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Mixed hemimicelles SPE based on CTAB-coated Fe_3O_4/SiO_2 NPs for the determination of herbal bioactive constituents from biological samples

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ABSTRACT

In this paper, a solid-phase extraction (SPE) method based on mixed hemimicelles of cetyltrimethyl ammonium bromide (CTAB) on silica-coated magnetic nanoparticles (MNPs) is developed for extraction and preconcentration of compounds from the biological samples. We selected rhein and emodin which are the major active anthraquinones of rhubarb as model analytes. A high performance liquid chromatography–fluorescence detection (HPLC/FLD) method was developed for the determination of rhein and emodin in urine and serum samples. The main factors influencing the extraction efficiency including the amount of surfactant, the concentration of MNPs, the shaking time and the desorption ability of organic solvents were investigated and optimized. No interferences were caused by proteins or endogenous compounds in urine and serum samples. Good linearities ($r^2 > 0.9995$) for all calibration curves were obtained, and the limits of detection (LODs) for rhein and emodin were 0.2 and 0.5 ng/mL in urine samples and 7 and 10 ng/mL in serum samples, respectively. Satisfactory recoveries (92.76–109.90% and 97.53–107.72% for rhein and emodin) in the biological matrices were achieved.

1. Introduction

Rhei Rhizoma (rhubarb) is a well-known traditional Chinese herbal medicine. Anthraquinone components are the main effective ingredients of rhubarb, which have many pharmacological activities including anti-bacterial [1,2], anticancer [3,4], antifungal [5], antioxidative actions [6,7] and protection of the damaged liver [8,9]. Therefore, it is necessary to determine the content of anthraquinones in biological samples for their pharmacological studies. Rhein and emodin are the major active anthraquinones [10,11]. Several analytical methods have been reported for the quantification of them in biological matrices, such as TLC [12], HPLC/UV [1], LC/MS [12], etc. Generally, for chromatographic analysis of pharmaceutical compounds in biological matrices such as urine, serum and plasma, sample pretreatment is required to clean up the sample before injection. A literature survey reveals that there have been a number of pretreatment methods for analysis of bioactive components in rhubarb, e.g., protein precipitation [12–14], liquid–liquid phase extraction (LLE) [10,15], column-switching [16], etc. However, these methods still have some limitations or shortcomings, for example, need of sample dilution, incomplete protein precipitation, drug co-precipitation, etc. Compared with LLE, solid-phase extraction (SPE) has many obvious advantages including high preconcentration factors, low consumption of organic solvents and ease to operate.

The absorption of ionic surfactants on metal oxides such as alumina, silica, titanium dioxide and ferric oxyhydroxides can form hemimicelles and admicelles [17–21]. Recently, new SPE methods based on mixed hemimicelles assembles has been proposed for the extraction and preconcentration of ionic [19], non-ionic [22,23], amphiphilic [18] compounds. In these methods, the sorbents were produced by the adsorption of ionic surfactants such as sodium dodecyl sulfate (SDS) or cetyltrimethyl ammonium bromide (CTAB) on the surface of mineral oxides [24]. Compared with the normal sorbents, one feature of these sorbents is that the outer surface of hemimicelles is hydrophobic and the admicelles is ionic, which offers different mechanisms for extraction. Moreover, the number of commercially available surfactants is very large, hence the degree of hydrophobicity and the charge of the sorbent can be easily modified according to the nature of analytes. Therefore, using mixed hemimicelles in SPE has many advantages, such as high extraction yields, high breakthrough volumes, and rapid elution of analytes. However, to our knowledge, mixed hemimicelles have not yet been applied in the analysis of biological samples.

Recently, many research groups have explored the application of several nanosized SPE adsorbents such as nanoparticles (NPs) and nanotubes. Magnetic nanoparticles are one of the most popular materials in analytical biochemistry, medicine, and biotechnology, and they have been increasingly applied to immobilize proteins, enzymes, and other bioactive agents [25]. Fe_3O_4 nanoparticles have

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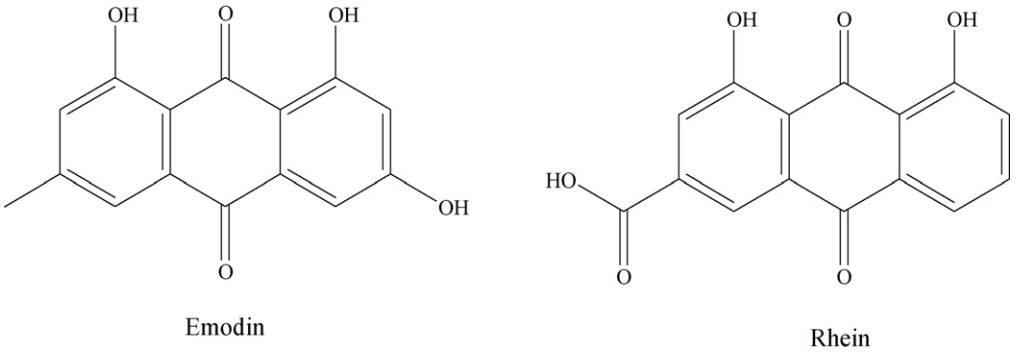

Fig. 1. The chemical structures of rhein and emodin.

attracted particular interest in separation science, for they can be easily isolated by using an external magnetic field placed outside of the extraction container. However, pure magnetic particles are prone to form aggregates and their magnetic properties can be altered in complex environmental and biological systems. To solve the above problems, a suitable protective coating on a magnetic core is often used. The core–shell magnetic nanoparticles have high surface areas. Silica has been considered as one of the most ideal shell materials due to its reliable chemical stability, biocompatibility and versatility in surface modification [26]. Hence, silica-coated Fe_3O_4 nanoparticles could be used for rapid separation. Mixed hemimicelles based silica-coated magnetic nanoparticles were proposed to preconcentrate phenolic compounds from environmental samples [27].

In this work, we combine the advantages of both mixed hemimicelles and silica-coated magnetic nanoparticles, and a mixed hemimicelles based nanosized SPE sorbent is proposed for the extraction and preconcentration of the two active components (rhein and emodin) of rhubarb from the serum and urine samples. We synthesized the Fe_3O_4/SiO_2 core/shell nanoparticles and modified them by coating with cationic surfactants (CTAB) to form the nanosized SPE sorbents. The factors influencing the formation of mixed hemimicelles and the recoveries of the analytes in biological samples were investigated. We found that, for the analysis of rhein and emodin in biological samples, the proposed mixed hemimicelle based nanosized SPE method is more sensitive than the conventional method.

2. Experimental

2.1. Instrumentation and chromatographic conditions

Liquid chromatographic system (Shimadzu, Kyoto, Japan) was consisted of two LC-20AT pumps, a CTO-10AS VP column oven and a RF-10A XL FLD system. They were connected via a communication bus module (Model CBM-20A), and controlled by a Shimadzu LC Solution workstation. A Shimadzu Shim-pack vp-ODS column (5 μm, 4.6 mm × 150 mm) was utilized. When methanol or acetonitrile/water was used as the mobile phase, the peaks of analytes showed band broadening and tailing. In order to improve the peak shape, acetic acid was used to modify the mobile phase. Acetic acid solution (0.1%, 0.3%, 0.5%, 0.8%)–methanol was tested as mobile phases. When the concentration of acetic acid solution was increased to 0.5%, narrow peaks, good symmetry and smooth baselines were obtained. So the mobile phase was composed of A (0.5% acetic acid, v/v)–B (methanol) with the gradient elution (0–5 min, isocratic conditions with 65% of methanol; 5–20 min, linear gradient from 65% to 90% of methanol). The other analysis

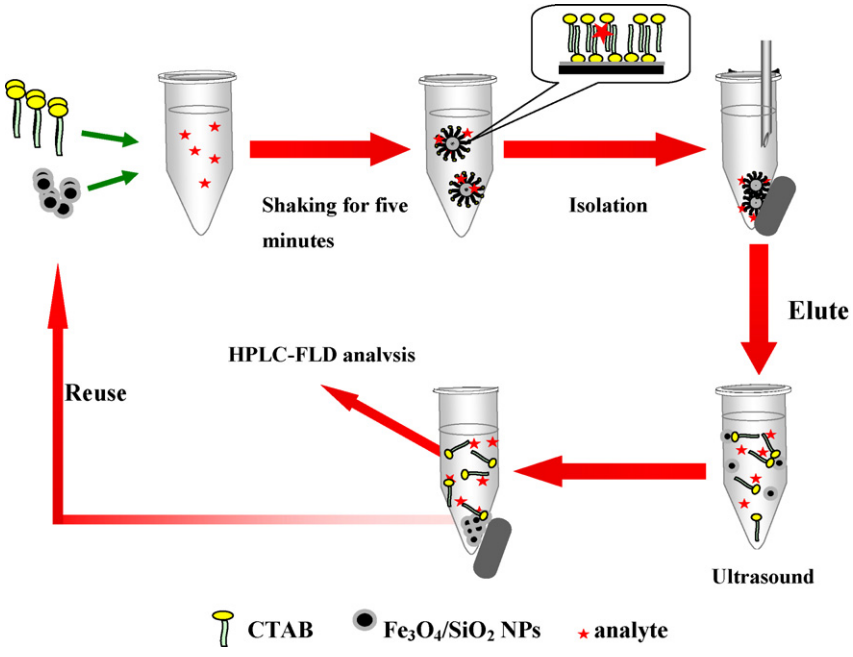

Fig. 2. Schematic illustration of the preparation of surfactants coated Fe_3O_4/SiO_2 MNPs and its application for enriching the active compounds as SPE sorbents.