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RESEARCH PAPER

Preparation and Characterization of Polymer Solid-phase Extraction Monolith Immobilized Metal Affinity Ligands

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Abstract: Based on the affinity interactions between Cu^{2+} metal chelate and -SH groups of aminothiols such as cysteine, a polymer solid-phase extraction (SPE) monolith was designed and synthetized by the tridentate ligand iminodiacetic acid immobilized on the surface of poly glycidyl methacrylate-based monolith and after modification with Cu^{2+}. Glutathione as a model compound was tested to evaluate the performance of the SPE monolith, and optimized to the enrichment conditions. Under the optimum conditions, the maximum absorption capacity for glutathione of this polymer SPE monolith was determined at 43.15 mg g^{-1}, and the polymer monolith was used for the extraction and enrichment of trace aminothiols in human plasma samples.

Key Words: Aminothiols; Metal chelate; Polymer monolith; Solid-phase extraction

1 Introduction

In recent years, monoliths are used as sorbent materials for solid-phase extraction (SPE) and became increasingly popular in the field of handling biological sample due to their unique properties[1]. For example, their high surface area can increase the loading capacity of the extraction sample; their good compatible with biomolecules and ease of preparation can provide the analyst many options in the choice of bioanalytical applications; their unique through-pores that provide a flow porous for liquid can reduce the resistance to mass transfer and improve the extraction efficiency; furthermore, their wide pH range of applications, good repetition and stability make monoliths as an ideal material for SPE[2–4]. Zhang *et al*[5] prepared poly (acrylamide-co-methylenebisacrylamide) monolith based immobilized enzyme reactor for bovine serum albumin (BSA) digestion using the glutaraldehyde technique. Later, a poly (glycidyl methacrylate-co-acrylamide-co-ethylene dimethacrylate) monolith and a poly (glycidyl methacrylate-co-ethylene dimethacrylate) (poly (GMA-co-EDMA)) monolith were prepared and modified with monomeric avidin using the glutaraldehyde technique for enrichment BSA by Sinz's research group[6].

Metal chelate is easily obtained by means of the coordination effect between transition metal ion and chelating agent of N, O and S atoms. Metal chelate has strong affinity for imidazole groups of histidine located on protein surface, thiol groups of aminothiols such as cysteine and indolyl groups of tryptophan. Now, metal-chelating affinity chromatography has been successfully applied to industrial production of proteins and peptides. Ma *et al*[7] prepared superparamagnetic silica-coated magnetite (Fe_3O_4) nanoparticles with immobilized iminodiacetic acid (IDA) and after charged with Cu^{2+} for the adsorption of BSA. Subsequently, Ensinger's research group demonstrated a novel biosensing platform for the detection of lactoferrin via metal-organic frameworks. In their work, firstly, monolayer of amine-terminated terpyridine (terPy) was covalently immobilized on the inner walls of the nanopore via carbodiimide coupling chemistry; secondly, Fe^{2+}-terPy

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complexes were obtained via the reaction of the terPy modified-nanopores with ferrous sulfate solution[8]. In addition, these materials were also used for the specific binding and recognition some peptides and drugs. Recently, Wang *et al*[9] designed and synthetized the superparamagnetic microspheres poly (methacrylate-co-divinylbenzene)-IDA-Cu^{2+} for affinity adsorption and purification of glutathione. Codd *et al*[10] prepared copper(II)-based and iron(II)-based metal chelate as affinity chromatography adsorbent for the isolation of the anticancer agent bleomycin from streptomyces verticillusculture using the IDA as organic ligand.

In this study, the advantages of strong affinity of metal chelate for thiol-containing groups were combined with the excellent performance of polymer monoliths. A monolith immobilized metal affinity ligand was designed and synthetized as adsorbent for SPE. Firstly, IDA (metal-chelating ligand) was covalently immobilized on the surface of poly (GMA-co-EDMA) monolith. Secondly, copper (II)-IDA (Cu^{2+}-IDA) complexes were obtained by treating the IDA modified-monolith with copper sulfate solution. Thiol-containing compounds such as glutathione as model compounds were investigated to evaluate the performance of the SPE monolith and optimize the enrichment conditions.

2 Experimental

2.1 Instruments and reagents

A homemade capillary electrophoresis (CE) setup with UV detector used in this work was similar to that described in literature[11]. Fused-silica capillary of 75 μm i.d. × 365 μm o.d. and 530 μm i.d. × 690 μm o.d. were purchased from Hebei Yongnian Optical Fiber Factory (Hebei, China). A high-speed TGL-16C centrifuge was purchased from Anting Scientific Instrument Factory (China). A QL-866 oscillator was obtained from Lindberg Instrument Manufacturing Co., Ltd (Haimen, China). Scanning electron microscope (SEM) was carried out on FEI Quanta 200 FEG SEM (Philips, The Netherlands).

Glycidyl methacrylate (GMA), ethylene dimethacrylate (EDMA) and azobisisobutyronitrile (AIBN) were purchased from Alfa Aesar (Ward Hill, MA, USA). Iminodiacetic acid (IDA), cysteine (Cys) and homocysteine (HCys) were purchased from TCI (Shanghai, China). Glutathione (GSH) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Human plasma was obtained from the healthy volunteers in Fifth People's Hospital of Guilin. Ultrapure water used for the preparation of solutions was produced by a Milli-Q water system (Millipore, Bedford, MA, USA).

2.2 Preparation of SPE monolith immobilized metal affinity ligands

A poly (GMA-co-EDMA) monolithic capillary column was prepared by the synthesizing method described previously in our previous work[12]. IDA was dissolved in 100 mL of deionized water until reaching saturation levels, and then the solution was adjusted to pH 11.0 with 1.0 M NaOH. At room temperature, the IDA aqueous solution was pushed through the poly (GMA-co-EDMA) monolith by a manual syringe for 30 min. After sealing the ends of the capillary column with a rubber septum, the monolith was modified by heating in a 70 ºC water bath for 4 h. This procedure was repeated 5 times. The monolith was then rinsed with deionized water until the elution solution was neutral. Then, a 0.5 M copper sulfate solution was pushed through the poly (GMA-co-EDMA-IDA) monoliths by a manual syringe for 5 h, and the monoliths were rinsed with water to remove the excess unbound Cu^{2+} until the elution solution was colorless. The monoliths functionalized in this fashion were referred to as "poly (GMA-co-EDMA-IDA-Cu^2) monoliths" in the text below. Figure 1 shows the photograph of poly (GMA-co-EDMA) monolith before and after modification. It can be seen that the original poly (GMA-co-EDMA) monolith was bright yellow, while it turned into blue after modification with IDA-Cu^{2+}.

The synthesis and surface modification of the polymer monolith immobilized metal affinity ligands are shown in Fig.2.

In a typical recipe[13], a syringe barrel was coupled seamlessly to one end of the pinhead of the syringe (both the syringe barrel and the pinhead were produced out of uniform molds), while at the other end of the pinhead, its metallic needle was replaced by a 3-cm long part cut from the prepared poly (GMA-co-EDMA-IDA-Cu^{2+}) monolith, the outside wall of which was coated uniformly with adhesive. The prepared device was available for the extraction top of the solid-phase and it was referred to as polymer SPE monolith immobilized metal affinity ligands.

2.3 Preparation of plasma sample

The standard solution of GSH, Cys and HCys (0.1 mM) were prepared by dissolving in the deionized water and were stored at 4 ºC in the dark. 50, 100 and 200 μL of the standard solution of GSH, Cys and HCys were added into 300 μL of

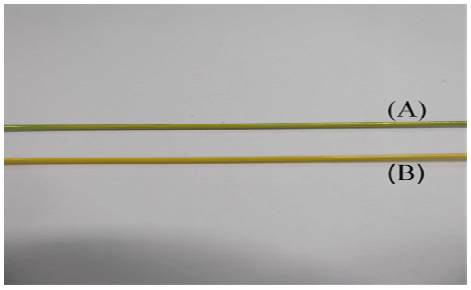

Fig.1 Photograph of poly (GMA-co-EDMA-IDA-Cu^{2+}) (A) and poly (GMA-co-EDMA) (B)