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Preparation and Evaluation of Silica-Based Ionic Liquid-Modified Stationary Phase for HPLC

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Abstract

A new high-performance liquid chromatography (HPLC) stationary phase was firstly synthesized based on the ionic liquid 2-ethyl-4-methylimidazole chloride. And the performances of the resulting stationary phase were determined by Fourier transform infrared (FT-IR), thermogravimetric analysis (TGA), and ^{13}C nuclear magnetic resonance (NMR) spectroscopy. The retention characteristics of the resulting stationary phase were evaluated by normal phase HPLC conditions to separate tanshinone I and tanshinone IIA in Danshen herb. The results demonstrated that the synthesized ionic liquid was bond-linked to the silica surface successfully and that IL-modified silica is more effective than unmodified silica when separating tanshinone I and tanshinone IIA in Danshen herb.

Introduction

Recognized as “green” chemistry, ionic liquids (ILs) have been widely used in analytical chemistry recently. In capillary electrophoresis (CE), ILs have been used as electrolyte additives for the separation of polyphenols (1), basic proteins (2), aromatic acids (3), and anthraquinones (4). Alkylimidazolium-based ILs have been used as a stable stationary phase for gas chromatography (5–8). They show unusual selectivity with “dual nature”, separating polar compounds as if they were polar stationary phases and nonpolar compounds as if they were non-polar stationary phases (5,6). In high-performance liquid chromatography (HPLC), ILs have been used as mobile phase additives for the separation of ephedrines (9), catecholamines (10), amines (11), and others (12,13). It was proposed that the imidazolium cations interact with the silanol groups on the surface of the silica particles, which suppresses the adverse effects of free silanols in liquid chromatography.

However, ILs have rarely been applied in the stationary phase of liquid chromatography. As mentioned in the literature (14), when ILs are diluted or immobilized on a support, they may not possess all the properties of neat ionic liquids (15,16). Nevertheless, their potential use as stationary phases for HPLC is an intriguing concept and deserves to be explored. In this work, 2-ethyl-4 methylimidazole combined with (3-chloropropyl)triethoxysilane was first prepared, and then the IL product was used to modify silica gel to get IL chemically modified silica (IL-silica) via the radical chain transfer addition

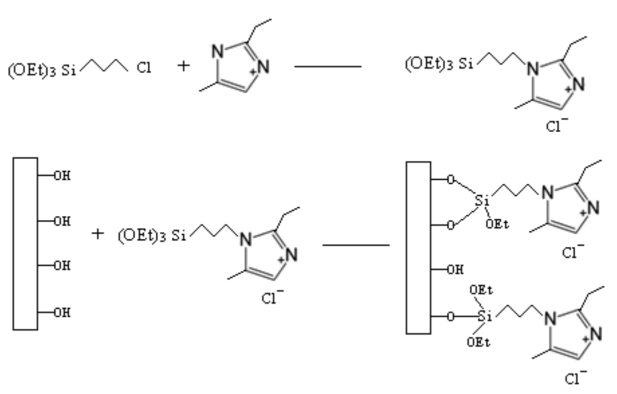

Figure 1. The preparation processes of IL-modified silica particles.

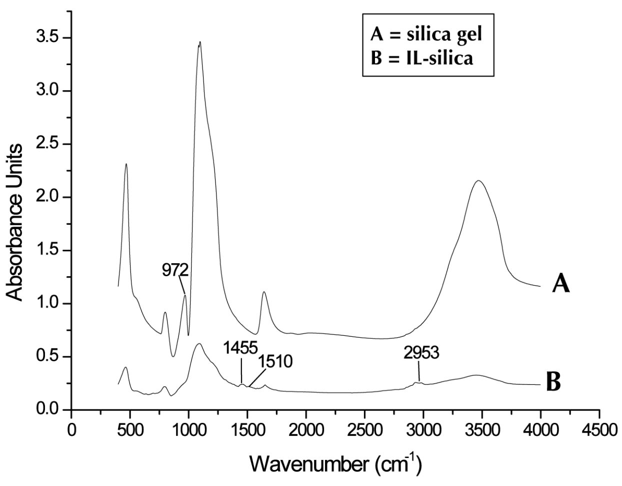

Figure 2. FT-IR spectrograms of unmodified silica particles (A) and IL-modified silica particles (B).

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reaction between allyl and thiol groups. IL-silica was characterized by thermogravimetric analysis (TGA) and Fourier transform infrared (FT-IR) and ^{13}C nuclear magnetic resonance (NMR) spectroscopies served and as stationary phase in HPLC to separate tanshinones from the traditional Chinese herb, Danshen.

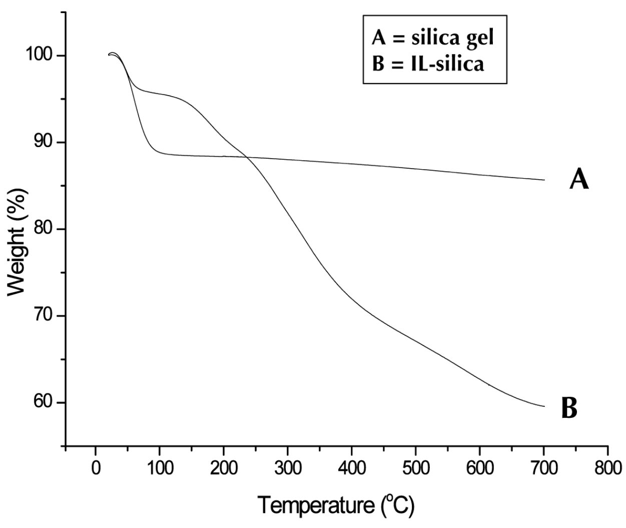

Figure 3. TGA curves of unmodified silica particles (A) and IL-modified silica particles (B).

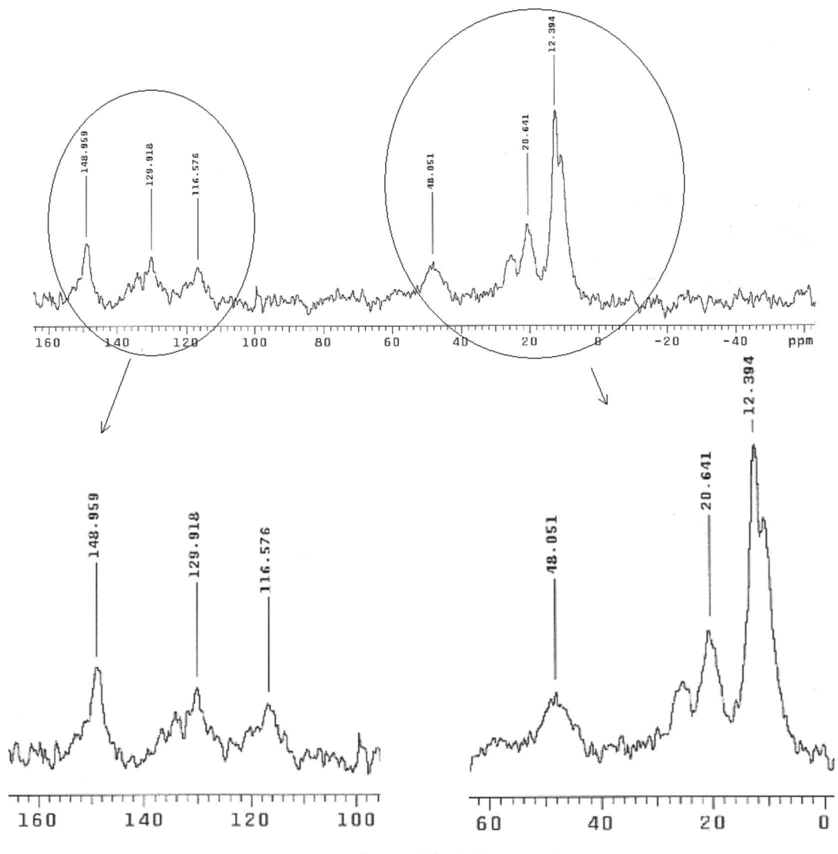

Figure 4. ^{13}C CP/MAS NMR spectrograms of IL-modified silica particles.

Experimental

Materials

The 15 µm Apex silica particles were purchased from Merck (Darmstadt, Germany). (3-Chloropropyl)triethoxysilane (95%) was purchased from Sigma Aldrich (St. Louis, MO) and 2-ethyl-4-methylimidazole (95%) was obtained from Aldrich (Milwaukee, WI). Authentic standards of tanshinone I and tanshinone IIA were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The Danshen powder was purchased from a local market in Korea. All other reagents were analytical-grade and purchased from Duksan Pure Chemicals (Ansansi, Korea). The water used was doubly distilled.

Preparation of solutions

Tanshinone IIA and tanshinone I solutions were prepared in methanol. Both of the concentrations of the standard solutes were 0.5 mg/mL, and the mixture of the two solutes was 0.25 mg/mL. All the solutions were prepared in dark brown calibrated flasks and stored at 40°C until analysis.

Instrumentation

The TGA unit (SCINCO thermal gravimeter S-1000) connected to a flow panel. The samples were heated at a rate of 5°C/min. All thermogravimetric measurements were performed in nitrogen atmosphere over the temperature range of 20–800°C.

The FT-IR measurements were carried out on a RFS 100/S spectrometer (Bruker, Karlsruhe, Germany) with a liquid nitrogen-cooled germanium detector equipped with a fiber-optic cable. The measured range was 4000–400 cm^{-1}. Each spectrum was accumulated over 5 min with 300 scans and a resolution of 4 cm^{-1}, and all spectra were obtained for the wrist area.

Solid state ^{13}C measurement for the IL-modified silica was performed on a Brucker Avance II spectrometer, using cross polarization and magic angle spinning (CP/MAS). A contact time of 5 ms and frequency of 125 MHz were employed.

The HPLC system was equipped with a Waters (Milford, MA) 600S solvent delivery system including 616 solvent delivery pump, 600S controller, and the 2487 UV dual channel detector and an injector (0.02-mL sample loop) of Rheodyne. The wavelength was fixed at 274 nm. The IL-modified silica particles were packed into a stainless steel column (250 × 4.6 mm) by the conventional slurry packing method using hexane as the solvent.

Preparation of ionic liquid stationary phases

(3-Chloropropyl)triethoxysilane (24.5 mL, 0.102 mol) and 2-ethyl-4-methylimidazole (11.359 g, 0.103 mol) were added to a clean and dry round-bottom flask containing a magnetic stir bar. Then toluene (40 mL) was added into the bottle as solvent. The reaction was carried out at 100°C for about 12 h. Obtained as slightly orange viscous oil, the product IL was washed several times with cold hexane.

15 µm diameter spherical silica particles (4 g, 0.3 cm^3/g pore volume, 60 Å pore diameter) and IL (0.235 g, 0.67 mmol) were added into a round-bottom flask, and toluene (45 mL) was also added as solvent. Then the mixture was heated to about 105°C until toluene refluxed. After reacting for 15 h, the product IL-modified silica particles were washed with toluene three times, then dried to be powder. The preparation process of IL-modified silica particles is shown in Figure 1.

Results and Discussion

FT-IR spectrum

FT-IR spectrograms of unmodified silica particles and IL-modified silica particles are shown in Figure 2. The spectrum of unmodified silica is stronger than that of the IL- modified silica particles generally, which is attributed to the lower weight percent of silica in IL-modified silica particles than in the unmodified silica. A band at around 972 cm^{-1} is observed in Figure 2A due to the stretching of the siloxane (≡Si-O) bonds of the silica backbone. In Figure 2B, the spectrum of IL-modified silica shows two characteristic peaks at 1456 and 1510 cm^{-1}, which are attributed to the C-N and C-C expansion peaks of the imidazole ring, respectively. And the peak at around 2953 cm^{-1} in Figure 2B is attributed to the C-H expansion peaks of the imidazole ring and allyl group. These results confirm the successful grafting of IL on the silica gel surface.

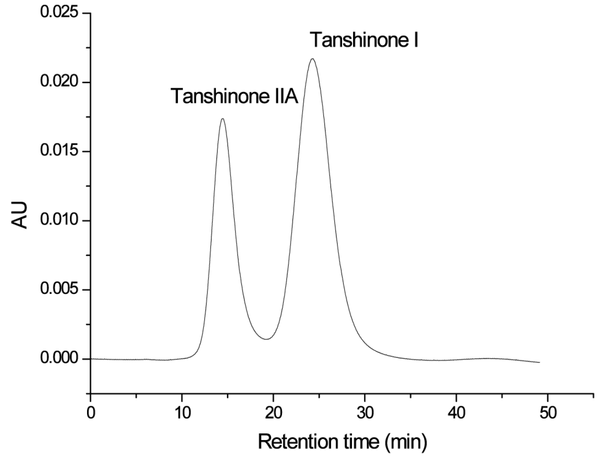

Figure 5. The chromatogram of tanshinone I (A) and tanshinone IIA (B) using the IL-modified silica packed column.

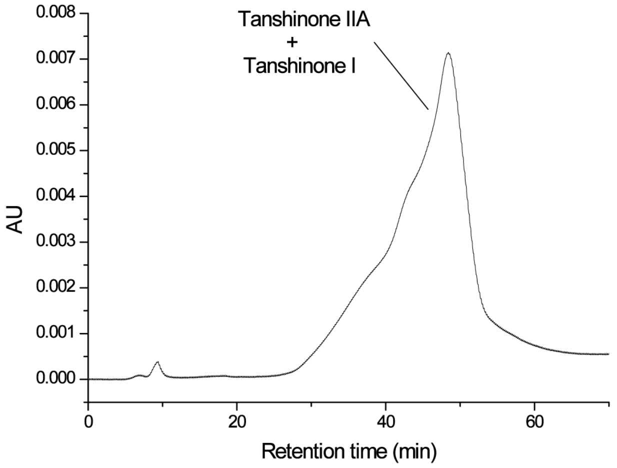

Figure 6. The chromatogram of tanshinone I (A) and tanshinone IIA (B) using the unmodified silica packed column.

Thermogravimetric analysis

TGA was performed on the silica particles modified with 2-ethyl-4 (trimethoxysilylpropyl)imidazolium bromide at two rates as well as on the unmodified particles in the temperature range of 20–800°C. The thermograms of Figure 3 shows that there is an initial weight loss at a temperature was less than 100°C for all samples, which is attributed to the removal of physically adsorbed water and/or any minor solvent residues remaining from the particle modification procedures.

At more than 100°C, there is no further change for the unmodified silica, whereas IL-silica continues weight loss due to the fracture of the bonds in IL. Three very distinct thermal events occur in the range of 150–250°C, 250–400°C, and 400–600ºC. The first and second of the regions are where the organic moieties at the surface are expected to thermally decompose. The third one is associated with decomposition of the residual methoxy side groups.

Nuclear magnetic resonance

^{13}C CP/MAS NMR spectrograms for the IL-modified silica particles are shown in Figure 4. The silica substrate doesn't exhibit any signal in ^{13}C NMR, so all of the six signals are related to IL. Three signals observed at 12.394, 20.694, and 48.051 ppm are related to the ethyl and methyl group bonded. And signals at 116.576, 129.918, and 148.959 ppm correspond to the imidazolium ring group bonds. These signals prove the successful bonding of IL to the silica surface.

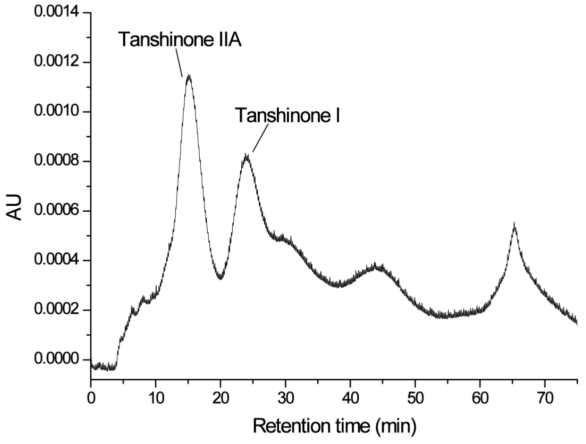

Figure 7. The chromatogram of real Danshen powder using the IL-modified silica packed column.

HPLC evaluation

The IL-modified silica particles were packed into a hollow column (4.6 × 250 mm) and tested under normal phase HPLC conditions. Figure 5 shows a chromatogram of the mixture of tanshinone I and tanshinone IIA using hexane/dichloromethane (87:13, v/v) as the mobile phase at the optimal flow rate of 0.5 mL/min. Obviously, it has been successfully applied to determine tanshinone I and tanshinone IIA, which are the main abietane-type diterpenes contained in Danshen herb.

In order to compare IL-modified silica with unmodified silica, a similar hollow column was packed with unmodified silica (15 µm), which was used to prepare the IL-modified silica. And the chromatogram, tested at the same HPLC conditions as IL-modified silica column, is shown in Figure 6. In it, there is a wide peak for the mixture of tanshinone I and tanshinone IIA, which means the two kinds of tanshinones cannot be separate. When comparing with Figure 5, the retention times for both of the tanshinones has been shortened, and the peaks are also sharper than in Figure 6. All of these provide that IL-modified silica is more effective than unmodified silica when separating tanshinone I and tanshinone IIA in Danshen herb.

The chromatogram of real Danshen powder on IL-modified silica column is shown on Figure 7. The composition of real Danshen is very complex, so the components cannot be separated completely. But tanshinone I and tanshinone IIA are the primary components in Danshen herb, so the corresponding peaks are obvious.

The synthesis of IL-modified silica on a HPLC column is still a very new field, and in recent research, the IL-modified silica column is usually used to separate simple organic and inorganic compounds. So, we will develop and apply it to separate real samples (like Danshen herb in this article) in the future.

Conclusions

First, IL material was synthesized using (3-chloropropyl)triethoxysilane and 2-ethyl-4-methylimidazole, and then the new HPLC stationary phase was synthesized based on the IL. IL was covalently immobilized on a silica substrate through *n*-alkyl ether, and the performances of the resulting stationary phase were determined by FT-IR, TGA, and ^{13}C NMR. And the results proved the successful bonding of the IL to the silica surface.

The retention characteristics of the IL-modified silica were evaluated by normal phase HPLC conditions to separate tanshinone I and tanshinone IIA in Danshen herb. Tanshinone I and tanshinone IIA were separated successfully with the hexane–dichloromethane (87:13, v/v) as mobile phase. Compared with unmodified silica at the same conditions, the results provide that IL-modified silica is more effective than unmodified silica when separating tanshinone I and tanshinone IIA in Danshen herb. And in the future, we will research more complex compounds and components in natural plan (like Danshen herb in this article) using IL-modified silica column.

Acknowledgments

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