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Restricted-access nanoparticles for magnetic solid-phase extraction of steroid hormones from environmental and biological samples

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ABSTRACT

Restricted-access materials based on non-ionic surfactant-coated dodecyl-functionalized magnetic nanoparticles were prepared and applied to extract steroid hormones from environmental and biological samples. The magnetic nanoparticles were synthesized by co-precipitation, and were functionalized with dodecyltriethoxysilane, giving dodecyl-grafted magnetic nanoparticles (C_{12}-Fe_3O_4). They were further modified with different non-ionic surfactants by self-assembly adsorption. Several types of non-ionic surfactants, Tween-20, 40, 60 and 85, and Span-40, 60 and 80, were investigated as the coatings. Tween surfactants coated C_{12}-Fe_3O_4, named as TW-20 (40, 60, 85)-C_{12}, exhibited good dispersibility in aqueous solution, which was a preferred character in extraction; besides, TW-20-C_{12} and TW-40-C_{12} showed good anti-interference ability and satisfactory reproducibility when they were used as magnetic solid-phase extraction (MSPE) sorbents. The factors that may influence the extraction, including the amount of magnetic nanoparticles, extraction and desorption time, the amount of salt addition, the type and volume of desorption solvent, the volume of methanol addition and pH of sample solution, were investigated in detail. High performance liquid chromatography–UV detection was employed for analysis of target analytes (steroid hormone compounds). The developed method was successfully used for the determination of the target analytes in environmental and urine samples. Both tested materials afforded good recovery, satisfactory reproducibility and low limits of detection for environmental samples, which indicates that the materials possessed anti-interference ability. However, compared to TW-40-C_{12}, TW-20-C_{12} nanoparticles provided better recovery in relatively complex biological samples, which may indicate that the latter one is more appreciated in complex samples.

1. Introduction

Analysts encounter an ongoing challenge to pursue efficient analysis in complex samples, due to low concentration of analytes and unavoidable interference of complex matrices to instrumental analysis. As a result, an appropriate sample pretreatment is necessary in analysis, which aims at cleaning up, isolating and/or concentrating analytes of interest.

In recent years, to afford operation convenience, enhance the extraction efficiency and save the cost, magnetic solid-phase extraction (MSPE) has gained much attention [1–3]. In MSPE, magnetic nanoparticles (MNPs), generally Fe_3O_4 or γ-Fe_2O_3, are used as sorbents. Compared to traditional SPE sorbents, MNPs possess high surface area and have unique magnetic properties. The equilibrium between the sorbents and sample solutions can be reached quickly once MNPs are introduced to the solution. After extraction, MNP sorbents enriched with analytes are easily isolated from suspension by exerting an exterior magnetic force. Hence, unlike traditional cartridge SPE, MSPE avoids column packing and possible blockage during use. However, bare MNPs were observed to aggregate easily, which may alter their stability and extraction capacity [4,5]. Derivatization has been a key to overcome some weakness of MNPs [6–8]. Recently, Chen et al. [9] reviewed applications of derivatized MNPs, e.g. silanized- and polymer modified-MNPs, in water samples. Nevertheless, modified MNPs still suffer from some defects. For instance, C_{18}-MNPs were difficult to disperse in water samples due to their high hydrophobicity and may lose their adsorption ability in complex matrices as they could be easily contaminated by sample matrix [6,8]. Hence, appropriately functionalized materials, being compatible with sample matrix, are still desired in MSPE.

Restricted access material (RAM) is a class of biocompatible SPE sorbents. Generally, RAM has interior support suitable for small molecules' retention and exterior surface with different groups to exclude macromolecules to the interior support. Therefore, it weakens or eliminates adsorption of uninterested macromolecules (e.g. proteins) without negative influence on extraction of target analytes, and a reliable cleanup of complex matrices can be achieved. Since its discovery, RAM has been satisfactorily used in

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environmental and biological analysis [10–13]. However, to our best knowledge, there are few papers drawing attention to RAM combining with MNPs. Cai et al. synthesized chitosan-coated C_{18}-MNPs to extract phthalate ester and perfluorinated compounds from environmental samples. The chitosan coating was demonstrated to have anti-interference ability to macromolecules by size exclusion or electrostatic repulsion [6,8]. Additionally, magnetic microspheres with mesoporous shell were reported to exclude proteins by size exclusion [14,15]. Very recently, MNPs modified with diol groups were also reported to generate RAM [16].

In previous work, ionic surfactants, e.g. cetyltrimethylammonium bromide (CTAB), octadecyltrimethylammonium (OTAB) and sodium dodecyl sulfate (SDS), were used to form hemimicelles and/or admicelles on the surface of MNPs by means of hydrophobic and/or electrostatic interactions [7,17]. The ionic surfactants provided functionality, e.g. hydrophobic and electrostatic groups, for extraction. Polyoxyethylene-containing non-ionic surfactant was reported to have anti-interference ability when used as SPE or high performance liquid chromatographic (HPLC) sorbent coating [18,19]. However, there is a lack of studies about non-ionic surfactant coating in MSPE.

In the present study, RAM-MSPE based on non-ionic surfactant coating was developed. Commonly used non-ionic surfactants, e.g. Tween- and Span-series, were chosen as the MNP coatings, which were supposed to shield macromolecules in complex sample matrix. Prior to coating, the MNPs were derivatized with C_{12}, which may interact with the target analytes. Steroid hormone compounds (ST-HRMs), a group of biological chemicals derivatized from cholesterol, were chosen as test analytes due to their potential negative effect on ecospecies and human beings.

2. Experimental

2.1. Chemicals and reagents

Four ST-HRMs, hydrocortisone (HC), 4-androstene-3,17-dione (AD), progesterone (PG) and testosterone propionate (TP) were obtained from Aladdin Chemical Reagent Co., Ltd. (Shanghai, China). Tween-20, 40, 60 and 85, and Span-40, 60 and 80, HPLC-grade methanol (MeOH), acetonitrile (ACN) and NaCl were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Acetone was purchased from Concord Co., Ltd. (Tianjin, China). HCl was from Kaifeng Dong Da Chemical Reagent Co., Ltd. (Henan, China). Dodecyltriethoxysilane was obtained from Hubei Wuhan University Silicone New Material Co., Ltd. (Wuhan, China). $FeCl_2{\cdot}4H_2O$ was purchased from Shanghai Gongxuetuan No. 2 Experiment Factory (Shanghai, China). Ultrapure water (pH 6.2) was produced by a Heal Fore NW system (Shanghai, China).

2.2. Apparatus

The determination of the four ST-HRMs was performed on a Hitachi (Tokyo, Japan) HPLC system. It consists of a Model L-2130 pump, a Rheodyne 7725i injector (Cotati, CA, USA) and an L-2400 UV–vis spectrophotometric detector. Data were collected and processed by T3000P (Hangzhou Hui Pu Technology Co., Ltd., Hangzhou, China) software. Chromatographic separation was performed on an ODS (4.6 × 250 mm, 5 μm) column from Shimadzu (Kyoto, Japan) at a temperature of 22 °C. A mixture of MeOH and water (78:22, v:v) was used as mobile phase. The flow rate was 1.2 mL min^{-1} and the injection volume was 20 μL. The UV wavelength was set at 242 nm. All the experiments were performed at least in triplicate.

The pH values were measured with a Mettler Toledo Delta 320 pH meter (Shanghai, China). Fourier transform infrared spectroscopy (FT-IR) was performed on AVATAR 360 (Thermo, USA). Thermogravimetric analysis (TGA) was carried out using a Setaram (France) TG-DTA analyzer. Transmission electron microscope (TEM) images were obtained on FEI Tecnal G^2 12 (Netherlands).

2.3. Sample preparation

Stock solutions of the four ST-HRMs (0.5 mg mL^{-1} of each analyte) were prepared separately in MeOH. They were stored at 4 °C. Water samples were prepared by spiking ultrapure water with the analytes at a known concentration (0.5 μg mL^{-1}) to study extraction performance under different conditions.

The genuine water samples were collected from Hanjiang River, Changjiang River and East Lake (Wuhan, Hubei, China). Tap water samples were from laboratory (Tongji School of Pharmacy, Huazhong University of Science and Technology, Wuhan, China). They were stored in brown glass containers at the temperature of 4 °C. Prior to extraction, they were adjusted to a desired pH without any other pretreatment.

The urine samples were collected from volunteers (both male and female). Before extraction, the urine was spiked with the stock solutions to give a desired concentration and diluted with pure water (1:10, v:v). The above solution was then adjusted to the desired pH and was subjected to the extraction process without any other pretreatment. For the blank urine sample, it was subjected to the same sample pretreatment procedures as the spiked one.

All the samples were freshly prepared daily. Each sample (4 mL) was used for extraction. The relative recoveries were determined as ratios of HPLC signal of the spiked extracted samples (real matrices) to that of standard solution at the same comparable concentrations.

2.4. Preparation of surfactant-coated MNPs

The preparation scheme of surfactant-coated C_{12}-MNPs is shown in Fig. 1. (1) Fe_3O_4 MNPs were prepared by an oxidative-coprecipitation method [20] and dried at 80 °C for 24 h. (2) Fe_3O_4 MNPs were functionalized with dodecyltriethoxysilane. Briefly, Fe_3O_4 MNPs (2.0 g) were dispersed in the mixture of anhydrous toluene (50 mL) and dodecyltriethoxysilane (10 mL) by ultrasonication for 20 min. Then, the above mixtures reacted at 120 °C for 16 h. The products, named as C_{12}-MNPs, were separated from the resulting solution by a magnet, washed with copious MeOH and dried for the following use. (3) Non-ionic surfactant modification was carried out at room temperature by self-assembly adsorption. For Tween-series surfactants (Tween-20, 40, 60, 85), 2.0 g C_{12}-MNPs were dispersed in 150 mL water containing desired surfactant (0.5%, w/w) under vigorous mechanical stirring for 4 h. Tween-20, 40, 60, 85 coated C_{12}-MNPs were named as TW-20-C_{12}, TW-40-C_{12}, TW-60-C_{12} and TW-85-C_{12}, respectively. For Span-series surfactants (Span-40, 60, 80), 2.0 g C_{12}-MNPs were dispersed in 150 mL water–MeOH (7:1, v:v) containing desired surfactant (0.5%, w/w) under vigorous mechanical stirring for 4 h. Span-40, 60, 80 coated C_{12}-MNPs were named as SP-40-C_{12}, SP-60-C_{12} and SP-80-C_{12}, respectively. Finally, the above MNPs were washed with pure water and dried at 80 °C for 12 h.

2.5. MSPE procedure

The procedure of MSPE was as follows. Certain amount of NaCl was dissolved in the sample solution (4 mL), which was contained in a 5 mL vial. Prescribed amount of surfactant-C_{12}-MNPs was then added into the above solution. After being sonicated for certain time, the MNPs were isolated from the suspension with a Nd–Fe–B magnet (50 mm × 50 mm × 10 mm), followed by adding ultrapure water (500 μL) into the vial to wash the remnant sample solution.