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Capillary Electrochromatography of Molecularly Imprinted Monolithic Column Using *p*-Hydroxybenzoic Acid as Templates

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Abstract: Molecularly imprinted polymers using *p*-hydroxybenzoic acid as templates was synthesized by an *in situ* polymerization reaction and rendered capillary monolithic column was used in capillary electrochromatographic mode. Good molecular recognition was achieved for *p*-hydroxybenzoic acid and good resolution of isomers can be realized.

Keywords: Molecularly imprinted polymer, capillary electrochromatography, monolith, *p*-hydroxybenzoic acid.

The combination of robust affinity stationary phases and miniaturized separation formats constitutes an important part of the development of analytical systems with enhanced throughput, sensitivity and robustness. The affinity in these systems can be provided by molecularly imprinted polymers (MIPs) which usually consist of highly cross-linked network polymers containing templates binding sites for the corresponding analytes. Capillary electrochromatography (CEC) has recently attracted interest as a new liquid phase analytical separation technique due to its combination of the high peak efficiency of CZE with the high selectivity of HPLC[1]. And MIP-CEC systems have been developed through different approaches to various drugs[2].

p-Hydroxybenzoic acid possesses antiviral, antibacterial, anti-inflammatory and antioxidant properties in phytopharmaceuticals[3] and qualification and quantification of *p*-hydroxybenzoic acid is important. In this paper, *p*-hydroxybenzoic acid imprinted polymer was synthesized by an *in situ* polymerization reaction and monolithic MIP capillary column was formed, the format of which shows high chromatographic performance. Separation of isomers of hydroxybenzoic acid was achieved on this monolithic column in CEC mode. *Rs* of two isomers reached to 5.0.

Experimental

Reagents and chemicals

3-(Trimethoxysilyl) propyl methacrylate (γ-MPS) was from Acros (Geel, Belgium).

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Methacrylic acid (MAA) was from Beijing Donghuan Chemical Reagent (Beijing, China). Ethylene glycol dimethacrylate (EDMA) was from Suzhou Anli Chemical & Engineering Co. Lt. (Suzhou, China). 2, 2'-Azobis (2-isobutyronitrile) (AIBN) was supplied by Special Chemical Reagent Factory of Nankai University (Tianjin, China). p-Hydroxybenzoic acid (4-HBA) was from Tianjin Tiantai Chemical Reagent (Tianjin, China). Salicylic acid (SA) was from Tianjin Medicine Reagent (Tianjin, China). The Tianjin Chemical Reagent (Tianjin, China) supplied HPLC-grade acetonitrile (ACN). Other analytical reagents were from Tianjin Chemical Reagent Co. Lt. (Tianjin, China). Fused-silica capillaries with 100 μm ID and 375 μm OD was purchased from Yongnian Optic Fiber Plant (Hebei, China).

Preparation of MIP capillary columns

A fused-silica capillary was flushed with 1 mol/L NaOH followed by water for at least 30 min each. Then the capillary was filled with a solution of 4 μL of γ-MPS in 1 mL of 6 mmol/L acetic acid, and the solution was kept in the capillary for 1.5 h. The capillary was then flushed with water and dried with a flow of nitrogen. Pre-polymerization mixture was prepared by mixing MAA（41 μL）, EDMA（362 μL）, toluene (622 μL), isooctane (156 μL) , 4-HBA (17.10 mg) and AIBN（3.6 mg）. The pre-polymerization mixture was sonicated for 10 min and introduced to the capillary. The ends of the capillary were sealed with soft plastic rubber. The capillary was submerged in a 60 ℃ water bath for 3 h. After polymerization, the capillary was flushed with acetonitrile and electrolyte, respectively, using a handhold syringe to remove any unreacted reagents. A detection window was created at the end of the continuous polymer bed by burning out 2-3 mm segment of the polyimide outer coating. A blank capillary column without imprint molecule was prepared in the same way.

Capillary electrochromatography

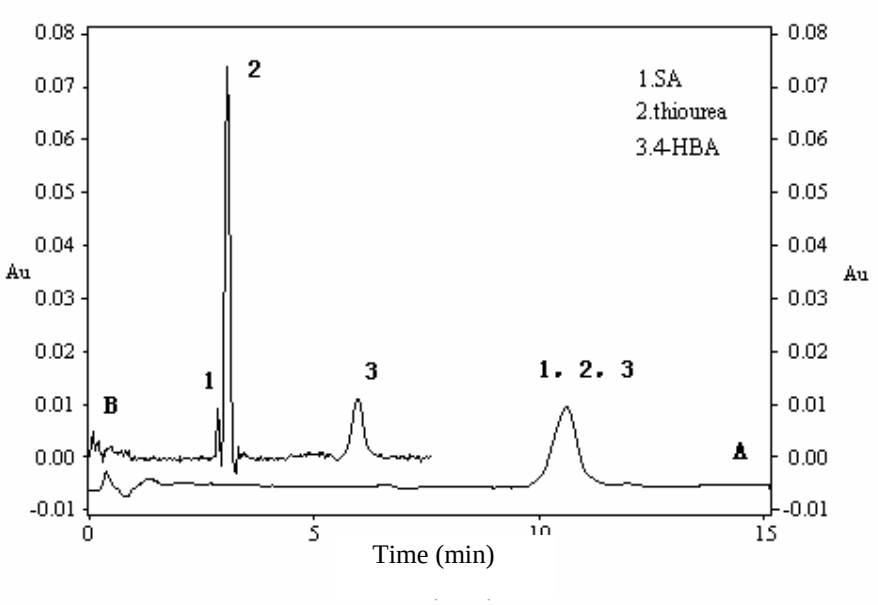
Electrochromatographic experiments were carried out on a Beckman P/ACE MDQ system (Beckman, Fullerton, CA, USA) equipped with a P/ACE system MDQ UV detector. An IBM personal computer with Beckman P/ACE system MDQ capillary electrophoresis software was used. The total length of the capillary was 31.2 cm and effective length (MIP-based stationary phase) was 20 cm. The column temperature was kept at 25 ℃. A pressure of 20 psi was applied to both vials during the separation. The electrolyte used was acetonitrile/50 mmol/L acetate (pH 5.0) (80/20,v/v). All the electrolyte was made using double distilled water and filtrated with 0.2 μm membrane. Sample was injected electrokinetically at 1 kV for 3 sec. and separation was performed at 15 kV.

The resolution was calculated using *R*s, according $Rs=(t_{R(2)}-t_{R(1)})/0.5(W_2+W_1)$, where t_R is the retention time and W is the width at the baseline between tangents drawn to inflection points for the peak.

Results and Discussion

The separation of 4-HBA, SA and thiourea on MIP monolithic column and blank column are shown in **Figure 1**. 4-HBA, SA and thiourea can not be separated on the blank column (**Figure 1-A**) with retention time 10.428 min, 11.036 min and 11.735 min, respectively. On MIP monolith, retention of 4- HBA varied dramatically, *i. e*., retention time of 4-HBA, SA and thiourea is 6.074 min, 2.918 min and 3.130 min, respectively, and the base-line separation of 4-HBA, SA and thiourea was achieved (**Figure 1-B**). It needs to be noted that elution order of 4-HBA on MIP column and blank column reversed. Neutral thiourea is commonly used EOF marker in CEC. 4-HBA was eluted after thiourea on the MIP column while before thiourea on the blank column. This can only be explained by strong imprinting effect of 4-HBA with MIP polymer and the blank column did not possess recognition sites complementary to the spatial structure of 4-HBA since it was synthesized without templates. Theory plates of 4-HBA was 13000 plates/m and *Rs* =5.0. It means that MIP-CEC format shows good column performance, resolution and less separation time than previous report[4,5]. Additionally, from difference in elution time of thiourea between MIP column and blank column, we can find variation of column permeability due to the introduction of imprinted molecule, i.e., 4-HBA, which is in good agreement with published paper6.

Figure 1 The chromatogram of 4-HBA, SA and thiourea on MIP blank column (A) and monolithic column (B).



In summary, isomers of hydroxybenzoic acid was rapidly separated on a MIP-imprinted monolithic capillary in CEC mode. The procedure of fabrication for capillary column is simple and low consumption of chemicals meets the demands of green chemistry.

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