths for HPLC. J. Liq. Chromatogr. & Rel. Technol. **2003**, *26*, 2881–2896.
6. Eeltink, S.; Svec, F. Recent advances in the control of morphology and surface chemistry of porous polymer-based monolithic stationary phases and their application in CEC. Electrophoresis. **2007**, *28*, 137–147.
7. Svec, F.; Huber, C.J. Monolithic materials, promises, challenges, achievements. Anal. Chem. **2006**, *78*, 2100–2107.
8. Ikegam, T.; Dicks, E.; Kobayashi, H.; Tanaka, N. How to utilize the true performance of monolithic silica columns. J. Sep. Sci. **2004**, *27*, 1292–1302.
9. Safrany, A.; Beiler, B.; Laszlo, K.; Svec, F. Control of pore formation in macroporous polymers synthesized by single-step [gamma]-radiation-initiated polymerization and cross-linking. Polymer. **2005**, *46*, 2862–2871.

10. Dulay, M.T.; Quirino, J.P.; Bennett, B.D.; Zare, R.N. Bonded-phase photopolymerized sol-gel monoliths for reversed phase capillary electrochromatography. J. Sep. Sci. **2002**, *25*, 3–9.
11. Kato, M.; Sakai-Kato, K.; Toyo'oka, T.; Dulay, M.T.; Quirino, J.P.; Bennett, B.D.; Zare, R.N. Effect of preparatory conditions on the performance of photopolymerized sol-gel monoliths for capillary electrochromatography. J. Chromatogr. A **2002**, *961*, 45–51.
12. Dulay, M.T.; Quirino, J.P.; Bennett, B.D.; Kato, M.; Zare, R.N. Photopolymerized sol-gel monoliths for capillary electrochromatography. Anal. Chem. **2001**, *73*, 3921–3926.
13. Kato, M.; Dulay, M.T.; Bennett, B.D.; Quirino, J.P.; Zare, R.N. Photopolymerized sol-gel frits for packed columns in capillary electrochromatography. J. Chromatogr. A **2001**, *924*, 187–198.
14. Zheng, J.; Syed, A.A.R.; Shahab, A.S.; Hou, J.G. Photopolymerized sol-gel monolithic column for capillary electrochromatography (CEC) and CEC coupled to atmospheric pressure photoionization mass spectrometry. J. Liq. Chromatogr. & Rel.Technol. **2007**, *30*, 43–57.
15. Morishima, K.; Bennett, B.D.; Dulay, M.T.; Quirino, J.P.; Zare, R.N. Toward sol-gel electrochromatographic separations on a chip. J. Sep. Sci. **2002**, *25*, 1226–1230.
16. Sakai-Kato, K.; Kato, M.; Toyo'oka, T. A protein-encapsulation technique by the sol-gel method for the preparation of monolithic columns for capillary electrochromatography. Anal. Chem. **2002**, *74*, 2943–2947.
17. Sakai-Kato, K.; Kato, M.; Toyo'oka, T. On-line drug-metabolism system using microsomes encapsulated in a capillary by the sol-gel method and integrated into capillary electrophoresis. Anal. Biochem. **2002**, *308*, 278–284.
18. Shao, H.; Deng, Q.; Lun, Z.; Yan, C.; Gao, R.Y. Preparation and evaluation of monolithic silica columns for capillary electrochromatography. Chin. J. Chromatogr. **2005**, *23*, 243–245.
19. Li, W.; Fries, D.P.; Malik, A. Sol-gel stationary phases for capillary electrochromatography. J. Chromatogr. A. **2004**, *1044*, 23–52.
20. Siouffi, A.M. Silica gel-based monoliths prepared by the sol-gel method, facts and figures. J. Chromatogr. A. **2003**, *1000*, 801–818.
21. Zhang, Y.P.; Fan, L.Q.; Lee, K.P.; Zhang, Y.J.; Choi, S.H.; Gong, W.J. Novel preparation of monolithic capillary columns for capillary electrochromatography by γ-ray irradiation. Microchim. Acta. **2007**, *158*, 353–360.
22. Gong, W.J.; Zhang, Y.J.; Zhang, Y.P.; Choi, S.H. Rapid preparation of monolithic columns for capillary electrochromatography separation. Chin. Chem. Lett. **2006**, *17*, 813–815.
23. Rathore, A.S.; Horvath, Cs. Effect of a predetection open segment in the column on speed and selectivity in capillary electrochromatography. Anal. Chem. **1998**, *70*, 3271–3274.
24. Hang, L.Y.; Ping, G.C.; Zhang, L.; Zhang, W.; Zhang, Y.K. Preparation and characterization of monolithic columns for capillary electrochromatography with weak electroosmotic flow. J. Sep. Sci. **2003**, *26*, 331–334.

25. Tanaka, N.; Nagayama, H.; Kobayashi, H.; Ikegami, T.; Hosoya, K. Monolithic silica columns for HPLC, Micro-HPLC, and CEC. J. High. Resol. Chromatogr. **2000**, *23*, 111–116.
26. Waguespack, B.L.; Hodges, S.A.; Bush, M.E.; Sondergeld, L.J.; Bushey, M.M. A direct comparison of the performance of ground, beaded and silica-grafted MIPs in HPLC and turbulent flow chromatography applications. Biosens. Bioelect. **2004**, *20*, 1098–1105.
27. Cabrera, K. Applications of silica-based monolithic HPLC columns. J. Sep. Sci. **2004**, *27*, 843–852.

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