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Ionic liquids monolithic columns for protein separation in capillary electrochromatography

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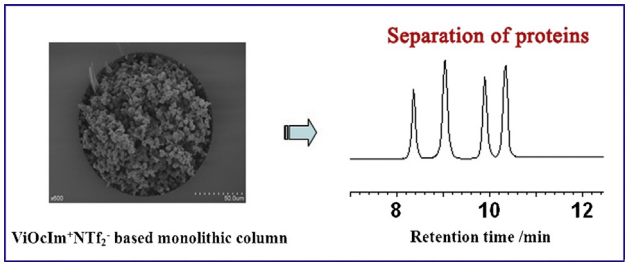
HIGHLIGHTS

- ILs-monolithic columns with different anions were prepared by two approaches.
- The performances of the resulting columns could be designed by tuning anions.
- $ViOcIm^+NTf_2^-$ based column exhibited the highest column efficiencies for proteins.
- ILs gave the columns potential to separate small molecules and macro biomolecules.

GRAPHICAL ABSTRACT



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ABSTRACT

A series of ionic liquids (ILs) monolithic capillary columns based on 1-vinyl-3-octylimidazolium ($ViOcIm^+$) were prepared by two approaches ("one-pot" approach and "anion-exchange" approach). The effects of different anions (bromide, Br^-; tetrafluoroborate, BF_4^-; hexafluorophosphate, PF_6^-; and bis-trifluoromethanesulfonylimide, NTf_2^-) on chromatography performance of all the resulting columns were investigated systematically under capillary electrochromatography (CEC) mode. The results indicated that all these columns could generate a stable reversed electroosmotic flow (EOF) over a wide pH range from 2.0 to 12.0. For the columns prepared by "one-pot" approach, the EOF decreased in the order of $ViOcIm^+Br^- > ViOcIm^+BF_4^- > ViOcIm^+PF_6^- > ViOcIm^+NTf_2^-$ under the same CEC conditions; the $ViOcIm^+Br^-$ based column exhibited highest column efficiencies for the test small molecules; the $ViOcIm^+NTf_2^-$ based column possessed the strongest retention for aromatic hydrocarbons; and baseline separation of four standard proteins was achieved on $ViOcIm^+NTf_2^-$ based column corresponding to the highest column efficiency of 479 000 N m^{-1} for cytochrome c (Cyt c). These results indicated that the property of ILs based columns could be tuned successfully by changing anions, which gave these columns potential to separate both small molecules and macro biomolecules.

Abbreviations: ILs, ionic liquids; RTILs, room temperature ionic liquids; PIL, polymeric ionic liquid; $ViOcIm^+Br^-$, 1-vinyl-3-octylimidazolium bromide; $ViOcIm^+BF_4^-$, 1-vinyl-3-octylimidazolium tetrafluoroborate; $ViOcIm^+PF_6^-$, 1-vinyl-3-octylimidazolium hexafluorophosphate; $ViOcIm^+NTf_2^-$, 1-vinyl-3-octylimidazolium bis-trifluoromethanesulfonylimide; CE, capillary electrophoresis; CEC, capillary electrochromatography; EOF, electroosmotic flow; GC, gas chromatography; HPLC, high performance liquid chromatography; SEM, scanning electron microscopy.

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1. Introduction

Capillary electrochromatography (CEC) which combines superiorities of the capillary electrophoresis (CE) and high performance liquid chromatography (HPLC) has been widely applied in the analytical separation sciences. As the core of CEC, capillary columns have been stimulated to develop by the growing demands for high-throughput analysis and microscale chromatographic separations. Monolithic columns, a novel separation media, have attracted great interests due to the merits of great permeability, fast mass transfer rate, high loading capacity, and ease of preparation [1]. To date, there have been numerous reports on organic polymer-based, inorganic silica-based and organic silica hybrid monolithic columns [2–5]. Organic monolithic columns prepared by in situ polymerization technology exhibits several significant advantages over the silica-based monolith, such as simpler and faster preparation, greater choices of surface functionalities, wider pH stability, as well as better biocompatibility [6]. And their applications in separation have been extended from small molecule to macro biomolecules, especially protein mixture. Nevertheless, they are suffered from apparent disadvantages, such as limited interactions between analytes and the monolithic matrix, high organic solvent content in running buffer and low separation efficiency for protein separation and so on [7–9]. Therefore, new alternatives are highly desirable to overcome these issues.

Ionic liquids (ILs) which compose of large asymmetric organic cations and inorganic or organic anions are salts with relatively low melting points compared to most traditional inorganic salts; some of them are even liquid around ambient temperature, called room temperature ionic liquids (RTILs). ILs have recently attracted great interest due to their unique properties such as chemical and thermal stability, non-flammability, non-detectable vapor pressures and chemical tunabilities [10]. Especially, ILs based on imidazolium cations have been proven to be versatile on account of their favorable characteristics, e.g., moisture- and air-stability, easy recycling and being a good solvent for a wider variety of organic and inorganic chemical compounds [11,12]. In addition, imidazolium are "designable" because structural modifications in both the cation (the 1- and 3-positions of the imidazolium ring) and anion permit the tuning of properties [13].

Over the years, ILs have been applied in the different fields of chemistry, such as organic chemistry, inorganic chemistry, electrochemistry, analytical chemistry, and so on [14–19]. The growing interest of ILs in chromatography field can be observed from the dramatic increase in the number of publications appeared during the last decade [20–26]. Especially in CEC field, ILs have usually been used as dynamic capillary coatings, physically adsorbed coatings and covalently linked coatings with the purpose of removing the deleterious effect of free silanols on the retention of basic analytes [27–30]. Recently, polymeric ionic liquid (PIL) has been used as the physically adsorbed coatings in CE for protein separation [29]. Although it is effective to obtain a better stability and a wider pH application range comparing to the traditional coatings (e.g. hydroxyethylcellulose, poly (ethylene oxide) and poly (ethylene glycol) methyl ether methacrylate) [31–33], the repeating wall modification steps between subsequent runs is time and labor consuming. In 2011, a new application of ILs in CEC has been reported by our group. Wang et al. has prepared a novel IL-monolithic capillary column via the thermal free radical copolymerization with IL (1-vinyl-3-octylimidazolium chloride, $ViOcIm^+Cl^-$), lauryl methacrylate (LMA) and ethylene dimethacrylate (EDMA) in 1,4-butanediol/methanol porogen system [34]. As "one-pot" approach, the preparation process dispensing with any additional modification steps is time and labor saving. The resulting column which could generate a stable reversed EOF in a wide pH range (2.0–12.0) not only effectively eliminates the wall adsorption of the basic analytes but also exhibits great separation efficiency and reproducibility. Considering these superior qualities, we have reasons to expect that ILs-monolithic capillary columns will continue its contribution to protein separation.

In this study, a twofold study is reported: (1) 1-vinyl-3-octylimidazolium ($ViOcIm^+$) based ILs-monolithic capillary columns with different counter ions (bromide, Br^-; tetrafluoroborate, BF_4^-; hexafluorophosphate, PF_6^-; and bis-trifluoromethanesulfonylimide, NTf_2^-) were prepared as Wang et al. [34]. (2) Firstly, $ViOcIm^+Br^-$ based monolithic capillary columns were prepared as above. Subsequently, anion-exchange was carried out by pumping salt solutions of the anion of interest through the columns for some time. Then all the columns obtained were evaluated chromatographically and applied in protein separation to investigate the impact of anions on separation performance in CEC.

2. Experimental

2.1. Instrumentation

CEC experiments were performed on a P/ACE MDQ CE system (Beckman-Coulter, USA) equipped with a UV detector. Data acquisition and processing were controlled by Beckman Chem Station software. Scanning electron microscopy (SEM) micrographs of the monoliths were obtained on a SU1510 SEM (Hitachi, Japan). The elemental (C, H, O, N) contents of the prepared monoliths were determined on Vario MACRO cube (ELEMENTAR, Germany) by using TCD detector.

2.2. Chemicals and materials

1-Vinylimidazole, LMA, γ-methacryloxypropyltrimethoxysilane (γ-MAPS) and ethylene dimethacrylate (EDMA) were obtained from Sigma (St. Louis, MO, USA). 1-Bromooctane was purchased from TCI (Tokyo, Japan). The free radical initiator 2,2-azobisisobutyronitrile (AIBN, 99%) was obtained from Tianjin Chemical Reagent Factory (Tianjin, China) and recrystallized in ethanol before use. Sodium tetrafluoroborate ($NaBF_4$), potassium hexafluorophosphate (KPF_6) and lithium bis-trifluoromethanesulfonylimide ($LiNTf_2$) were purchased from Fluka (Fluka, Buchs, Switzerland). A fused-silica capillary (100 μm i.d., 375 μm o.d.) was purchased from the Yongnian Optic Fiber Plant (Hebei, China). Doubly deionized water (DDW, 18 MΩ cm^{-1}) produced using a Milli-Q system (Millipore Corporation, USA) was used throughout the experiments. Chromatographic grade of acetonitrile (ACN) were purchased from Tianjin Chemical Plant (Tianjin, China), and the other chemicals were at least of analytical grade.

2.3. Synthesis of ILs

$ViOcIm^+Br^-$ was synthesized according to the protocol reported by Hsieh et al. [35]. Briefly, 1-bromooctane (9.270 g, 0.048 mol) was added dropwise to 1-vinylimidazole (3.760 g, 0.040 mol). The mixture was heated to 70 °C under stirring for 50 h. Phase separation occurred and the viscous brown liquid obtained was washed with ethyl acetate. Then the product was filtered and dried in a vacuum oven until constant weight.

Different counter ion ILs were prepared by modifying the procedures described in the literature [36]. Simple anion-exchange reactions were conducted to replace the bromide ions. 2.880 g of $ViOcIm^+Br^-$ was dissolved in 10 mL of distilled water, and 1.096 g of $NaBF_4$ in 10 mL water was slowly added. After stirring for 12 h at room temperature, the resulting viscous brown liquid was washed thoroughly with distilled water and dried in a vacuum oven until