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Polyethylene glycol modified magnetic carbon nanotubes as nanosorbents for the determination of methylprednisolone in rat plasma by high performance liquid chromatography

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ABSTRACT

In this paper, polyethylene glycol modified (PEGylated) magnetic carbon nanotubes were developed as solid-phase extraction nanosorbents for the determination of methylprednisolone in rat plasma. The procedure mainly involved two steps including preparation of PEGylated magnetic nanosorbents and bioanalysis. Monodisperse magnetites (Fe_3O_4) anchored onto multi-walled carbon nanotubes (MWCNTs) were synthesized by a facile solvothermal synthesis method. The obtained MWCNTs-Fe_3O_4 nanomaterials were further non-covalently functionalized by a surfactant phospholipids-polyethylene glycol (DSPE-PEG). Owing to dispersibility and high enrichment ability, water-soluble PEGylated MWCNTs-Fe_3O_4 nanomaterials can provide more efficient way for the extraction of methylprednisolone than only MWCNTs-Fe_3O_4 used. The methylprednisolone could be easily extracted via π–π stacking interactions with PEGylated MWCNTs-Fe_3O_4. The captured methylprednisolone/nanosorbents were isolated from the matrix by placing a magnet, and desorbed by the elution solvent composed of acetonitrile. Extraction conditions such as amount of nanosorbents added, adsorption time, desorption solvent, and desorption time were investigated and optimized. The method recoveries were obtained from 88.2% to 92.9%. Limits of quantification and limits of detection of 0.01 and 0.005 μg/mL were acquired, respectively. The precision ranged from 4.2% to 7.8% for within-day measurement, and for between-day variation was in the range of 5.5–9.0%. Moreover, the analytical performance obtained by PEGylated magnetic MWCNTs was compared with that of magnetic MWCNTs. The results indicated that the approach based on PEGylated magnetic MWCNTs was useful for the analysis of methylprednisolone in the complex plasma.

1. Introduction

Methylprednisolone is effective when used in high doses within 8 h of acute spinal cord injury, which was initially reported in a series of National Acute Spinal Cord Injury Studies (NASCIS) in the 1990s [1]. However, overdose of methylprednisolone can lead to serious side effects, such as suppression of the immune system, gastric bleeding, acute corticosteroid myopathy and so on [2–13]. Thus, monitoring the plasma concentrations of methylprednisolone is highly desired. Many preparation methods have been developed for the analysis of methylprednisolone in plasma, mainly including liquid–liquid extraction [14] and solid-phase extraction [15].

In recent years, more and more studies focused on sample separation by nanomaterials because nano-size materials can promise much greater extraction capacity and efficiency due to significantly higher surface area-to-volume ratio [16–21]. Compared to liquid–liquid extraction and solid-phase extraction, the sampling technique based on nanomaterials is a harmless, efficient and low-cost. Many nanomaterials have been employed as solid-phase extraction nanosorbents for the analysis of drugs, peptides, proteins, contaminations and so on [22–32]. Among the popular nanosorbents, magnetic carbon nanotubes have become the most attractive nanocomposites. Carbon nanotubes with extremely large surface areas allow efficient loading of multiple molecules alongside the nanotube wall. The compounds containing aromatic structure can be adsorbed onto the polyaromatic surface of carbon nanotubes through π–π stacking interactions. However, as we know, only carbon nanotubes used are difficult to operate and the boring centrifugation processes are often required for further

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separation. Combination of carbon nanotubes with magnetic Fe_3O_4 nanoparticles can overcome the disadvantage. Due to the magnetic Fe_3O_4 ingredients, the multifunctional nanocomposites could facilitate the separation and recovery of the solid material from the solution by a magnetic field after extraction and enrichment. Lately, Morales-Cid et al. utilized MWCNTs-Fe_3O_4 to preconcentrate (fluoro)quinolones in human plasma [33]. Zhang and Shi developed hydroxylated MWCNTs modified with Fe_3O_4 nanoparticle for the determination of aconitines in human serum samples [29]. Our group employed magnetic MWCNTs with near-infrared radiation-assisted desorption for the determination of tissue distribution of doxorubicin liposome injects in rats [23]. The results demonstrated that the preconcentration ability of magnetic MWCNTs was acceptable and sample preparation operation was simplified. Even so, there are still inadequacies, especially for the drug analysis in the complex matrix by magnetic MWCNTs. In magnetic solid-phase extraction, it is important that nanosorbents are dispersed homogeneously in the sample solution, which can result in rapid extraction dynamics. Unfortunately, magnetic MWCNTs with poor solubility can hardly be expected to disperse uniformly in the complex plasma. Therefore, the functionalization of magnetic MWCNTs nanomaterials is necessary to render high aqueous solubility and stability in physiological solutions including plasma. DSPE-PEG compounds have been widely used to noncovalently functionalize carbon nanotubes because the two hydrocarbon chains of the lipid can strongly anchor onto the nanotube surface [34–36]. The PEGylated carbon nanotubes were stable in water and physiological buffers without aggregating and precipitating out of the solution [34,35].

Herein, we developed the PEGylated magnetic MWCNTs as solid-phase extraction nanosorbents for the determination of methylprednisolone in rat plasma. Different extraction parameters (amount of nanosorbents added, adsorption time, desorption solvent, desorption time) were investigated using blank plasma samples spiked with methylprednisolone and the results were discussed in detail. To demonstrate the validation of the proposed method, the quantification limit, detection limit, linearity, precision and accuracy were also studied.

2. Materials and methods

2.1. Material and chemicals

Methylprednisolone, dexamethasone (internal standard, I.S.), *n*-hexane, methanol and acetonitrile (ACN) were all >99% purity and purchased from Sigma–Aldrich Chemical Company (St. Louis, MO, USA). Pristine MWCNTs with 40–60 μm in length and 20–40 nm in diameter (95% purity in MWCNTs) were obtained from Shenzhen Nanotech Port Co., Ltd. (Shenzhen, China). Aqueous solutions were prepared using Milli-Q water by Milli-Q system (Millipore, Bedford, MA, USA). The other reagents including $FeCl_3{\cdot}6H_2O$, ethylene glycol, (polyethylene glycol)-5000 (PEG5000), and bicarbonate of ammonia were analytical grade and acquired from Sinopharm Chemical Reagent Co., Ltd. (China, Shanghai). 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy (polyethylene glycol)-5000] (DSPE-PEG5000) was bought from Avanti Polar Lipids Inc. (Alabaster, AL, USA).

A stock solution (1 mg/mL) of methylprednisolone was prepared by dissolving compound in 5 mL methanol and kept at 4 °C for use in two days. The working solutions of methylprednisolone (0.1, 1, 10, 50, 100, and 200 μg/mL) were obtained through diluting the stock solution with methanol. I.S. working solution was prepared with methanol at the concentration of 100 μg/mL. Drug-free plasma collected from healthy rats was stored at −20 °C for use in two days.

2.2. Preparation of PEGylated magnetic MWCNTs

The PEGylated magnetic MWCNTs nanomaterials were synthesized according to our previous report [36]. Firstly, 1 g of pristine MWCNTs was dispersed into 40 mL of concentrated sulfuric and nitric acids (1:3, v/v) with refluxing for 6 h. The oxidized MWCNTs solution was rinsed with 500 mL of distilled water for five times until the pH 7.0. The resulting MWCNTs were separated from the solution by vacuum filtration using 0.22 μm hydrophilic filter membrane and dried in vacuum at 60 °C for further use. Then, 40 mL ethylene glycol solution containing 0.2 g of $FeCl_3{\cdot}6H_2O$ was constantly stirring for 30 min. Subsequently, 0.3 g of oxidized MWCNTs was added. After sonication for 1 h, 1.25 g bicarbonate of ammonia (NH_4HCO_3) and 0.5 g PEG5000 were added with stirring for 30 min. The mixture was sealed in a Teflon-lined stainless steel autoclave and maintained at 200 °C for 8 h. The black products were rinsed with 20 mL of ethanol for five times through an Nd–Fe–B magnet and dried at 60 °C for 12 h. Finally, PEGylated magnetic MWCNTs nanomaterials were prepared as follows. 10 mg of magnetic MWCNTs was mixed with 10 mg of DSPE-PEG5000 in 10 mL aqueous solution for 1 h of sonication at 20 °C. The PEGylated magnetic MWCNTs products were rinsed with pure water for several times through an NdFeB magnet and finally resuspended in pure water.

2.3. Apparatus and measurement

Scanning electronic microscope (SEM) images were recorded on a Philips XL30 electron microscope (Netherlands). Transmission electron microscopy (TEM) images were obtained by a JEOL JEM-2010 microscope (Japan). The magnetic properties of magnetic MWCNTs and PEGylated magnetic MWCNTs were measured by a vibrating sample magnetometry (VSM, model-155, Digital Measurement System) at room temperature.

The iron content in PEGylated magnetic MWCNTs was performed by flame atomic absorption spectroscopy (Hitazhi, Japan). An iron hollow cathode lamp was used as a light source. The excitation current, slit width and absorption wavelength of iron were set at 15 mA, 0.2 nm and 248.3 nm, respectively. The calibration of iron was $y = 0.034x + 0.008$ ($r^2 = 0.998$), where y was absorption value of iron and x was the concentration of iron concentration (μg/mL).

The HPLC method was also used to quantify the methylprednisolone in the extracted samples with the mobile phase of ACN–water (40:60, v/v) through an Agilent ZORBAX Eclipse XDB C_{18} column (5 μm, 4.6 mm × 150 mm) at room temperature. The flow rate was kept at 1.0 mL/min. The injection volume of 20 μL was selected. The classic detection wavelength for methylprednisolone was set at 250 nm.

2.4. Optimization of extraction conditions by PEGylated magnetic MWCNTs

Many parameters that affect the extraction efficiency, such as amounts of nanosorbents added, adsorption time and desorption solvents, were studied. 100 μL of drug-free plasma with 10 μL of methylprednisolone (200 μg/mL) and 10 μL of I.S. solutions (100 μg/mL) was placed in a 1.5 mL Eppendorf tube (EP), and adjusted to 500 μL using pure water. Then, 10, 20, 30, and 50 μL suspension of nanosorbents (2 mg/mL) was appended to plasma liquid, respectively. The mixture was continuously ultrasonicated (200 W, 59 kHz) with 5 min in ice bath and kept undisturbed for 20 min to achieve the adsorption equilibrium of the target analytes between nanosorbents and the sample solution. The captured methylprednisolone/nanosorbents were collected and rinsed with water by placing a magnetite in the bottom of EP tube. According to our results in previous experiments, complete removal of water