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Solid-phase extraction based on ground methacrylate monolith modified with gold nanoparticles for isolation of proteins

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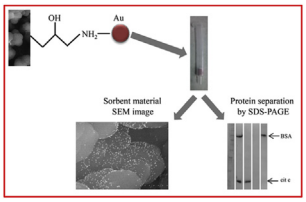
HIGHLIGHTS

- SPE sorbent with AuNPs immobilized onto the polymeric monolith was prepared.
- High recovery efficiency and satisfactory reusability of sorbent were demonstrated.
- Selective separation of proteins is easily achieved according to their pI values.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, a novel polymeric material functionalized with gold nanoparticles (AuNPs) was prepared as solid-phase extraction (SPE) sorbent for isolation of proteins. The sorbent was synthesized from a powdered poly(glycidyl-co-ethylene dimethacrylate) monolith, and modified with ammonia, followed by immobilization of AuNPs on the pore surface of the material. To evaluate the performance of this SPE support, proteins were selected as test solutes, being the extraction conditions and other parameters (loading capacity and regenerative ability of sorbent) established. The results indicated that this sorbent could be employed to selectively capture proteins according to their pI, on the basis of the strong affinity of these biomacromolecules towards to AuNPs surface. The applicability of this sorbent was demonstrated by isolating protein species of interest (bovine serum albumin, cytochrome c and lectins in European mistletoe leaves), followed by SDS-PAGE analysis.

1. Introduction

Porous polymer monoliths are a category of materials developed during the last two decades, which have attracted much interest [1]. Their simple in situ preparation, high permeability, stability along wide pH-ranges, and versatile surface chemistries have made these materials to be a competitive alternative to the conventional packed chromatographic columns [2]. Due to their singular porous structure, polymer monoliths provide rapid conventional mass transport due to its high permeability, and consequently, low backpressure compared to packed columns. As a result, these stationary phases have been used in HPLC [3] and capillary electroseparation techniques [4]. Recently, the application of polymeric monoliths has undergone a rapid growing in the field of sample pretreatment [5,6]. Thus, the performance of extraction efficiency of organic monoliths could be modified by tailoring its pore structure and surface chemistry.

Glycidyl methacrylate (GMA) material has been used as "reactive support" to provide monoliths with different chromatographic properties (ion exchange, hydrophobic/hydrophilic, chiral, etc.)

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[7–10]. GMA-based monoliths have been also used for holding silver and gold nanostructures to generate a good surface enhanced Raman spectroscopy (SERS) performance [11–13]. In addition, GMA-based materials in powder have been demonstrated to be helpful in ultrasensitive SERS detection [12,13] as an "entrapment support" to immobilize biomacromolecules [14]. The powder material could be easily processed into a thin packed column and contains active epoxy groups that can susceptible of undergoing functionalization.

Gold nanoparticles (AuNPs) have unique properties such as special stability, quantum and surface effect, and great biocompatibility [15]. Since AuNPs have high surface-area-to-volume ratios, easy chemical modification and strong affinity for thiol-containing ligands, they have already been used for extracting and enriching analytes from complex matrices [16–19]. Thus, Tseng et al. [17] used AuNPs to selectively extract thiol-containing compounds as a result of the specific formation of Au–S bonds. In a similar way, they have also developed methods for determining aminothiols in human urine and protein-bound aminothiols in human plasma [18,19]. Also, the AuNPs have been suspended in the BGE [20] or attached [21] on the inner wall of capillary in electrodriven separation techniques. In spite of its excellent properties, the combination of AuNPs with organic monoliths has been slightly explored. Thus, Svec's research group [22–24] has studied the attachment of AuNPs on the pore surface of reactive polymer-based monolithic supports. In particular, a GMA-based monolithic capillary column was functionalized with cysteamine to afford a pore surface with thiol groups, to which the AuNPs were attached. These capillary monolithic columns have proved to be useful for the capture and separation of cysteine-containing peptides [23] and as platforms to facilitate further variations in surface functionalities [22,24]. However, the extension of this novel hybrid material as extraction phase has been scarcely reported [13].

SPE is a powerful tool to preconcentrate and purify analytes of interest from a great variety of sample matrices. In particular, this technique is among the widely employed alternatives for protein separation and preconcentration in protein analysis schemes and proteomics techniques [25]. However, a limited choice of sorbents for protein species is available at present, consequently, the development of novel sorbent materials with satisfactory extraction efficiency and selectivity for protein species is highly desirable.

The aim of this study was the development of a novel SPE sorbent based on the modification of a polymer monolith with AuNPs. For this purpose, a GMA-based monolith was first synthesized, ground and subsequently amino groups were introduced onto the surface of the material by reacting epoxy groups with ammonia. Then, the AuNPs were immobilized onto the amino-functionalized GMA powder material surface on the basis of the strong interaction between the amino group and AuNPs [26,27]. The prepared materials were filled into an empty plastic SPE column tube and its extraction efficiency was evaluated by using proteins as test solutes. The conditions for the extraction of proteins (bovine serum albumin and cytochrome c) were optimized. Loading capacity and regenerative ability of the SPE monolithic material was also evaluated. The ability of the developed sorbent to isolate proteins in several samples was also evaluated.

2. Experimental

2.1. Chemicals and reagents

Glycidyl methacrylate (GMA), ethylene dimethacrylate (EDMA), 3-(trimethoxysilyl) propyl methacrylate, acetonitrile (ACN) and methanol (MeOH) were purchased from Scharlab (Barcelona, Spain). Azobisisobutyronitrile (AIBN) was from Fluka (Buchs, Switzerland). AuNP suspension (particle size, 20 nm, stabilized with sodium citrate) and Coomassie Blue were from Alfa Aesar (Lancashire, United Kingdom). Monosodium and disodium phosphate (NaH_2PO_4 and Na_2HPO_4, respectively) and orthophosphoric (H_3PO_4) acid were from Merck (Darmstadt, Germany). Ammonium persulfate, acrylamide, bisacrylamide, bovine serum albumin (BSA), cytochrome c (cyt c), gold (III) chloride trihydrate, hydrochloric acid (HCl), 2-mercaptoethanol, nitric acid (HNO_3), lactose, sodium bromide, sodium dodecyl sulfate (SDS), tetramethylethylenediamine, tris(hydroxymethyl)-aminomethane (Tris) and trisodium citrate were obtained from Sigma–Aldrich (St. Louis, MO, USA). A molecular-weight-size protein standard (6.5–200 kDa) was also provided by Sigma–Aldrich. Deionized water (Barnstead deionizer, Sybron, Boston, Mass., U.S.A.) was used in all procedures.

Stock solutions of BSA and cyt c (1 mg mL^{-1}) were prepared by dissolving appropriate amounts of each protein in deionized water, and working standard solutions were obtained by dilution of the stock solutions. Phosphate buffer solutions (PBS) of 20 mM at several pH values were prepared by mixing appropriate amounts of Na_2HPO_4, NaH_2PO_4 and H_3PO_4 according to the required pH.

2.2. Instrumentation

SEM/backscattered electron (BSE) images and energy dispersive X-ray (EDAX) analysis were obtained with a Philips XL 30 ESEM integrated with backscattered electron detector and a QUANTAX 400 energy dispersive spectrometer (Bruker, Germany). For these measurements, the monoliths were coated with a very thin-layer of conductive carbon instead of the more usual Au/Pd coating.

Elemental analysis of synthesized material was performed using an EA 1110 CHNS elemental analyzer (CE Instruments, Milan, Italy). The determination of Au in synthetized materials and Bradford protein assay were carried out by measuring in UV–vis with an 8453 diode-array UV–vis spectrophotometer (Agilent Technologies, Waldbronn, Germany). For Bradford's assay [28], a calibration curve up to 1 mg mL^{-1} of BSA and cyt c was prepared in the elution solvent (see Section 3.2). SDS-PAGE experiments were performed using a vertical minigel Hoefer SE260 Mighty Small system (Hoefer, MA, USA).

2.3. Preparation and functionalization of GMA-based monolith

The GMA-*co*-EDMA monolithic material was based on a previous work [10]. Briefly, a polymerization mixture was prepared in a 10 mL glass vial by weighing GMA (20 wt%), EDMA (5 wt%), and a binary porogenic solvent mixture containing cyclohexanol (70 wt%) and 1-dodecanol (5 wt%). AIBN (1 wt% with respect to the monomers) was added as initiator. This mixture was sonicated for 5 min and then purged with nitrogen to remove oxygen for 10 more minutes. The polymerization was carried out in an oven at 60 °C for 24 h. Next, the polymeric material was washed with methanol to remove the porogenic solvents and possible unreacted monomers. Then, the monolithic bulk material was ground with a mortar and sieved with a steel sieve with sizes $\leq$100 μm. The synthesized powder porous material was treated with aqueous 4.5 M ammonia in a round bottomed-flask at 60 °C (water bath) for 2 h under continuous stirring. Upon completion of the reaction, the material was washed with ultra-pure water to remove the excess of ammonia until the eluent was neutral. The poly(GMA-*co*-EDMA) powdered material functionalized with amino group was obtained for further use.