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Development and characterization of hydrophobic organic monolithic columns for use in capillary electrochromatography

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ABSTRACT

Monolithic poly(octadecyl methacrylate-*co*-ethylene dimethacrylate) capillary columns for use in capillary electrochromatography (CEC) were developed and characterized by porosimetric measurements and scanning electron microscopy (SEM). The stationary phases were prepared using 2-acryloylamido-2-methylpropanesulfonic acid (AMPS) as the ionizable monomer and 2,2′-azobisisobutyronitrile (AIBN) as the initiator of thermal polymerization, which occurred at 60 °C. The porogenic solvents used in this work were amyl alcohol and 1,4-butanediol, in the proportion 65:35 (v/v). The ratio between monomers and porogenic solvents was varied in a range of 60 to 80% (v/v) of porogenic agents. The porosimetry showed that the increase in content of porogenic solvents caused an increase in pore surface area, but a relationship with the separation efficiencies was not observed. SEM agreed with the conclusions made through porosimetry, where decreases in globule size could be noted with increases in porogenic solvents.

1. Introduction

Capillary electrochromatography (CEC) is a separation technique defined as a hybrid of high performance liquid chromatography (HPLC) and capillary electrophoresis (CE). The advantages of these two techniques are combined, corresponding to the high selectivity of HPLC and the high efficiency of CE, and result in faster analyses [1–3].

There are three types of columns that can be used in CEC: particulate, wall-coated open-tubular [4,5] and monolithic.

The monolithic phase can be defined as a continuous separation medium and can be considered a single particle, molded within the capillary and chemically linked to its walls, without the need of sintering frits to retain the stationary phase. This single particle is quite porous, composed of small domains and large channels, called macropores, where the mobile phase flows. In the small domains, there are micro- and mesopores whose surface area is the region that permits differential interactions between the analytes and the stationary phase. According to IUPAC [6], macropores have diameters greater than 50 nm, in micropores the diameters do not exceed 2 nm and the mesopores exhibit diameters in the range of 2 to 50 nm. Monoliths, generally, consist of interconnected microglobules that partially aggregate into larger clusters, creating the monolithic bed [7,8].

The simple preparation process of the monolithic phases, in a single step, enables the incorporation of different ionic species, charged positively or negatively and needed to generate the desirable electroosmotic flow (EOF), plus other species with the ability to control functionality (incorporation of various hydrophobic or hydrophilic molecules or ones that present specific interactions) and the porous properties [9–13].

There are two types of monoliths: silica-based inorganics and polymer-based organics. The preparation of the latter is easier and there are a wide variety of monomers available to synthesize the polymers with different functionalities [4,7,14]. The silica-based monolithic columns are prepared through sol–gel technology [15–18] while the organic monolithic columns can be based on acrylates, methacrylates, acrylamides or styrenes [19–37]. Recently, a new class of monoliths has been introduced: hybrid organic-silica monoliths [38].

The performance of organic monoliths is determined by their bed structure morphology and porosity. These characteristics are influenced by initiation method (thermal initiation or photoinitiation), nature of the porogenic solvents and/or their ratios and chemical properties or amount of a monomer used in preparation of organic monoliths in capillary columns. Porogens and monomers, as well as the initiation method, can influence monolith morphology, pore size distribution and separation performance. The literature reports various studies about the effects of the nature of the porogenic solvents and the effects of monomer to porogen ratio on porosity and monolith morphology. According to some papers, the variation of both the nature and the proportion of the porogenic solvents, generally, is a more effective way to control the pore size than the ratio of monomers relative to porogenic solvents [39–44]. It has been noted that increasing the amount of porogenic solvent causes a decrease in the pore surface area and a consequent increase in the pore size [12,25,45–47].

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Li et al. [40] prepared poly(bisphenol A dimethacrylate) (BDMA) monolithic columns using decanol and toluene as porogens and they observed that the porosity of the monoliths was very sensitive to the ratio of toluene and decanol. They found that monoliths with larger microglobules and microglobule clusters had low back pressure, while monoliths composed of microglobules of smaller size had high back pressure.

Sáfrány et al. [8] prepared monoliths based on diethylene dimethacrylate as the single monomer in the presence of methyl and 2-propyl alcohols as the porogenic solvents, for providing larger pores. Nevertheless, this research group did not find a direct relation between the proportion of monomers and the surface areas. It was only possible to set a general relationship between the porosimetric results and the microscopic ones obtained by SEM, i.e., between the pore surface area and the globule size belonging to the monolithic structure.

The influence of the amount of monomer on chromatographic parameters, such as the efficiency of separation was documented in a study by Ueki et al. [48]. The authors prepared octadecyl methacrylate-based monoliths, which were used in separations by HPLC. They used as porogenic solvents 80:20 (v/v) isoamyl alcohol and 1,4-butanediol, which afforded a highly permeable stationary phase and efficient and repeatable separations. The proportion of monomers, relative to porogenic solvents, was varied in the range of 10 to 40%, with the optimum condition being 25% of monomers, i.e., this proportion resulted in higher values of efficiency.

In this work, several monolithic stationary phases for CEC were prepared using the monomer octadecyl methacrylate with high hydrophobicity as precursor monomer and ethylene dimethacrylate as cross-linking agent. The effect of the proportion of the two monomers and of the porogenic agents on the porosity and on the electrochromatographic properties was investigated separately for each composition. The resulting monoliths were morphologically characterized by scanning electron microscopy and nitrogen-adsorption/desorption techniques.

2. Experimental

2.1. Chemicals and materials

The sodium hydroxide solution was from Agilent (Waldbronn, Germany); 3-(trimethoxysilyl)propyl methacrylate (TMSPM), N,N-dimethylformamide (DMF), ethylene dimethacrylate (EDMA), octadecyl methacrylate (ODMA), amyl alcohol and 1,4-butanediol were purchased from Aldrich (Steinheim, Germany); 2,2′-azobisisobutyronitrile (AIBN), 2-acryloylamido-2-methylpropanesulfonic acid (AMPS), ethylbenzene, propylbenzene, butylbenzene and pentylbenzene were from Fluka (Steinheim, Germany); HPLC grade acetonitrile (ACN) was purchased from J.T. Baker (Phillipsburg, NJ, USA); tris(hydroxymethyl) aminomethane (Tris) was from Fluka (Düsseldorf, Germany); thiourea was from Riedel-de Haën (Düsseldorf, Germany), and methyl alcohol was from Carlo Erba Reagents (Rodano, Italy). The water used for sample and mobile phase preparation was purified with a Milli-Q deionization system (Millipore S.A.S., Molsheim, France).

Polyimide coated fused silica capillaries with 75 μm inner diameters were from Agilent (Portland, USA).

2.2. Instrumentation

CEC experiments were performed with a CE instrument from Agilent Technologies (Waldbronn, Germany) equipped with a UV–visible diode-array detector. Data acquisition and processing were performed using HP Chemstation software.

A gas chromatograph oven, Hewlett Packard 5890A, was used for initiating the thermal polymerizations. A LC-10AD HPLC pump from Shimadzu (Kyoto, Japan) and a syringe microchromatography pump (ISCO 260D) were used to condition the columns.

2.3. Pretreatment of the capillary

Using a glass syringe, the capillary was washed and filled with 1 mol L^{-1} sodium hydroxide solution, sealed with glass connectors and kept in an oven at 95 °C for 2 h. Next, the capillary was flushed with filtered deionized water to neutrality, then with methyl alcohol and dried under purging nitrogen gas for 1 h [49].

A solution of 50% (v/v) of TMSPM in DMF was used to fill the capillary. Both ends were sealed and it was heated in an oven at 100 °C for 8 h. After, the capillary was washed with DMF and filtered deionized water. Finally, it was again dried with flowing nitrogen [49].

2.4. Preparation of monolithic columns

Initially, the AIBN and the AMPS were weighed. For each synthesized phase, about 8.0 mg of initiator agent was added. Next, the solvents were put into a covered glass vial and, in another vial, the monomers (ODMA and EDMA) were placed under a nitrogen stream, to avoid contact with air. The AMPS and the AIBN were added to the vial containing the monomers and, finally, the porogenic solvents were added. Table 1 indicates the different compositions of the stationary phases that filled the capillary columns. The ratio monomer: porogenic solvent was changed in a range of 60 to 80% (v/v) of porogenic solvents. The proportions of precursor monomer (ODMA), cross-linker (EDMA) and charged monomer (AMPS) were maintained at 59.4:40.0:0.6 (w/w/w) (proportion adapted from work of Jiang et al. [49]). The vial was closed and sonicated for 1 h to homogenize the solution and to solubilize the solid reagents.

After solubilization of the monomers with the porogenic solvents, the capillaries were filled with the mixtures using a glass syringe. Both ends were sealed with connectors and the remaining solution was left in the closed vials to evaluate the polymerization process outside the capillary. The capillaries and the vials were kept in an oven at 60 °C for 24 h for polymerization.

After the polymerization, the capillaries were washed with 70:30 (v/v) acetonitrile:water using the HPLC pump to remove the residual porogenic solvent. The acetonitrile and the water were previously passed through a 0.45 μm filter and degassed in an ultrasonic bath.

For creating the detection window, aluminum foil was used to delimit a portion of the capillary, about 8 cm from the extremity of the capillary. The open portion was burned with a lighter to remove the polyimide coating and form the silica window. The burn afforded by the lighter removed the polyimide and decomposed the polymeric material present in the region of detection window, which became clear to the UV light of the detector. After preparation of the window, the capillary was washed with a solution of buffer electrolyte (30:70 (v/v) 25 mmol L^{-1} Tris, pH 8.0:acetonitrile) using the HPLC pump, in order to eliminate fragments produced by the decomposition of the polymer in the detection window zone.

2.5. Physical characterization of the monoliths

2.5.1. Optical microscopy

Evaluation of column filling was made by optical microscopy with a Motic BA300 microscope (Diadema, Brazil).

2.5.2. Scanning electron microscopy

Morphological evaluations of the monolithic columns were made by scanning electron microscopy (SEM) using a Jeol GSMT-300 instrument (Tokyo, Japan). For the analysis by scanning electron microscopy, the extremities of the capillary were cut off and the capillary tube was fixed to the sample holder, through the use of double-sided carbon

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