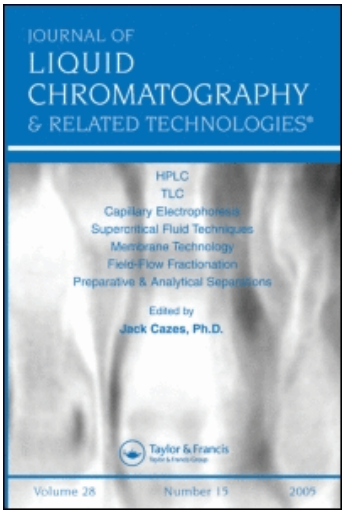

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Photopolymerized Sol-Gel Monolithic Column for Capillary Electrochromatography (CEC) and CEC Coupled to Atmospheric Pressure Photoionization Mass Spectrometry

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Abstract: A sol-gel photopolymerization procedure was adopted to prepare the monolith in a 100 μm I.D. UV-transparent capillary using methacryloxy-propyltrimethoxysilane as the monomer. The chromatographic behavior of the column is evaluated with two mixtures of neutral compounds, namely 16 polycyclic aromatic hydrocarbons (PAH), and 11 alkyl phenyl ketones. The feasibility of applying this column for the CEC-APPI-MS separation and detection of these compounds was investigated.

Keywords: Monolith, Sol-gel, Capillary electrochromatography, Mass spectrometry, Atmospheric pressure photoionization, Polycyclic aromatic hydrocarbons

INTRODUCTION

Capillary electrochromatography (CEC), one of emerging separation techniques, is often presented as a hybrid of high performance liquid

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chromatography (HPLC) and capillary electrophoresis (CE). This technique has attracted a growing interest in the past 20 years due to the fact that CEC combines high separation efficiency with excellent selectivity. Similar to the other chromatographic systems, the CEC column or capillary is the key component because it serves not only as a chamber to transport the mobile phase, but also as a separation channel. Therefore, the development of column techniques is critical for CEC. According to the existing state of stationary phases, the CEC column formats can be defined as packed column, open-tubular, or monolithic column. Among all these three column formats, the application of packed columns has the longest history and till now, it is still the dominant published CEC works.[1–4] One of the major reasons for the high popularity of packed columns is their ability to transfer some well established HPLC stationary phases directly into CEC.[3–6]

Although, the use of packed columns in CEC has achieved great success, it has also suffered from several drawbacks, such as a time consuming fabrication procedure, poor permeability, and fragility. The most serious drawback is the bubble formation in the packed column arising from the on-column frits, which often leads to poor baseline, irreproducible retention times, and even current breakdown. These limitations associated with packed columns have stimulated the development of alternative column technologies. Among various approaches, the use of monolithic or continuous bed columns has attracted a growing interest.

A monolithic column is typically a continuous, porous solid anchored to the capillary wall, which is prepared through *in situ* polymerization or consolidation inside the column. The stationary phase could be attached on the monolithic surface or embedded in the monolithic matrix. Thus, no frits are necessary to keep the stationary phase in place, and the problem of bubble formation arising from the frits in packed columns could be avoided. Moreover, these monolithic columns provide a number of other positive aspects, such as high surface areas and adsorption capacities, high permeability, and valuable flexibility on the surface chemistry. Based on the fabrication process and their matrix, traditional monolithic CEC columns can be classified as inorganic based, organic based, and particle loaded types.[7] Some unique advantages for each class of monoliths have been noticed. For example, inorganic monoliths showed good mechanical characteristics and excellent support for EOF, while organic monoliths offered more flexibility in terms of "fine tuning" the surface chemistry.[7] In addition, the particle loaded monoliths provided an alternative approach to apply the HPLC stationary phase for CEC.[8]

Recently, the research group of Zare[9,10] reported the use of methacryloxypropyl-trimethoxysilane (MPTMS) for the preparation of monolithic stationary phases. The MPTMS is a hybrid organic silane containing both methacrylate and alkoxysilane groups. Thus, the MPTMS based monoliths synthesized through a sol-gel photopolymerization procedure (Figure 1a),

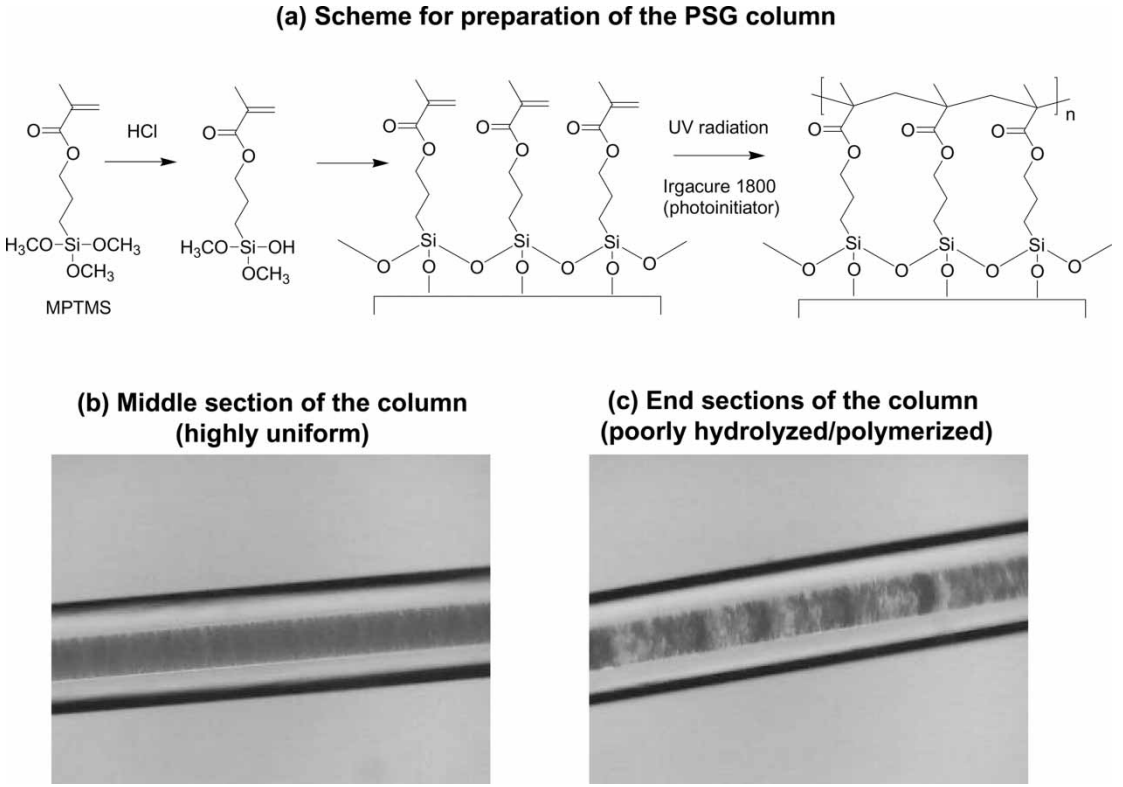

Figure 1. (a) Scheme for preparation of the PSG column. Microscopic images showing middle (b) and end (c) sections of the PSG column.

combine both the positive aspects of inorganic and organic monoliths. Besides direct usage for CEC separation, the same monoliths have been used for the sample on-line stacking,[11] or utilized as matrices for further derivatization.[12,13] Furthermore, bioactive enzymes, e.g., pepsin and trypsin have been immobilized on the monolithic surface and served as a "microreactor".[14,15]

On-column UV detection is still the most commonly adopted detection method for CEC. However, several limitations have been recognized, such as limited detection sensitivity due to a small column inner diameter, and poor specificity. These drawbacks have hindered the further application of CEC-UV, especially for complex samples. As one of the recently developed alternative techniques, CEC coupled to mass spectrometric (MS) detection is gaining popularity because MS offers excellent sensitivity, specificity, and valuable structural information.[16–18] Among all of the atmospheric pressure ionization (API) techniques used in MS, electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) have been commonly adopted because of their versatility for a broad range of polar to medium polar compounds. However, atmospheric pressure photoionization (APPI) is a newly introduced ionization technique, which is able to efficiently ionize non-polar compounds (e.g., polyaromatic hydrocarbons or PAHs) that are not readily ionized by ESI and even APCI. The coupling of HPLC with APPI-MS showed high sensitivity and low noise level, along with a good linear dynamic range.[19–31] However, only very recently, the feasibility of coupling CE with APPI-MS was demonstrated by several research groups.[19,32–34]

In this study, the MPTMS based monoliths were synthesized and their chromatographic performances were evaluated under both UV and APPI-MS detection for analysis of two mixtures of neutral compounds, namely PAHs and alkyl phenyl ketones. To our knowledge, this is the first attempt to couple the output of monolithic CEC columns to APPI-MS.

EXPERIMENTAL

Reagents and Chemicals

Methacryloxypropyltrimethoxysilane (MPTMS), thiourea, and 11 alkyl phenyl ketones were purchased from Aldrich (Milwaukee, WI). The 16 PAH mixtures were obtained from ChemService (West Chester, PA) as 200 $\mu g/mL$ solutions in acetonitrile. HPLC grade acetone, acetonitrile (ACN), methanol (MeOH), acetic acid (HOAc), and toluene were supplied by Fisher (Springfield, NJ). Irgacure 1800 was a gift from Ciba (Tarrytown, NY). Ammonium acetate (NH_4OAc), sodium hydroxide (NaOH), and hydrochloride acid (HCl) were purchased from Sigma (St. Louis, MO). Water was purified by a Barnstead Nanopure II Water system (Dubuque, IA).

Fabrication of the Photopolymerized Sol-Gel (PSG) Column

The 100 μm internal diameter fused silica capillaries (Polymicro Technologies Phoenix, AZ) with UV transparent PTFE coating were utilized to fabricate the PSG monolithic column based on a modified procedure developed by Kato et al.[10] In brief, the inner surface of the capillary was cleaned by rinsing with acetone and water for 30 min each. Then, 1 M NaOH and 0.1 M HCl, were utilized to flush the capillary to activate silanol groups. This included two processes, in which a small portion of silicon (−O-Si-O-) on the surface was converted into $-SiO^-$ with NaOH; then, these $-SiO^-$ groups were protonated by HCl. After cleaning the capillary with water and acetone, a solution containing MPTMS/acetone 1:1 (v/v) was used to treat the capillary for 1 h to vinylize the inner surface. The resulting capillary was flushed with acetone for 30 min and purged to dryness with N_2. Next, 750 μL MPTMS, 22.5 μL 1.0 M HCl, and 225 μL water were mixed in a glass vial and stirred in the dark for 0.5 h (mixture 1). A portion of 30 μL of the resulting mixture 1 was further diluted with 170 μL toluene, followed by stirring in the dark for another 0.5 h (mixture 2). About 9 mg of the photoinitiator, Irgacure 1800, was then added to the mixture 2 and stirred in the dark for 2.5 h (mixture 3). By applying vacuum or using a hand held syringe, the resulting mixture 3 was slowly introduced into the capillary. After the full length of the capillary was filled, both ends of the capillary were sealed. The photopolymerization was initiated by irradiating the capillary with a 15 W UV lamp (wavelength 365 nm, Fisher, Springfield, NJ) for 20 min.

Followed by the irradiation, the capillary was flushed with methanol for 3 h using a HPLC pump. Since both of the PTFE coating and the monolithic bed are UV-transparent, there is no need to fabricate the UV detection window. Before the CEC experiment, the column was preconditioned with the desired mobile phase for 1 h using the HPLC pump. Further conditioning was conducted electrokinetically on the CE instrument by gradual increments of the applied voltage until a stable current and baseline were achieved. Typically, separation voltage was set at 10 kV, employing a voltage ramp of 3 kV/s. During the CEC-APPI-MS separation, a 4-bar external pressure was applied to the inlet buffer vials. The selective ion monitoring (SIM) mode was utilized to monitor the desired molecular ions, $[M]^+$.

CEC-MS Instrumentation, Parameters, and Conditions

All CEC-MS experiments were carried out with an Agilent 3D capillary electrophoresis instrument interfaced to a single quadrupole mass spectrometer (Palo Alto, CA). The APPI source was utilized. An Agilent 1100 series HPLC pump equipped with 1:100 splitter was used to deliver the sheath

liquid at 5.0 μL/min, which consists of MeOH/H_2O 90:10 (v/v) and 5 mM NH_4OAc. Typical settings for the MS spray chamber were listed as follows: krypton lamp (10 eV); vaporizer temperature, 200°C; capillary voltage, 1000 V; fragmentor voltage, 80 V; drying gas flow rate, 8.0 L/min; drying gas temperature, 250°C; nebulizer pressure, 4 psi.

Preparation of Standard Analytes

Stock solutions of individual alkyl phenyl ketones were prepared by dissolving 10 mg of each homologue in 1 mL of ACN and stored at −20°C. The mixture of alkyl phenyl ketones was prepared by taking 100 μL aliquot from each stock solution. A typical 40 μL injection aliquot was prepared by taking 20 μL of the stock mixture and then diluting with 20 μL of triply deionized water. For the PAHs, the injected sample was prepared by directly diluting 20 μL of a commercially available test mixture with the same volume of triply deionized water.

Safety Precautions

Since several PAHs are suspected carcinogens, caution was exercised with these compounds. All handlings were performed in a ventilated hood with appropriate clothing, mask and gloves, to avoid inhalation or skin contact. The samples were stored in a closed container at −20°C, and care was taken to dispose of the PAH waste solutions appropriately.

RESULTS AND DISCUSSION

Preparation of PSG Monoliths

As shown in Figure 1a, the sol-gel photopolymerization procedure involves three steps. In the first step, the alkoxysilane groups are hydrolyzed using HCl (step 1, Figure 1a). Next, the condensations between the resulting silanol groups and alkoxysilane occur (step 2, Figure 1a). Finally, the photopolymerization of the methacryloxy groups is initiated with the presence of Irgacure 1800 (step 3, Figure 1a). The reaction conditions, including concentration of HCl and the ratio of monomer to porogen have been investigated in a previous study.[10] Using the optimized conditions from the literature, we focused on the improvement of homogeneity and robustness of the monolithic column. In particular, we have noticed that although the whole monolith is covalently bonded, the stability of the monolithic bed, especially under high back pressure, still could be undermined due to the presence of small gaps between the monolithic bed and capillary wall. To overcome this problem,

fused silica capillaries with their inner surface pretreated with MPTMS were utilized in this study. Since the methacryloxy groups on the vinylized surface could be copolymerized along with the bulk monolith, they can serve as "anchors" to position the bulk monolith in place. As displayed in Figure 1b, a highly homogenous monolith bed in bulk was obtained using the aforementioned procedure with no crack in the middle. Although, sometimes small inhomogeneous sections can be found close to the column ends (Figure 1c), they can be easily overcome by discarding the corresponding portions. Furthermore, replacing the polyimide coated capillaries with the PTFE coating avoids the need to fabricate the UV detection window, which in turn, enhance the robustness of the monolithic columns.

Evaluation of Column Performance Under CEC-UV

The performance of the PSG monolithic column was evaluated using 16 PAHs and alkyl phenyl ketones as test solutes. Using a reversed-phase eluent containing 50% (v/v) ACN, an electroosmotic flow (EOF) mobility of $10.0\ cm^2/min \cdot kV$ was obtained. In addition, separation efficiency as high as 120,000 plates/m was achieved for the non-retained analyte, thiourea. As displayed in Figure 2, 15 out of 16 PAHs were separated with partial resolution of benz[a]anthracene/chrysene, benzo[k]fluoranthene/benzo[a]pyrene, benzo[ghi]perylene/indeno[1,2,3-cd]pyrene within 35 min. The elution order of PAHs showed strong correlation of chromatographic retention to the hydrophobicity and numbers of aromatic rings (Figure 2 inset). For example, the less hydrophobic 3-ring anthracene ($\log P = 4.680$) is less retained than the 5-ring benzo[a]pyrene ($\log P = 6.402$). In addition, note that the resolution for these aromatic compounds is severely undermined by peak tailing for PAHs, especially for the later eluting PAHs. This deleterious peak tailing could be associated with π-π interaction between the PAH and methacryloxy residues in the bulk PSG monolith.

A mixture of the 11 alkyl phenyl ketones was resolved within 25 min (Figure 3). A strong dependence on the length of alkyl chain length was observed for the elution order of alkyl phenyl ketones. The excellent correlation ($R^2 = 0.997$) between the values of $\log k'$ versus the number of carbons provides strong evidence for the reversed-phase behavior for the prepared PSG monolithic column. Furthermore, the PSG showed better efficiency and resolution along with no peak tailing, even for the highly retained alkyl phenyl ketones.

CEC-APPI-MS Separation Using PSG Columns

Considering that PAHs and alkyl phenyl ketones are non-polar and lack ionizable groups, ESI is not a suitable ionization source for MS detection in this study. APPI is a much better choice, since it has demonstrated an

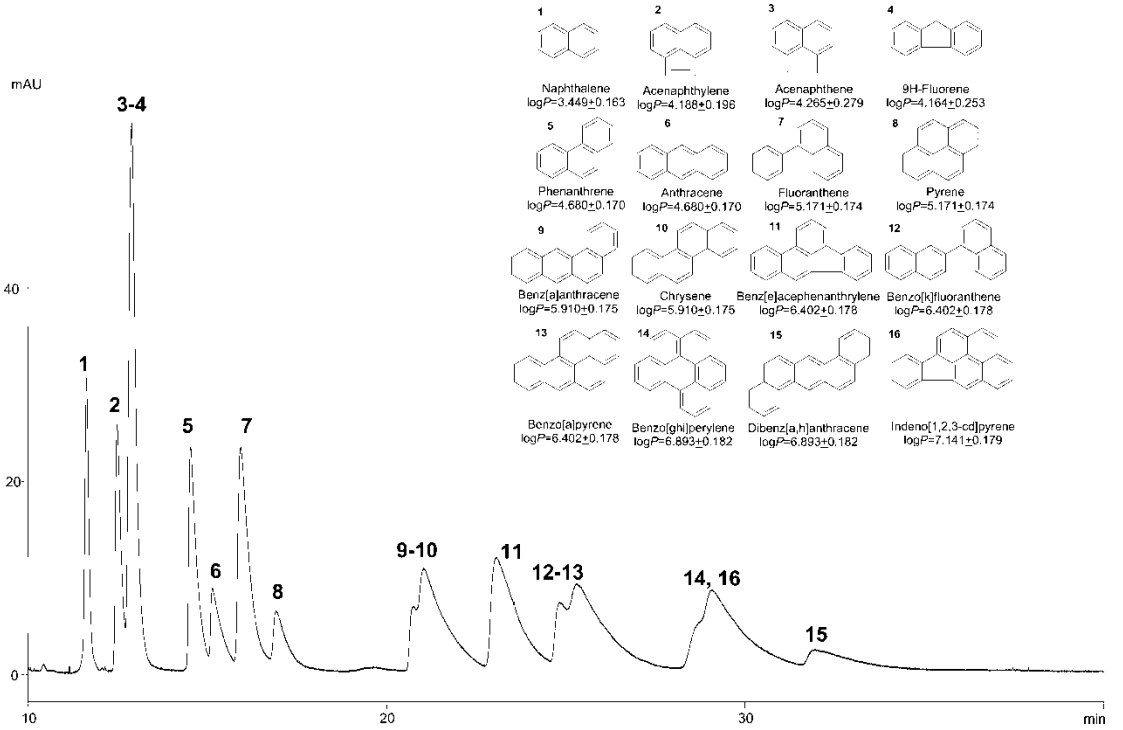

Figure 2. Electropherogram of CEC-UV separation of a test mixture of 16 PAHs using the PSG column. The peak identification is listed in the inset. Conditions: 100 μm (I.D.) 30 cm long PSG capillary, mobile phase ACN/H_2O 50:50 (v/v) containing 5 mM NH_4OAc at pH 8.0, 10 kV, 25°C, UV detection at 214 nm.

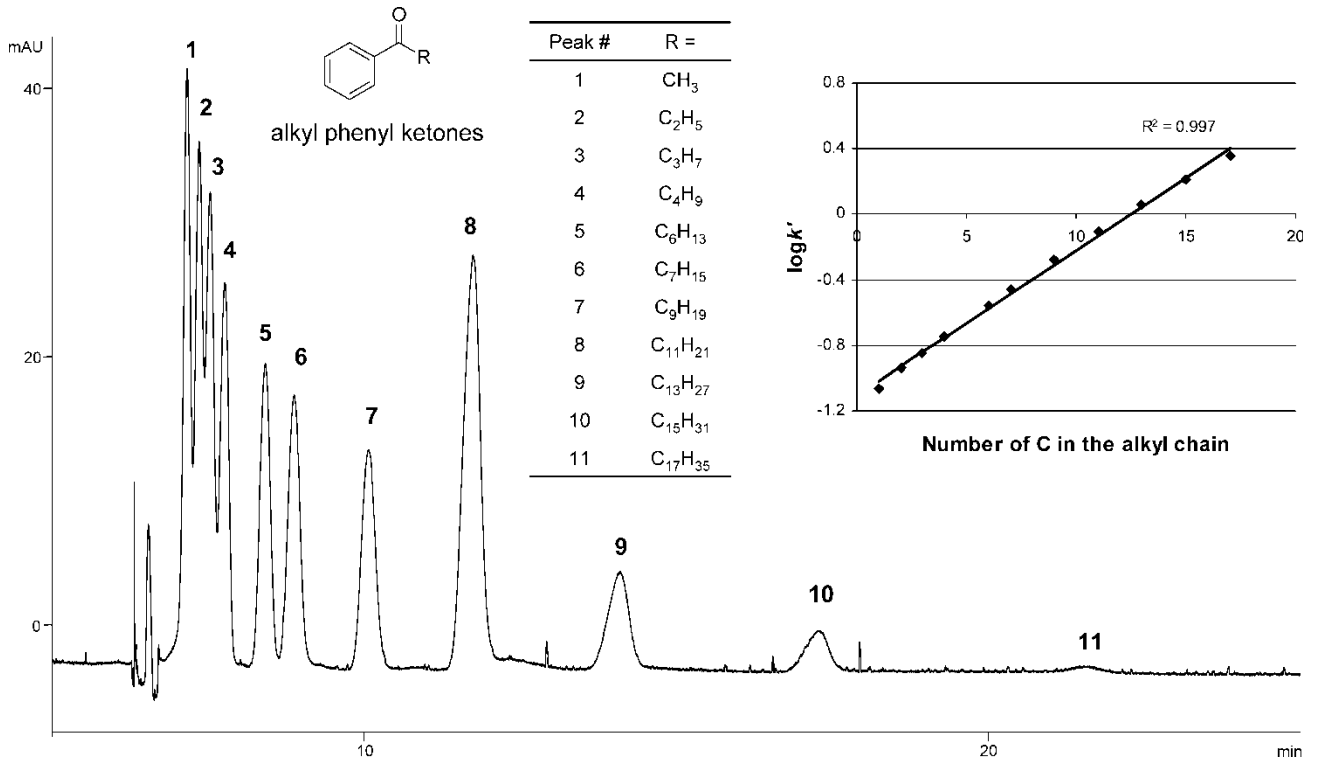

Figure 3. Electropherogram of CEC-UV separation of a test mixture of 11 alkyl phenyl ketones using the PSG column. The peak identification is listed in the inset table. Conditions are same as Figure 2.

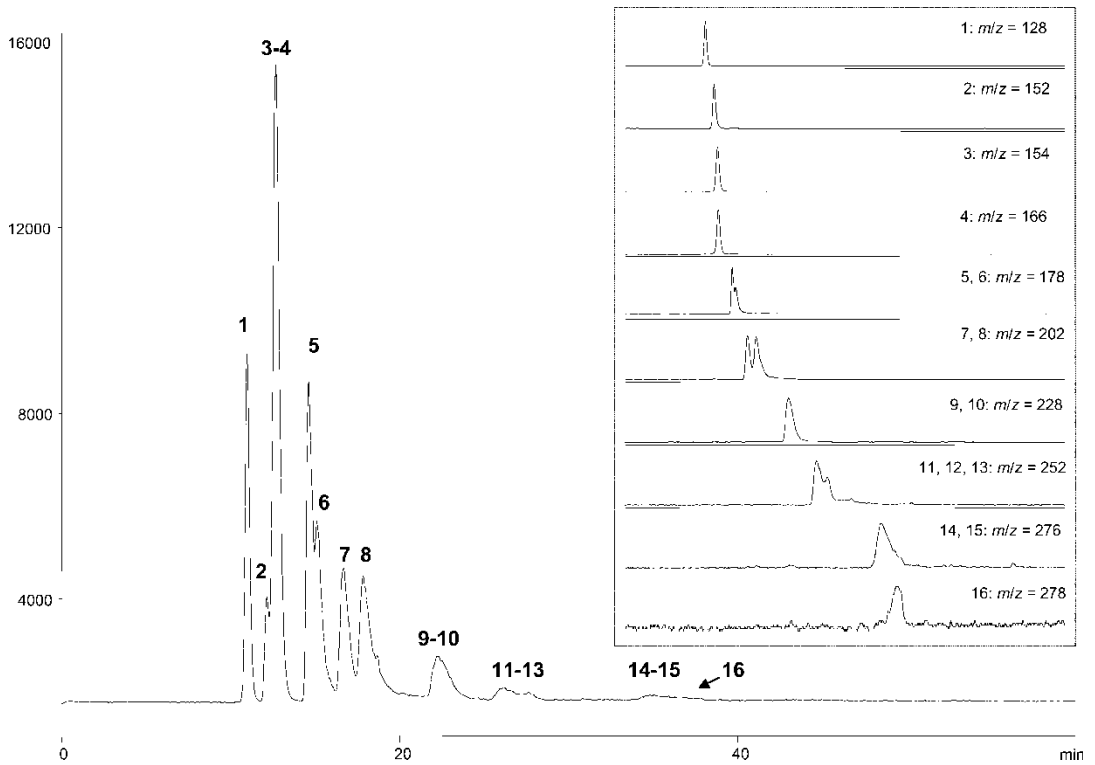

Figure 4. Electropherogram of CEC-APPI-MS separation of PAHs using the PSG column. Conditions: 100 μm (I.D.) 65 cm long PSG capillary, mobile phase ACN/H_2O 50:50 (v/v) containing 5 mM NH_4OAc at pH 8.0, 10 kV, 25°C. For the other conditions see experimental section.

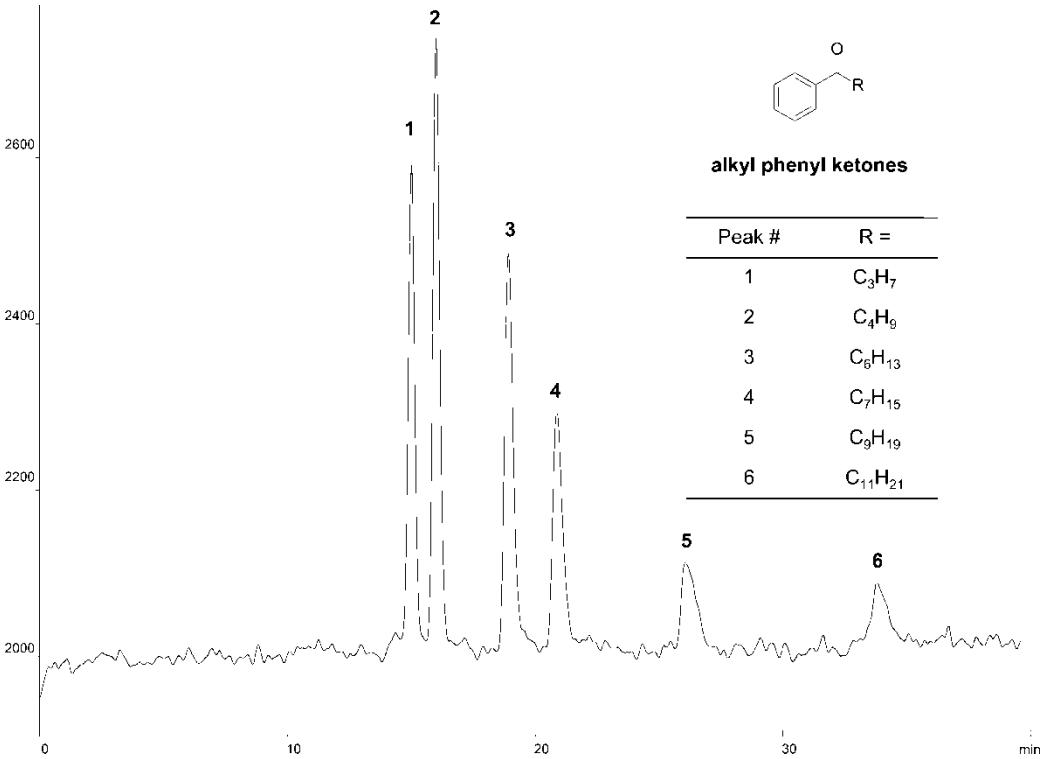

Figure 5. Electropherogram of CEC-APPI-MS separation of alkyl phenyl ketones using the PSG column. The conditions are same as Figure 4 or described in experimental section.

excellent capability for LC-MS analysis of non-polar compounds. Therefore, in this study, the feasibility of coupling monolithic CEC with APPI-MS detection was explored. To suppress the possible bubble formation at outlet side of the PSG monolithic column caused by high temperature in the spray chamber, several different approaches were tested. Initially, we tried to use the internally tapered capillaries to fabricate the PSG monolithic column. However, several drawbacks were noticed. For example, due to the high back pressure of the taper, the flushing of the capillary with the sol-gel solution was not achievable by applying a vacuum or using a hand held syringe. In addition, the column outlet tends to clog due to the small diameter of the tapered channel. As a result, an untapered column configuration was adopted in this study. In addition, a 3-bar pressure was applied at the inlet of the PSG monolithic column during the CEC-APPI-MS separation, to suppress the deleterious bubble formation.

As expected, the APPI-MS provided excellent ionization efficiency for the PAHs. Furthermore, the use of the highly selective SIM mode of MS enhanced the detection specificity and lowered the background noise. As a result, CEC-APPI-MS showed a significant improvement in terms of detection sensitivity. For example, naphthalene showed 12-fold higher S/N with APPI-MS (S/N 740) than with UV detection (S/N 60). Moreover, it is worth mentioning that the elution order of PAHs can be easily determined from the extract ion chromatogram (Figure 4 inset) without the need for tedious spiking. As shown in Figure 4, a total of 12 of 16 PAHs were resolved within 40 min. Compared to CEC-UV (Figure 2), slight loss of resolution was observed for some of PAHs. This could be attributed to the extra band broadening (average efficiency 11000 plate/m) caused by applied external pressure at the inlet. We also investigated the possibility to enhance the resolution of several PAHs by lowering the ACN content in the mobile phase to 40% (v/v). However, the run time was significantly increased to over 100 min (data not shown).

Next, we also investigated the CEC-APPI-MS analysis of 11 alkyl phenyl ketones with a PSG monolithic column (Figure 5). Interestingly, only 6 ketones with a medium length of alkyl chain (C_3–C_{11}) were detected. The absence of acetophenone and propionophenone could be associated with the poor stability of their $[M]^+$ ions, which could undergo fragment or rearrangement reactions. On the other hand, the decreasing ionization efficiency for longer alkyl chain ketones could be the reason why hexadecanophenone (C_{15}) and octadecanophenone (C_{17}) showed no peaks.

CONCLUSIONS

A fused silica capillary with transparent coating and vinylized inner surface has been utilized to prepare the PSG monolithic columns, which shows improved homogeneity and robustness. The performance of these columns

was evaluated using PAH and alkyl phenyl ketones as test mixtures. Excellent separation efficiency and reversed-phase retention behaviors were observed. It is also observed, that the π-π interaction between the aromatic analytes and the methacryloxy residues led to some degree of peak tailing, especially for the highly hydrophobic PAHs, which resulted in a loss of resolution. Next, the combination of monolithic CEC and MS detection via APPI interface for separation and detection of PAHs and alkyl phenyl ketones was demonstrated. For these non-polar analytes, APPI-MS provided excellent ionization efficiency for the PAHs, which in turn improved their detection sensitivity. Compared with UV detection, the APPI-MS provided 12-fold better S/N of naphthalene. In addition, excellent detection specificity was achieved due to the use of SIM and EIC. In contrast to PAHs, the alkyl phenyl ketones with only a moderate length of alkyl chain were detected under the experimental condition. Further investigations that include exploration of fragmentation pattern and adding dopant in the sheath liquid to improve the ionization efficiency in APPI-MS are under investigation.

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