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Short communication

Preparation of an internal surface reversed-phase restricted-access material for the analysis of hydrophobic molecules in biological matrices

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ABSTRACT

Restricted-access materials (RAMs) have been broadly used for sample pretreatment in the chromatographic analysis of biological samples. In the present work, a novel internal surface reversed-phase (ISRP) RAM was prepared via surface-initiated atom transfer radical polymerization (SI-ATRP). Octadecyl and 4-(chloromethyl)phenyl were immobilized on silica using a one-pot synthesis approach to form a reversed-phase layer to retain small hydrophobic molecules, allowing the modified silica to serve as a macro-initiator. Then, poly(glycidyl methacrylate) (pGMA) was grafted onto the surface via SI-ATRP, and the epoxy groups were further hydrolyzed to form an external hydrophilic layer. The properties of this ISRP-RAM for the retention of small molecules and the exclusion of proteins were evaluated using solid-phase extraction (SPE). The removal efficiencies of bovine serum albumin (BSA) and lysozyme were 94.9% and 93.5%, respectively. The recoveries of five drugs, puerarin, *p*-hydroxybenzaldehyde, loratadine, nifedipine and diazepam, were 93.2–116%. Furthermore, the ISRP-RAM was employed for the SPE of five phthalate esters (PAEs) from bovine milk prior to high-performance liquid chromatography (HPLC) analysis. The results indicate that the prepared ISRP-RAM is qualified for practical bioanalysis.

1. Introduction

For the analysis of substances in biological matrices, a sample preparation step for protein removal and analyte enrichment is often required [1]. As a specific type of material for solid-phase extraction (SPE), restricted-access materials (RAMs) have attracted significant attention in this field [2]. RAMs generally possess an interior phase for retarding small molecules and an external hydrophilic layer for excluding proteins. Protein exclusion is commonly performed by a physical diffusion barrier based on pore size or by a chemical diffusion barrier formed by a hydrophilic polymer network or by proteins. Recently, the modification of adsorbent surfaces using hydrophilic polymers for the preparation of RAMs has been receiving increasing attention [3–6]. As a new surface modification method, surface-initiated atom transfer radical polymerization (SI-ATRP) has several advantages, such as a high polymer grafting density and controllable polymer chains. This technique has recently been employed to prepare RAMs. Functional groups that could bind small molecules were first grafted onto solid supports by SI-ATRP to form the inner layer; then, the external poly(glycidyl methacrylate) (pGMA) layer was further grafted on the surface via a second round of SI-ATRP, and a hydrophilic structure was formed after hydrolysis, which created a diffusion barrier for proteins [7,8].

To date, reversed-phase RAMs are still the most widely used RAMs in practice. However, the current methods for preparing reversed-phase RAMs are quite complex. For these methods, a step for the selective removal of the modifying hydrophobic groups on the external surface is essential; alternatively, more than four steps are involved in the preparation process [1,5,8,9]. Therefore, developing simple and versatile methods for preparing RAMs remains a significant field of research.

Phthalate esters have been widely used in industrial production [10,11]. These materials have been linked to hormone disruption, which can affect development and fertility [12]. Currently, monitoring of PAEs in food, including milk, is very important. As a biological sample, sample pretreatment is mandatory for the determination of PAEs in milk. To date, the most widely used sample preparation methods for the analysis of PAEs in milk are liquid–liquid extraction (LLE) [13], solid-phase extraction (SPE) [14] and solid-phase

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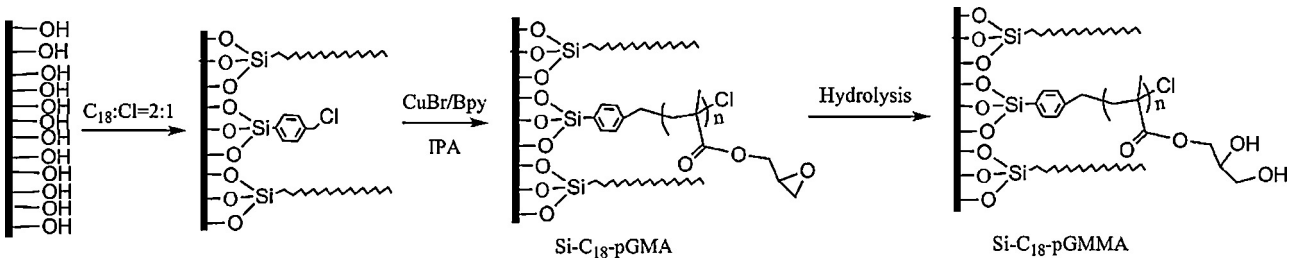

Fig. 1. Synthesis of the ISRP-RAM.

microextraction (SPME) [15]. To the best of our knowledge, there have been no reports on sample pretreatment using RAMs for the analysis of PAEs in milk. In this study, a novel internal surface reversed-phase (ISRP) RAM was prepared via SI-ATRP. The material's performance in terms of the retention of small hydrophobic molecules and the exclusion of proteins were evaluated. Additionally, an offline SPE–HPLC method for determining five phthalate esters (PAEs) in bovine milk was established based on this RAM as an example of a practical application.

2. Materials and methods

2.1. Immobilization of octadecyl and ATRP initiator on silica gel

Silica was activated by rehydroxylation in 6 mol/L hydrochloric acid under reflux for 4 h at 120 °C, followed by washing with water, methanol and acetone in sequence, and dried under vacuum at 120 °C for 8 h. The activated silica (1 g) was dispersed in a solution of anhydrous toluene (20 mL) containing pyridine (1 mL). Then, a mixture of octadecyltrichlorosilane and 4-(chloromethyl)phenyltrichlorosilane in toluene was added dropwise into the silica suspension. The reaction was performed at 120 °C for 24 h under a nitrogen atmosphere with gentle stirring. The modified silica was collected by filtration, then rinsed with dry toluene, methanol and acetone in sequence and dried under vacuum at 40 °C for 8 h.

2.2. Grafting of pGMA on the silica by SI-ATRP

For the immobilization of pGMA brushes on the surface of the initiator-modified silica, the reaction was performed using a [GMA]/[CuBr]/[$CuBr_2$]/[bpy] feed ratio of 100:1:0.2:2 in isopropanol (10 mL). The mixture was de-oxygenated by three freeze–pump–thaw cycles, and high-purity nitrogen was introduced after each evacuation stage. The reaction was performed at 40 °C for 24 h under a nitrogen atmosphere with gentle stirring. Then, the modified silica was collected by filtration. Next, the copper was removed by immersing the particles in 20 mL of 0.3 mol/L disodium ethylene diamine tetraacetate for 4 h at 60 °C with stirring. Finally, the sample was dried under vacuum, and pGMA-coated silica was obtained.

2.3. Hydrolysis of pGMA

The pGMA was hydrolyzed by a method described in a previous study [8], with minor modifications. Briefly, the pGMA-coated silica was dispersed in a solution of tetrahydrofuran (10 mL), and H_2SO_4 (0.1 mol/L) was added dropwise into the suspension. The reaction was performed at 60 °C for 8 h with stirring. After hydrolysis, a hydrophilic polymer [poly(glycerol monomethacrylate), p(GMMA)] was formed. Finally, the modified silica was washed with deionized water and methanol and then dried under vacuum. The ISRP-RAM was thus obtained.

3. Results and discussion

3.1. Preparation of ISRP-RAM by SI-ATRP

To date, reversed-phase RAMs are still the most broadly used RAMs. C_{18}-bonded silica is the most widely used reversed-phase material both in chromatographic separation and sample pretreatment. Thus, in this study, a novel reversed-phase RAM was prepared based on SI-ATRP. First, octadecyl and 4-(chloromethyl)phenyl (as an initiator for ATRP) were immobilized on the silica using a one-pot synthesis method via the silylation reaction. In general, any alkyl halide with activating substituents on the *R*-carbon, such as aryl, carbonyl or allyl groups, can potentially be used as an ATRP initiator [16]. In the present work, 4-(chloromethyl)phenyl was immobilized on the surface of the silica as the ATRP initiator (Fig. 1) for two reasons: (1) it is one of the most broadly used initiators in the surface modification of silica via ATRP and (2) the benzyl group can serve as a functional ligand in reversed-phase media. After the immobilization of the initiator, pGMA chains were grafted to the silica surface by SI-ATRP, and an epoxy group in the pGMA chain was hydrolyzed to a diol to form an external hydrophilic layer, which created a diffusion barrier for proteins (Fig. 1). The internal hydrophobic layer of this RAM was composed of octadecyl and benzyl groups, which allow it to serve as a universal reversed-phase RAM for the analysis of hydrophobic small molecules. The external surface was covered with a hydrophilic network consisting of polymeric chains with diols. Therefore, the RAM prepared in the present work has a dual surface topochemistry and should belong to the group of ISRP supports [5,9].

The preparation of ISRP materials usually consists of the following procedures [5]: the entire surface of the silica is chemically modified with hydrophobic ligands; then, the ligands located on the external surface are exclusively removed. Thus, hydrophilic groups or silanol groups are solely exposed on the external surface. Finally, neutral hydrophilic groups such as glycerylpropyl are covalently coupled to the silanols. The selective removal of the modifying hydrophobic groups on the external surface is vital during the preparation of this type of RAM. Gasparrini et al. [5] reported a novel method for preparing ISRP based on the following four steps: (a) amination of the silica surface, (b) activation of the aminopropylated silica with 1,6-diisocyanatohexane, (c) modification of the silica with polyvinyl alcohols and (d) deactivation of the residual pendant isocyanate groups on the internal surface with hexylamine. Xu et al. [8] prepared a reversed-phase RAM using ATRP; five steps were involved in their protocol: (1) amination of the silica surface, (2) immobilization of the initiator, (3) grafting of poly(styrene-*co*-divinylbenzene) to form hydrophobic polymer layers by ATRP, (4) grafting of pGMA to form hydrophilic polymer layers by second ATRP and (5) hydrolysis of the epoxy