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Preparation and application of covalently bonded polysaccharide-modified stationary phase for *per* aqueous liquid chromatography

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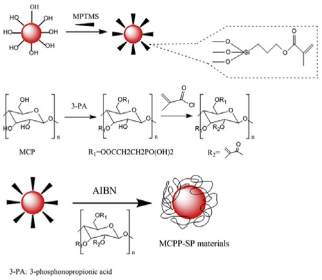
HIGHLIGHTS

- A new bonded plant polysaccharide-modified stationary phase was papered.
- *Per* aqueous liquid chromatography (PALC) that was a green LC analytical method was studied.
- The new stationary phase exhibited stronger retention efficiency for various polar compounds in the PALC mode.

GRAPHICAL ABSTRACT



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ABSTRACT

The mixed phosphorylated/methacryloyl polysaccharide derivative was prepared and immobilized onto porous silica surface through the radical polymerization. The successful immobilization of polysaccharide on the silica support was confirmed by FT-IR spectra, elemental analysis and transmission electron microscopy (TEM), and so on. The new stationary phase (MCPP-SP) showed both hydrophilic interaction liquid chromatography (HILIC) and *per* aqueous liquid chromatography (PALC) characteristics. The chromatographic behaviors were evaluated by investigating the effects of water content, column temperature, mobile phase pH and salt concentration, and a typical PALC retention feature of MCPP-SP based column was observed at high percentage of water content. Compared with C18 column, using MCPP-SP column, separation of polar compounds including synthetic pigments and sulfa compounds in the PALC mode was successfully accomplished. The results demonstrated that MCPP-SP column exhibited stronger retention efficiency for various polar compounds. PALC as a green chromatography analytical method was suitable for the replacement of HILIC.

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1. Introduction

As a branch of high performance liquid chromatography (HPLC), hydrophilic interaction liquid chromatography (HILIC) has found many useful applications for separation of highly polar compounds that show no or little retention in reversed-phase liquid

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chromatography (RPLC) [1–5]. However, a high percentage of acetonitrile (ACN) (70–95%) is often used in the HILIC mode [6–8]. ACN is ranked as hazardous solvent and has negative influence on the environment. To solve the problem, the first option can be to use ethanol instead of acetonitrile. Ethanol, which is produced in large amounts and is biodegradable, has about the same elution strength as acetonitrile, costs much less and appears an attractive candidate [9]. A second option is to take advantage of the hydrophobic character of siloxane groups at the surface of silica by using water-rich mobile phases in HPLC analysis [10]. This mechanism is called *per* aqueous liquid chromatography (PALC) by Sandra et al. [10].

PALC is a green LC analytical method [11]. In the PALC mode, mobile phases contain a high percentage of water (70%–100%), and stationary phase is reversed to non-polar to separate polar compounds [12]. It can reduce the use of harmful solvents to help the realization of green liquid chromatography, protect the environment and greatly decrease the cost of determination. Gritti et al. [9] found that PALC could be a suitable mode of chromatography as a replacement of HILIC processes for the analysis and separation of polar compounds, highly consuming in acetonitrile. Li et al. [11] prepared a new carbon nanoparticles-silica column, which could provide the similar retention for polar compounds in the HILIC and PALC modes. The new column showed good separation selectivity for polar compounds and hydrophilic compounds. Pereira et al. [10] also illustrated the features of PALC with the analysis of catecholamines, nucleobases, acids, and amino acids.

In this work, we prepared a new bonded polysaccharide-modified stationary phase, which was used in the PALC mode. Plant polysaccharide isolated from *Momordica charantia* L. (MCP) with good stability and solubility in water has been demonstrated [13,14]. Cheng reported that MCP was composed of glucose, galactose, arabinose, rhamnose, and mannose with molar ratio of 24.84:27.94:16.47:24.03:6.72 [15]. The main glycosidic bond configuration was β-configuration. Furthermore, we synthesized phosphorylated derivative of MCP (MCPP), and phosphorylated modification could improve the water solubility of MCP. Then, MCPP was effectively immobilized onto the surface of porous silica particles by the radical co-polymerization reaction, which was stable enough while contacting with water-rich mobile phase for a long time. Due to the existence of hydroxyl and phosphate groups on MCPP, the stationary phase showed both PALC and HILIC characteristics and a weaker ion exchange interaction. The chemical structure of the stationary phase was analyzed by elemental analysis, FI-IR spectra, TEM and scanning electron microscopy (SEM). The water content, column temperature, pH and ionic strength of mobile phase were fundamental parameters that need to be investigated in the retention mechanism of polar compounds under the PALC mode. In the PALC mode, highly aqueous eluents with the range of 90–100% were mainly studied. This not only helped to reduce the use of hazardous solvents, but also made green LC possibilities.

2. Experimental

2.1. Materials and reagents

The crude MCP from *Momordica charantia* L. was obtained from Shaanxi Lixin Biotechnology Co. (China). Spherical silica (5 μm particle size, 10 nm pore size, 380 m^2/g surface area) was purchased from Fuji Silysia Chemical (Aichi, Japan). 3-(Methacryloyloxy)-propyltrimethoxysilane (MPTMS), methacryloyl chloride and 3-phosphonopropionic acid were obtained from Alfa Aesar (Shanghai, China). α,α′-azodiisobutyronitrile (AIBN), 4-dimethylamino pyridine (DMAP) and *N*,*N*-dimethylformamide (DMF) were from Aladdin Chemistry Co., Ltd. (Beijing, China). Papain was from Beijing Huamei Biotechnology Co., Ltd (China). SephadexG-100 was from Pharmacia Co. (Sweden).

Sodium benzoate, potassium sorbate, caffeine, vitamin B_3, melamine, thymine, uracil, cytosine, thymidine, cytidine, and uridine were from Aladdin Chemistry Co., Ltd. (Beijing, China). Nine synthetic pigments were purposed from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Ten sulfa compounds were from Dr. Ehrenstorfer (Augsburg, Germany). ACN of HPLC grade was from Merck (Darmstadt, Germany). Deionized water (>18 MΩ cm^{-1}) from a RiOs system was used throughout the experiments (Merck Millipore, American).

2.2. Purification of MCP

The crude MCP was purified as follows: the protein was removed by the Sevage method [16], combined with papain (150 U/mL). After centrifugation, the supernatant was dialyzed with distilled water for 48 h. Through Sephadex G-100 column, the purified MCP was obtained. The molecular weight (Mw) of MCP was 8.5 kDa. The content of MCP was measured by Vitriol–Phenol taking anhydrous glucose as standard control.

2.3. Phosphorylated modification of MCP

3-phosphonopropionic acid (0.5 g) was mixed with 10 mL of anhydrous DMF. Then, 40 mg of DMAP as catalyst was added and stirred for 2 h. 0.5 g of MCP dissolved in 10 mL of anhydrous DMF was added dropwise into the reaction mixture. The mixture was stirred continuously for 24 h at room temperature. The products were dialyzed (molecular weight cutoff 3 kDa) by distilled water for 24 h to remove DMF, DMAP, and so on. MCPP was obtained after lyophilizing.

2.4. Preparation of methacryloyl MCPP derivative

MCPP (0.5 g) was dissolved in anhydrous DMF (35 mL) at room temperature with stirring for 30 min, and 0.5 mL of methacryloyl chloride was added into the mixture. The reaction mixture was stirred under N_2 atmosphere for 10 h at 60 °C. Then, the mixture was precipitated with ethanol. After centrifugation, methacryloyl MCPP derivative was obtained after lyophilizing in vacuum.

2.5. Immobilization of MPTMS on silica surfaces

Spherical silica (2.0 g) was suspended in 30 mL of anhydrous toluene, and 2.0 mL MPTMS was added with a stirring. Then, 0.5 mL of $NH_3 \cdot H_2O$ was added dropwise. The reaction mixture was stirred under N_2 atmosphere for 20 h at room temperature. After centrifugation, the mixture was washed with dichloromethane, acetone and methanol, respectively. Then the products were dried under vacuum at 60 °C overnight. MPTMS-bonded silica was obtained.

2.6. Polymerization

Methacryloyl MCPP (0.5 g) was dissolved in anhydrous DMF (30 mL) at room temperature with stirring for 30 min, and 2.0 g of MPTMS-bonded silica was added into the mixture. Then, the polymerization was performed by the initiating of AIBN (1.0 wt% of monomer) under N_2 atmosphere at 70 °C for 24 h. The final product was filtered, intensively washed with water and methanol respectively, and then dried under vacuum at 60 °C overnight. The new bonded polysaccharide-modified stationary phase (MCPP-SP) was obtained. The routes for the synthesis of the new stationary phase were shown in Fig. 1.

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