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Polymer monoliths with silver nanoparticles-cholesterol conjugate as stationary phases for capillary liquid chromatography

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ABSTRACT

Monoliths in fused-silica capillaries were prepared by an *in-situ* polymerization using glycidyl methacrylate (*GMA*) as a functional monomer and in the presence of different crosslinkers such as ethylene dimethacrylate (*EDMA*), trimethylolpropane trimethacrylate (*TRIM*), 1,6-hexanediol dimethacrylate (*HEDMA*), pentaerythritol tetraacrylate (*PETeA*), triethylene glycol dimethacrylate (*TriEDMA*), while azobisisobutyronitrile (*AIBN*) was an initiator. The monoliths' surfaces were modified chemically with cystamine dihydrochloride, followed by immobilization of Ag-NPs the surface of which was functionalized with cholesterol cysteamine (*Chol-SH*). Such a new material enabled the separation of proteins in a reversed phase mode. The influence of the kind of a cross-linker and composition of the porogenic solutions on the porosity and separation properties of the obtained polymers was investigated and discussed. It was found that the amount of Ag-NPs embedded on the monoliths' surfaces depended on the porous properties of the monoliths, which was directly connected with the kind of the cross-linkers used. For the determination of the amount of the attached Ag-NPs the thermogravimetric analysis was used and additionally the ISEC method as a comparative method was also proposed. It was found that the maximum amount (about 20% by weight) of the 10 nm Ag-NPs was attached to the poly(GMA-EDMA) and poly(GMA-TRIM) monoliths.

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1. Introduction

Modern biomedical, biochemical, and pharmaceutical research, as well as *on-line* monitoring of environmental pollution make it necessary to develop more and more sophisticated and sensitive analytical tools and methods, both at the stage of sample preparation, as well as the final quantitative determination. On one hand, miniaturization of analytical devices by, among others, the use of capillary columns is an important the way to develop separation techniques including chromatographic ones [1–4], on other hand, the preparation of new stationary phases including those in the form of porous polymeric materials proved its usefulness in seeking for selectivity improvement and general chromatographic performance [5–7].

Introduced in 1990s, silica-based and organic-based monoliths have been constantly developed and currently they are successfully used in liquid chromatography and related techniques [8–13]. The popularity of monolithic columns is primarily due to their unique porous structure consisting of micro- and mesopores responsible for chromatographic phenomena and macropores responsible for the speed mass transfer. However, a long-term and time consuming optimization is to be performed to obtain monolithic beds with a bimodal pore size distribution and what is worse, it is impossible to achieve the universal separation column for many applications. Thus, the columns must be individually optimized to obtain different chemical properties: hydrophobic, hydrophilic or ionic. To overcome these limitations the post-polymerization modification of monoliths surface can be performed using such methods as grafting [14–16], chemical modification of reactive groups [17–19], hypercrosslinking [20,21] or embedding the nanoparticles (NPs) [22–29].

The use of NPs, particularly Ag-NPs in the combination with microcolumns (open-tubular, packed and monolithic ones) creates great opportunities in development of separation techniques, mainly chromatographic and electrochromatographic. The NPs can be used for chemical modification of the inner surface of silica capillary or of the surface of the chromatographic stationary phases including monolithic beds in order to improve their characteristics or give them a new, more favorable properties. Additionally, a great advantage of the use of NPs is their high surface-to-volume

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ratio, which facilitates mass transfer and increases the efficiency of the separation methods. An example of modification of the inner capillary wall with Ag-NPs was demonstrated by Přikryl et al. [30]. They formed a 10 μm layer of Ag-NPs photodeposit inside the capillary detection window in the CE-SERS method to detect rhodamine. Additionally, in the CE-SERS system the Ag-NPs suspended in the running buffer were successfully used as a pseudostationary phase [31]. Qin et al. used poly(ethylene oxide)-stabilized Ag-NPs as a pseudostationary phase for the separation of proteins in a classical CE-UV system [32]. Another way of Ag-NPs application is to incorporate them into the surface of a monolithic bed. For example, Pan et al. incorporated Ag-NPs onto the surface of poly(glycidyl methacrylate-co-ethylene dimethacrylate) (poly(GMA-EDMA)) copolymers in fused silica capillaries (530 μm i.d., 690 μm o.d.) and used such columns for the determination of pesticides [33]. Similar studies were carried out by Sedlacek et al., who obtained poly(GMA-EDMA) containing Ag-NPs, but in a stainless-steel column and used such a system for the elimination of excess radioiodine from the radiolabeled pharmaceutical products [34]. Poly(GMA-EDMA) monolith was not the only porous material to be embedded with Ag-NPs. Other acrylic porous polymer monoliths based on trimethylolpropane ethoxylate triacrylate (poly(GMA-SR454)) [35] or lauryl methacrylate-co-ethylene dimethacrylate-co-[2-(methacryloyloxy)ethyl] trimethyl ammonium chloride (poly(GMA-EDMA-META)) have also been used [36]. Moreover, Zhu et al. immobilized Ag-NPs on porous silica monoliths and demonstrated the separations of polyaromatic hydrocarbons using such a stationary phase [37,38]. Additionally, Ag-NPs have been also used in matrix-assisted laser desorption/ionization mass spectrometry of peptides [39] or as a nanocomposite material with polyaniline for microextraction of furosemide from urine samples [40]. A very interesting approach was to use them as hydrophobic affinity probes (the surface of Ag-NPs were modified by the dodecanethiol and octadecanethiol) for the isolation and preconcentration of peptides and proteins from biological samples prior to AP-MALDI-MS and MALDI-TOF-MS analysis [41]. All of the above-mentioned interesting research showed the high potential of Ag-NPs in different analytical techniques and for variety of analytes. Moreover, application of these materials in analysis of biologically active compounds may have an influence not only on the development of new research methods but can be potentially important in routine analysis.

This work, which is a continuation of our previously reported research on cholesterol-based monolithic materials [42–44] presents our new results of preparation of capillary monolithic columns modified with silver nanoparticles functionalized with cholesteryl cysteamine (*Chol-SH*). To provide optimum column properties (permeability and amount of silver attached) the columns prepared with different cross-linking monomers were compared and their properties discussed. Additionally, the effect of different composition of porogenic solvents have been also evaluated. Porous structure and properties of the obtained columns were characterized by SEM and pore size distribution was determined using nitrogen adsorption/desorption isotherm, mercury intrusion porosimetry (MIP) and inverse-size exclusion chromatography (ISEC). The chromatographic performance in the RP mode was also evaluated with test mixtures of alkylbenzenes and proteins.

2. Experimental

2.1. Chemicals and reagents

The following chemicals were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany): thiourea, benzene, toluene, ethylbenzene, propylbenzene, butylbenzene, ribonuclease A (from bovine pancreas ≥70 Kunitz units/mg protein), cytochrome C (from equine heart, ≥95%) and myoglobin (from equine skeletal muscle, 95–100%), glycidyl methacrylate (*GMA*, ≥97% (GC)), ethylene dimethacrylate (*EDMA*, 98%), trimethylolpropane trimethacrylate (*TRIM*, technical grade), 1,6-hexanediol dimethacrylate (*HEDMA*, ≥90%), pentaerythritol tetraacrylate (*PETeA*), triethylene glycol dimethacrylate (*TriEDMA*, 95%), 3-(trimethoxysilyl)propyl methacrylate (*γ-MAPS*, 98%), 1-dodecanol (*1-Dod*, 98%), cyclohexanol (99%), trifluoroacetic acid (*TFA*, 99%), cystamine dihydorchloride (96%), tris(2-carboxylethyl)phosphine hydrochloride (*TCEP*) solutions (0.5 mol/L, pH 7 adjusted with ammonium hydroxide), silver colloids with particle sizes of 10 nm (0.02 mg/mL in aqueous buffer, contains sodium citrate as a stabilizer), cholesteryl chloroformate (95%), trimethylamine, cysteamine (≥98%). Proteins were dissolved in water at concentrations ranging from 1.0 to 2.0 mg/mL. Free radical polymerization initiator, 2,2′-azobisisobutyronitrile (*AIBN*) was from Fluka (Buchs, Switzerland). Acetonitrile (HPLC ultra gradient grade), acetone (technical grade), dichloromethane (99.8%), toluene (99.9%) and anhydrous ethanol (99.8%) were from VWR International (Gdańsk, Poland). Deionized water was produced in our laboratory using the Milli-Q ultrapure water system (Millipore, Bedford, MA, USA). Polyimide-coated fused silica capillaries were purchased from Polymicro representative CM Scientific Ltd. (Silsden, United Kingdom).

2.2. Instrumentation

All chromatographic experiments were performed using a lab-made capillary LC system consisting of a pump delivering a mobile phase (Agilent 1260 Cap Pump with degasser, Agilent Technologies, USA), 10-port valve with a microelectric actuator (C72MX-4694EH, Vici Valco Instruments Inc. Co., Houston, TX, USA) equipped with a 50 nL external capillary loop, a set of connecting capillaries (TSP-series of various diameters, Polymicro Technologies; USA). On-column detection was performed using the Spectra-100 UV–vis (Thermo Separations Products, San Jose, CA, USA) detector. For control of the process and data collection the Clarity software was used (Data Apex, Prague, Czech Republic). A syringe pump NE-1002X (New Era Pump Systems, Farmingdale, NY, USA) was utilized for pumping reagents through the column during modification of monoliths with cystamine dichydrochloride and TCEP. Ag-NPs colloids and *Chol-SH* solutions were pumped through the monolithic capillary columns using a high pressure LC pump (LC-20AD, Shimadzu, Kyoto, Japan) and a manual six-port sample injection valve (model 7125 Rheodyne, Rohnert Park, USA) with a 300 μL loop. An air-driven constant pressure HPLC pump from Knauer (Knauer GmbH, Berlin, Germany) was used for column flushing after synthesis. Nitrogen adsorption/desorption isotherms were measured using Gemini VI surface area and pore analyzer (Micromeritics, Norcross, GA, USA), and the surface areas of the obtained materials were calculated using Brunauer-Emmett-Teller theory. Additionally, pore size distribution was determined using AutoPore IV 9510 mercury intrusion porosimetry analyzer (Micromeritics, Norcross, GA, USA). The scanning electron micrographs were taken using Quanta 3D FEG scanning electron microscope (FEI, Hillsboro, OR, USA) after coating the specimens with a conductive gold layer (monoliths without nanoparticles) or without coating (monoliths with nanoparticles). Additionally SEM-EDX measurements were performed using Leo 1430 VP apparatus (Leo, Elektronenmikroskopie GmbH, Oberkochen, Germany). The silver content in the monolithic columns was measured using Simultaneous Thermal Analyzer-STA 445 F5 Jupiter (NETZSCH-Gerätebau GmbH, Germany) under nitrogen atmosphere (flow rate 60 mL/min) in a temperature range of 35–870 °C, at a heating rate of 10 °C/min.