

Preparation of Monolithic Column and Its Application in p-CEC

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Abstract: A novel monolithic stationary phase having long alkyl chain ligands was introduced and evaluated in pressurized-capillary electrochromatography of small neutral and charged compounds. The monolithic column was prepared by the *in situ* copolymerization of ethylene dimethacrylate, 1-hexadecene, allyl alcohol and 2-acrylamido-2-methyl-1-propanesulfonic acid in a quaternary porogenic solvent mixture consisting of 1, 4-butanediol, cyclohexanol, dodecanol and water.

Keywords: Monolithic column , p-CEC , aromatic compounds.

Capillary electrochromatography(CEC) is increasingly recognized as a powerful micro-column separation technique due to its hybrid nature, which combines the differential electromigration of electrophoresis to the differential partition of chromatography. In addition, the column efficiency in CEC is further improved by the plug-like flow profile of the mobile phase generated by the electroosmotic flow EOF). In principle, these sound features make CEC well suited for the separation of a wide variety of natural and charged species. Supplementary pressure can be applied to a column in CEC by using HPLC pumps to overcome the dry-out and bubbles formation. This technique is known as pressurized CEC(p-CEC), where a pressure gradient or a solvent-gradient is applied along with the electric field. The coupling of hydraulic pressure in HPLC with high voltage in CEC results in many advantages of p-CEC over CEC. However, CEC(or even p-CEC) becomes widely accepted and found its important applications, the column technology which is the heart of the separation process must be improved. Thus far, major advances have been made in the area of stationary phases, specially for the CEC¹⁻³.

Although these microparticulate stationary phases in capillaries usually necessitate the fabrication of retaining frits at both ends of the column, the frits break easily and are sites of bubble formation, which disrupts the CEC process. To overcome these problems, fritless columns with monolithic stationary phases based on either porous polymers^{1,4-6} or porous silica-based monoliths⁷⁻⁹ have been introduced. *In situ* polymerization of polymer-based monoliths has several advantages over silica-based ones, including the easy preparation and introduction of functional groups in the stationary phase. In the

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result, this type of column is suitable for much large range of chemicals. Monoliths are characterized by a bimodal pore structure¹⁰, comprising large canal-like pores¹¹ for mobile phase flow and a domain of small pores for solute adsorption. Several groups have taken a lot of efforts in this field. However, this technique is still virtually in its infancy. Most applications have been concentrating on small natural compounds. Many questions remain to be solved.

In order to circumvent this impediment, we introduced the columns, containing a novel separation medium, consisting of a rigid macroporous monolith prepared by *in situ* polymerization process. The hexadecane moiety (with a C₁₆ alkyl chain) acts as the retentive ligand, and allyl alcohol makes the monolith to be hydrophilic, the AMPS moiety is to provide the monolith with charge which is necessary to support a strong EOF. For evaluating the monolithic column, we separated several aromatic compounds on p-CEC by different supplying voltages.

Experimental

Chemicals and Apparatus

Ethylene dimethacrylate(EDMA), 1-hexadecene, and 2-acrylamido-2-methyl-1-propane sulfonic acid(AMPS) were purchased from Aldrich(Milwaukee, WI, USA). 2,2-Azobisisobutyronitrile(AIBN), and other reagents were purchased from the Shanghai Medicine Groups(Shanghai, China). Fused-silica capillary(100 μ m i.d.) was purchased from the Hebei Yongnian(Hebei, China).

p-CEC was performed on a Trisep-2010GV CEC system(Unimicro Technologies, Inc., Pleasanton, CA, USA) which comprised a solvent delivery module, a high voltage power supply, a variable wavelength UV detector, and a data acquisition module.

Preparation of Monolithic Columns

Free radical initiator(AIBN, 1wt% with respect to the monomers) was added to a polymerization mixture, consisting of 40% monomers, a mixture of EDMA, 1-hexadecene, allyl alcohol, and AMPS in various ratios; and 60% of a porogenic solvent mixture of 1,4-butanediol, cyclohexanol and 1-dodecanol(various ratios), and water(8 wt% with respect to porogen). AMPS charges of below 2.0 wt% of the monomer mixtures were added as an aqueous solution, such that the total water content remained constant. The homogeneous mixtures were sonicated for 10 min to obtain a clear solution, and then purged with nitrogen for 10 min to remove oxygen and drawn into a 100 μ L syringe. A 50 cm length of modified capillary was attached to the syringe inlet and filled with the polymerization mixture to a total length of 30 cm. The ends of the capillary were plugged with a piece of rubber tubing, and the capillary was submerged in a 60 °C bath for 24 h. After the polymerization completed, the monolithic column was washed with methanol using HPLC pump. A detection window was created at 1-2 mm at the end of the polymer bed using thermal wire stripper.

Result and Discussion

Preparation of Monolithic Columns

Upon free radical polymerization, a porous polymer consisting of a cross-linked network (EDMA as a cross-linker) with a hydrophobic moiety (1-hexadecene, as a C₁₆ alkyl chain) can be prepared. In order to render the hydrophobic polymer to be a useful stationary phase suitable for use in CEC, allyl alcohol as a hydrophilic moiety, a charged monomer must be copolymerized with EDMA to support a relatively strong EOF.

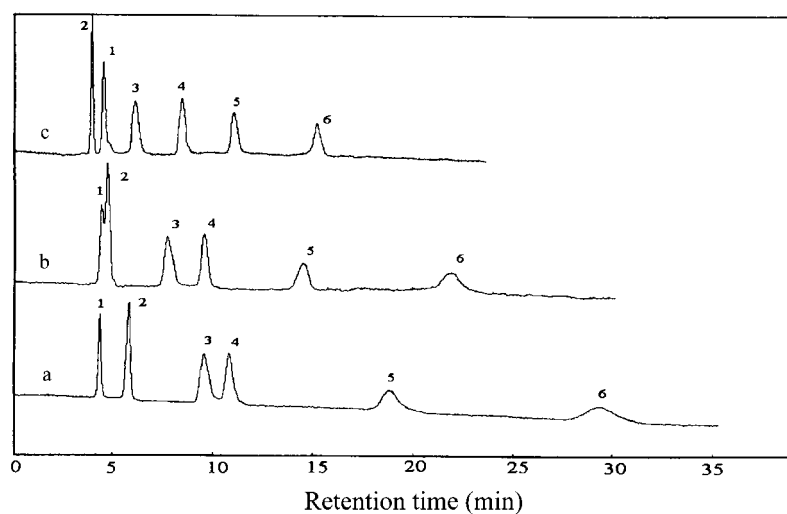
To produce a permanent porosity in the monolithic polymer, the copolymerization is usually performed in the presence of porogens, which produce phase separation of the solid polymer from the liquid porogens. In this study, a porogenic solvent mixture, consisting of 1,4-butanediol, cyclohexanol, 1-dodecanol and water, was found to be able to yield a suitable, rigid porous chromatographic monolith. While 1-dodecanol well dissolved the monomer of 1-hexadecene, a small amount of water was added to dissolve the charged, hydrophilic AMPS.

Chromatographic Properties of the Monoliths

The monolithic columns are chemically very stable. Therefore, the mobile phase parameters such as apparent pH, electrolyte concentration, type and content of organic solvents can be varied broadly without adverse affect on to the stability of the monolithic stationary phase.

Figure 1 indicates that the column efficiency was higher, and the retention time was shorter with the increase of the applied voltage. The total flow of the mobile phase is higher than the pure pressure-driven flow as a result of the superimposed EOF on the pressure-induced flow. The movement of benzoic acid was opposite to the EOF, so with the increase of voltage, the peak 1 and 2 changed their order.

Figure 1 Effect of applied voltage of p-CEC on their chromatographic properties



Conditions: capillary column, 100 μ m(i. d.) \times 20 cm effective length; stationary phase, 1.0 wt% AMPS in polymer mixture; mobile phase, 80/20 v/v mixture of methanol and 10 mmol/L phosphate buffer pH 7; UV detection at 254 nm; Peaks: (1) benzoic acid, (2) thiourea, (3) aniline, (4) toluene, (5) naphthalene, (6) fluorene; Supply voltage: a = 0, b = 10 kV, c = 20 kV.

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