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Highly efficient Rod-shape Poly (methacrylic acid -co- styrene-co-divinylbenzene) High-performance Liquid Chromatographic Column

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Abstract: A highly efficient copolymer (methacrylic acid – co – styrene – divinylbenzene) *in situ* making high-performance liquid chromatographic column was prepared and evaluated. The column efficiency calculated in terms of height equivalent to a theoretical plate was 25 μm and rapid separations of small molecules such as substituted aromatic hydrocarbons and aromatic acids were achieved within five minutes.

Keywords : Highly efficient column, poly (methacrylic acid -co- styrene-co-divinylbenzene), *in situ* column, substituted aromatic hydrocarbon, aromatic acid.

Introduction

The development of a highly efficient *in situ* making copolymer high-performance liquid chromatographic (HPLC) column, the so-called rod shape continuous column is the key problem in separation of enantiomers, proteins, peptides, polymers and small molecules as well because of its excellent permeability and efficiency. Modified poly (glycidyl methacrylate-co-ethylene dimethacrylate)[1,2] and poly (styrene–co–divinylbenzene)[3~6] rod-shape column were prepared by *in situ* polymerization with the confines of the stainless-steel tube.The height equivalent to a theoretical plate (HETP) reported was 250 μm for poly (styrene–co–divinylbenzene) column. Proteins[1-3], substituted aromatic hydro carbons, peptides[4] and polymers[5,6] had been separated.

A new method for making a highly efficient *in situ* HPLC column is presented in this paper. The HEPT was 25 μm and rapid separations of small molecules such as substituted aromatic hydrocarbons and aromatic acids in reversed-phase chromatography were achieved within five minutes.

Experimental

Materials

The styrene (90%, Fluka) and divinylbenzene (70~85%, Fluka) were washed with 10% aqueous sodium hydroxide and water, dried over anhydrous magnesium sulfate, and passed through neutral aluminium oxide column. Methacrylic acid (Beijing Chemical

Plant, Beijing, China) was distilled under vacuum to remove stabilizer. L-mandelic acid was purchased from Sigma and S-naproxen was a gift from Hangzhou Minsheng Pharmaceutical Factory. Azobisisobutyronitrile (AIBN), dodecanol, substituted aromatic hydrocarbons, biphenyl, naphthalene and benzoic acid were all of analytical grade. Acetonitrile was HPLC grade from Shandong Yuwang Chemical Plant and water was twice distilled.

Preparation of Rod-shape Copolymer Column

The rod-shape membrane column was prepared by an *in-situ* polymerization within the confines of the stainless-steel tube of a 50×8 mm I.D. chromatographic column. Styrene (3.0 volume), divinylbenzene (3.0 volume), methacrylic acid (1.0 volume), dodecanol (8.0 volume) and AIBN (1.0 %, w/v, respect to monomers) were dissolved. The stainless-steel tube was filled with the above mixture and then sealed at both ends with rubber nut plugs. The polymerization was allowed to proceed at 60℃ for 24 hr within the stainless-steel tube. The rubber nut plugs were replaced by the column end fittings and the column was attached to the HPLC system. Methanol (100 ml) was pumped through the column at a flow-rate of 1.0 ml/min to remove the dodecanol and other soluble compounds present in the polymer rod after the polymerization was completed.

Chromatography

Chromatography was carried out using a LC-890A system from Beijing Xingda Technology Development Company (Beijing, China) consisting of two LP-05C pumps equipped with a LC-830 UV-VIS detector (Soma Optic LTD, Japan). The samples were injected through a Rheodyne 7125 valve loop injector, and monitored at 254 nm. The system was controlled by a JS-3030 Chromatography Operation Station (Jiangshen Separation Technology Company, Dalian, China).

Result and Discussion

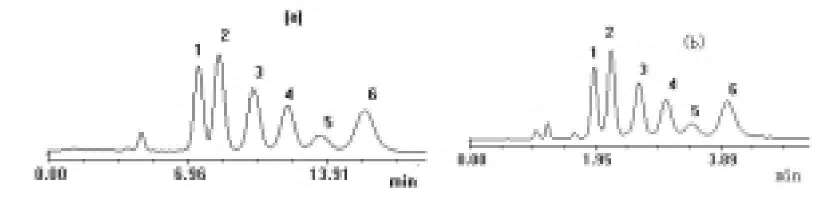
Permeability of the copolymer (methacrylic acid–co–styrene–divinylbenzene) in situ column

Scanning electron micrograph of poly (styrene–co–divinylbenzene) shown in ref.3 revealed that it was a macroporous matrix and clusters of globules were separated by large pores and these large pores form a network. This leads to excellent permeability. Copolymer (methacrylic acid–co–styrene–divinylbenzene) prepared under similar polymerization conditions should have similar pore structrure and this was confirmed by the good permeability. The pressure reached only 1.0 MPa when the flow-rate was 1.0 ml/min with acetonitrile-water (80/20,v/v).

Chromatographic performance of the macroporous column

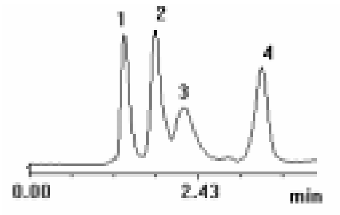
The entire volume of the chromatographic column was occupied by the rod-shape macroporous *in situ* separation medium and there was no path between the packed particles which exists in the conventional packed HPLC column, all of the mobile phase had to flow through the large pores inside the macroporous separation medium. Therefore, the diffusional length for the solutes was very short. Meanwhile, according to the theory[7], mass transfer was accelerated by convection. So high efficiency in terms of HETP of the macroporous column was 25 μm at a linear velocity of 2.0 ml/min. That means that 2,000 plates were achieved for the 50×8 mm i.d. column. **Figure1.** shows the rapid separation of substituted aromatic hydrocarbons. The chromatogram obtained at flow-rate 2.0 ml/min was almost the same as that at 0.5 ml/min. The elution order and capacity factors reflect that the separation mode was reversed-phase and the hydrophobicity of this copolymer (methacrylic acid–co–styrene–divinylbenzene) column was weaker than that of poly (styrene-co-divinylbenzene) column in ref. 4.

Figure 1 Separations of substituted alkylbenzenes at different flow-rates



mobile phase: acetonitrile-water (80/20,v/v). flow-rate : (a) 0.5ml/min, (b) 2.0 ml/min. UV detection : 254nm, analyte：1. benzene, 2. toluene, 3. *iso*-propylbenzene, 4. *iso*-butylbenzene, 5.naphthalene, 6. biphenyl.

Figure 2 Separation of aromatic acids



mobile phase:acetonitrile-water (80/20,v/v).flow-rate : 1.5 ml/min. UV detection: 254 nm. ：1.L-mandelic acid, 2.benzoic acid, 3.S-naproxen, 4.*n*-propylbenzene.

Figure 2. shows the great separation potential of this copolymer (methacrylic acid–co–styrene–divinylbenzene) *in situ* column. Three aromatic acids were separated within 4 minutes with isocratic elution. Separations of other aromatic molecules and proteins are ongoing.

Acknowledgments

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