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# Poly(1-allylimidazole)-grafted silica, a new specific stationary phase for reversed-phase and anion-exchange liquid chromatography

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ABSTRACT

A new specific stationary phase based on poly(1-allylimidazole)-grafted silica has been synthesized and characterized, by infrared spectra, elemental analysis, thermogravimetric analysis and X-ray photoelectron spectroscopy. The results of test showed that poly(1-allylimidazole) can effectively mask the residual silanol groups and reduce the adverse effect of residual silanol. Using this stationary phase, phenol compounds, aniline compounds, and polycyclic aromatic hydrocarbons were successfully separated with symmetric peak shapes in the reversed-phase chromatography. Inorganic anions ($IO_3^-$, $BrO_3^-$, $Br^-$, $NO_3^-$, $I^-$, $SCN^-$) were also separated completely in the anion-exchange chromatography using sodium chloride solution as the mobile phase. The effects of pH and the concentration of eluent on the separation of inorganic anions were studied. The separation mechanism appears to involve the mixed interactions of hydrogen bonding, hydrophobic, π–π, electrostatic, and anion-exchange interactions.



## 1. Introduction

In liquid chromatography, the most popular stationary phases are silica-based stationary phases, because silica has some favorable physical characteristics, such as high mechanical strength, appropriate surface area and narrow mesopore size distributions [1,2]. Silica accounts for about 90% of the chromatographic supports, especially in HPLC, and it is also used in capillary columns for GC and CE. However, the adverse effect of residual silanol is always a problem difficult to solve. The reason is that the coverage of silanol groups in chemically bonded phases is less than 60% due to the steric hindrance and other effects [3]. Peak-tailing and poor reproducibility often exist in chromatographic process, especially for the separation of basic compounds. And the retentions of acid compounds can also be affected by residual silanol due to electrostatic exclusion phenomena [4] and hydrogen bonding.

To eliminate these undesirable properties, many end-capping reagents and methods have been developed. The most commonly used reagents are trimethylchlorosilane [5,6] and hexamethyldisilazane [7]. However end-capping cannot eliminate all residual silanol, unfavorable effects of residual silanol still exist to some degree. Alternatively, *n*-alkyl-bonded silica with embedded polar functional groups, containing nitrogen atoms, amide [8–14], carbamate [15–17] and urea groups [18,19], has been developed as another approach to improve peak shape and reproducibility. The new family, the so-called embedded polar phase, shows excellent performance for the separation of basic compounds compared with conventional *n*-alkyl-bonded phases [20,21]. The mechanism is often interpreted as the action of hydration layer on silica surface or the competitive interaction between its polar functional groups and silanol on silica [22].

Recently, poly(4-vinylpyridine)-grafted silica stationary phase, which reduces the undesirable silanol effect on basic compounds in HPLC by forming hydrogen bonds and providing exclusion effect on basic compounds, has been developed by Ihara et al. [23] Ihara and co-workers [24]. *N*-alkyl imidazole is usually prepared as ionic liquids. Anion-exchange stationary phases based on imidazolium have been applied in HPLC [25,26]. In fact, *N*-alkyl imidazole is a class of alkali, because the nitrogen in the 3-place of *N*-alkyl imidazole can combine with $H^+$ to form a cation of the imidazole ring and can also generate a hydrogen bond with the hydrogen of silanol by lone pair electrons. A π conjugative system of the molecule in *N*-alkyl imidazole may interact with some molecules having π conjugative systems. The π–π interaction may help to realize the chromatographic separation.

Based on these considerations, the primary purpose of this research was to synthesize and characterize a novel stationary phase with poly(1-allylimidazole) groups grafted onto the silica surface, with its chromatographic property examined by reversed-phase and anion-exchange chromatographic tests, and the masking residual silanol evaluated by chromatographic tests.

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## 2. Experimental

### 2.1. Apparatus and reagents

All chromatographic tests on the poly(1-allylimidazole)-grafted silica stationary phase were performed on a self-assembled HPLC system, including a Shimadzu LC-10AT pump (Kyoto, Japan), a 20 μl sample loop and a Perkin-Elmer 785A (Boston, MA, USA) UV–vis detector. Data collection and treatment were carried out by using HW-2000 chromatographic work station from Qianpu Software Ltd. (Shanghai, China). All chromatographic tests on ODS column were performed on an Agilent 1100 series (Santa Clara, CA, USA) with a 20 μl sample loop and a UV–vis detector. The column (200 mm × 4.6 mm I.D.) was packed with poly(1-allylimidazole)-grafted silica. Spherical silica porous particles of 5 μm, with an average pore diameter of 9 nm and a specific surface area (BET) of 390 $m^2 g^{-1}$, which were made in our laboratory, were used as the support. ODS column (200 mm × 4.6 mm I.D.) was packed with commercial C18 silica gel (5 μm, Chromatorex, Fuji Silysia Chemical Ltd., Japan). 1-Allylimidazole (97%) was purchased from Acros (Morris Plains, NJ, USA). Azobisisbutyronitrile (AIBN) was recrystallized in ethanol before use. 3-Mercaptopropyltrimethoxysilane (98%) was obtained from Jingzhou Jianghan Fine Chemical Co. (Hubei, China). Organic compounds including phenols, anilines, benzene and polycyclic aromatic hydrocarbons (PAHs) used in reversed-phase chromatographic tests and inorganic salts including potassium iodate, potassium bromate, potassium bromide, potassium nitrate, sodium iodide, potassium thiocyanate, and sodium chloride used in anion-exchange chromatographic tests were analytical reagents. All other compounds used in experiments were analytical grade and were used without further purification.

### 2.2. Synthesis of poly(1-allylimidazole)-grafted silica stationary phase

Poly(1-allylimidazole) with a terminal trimethoxysilyl group ($AIm_n$), was synthesized by the modification of the telomerization method [23,27] of 1-allylimidazole with 3-mercaptopropyltrimethoxysilane. The synthetic procedure of $AIm_n$ was as follows: 0.68 ml of 3-mercaptopropyltrimethoxysilane and 7.5 ml of 1-allylimidazole were mixed in a 50 ml flask. After the addition of 0.075 g of AIBN, $N_2$ was bubbled for 25 min at room temperature. The mixture was stirred under nitrogen atmosphere at 60 °C for 24 h. The product was liquid. Because it was difficult to purify the product, so the product was not purified.

Second, the resultant poly(1-allylimidazole) was grafted onto silica by using the reaction of trimethoxysilyl group and silanol on the silica surface. 2.5 g of dry silica was placed in a reaction flask containing the resultant $AIm_n$ mixture. The residual 1-allylimidazole was used as the solvent for the grafting process. The mixture was stirred under nitrogen atmosphere at 120 °C for 68 h, and then, the reaction was stopped and the modified silica was cooled to room temperature, transferred to a vacuum glass filter, and washed with chloroform and methanol in turn.

### 2.3. Characterization of poly(1-allylimidazole)-grafted silica

Diffuse reflectance infrared Fourier transform (DRIFT) spectra of the samples in the range of 4000–400 $cm^{-1}$ were obtained on a Thermo Nicolet 5700 FTIR spectrophotometer (Madison, WI, USA). Elemental analyses were performed on a Elementar Vario EL (Hanau, Germany), to determine contents of carbon, hydrogen, and nitrogen in poly(1-allylimidazole)-grafted silica. The average concentration of imidazolium groups grafted onto the surface of silica can be calculated through the nitrogen content of elemental analyses. Thermogravimetric curves were obtained on a PET series Thermal Analyzer from Perkin-Elmer Instruments with a heating rate of 10 °C $min^{-1}$ under nitrogen. X-ray photoelectron spectroscopy (XPS) was used to evaluate the nitrogen and carbon. The spectrum were recorded on an Escalab 210 Axis Ultra photoelectron spectrometer (VG Scientific, UK) using an Mg Kα excitation souse.

### 2.4. Column packing

Two columns (200 mm × 4.6 mm I.D.) were made from stainless-steel tube and were downward packed using a constant packing pressure of 40 MPa. The column of poly(1-allylimidazole)-grafted silica was packed using 10% (w/v) stationary phase slurries in $CCl_4$, with hexane as the propulsive solvent. ODS column was packed using 10% (w/v) C18 silica gel slurries in ethyl bromide, with methanol as the propulsive solvent.

### 2.5. Chromatographic conditions

Mobile phases were filtered through a 0.45 μm nylon membrane filter and were degassed ultrasonically before use. All tests were performed using methanol–water as the mobile phase with UV detection at 254 nm in the reversed-phase chromatography. The content of methanol was in the range of 30–90% (v/v) in methanol–water mobile phase. All tests were performed using sodium chloride solution as the mobile phase with UV detection at 214 nm in the anion-exchange chromatography. The concentrations of sodium chloride solution varied from 50 to 250 mmol $l^{-1}$ in the eluent. pH of a 100 mmol $l^{-1}$ sodium chloride eluent was modulated from 3.5 to 7.5 with hydrochloric acid and sodium hydroxide using a calibrated Sartorius PB-10 pH meter (Goettingen, Germany). All the test mixtures were separated at room temperature, at a flow rate of 1 ml $min^{-1}$. The column dead time was obtained from the mobile phase signal in the UV detection.

## 3. Results and discussion

### 3.1. Synthesis of poly(1-allylimidazole)-grafted silica stationary phase

The preparation process of poly(1-allylimidazole)-grafted silica stationary phase (Sil-$AIm_n$) consists of a two-step reaction. The synthesis route of preparing Sil-$AIm_n$ is schematically described in Fig. 1, i.e., the free radical vinyl polymerization of 1-allylimidazole started from the mercaptopropyl group, and the silanization of unmodified silica with $AIm_n$ to form Sil-$AIm_n$, the target stationary phase.

### 3.2. Characterizations of stationary phase

#### 3.2.1. Infrared analysis

The FT-IR spectra of bare silica and poly(1-allylimidazole)-grafted silica are shown in Fig. 2. In all spectra, bands around 1110 $cm^{-1}$ which are assigned the stretching vibrations of the siloxane (Si–O) groups of the silica backbone are observed. 1632 $cm^{-1}$ band in Fig. 2(a) is attributed to the bending vibrations of the water molecules, adsorbed on the surface of bare silica by hydrogen bonding with silanol, and its intensity decreases in the spectrum of the grafted silica Fig. 2(b). Broad bands around 3448 $cm^{-1}$, the stretching vibrations of O–H bonds of the geminal and vicinal silanols, and water molecules adsorbed, decrease greatly in the spectrum of the grafted silica Fig. 2(b), which is the result of the forming of hydrogen bonding between silanol and the nitrogen in imidazole, and is also due to the decrease of silanol after being bonded. Another important feature is that, after modification, the intensity of the shoulder at 973 $cm^{-1}$, which is attributed to free silanol on the silica surface,