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Carbon nanotubes reinforced hollow fiber solid phase microextraction for the determination of strychnine and brucine in urine

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ABSTRACT

A mixed matrix membrane (MMM), based on carbon nanotubes (CNTs) and hollow fiber (HF), was prepared and combined with solid phase microextraction (SPME) mode to determine strychnine and brucine in urine. This MMM was prepared by dispersing CNTs in water via surfactant assistance, and then immobilizing CNTs into the pores of HF by capillary forces and sonification. The prepared carbon nanotubes reinforced hollow fiber (CNTs–HF) was subsequently wetted by a few microliters of organic solvent (1-octanol), and then applied to extract the target analytes in direct immersion sampling mode. After extraction, analytes were desorbed via ultrasonic-assisted effect, and then detected via high-performance liquid chromatography (HPLC). To achieve the highest extraction efficiency, main extraction parameters such as the type and amount of surfactant, the diameter and doping level of CNTs, extraction time, desorption condition, pH value, stirring rate and volume of the donor phase were optimized. Under the optimum extraction conditions, the method showed good linearity ranges with correlation coefficients higher than 0.9990, good repeatability and batch-to-batch reproducibility with relative standard deviations (RSDs) less than 6% and 5% for strychnine and brucine, respectively, and low limits of detection (0.7 and 0.9 μg L^{-1} for strychnine and brucine, respectively). The recoveries were in the range of 83.81–116.14% at three spiked levels. The developed method was successfully applied to real urine sample with mean relative recoveries of 94.28% and 91.30% for strychnine and brucine, respectively. The developed method shows comparable results against reference methods and is a simple, green, and cost-effective microextraction technique.

1. Introduction

Strychnine and brucine are structurally related monoterpenoid indole alkaloids and mainly exist in the seed *Strychnos nux-vomica* L. (Semen Strychni), a traditional Chinese medicine which has been used in clinical practice for thousands of years [1]. These alkaloids when ingested could stimulate the central nervous system and make the sensory organs more sensitive [2]. So at low doses (such as at 10 mg daily dose of strychnine and 5 mg of brucine) they are often used to treat nervous diseases and vomiting as well as arthritic and traumatic pains [3]. However, strychnine and brucine are highly toxic and the margin between therapeutic and toxic doses is very narrow as it was reported to be fatal to man at doses of 30–90 mg [4,5]. Therefore, establishing a simple, direct and sensitive technique to monitor trace levels of strychnine and brucine in biofluid is of great importance for toxicological research, clinical study, forensic analysis and drug abuse.

Sample pretreatment plays an irreplaceable role in analyzing trace levels of analytes especially in complex matrices [6]. Recently, hollow fiber liquid-phase microextraction (HF-LPME) has shown its superiorities in measuring trace alkaloids from *S. nux-vomica* L. in urine [7,8]. However, organic liquid membrane immobilized in the wall pores of hollow fiber has poor stability and would run off after relatively long extraction time, which limits the extraction efficiency. Moreover, an assessment of permeability and selectivity has shown asymptotic limitations on the separation capability of pure polymeric membranes [9]. Consequently, developing novel membrane systems is of great importance. MMMs which combine polymeric materials with inorganic fillers such as zeolites and fullerene have attracted much interest

Abbreviations: CNTs–HF, carbon nanotubes reinforced hollow fiber; CNTs–HF-SPME, carbon nanotubes reinforced hollow fiber solid-phase microextraction; HF, hollow fiber; HF-SPME, hollow fiber solid-phase microextraction; HPLC, high-performance liquid chromatography; HPLC-PDA, high-performance liquid chromatography photodiode array detection; MMM, mixed matrix membrane; MWCNTs, multi-walled carbon nanotubes.

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[10,11], due to their excellent thermal, electrical and structural properties; carbon nanotubes which are essentially graphene sheets rolled into tubes as single-walled or multiple-walled structures, can be interesting materials for membrane systems [12]. Incorporating CNTs in a membrane system may offer several advantages during the extraction. Theoretical studies have suggested that CNTs have high flux, which are attributed to the smooth CNT surface, frictionless rapid transport and molecular ordering. Besides being excellent transporters, CNTs are also effective sorbents, particularly for organics [13,14]. Together these can increase the selective partitioning and permeation of the solute. However, CNTs tend to agglomerate into bundles when dispersed in either water or organic solvents due to strong van der Waals interactions, so the realization of their potential has been limited [15]. Moreover, the suitable interfacial interactions between CNTs and polymers contribute greatly to the improvement in limits of detection and enrichment factors (EFs). Some solutions like functionalization of the surface of CNTs [16], addition of surfactants [17] and sol–gel technology [18] have been used to improve the dispersibility of CNTs and strengthen the compatibility between CNTs and polymers. Recently, a novel extract material, carbon nanotubes reinforced hollow fiber (CNTs–HF) was prepared by filling CNTs in the wall pores of hollow fiber using sol–gel technology, and then combined with SPME mode to detect trace analytes in relatively complicated matrices [19,20]. However, this preparation method of CNTs–HFs showed some limitations. To begin with, CNTs should be oxidized to increase dispersibility in sol–gel, which would destroy their effective properties. Furthermore, it needed relatively long time to prepare the sol solution. Besides that, the preparation process involved various factors such as pH, the molar ratios of precursor, organic solvent and water, and the type and amount of catalytic agent which would affect its performance and make the batch-to-batch reproducibility poor. Dispersing CNTs by the surfactant is particularly attractive, as it preserves the delocalized Π-electron network of the nanotube sidewall, which is of great importance to achieve the high extraction efficiency of CNTs [20]. Surfactant can interact with CNTs through various interactions, like hydrophobic interaction between hydrophobic chain of surfactant and side walls of CNTs, or Π–Π interaction of benzene rings on surfactants with CNT surfaces, or Lewis acid–base interaction between MWCNTs as Lewis base and surfactant as Lewis acid [19]. At present, a novel MMM named CNTs–HF was prepared by using surfactant, and then applied to SPME to determine strychnine and brucine at trace levels in urine. The main parameters affecting the extraction efficiency are optimized.

2. Experimental

2.1. Chemicals and materials

Certified standards of strychnine, brucine and berberine hydrochloride (internal standard, I.S.) were obtained from the National Institute for the Control of Pharmaceutical Products (Beijing, China) with purity higher than 98% and their chemical structures are as shown in Fig. 1. HPLC-grade acetonitrile was purchased from Merck Co. (Darmstadt, Germany) while hexadecyltrimethyl ammonium bromide (CTAB) and other analytical grade chemicals were bought from Tianjin Chemical Reagent Co. (Tianjin, China). Deionized water was prepared by using an OKP purification system (Model: Exceed-AC-16, Shanghai Laikie Instrument Co. Ltd., China) and then used to prepare mobile phase and sample solution. Accurel Q3/2 polypropylene hollow fiber membrane (200 μm thick wall, 600 μm inner diameter and 0.2 μm average pore size) was provided by Membrana GmbH (Wuppertal, Germany). MWCNTs were purchased from Chengdu Organic Chemical Co. Ltd., Chinese Academy of Sciences (Chengdu, China). MWCNTs with mean diameters of 8–15 nm and 30–50 nm, lengths of 0.5–2 μm and purity of higher than 95% were used.

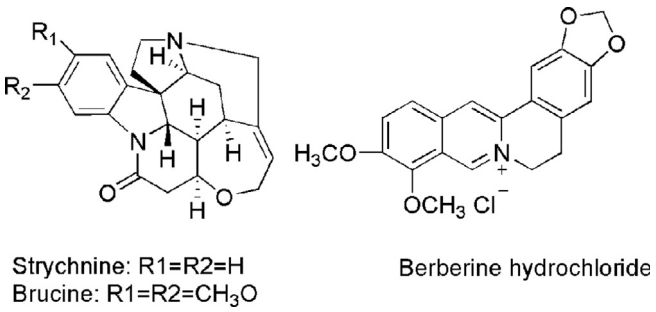

Fig. 1. The structures of the analytes and I.S.

2.2. Apparatus and chromatographic conditions

Waters Corp. series HPLC coupled with UV detection with a PDA detector (Mode 2996) was used. Data analysis was done by a Waters Millennium^{32} software for peak identification and integration. Chromatographic separation of the analytes was achieved on a Kromasil C_{18} column (5 μm, 4.6 mm × 250 mm i.d.; Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, China). The mobile phase made up of acetonitrile and 0.1% phosphoric acid (the ratio starting with 18:82 and decreasing to 11:89 within 13 min and then increasing to 70:30 in the next 5 min, finally maintaining this ratio till the end) was filtered by a Milli-Q filtering system, degassed with helium (He) and delivered by a Waters quaternary pump (Model Delta 600E). The flow rate of the mobile phase was 1.0 mL min^{-1} and signals were monitored at 254.4 nm for strychnine and 263.8 nm for brucine and I.S. The temperature of the column during analysis was maintained at 25 °C.

2.3. Dispersion of CNTs

To prepare surfactant stabilized MWCNTs dispersions, 0.5 mg mL^{-1} of 3 mL CTAB was prepared via ultrasonic-assisted effect, to which 12.0 mg of MWCNTs was added and bath sonicated for an hour. Finally, the CNTs dispersion was centrifuged at 4000 rpm for another hour to precipitate large bundles [21].

2.4. Preparation of CNTs -reinforced hollow fiber

The polypropylene hollow fiber was cut manually into segments of 4 cm, ultrasonically cleaned with acetone for 10 min to remove any impurities, and then dried in air. In order to prepare the nanotube immobilized membrane, hollow fiber segments were put into the aqueous CNTs dispersion; CNTs would be held in the wall pores of hollow fiber via sonification and capillary forces after sonication at room temperature for 3 h. After that, deionized water was used to remove MWCNTs on the surface and the inner lumen of hollow fibers. Finally, the prepared CNTs–HF was dried under 80 °C in a drying cabinet for 1.5 h. To study the effects of surfactant on the extraction capability, hollow fiber with different types and concentrations of surfactant was also prepared by the above procedure. Fig. 2 shows the scanning electron microscopy image of MWCNTs held in the wall pores of hollow fiber.