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Fabrication of an ionic liquid-based macroporous polymer monolithic column via atom transfer radical polymerization for the separation of small molecules

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ABSTRACT

A polymer monolithic column was prepared in a stainless steel column (50 × 4.6 mm i.d.) via atom transfer radical polymerization technique using triallyl isocyanurate and ionic liquid (1-allyl-3-methylimidazolium chloride) as co-monomers, ethylene dimethacrylate as cross linking agent, polyethylene glycol 200, 1,4-butanediol, and N, N- dimethylformamide as porogen system, CCl_4 as initiator, and $FeCl_2$ as catalyst. The optimized polymer columns were characterized by scanning electron microscope, nitrogen adsorption-desorption instrument, mercury intrusion porosimetry, infrared spectrometer, and thermogravimetric analysis technique. Respectively, all of these factors above could illustrate that the optimized columns had relative uniform macroporous structure and high thermal stability. A series of basic and acidic small molecules, isomers, and homologues were used to evaluate the performance of these monoliths and enhanced column efficiency was obtained.

1. Introduction

Over the past two decades, compared to the silica-based columns, there has been a strong interest in polymer monolithic columns[1,2] due to their advantages of time-saving preparation, enhanced permeability, reduced mass transfer resistance, and better pH tolerance [3–5]. Polymer monoliths have been successfully used for the separation of large biomolecules, such as proteins, oligonucleotides, and peptides [6–11].

In contrast, it is unfavorable for the chromatographic performance of polymer monolithic columns for the separation of small molecules as a result of low mesopore volume and inhomogeneity of the structure [12,13]. Several methods have been proposed to solve this problem, such as applying a single cross-linker [14], using various living-control radical polymerization [15–17], adding nanostructures [18,19], and introducing click chemistry reaction [9,20,21], which mainly focused on the improvement of the performance of capillary monoliths. To some extent, all these methods led to varying degrees of improvement of column efficiency. The highest efficiency of obtained monoliths exceeded 100,000 plates/m for capillary liquid chromatography (CLC). However, there are still many difficulties to overcome. Many of click chemistry reactions are easily to tolerate conditions that will be damaging for processes currently used for the preparation of monoliths with some reactive chemistries [5]. As for nanostructure substance, because of its poor dispersion in the polymer matrix, it is suitable to be attached on the pore surface of the well-defined monolith in case of being buried and inaccessible, which is more time-consuming and complex operation compared to one-pot polymerization [5,22]. In addition, in terms of capillary monolith column, it also brings difficulties to the process of analysis for the too low capacity and poor reproducibility. For routine monolithic column (50 × 4.6 mm i.d.), there is not any prominent progress in recent time. The attempts mainly concentrated on the variation of monomers, cross-linkers, and porogen solvent mixture, the adjustment of the polymerization conditions (including temperature, time, and polymerization technique) [23], but these methods have not improved the applicability of the polymer monoliths to the desired degree. In order to develop a uniform and stable system with simple optimization of polymerization conditions, especially focus on improving the homogeneity of the structure and enhancing column efficiency, the green solvent ionic liquid (IL) as co monomer was proposed in the present work.

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Ionic liquids (ILs) are molten salts containing relatively asymmetric organic cations and inorganic or organic anions, whose physical and chemical properties are convenient to be changed by controlling the cations and anions [24]. Furthermore, contributed to the properties of high thermal stability, low volatility, good adjustability, high electrolytic conductivity, and miscibility [25], IL has been widely applied in chromatography [26–28] and aroused considerable scientific interest in the fabrication of monolithic column. IL-based monolithic columns [29,30] have been successfully utilized to separate a variety of analytes especially complex biological samples by CEC., These success mainly benefited from the property of good electrolytic conductivity of IL to generate a stable reversed EOF in a wide pH range for the CEC mode. However, it didn't help improve the morphology and enhance the separation of small molecules in HPLC. Our group [31,32] employed ILs as function monomer for the preparation of polymer monolithic columns via *in situ* free radical polymerization. The obtained monolithic columns, as the station phase for reversed-phase liquid chromatography (RPLC), exhibited good separation performance for small molecules. It demonstrated that the addition of IL made the structure more uniform with larger surface area. Furthermore, the conditions of polymerization could be optimized to get better mechanical and thermal stability, greater performance improvement and higher repeatability.

The combination of the unique advantages of porous polymer monoliths, and the specific features of IL might present a promising improvement in the performance of monoliths for HPLC. Herein, IL (1-allyl-3-methylimidazolium chloride, $AMIM^+Cl^-$) and a high concentration of crosslinking monomer triallyl isocyanurate (TAIC) build up a co-monomers system, with ethylene dimethacrylate (EDMA) as single cross-linker. The atom transfer radical polymerization (ATRP) technique was introduced to prepare the monolith. After the optimization of porogen system and polymerization conditions, a series process of characterization were carried out respectively including scanning electron microscopy, infrared spectrometer, nitrogen adsorption/desorption measurement, mercury intrusion porosimetry and thermal gravimetric analysis. Besides, the separation ability of these columns was estimated comprehensively by separating a series of basic and acidic small molecules, isomers and homologues by HPLC.

2. Experimental

2.1. Materials and methods

Triallyl isocyanurate (TAIC) was purchased from Shanghai Huangguan Chemical company (Shanghai, China). Ethylene dimethacrylate (EDMA) was obtained from Maya-Reagent (Zhejiang, China). Polyethylene glycol 200 (PEG-200), 1,4-butanediol, N, N-dimethylformamide (DMF), CCl_4, and $FeCl_2$ were supplied by Tianjin Guangfu Fine Institute of Chemistry (Tianjin, China). Meanwhile, 2,2-Azobisisobutyronitrile(AIBN), HPLC-grade methanol (MeOH), acetonitrile (ACN), and KBr were produced by Kermel Chemical Reagent Factory (Tianjin, China). The analytes were provided by the National Institute for the Control of Pharmaceutical and Biological Products of China (Beijing, China). Besides, ultrapure water was used for all experiments and all medias were filtered through a 0.45 μm membrane before use.

2.2. Instruments

All chromatographic experiments were performed on an 1100 system from Agilent Technologies (USA). Scanning electron microscopy (SEM) images of the monoliths were carried out on Hitachi S-3400 SEM instrument (Hitachi High Technologies, Japan). The Fourier transform infrared spectroscopy (FT–IR) spectra were recorded on an FTIR–8400S IR apparatus in the region of 400–4000 cm^{-1} (Shimadzu, Japan). The pore type was determined by the nitrogen adsorption/desorption measurment on a TriStar II 3020 instrument (Micromeritics, USA) while pore size measurement was performed on an AutoPore IV 9500 instrument (Micromeritics, USA). Thermal gravimetric analysis (TGA) was carried out on a Setsys 16/18 instrument (Setaram, Caluire, France). Molecular weight data for ILs was obtained by MSD Trap XCT instrument with electrospray ionization (ESI) source which was bought from Agilent Technologies (USA).

2.3. Preparation and optimization of the poly(IL-co-TAIC-co-EDMA) monoliths

2.3.1. Synthesis of the IL ($AMIM^+Cl^-$)

Allyl chloride (9.95 g, 0.13 mol) was added dropwise to 1–methylimidazole (8.21 g, 0.1 mol). The mixture was heated at 55 °C under stirring for 11 h. When phase separation occurred, the brown viscous liquid was washed with ether (60 mL for three times). Then the product, IL (1-allyl-3-methylimidazolium chloride, $AMIM^+Cl^-$), was filtered and dried in a vacuum oven until constant weigh (9.39 g, yield: 59.16%). Thus, the obtained IL was assayed by mass spectrometer. The reaction equation was shown in Scheme1(a).

2.3.2. Preparation of the poly(IL-co-TAIC-co-EDMA) monoliths

The processes of preparation columns were as follows: IL and TAIC were used as co-monomers, EDMA as cross linking agent, 1,4-butanediol, PEG200, and DMF as tri-porogens, CCl_4 as initiator, $FeCl_2$ as catalyst. All the reagents were put into a tube followed mixing and degassed by sonication for 15 min, and then the mixed solution was poured into a stainless-steel column (50 × 4.6 mm i.d.). The column was sealed and allowed to react in a water bath for 24 h. The resulting monolithic column was washed online with methanol by an HPLC pump to remove the porogens. The polymerization scheme was illustrated in Scheme 1(b).

2.3.3. Optimization of the poly(IL-co-TAIC-co-EDMA) monoliths

In order to find the proper porogens then to improve the morphology and structure of the monolithic column, several candidates, such as propanol/1,4-butanediol, DMF/1,4-butanediol, 1,4-butanediol/PEG200, DMF/PEG200 and DMF/PEG200/1,4-butanediol were proposed according to their solubility and polarity, respectively. The pre-experiment showed that the ternary porogenic system DMF/PEG200/1,4-butanediol had prior effects on the property of the monolithic columns to that of single or double porogens. Table 1 listed the representative conditions and some of the obtained results, containing the effect of polymerization temperature on the property of monolithic column.

Furthermore, compared to the IL-based monolith prepared via ATRP, columns H and I were prepared via *in situ* or without IL addition, respectively, with the details listed in Table 2.

2.4. HPLC procedures

An Agilent 1100 system consisted of a quaternary pump with an online vacuum degasser, an autosampler with variable injection capacity from 0.1 to 100 μL, and UV detector. All sample solutions injected in the chromatographic system were filtered through a millipore membrane (0.45 μm) to remove particles and large aggregates. The chromatographic conditions were as follows: mobile phase: ACN/water; UV wave length: 254 nm; temperature: 25 °C; concentration of sample: 0.01 mol L^{-1}; sample injection volume: 5.0 μL.