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Bifunctional magnetic nanoparticles for analysis of aldehyde metabolites in exhaled breath of lung cancer patients

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ABSTRACT

We report here the preparation of dual-functionalized magnetic nanoparticles, with the nanoparticles as extraction sorbents, a magnetic solid phase extraction method was developed and applied for the analysis of trace amount of aldehydes in human exhaled breath condensate. In the material, octyl-functionalized internal surface provided hydrophobic groups for extraction, non-ionic surfactant (Tween-20)-coated outer surface offered hydrophilic network structure to prevent the access of macromolecules, strong magnetic property of nanoparticles simplified the analytical procedure. The experimental results showed that the prepared nanoparticles exhibited good dispersibility in aqueous solution and excellent extraction efficiency toward aldehydes. Six aldehydes were derivatized with 2,4-dinitrophenylhydrazine and then the formed hydrazones were extracted by the nanoparticles and analyzed by high-performance liquid chromatography–photo diode array detector. Under the optimal conditions, the method provided low limits of detection (2.9–21.5 nmol L^{-1}), satisfactory reproducibility (relative standard deviations, 2.9–13.1%) and acceptable recoveries (73.7–133.1%). The developed method was applied successfully to determine the aldehydes metabolites in the exhaled breath condensate samples of healthy people and lung cancer patients. The dual-functionalized material is suitable for biological sample analysis. The proposed method provides an alternative approach for quantification of aldehyde metabolites in complex biological samples.

1. Introduction

Recently, great attention has been paid to the synthesis of magnetic nanoparticles (MNPs) due to their potential applications in magnetic resonance imaging [1], drug delivery [1,2], hyperthermia treatment [3] and analytical applications [4–6]. Besides the above applications, MNPs have been used successfully in sample preparation. A novel sample preparation method named magnetic solid-phase extraction (MSPE) has been developed based on the MNPs [7–9]. Compared with traditional solid-phase extraction sorbents, the outstanding potential advantages of MNPs rely on large surface areas, high extraction efficiencies, use of reduced amounts of sample and of toxic organic solvents. Moreover, it can be easily and quickly isolated by applying an external magnetic field placed outside the extraction container without additional centrifugation or filtration of the sample. In addition, magnetic separation can avoid the time-consuming process of loading large-volume of samples and the problem of high back pressure induced by SPE column.

Bare MNPs were observed to aggregate easily, which may alter their stability and extraction capacity. In order to resolve the problem, a proper surface functionalized modification (with alkyl, metal and others groups) are commonly adopted for Fe_3O_4 nanoparticles in MSPE [10,11]. Octadecyl hydrocarbons (C_{18}) are some of the most widely used alkyl-bonded silica materials in MNPs. Unfortunately, these MNPs are difficult to disperse in water samples because of their strong hydrophobic surface. As a result, its extraction efficiency is reduced accordingly [12]. Thus, taking into account this, excellent water dispersion and surface functionalization of nanomaterials are crucial for their bio-application [13].

Meanwhile, sample preparation methods using restricted access materials (RAM) have received considerable interest in the field of biomedical analysis for its potential to isolate and enrich trace analytes in the presence of abundant proteins [14,15]. Non-adsorptive outer surface of the RAMs provides size exclusion and hydrophilic barrier to macromolecules, while smaller molecules can penetrate and be adsorbed by hydrophobic inner surfaces. The incorporation of restricted access properties to magnetic particles is an important progress in sample preparation for bio-analysis due to the combination of both advantages. However, to our best knowledge, only a few researchers paid attentions to this issue. MNPs modified with diol groups were reported by Wan and coworkers, the RAM was

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used for the extraction of therapeutic agents from biological fluids [16]. And ionic surfactants (CTAB) [17] and non-ionic surfactant (Tween) [18] coated magnetic nanoparticles were prepared and applied successfully to the analysis of biological samples.

In the field of human bio-monitoring, the measurement of metabolites in human exhaled breath represents a non-invasive diagnosis approach through assessing volatile organic compounds (VOCs) generated in the organism [19,20]. As lung cancer is the most common cause of cancer-related deaths, biomarkers that could enable early recognition of this disease can play an important role in the diagnosis and treatment of lung cancer. During recent years, the noninvasive detection of lung cancer by analysis of exhaled breath has received the greatest attention. VOCs like hydrocarbons, alcohols, aldehydes and ketones have been found in human exhaled breath and some of them have been proposed as cancer biomarkers [21–23]. Pauling et al. detected about 250 volatile organic compounds (VOCs) in exhaled breath from healthy subjects [19]. Some researchers focused on the determination of aldehydes in breath, serum and urine samples of lung cancer patients [24–28]. However, several main problems still challenge the existing analytical method of EBC. The low concentrations of metabolites in exhaled breath are beyond instrument limits of detection [29], the co-existed protein (average level: 12.1–13.7 $\mu g\,mL^{-1}$ [30]) in EBC leads to possible matrix interference. In addition, the dilution of sample with condensed water vapor [31] and possible contamination by saliva [32] may affect the qualitative analysis of EBC. Therefore, chemical derivatization together with sample pre-concentration are necessary before chromatographic and mass spectrometric analysis [25,33]. The most extensively used preconcentration method for the extraction of VOCs in breath analysis is solid phase microextraction, it is attractive due to its solvent-free, simplicity and rapidity [34–36]. But the extraction fiber is fragile and has a limited lifetime.

In the present study, novel non-ionic surfactants-modified magnetic nanoparticles were synthesized and used as a selective magnetic sorbents for the extraction of aldehydes from human exhaled breath condensates, HPLC/PDA was employed for the subsequent analysis. Non-ionic surfactant (Tween-20) was chosen as the coating of MNPs, the hydrophilic outer surface was supposed to reduce the adsorption of macromolecules in EBC sample matrix. In the inner surface of MNPs, octyl-functionalized modification provided hydrophobic groups for the extraction of the target analytes. Aldehydes were chosen as test analytes due to their potential disease diagnostic value for lung cancer. The experimental parameters that may influence the derivatization and extraction were investigated. Under the optimal conditions, the MSPE-HPLC method was validated and applied in the real EBC sample analysis.

2. Experimental

2.1. Chemicals and reagents

n-Butanal (98%) and n-octanal (98%) were obtained from Dixiai Chemical (Shanghai, China). n-Nonanal (97%) was obtained from Alfa Aesar (Tianjin, China). n-Hexanal (98%) and n-heptanal (97%) were obtained from ABCR GmbH & Co. KG (Germany). n-Pentanal (98.5%) was purchased from Amethyst Chemicals (Beijing, China). 2,4-Dinitrophenylhydrazine (2,4-DNPH, 99.6%) was purchased from CHEM SERVICE (West Chester, PA) and recrystallized once in acetonitrile–water (1:5) solution before use. Chlorodimethyloctylsilane (97%) and 1-hydroxypyrene (98%, internal standard, IS) were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI, USA). HPLC-grade methanol was purchased from Fisher Chemicals (Fair Lawn, NJ, USA). Formic acid (96%) was purchased from TEDIA (Tedia Company, Inc., Fairfield, OH, USA). Tween- 20 was purchased from Shanghai Chemical (Shanghai, China) with analytical grade. Bovine serum albumin (BSA) and α-salivary amylase were obtained from Rujie Biotech. Co. (Shanghai, China). Ultra-pure water was used in all experiments (arium® pro Ultrapure Water Systems, Sartorius Stedim Biotech, Gottingen, Germany). Rtube™ was purchased from Respiratory Research, Inc. (Charlottesville, VA, USA).

2.2. Chromatographic conditions

The Shimadzu LC-2010A System (Shimadzu Corporation, Tokyo, Japan) equipped with a LC-20AT quaternary pump, SIL-20A autosampler, CTO-10ASVP column oven and a SPD-M20A photo diode array detector was used in this study. The analytes were separated on Venusil, XBP C18 column (250 mm × 4.6 mm, 5 μm), which was obtained from Agela Technologies Inc. (Beijing, China). The mobile phase was formed by methanol (mobile A) and water (mobile B) at a flow rate of 1 mL min^{-1}. The linear gradient elution was as follows: 75% A for 6 min; linear to 93% A within 6 min and maintained 5 min, got back to 75% A and equilibrated for 3 min. The column temperature was 40 °C and the detection wavelength was set at 360 nm and the injection volume was 10 μL.

2.3. Sample preparation and collection

2.3.1. Preparation of standard solutions

Two sets of the standard solutions were prepared. Standard stock solution of six aldehydes was prepared separately in methanol at the concentration of 50 mmol L^{-1} and stored at −20 °C. The daily standard working solution was prepared by mixing and diluting with ultra-pure water according to demand. A series of calibration standards containing known concentrations of IS (1-hydroxypyrene, 11.4 μmol L^{-1}) were prepared in EBC samples.

2.3.2. EBC sample preparation

Six EBC samples of lung cancer patients and eight EBC samples of volunteers were obtained from Jingzhou Center Hospital, Hubei, China and Huazhong normal University, separately. Ethical approval for the study was obtained from the Ethnics Committee of Jingzhou Center Hospital prior to the collection and analysis of human EBC samples. EBC samples were collected by a commercially available, FDA-approved, disposable EBC collection system, RTube™ technique. The subjects breathed at normal frequency and tidal volume through the mouthpiece for 10 min, typically yielding 0.7–2.5 mL of EBC. The EBC samples were stored at −20 °C in the freezer before use. In EBC analysis, 500 μL EBC was diluted with ultra-pure water according to demand.

2.4. Preparation of C8-functionalized magnetic particles

Firstly, preparation of amine-functionalized magnetic nanoparticles was performed as follows [11]: a solution of 1,6-hexanediamine (3.6 g), anhydrous sodium acetate (4.0 g) and $FeCl_3{\cdot}6H_2O$ (1.0 g) as a ferric source in glycol (30 mL) was stirred vigorously at 50 °C to give a transparent solution. Then this solution was transferred into a Teflon lined autoclave and reacted at 200 °C for 12 h. The products were then washed with water and ethanol to effectively remove the solvent and unbound 1,6-hexanediamine, and then dried at 50 °C before use. Subsequently, the amine- Fe_3O_4 MNPs was modified with chlorodimethyloctylsilane. A 10 mg amount of the obtained powder was dispersed in 1 mL of anhydrous pyridine with the aid of sonication. Then, 0.1 mL chlorodimethyloctylsilane was added to the above solution with vibrating, and the reaction was allowed to proceed for 12 h at room temperature. Finally, the magnetic nanoparticles were rinsed with