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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.

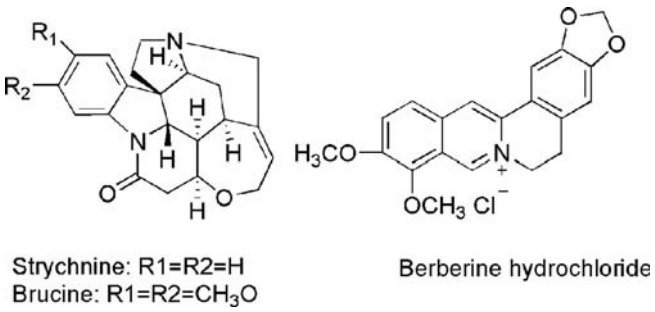

Fig. 1. The structures of the analytes and I.S.

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.

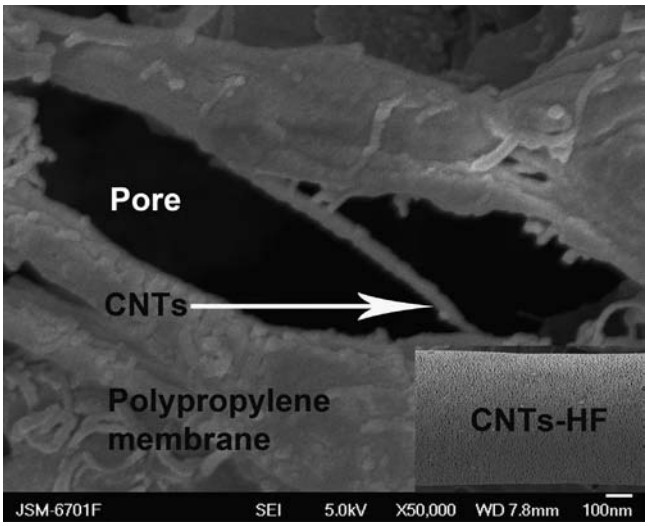

Fig. 2. Scanning electron microscopic image of carbon nanotubes reinforced hollow fiber.

2.5. Preparation of standard solutions

The stock solutions of strychnine, brucine and I.S. dissolved in methanol were stored at 4 °C. Working solutions at different concentrations were prepared daily by diluting the stock solutions with deionized water.

2.6. Preparation of real samples

Urine provided by a volunteer was gathered daily at a set time and then stored at −20 °C. It was diluted to half with deionized water and adjusted to pH value of 14 by sodium hydroxide.

2.7. CNTs–HF-SPME procedure

A 50-μL microsyringe with a cone tipped needle was used for SPME. The syringe needle was first pierced through the silicon septum in the screw cap and then inserted into the lumen of CNTs–HF. Then, the CNTs–HF was impregnated in 1-octanol for 1.5 min. After that, the end was sealed with heated tweezers, and then ultrapure water was used to remove excess 1-octanol on the surface. Finally, the prepared device was introduced into the sample solution for extraction. The extraction process was performed for 70 min at 1050 rpm of agitation rate. After extraction, the syringe together with CNTs–HF was taken out of the sample solution. The hollow fiber section was firstly cleaned with ultrapure water, then wiped up with filter paper and finally put into an end-sealed pipette tip with 80 μL methanol to desorb analytes ultrasonically for 25 min. Finally, 60 μL of the desorbed solution was drawn out, concentrated and diluted to 25 μL, of which 20 μL was injected. To eliminate the possible carry-over effect, fresh CNTs–HF was used in each experiment.

3. Results and discussion

3.1. Optimization of extraction procedure

In order to achieve high extraction efficiency and enrichment factor, the important parameters were optimized. In these optimization experiments, 100 μg L^{-1} of analytes and 50 μg L^{-1} of I.S. were used and each experimental condition was operated in four parallel experiments.

3.1.1. Dispersion of MWCNTs

In the present experiment, the primary and prerequisite step is to obtain well dispersed aqueous solution of MWCNTs, and surfactant-assisted dispersion was applied in order not to destroy effective properties of CNTs. In the preliminary experiments, three different types of surfactants at various concentrations were compared. They are *p*-octyl polyethylene glycol phenylether (Triton X-100, nonionic surfactant, critical micelle concentration (CMC) value: 0.125 mg mL^{-1}), hexadecyltrimethyl ammonium bromide (CTAB, cationic surfactant, CMC value: 0.30 mg mL^{-1}) and sodium dodecylbenzenesulfonate (SDBs, anionic surfactant, CMC value: 0.35 mg mL^{-1}). It is generally recommended in the literature to use surfactant concentrations equal to critical micelle concentration values [22], so CNTs-HFs prepared by each surfactant at five different concentrations centering the CMC were studied in this experiment. Clearly, the concentration ranges of Triton X-100, SDBs and CTAB were 0.03–0.25 mg mL^{-1}, 0.09–1.40 mg mL^{-1} and 0.10–0.50 mg mL^{-1}, respectively. Finally, 0.5 mg mL^{-1} of CTAB provided the best dispersing assistance for the CNTs. This is because MWCNTs as Lewis base can have additional Lewis acid–base interaction with amine head group of CTAB as Lewis acid besides the hydrophobic interaction [21]. What is more, the diameter of CNTs also poses an effect on CNT interactions. In this experiment, we compared two specifications of CNTs which had the same length but different diameters. The results showed that the CNTs with smaller diameter were easier to be dispersed. It can be noted that the contact surface between CNTs becomes bigger as their diameters increase. So the interaction between the CNTs is stronger for the bigger diameters. Accordingly, the stronger interactions between CNTs would cause higher possibility of forming aggregates while dispersing them in water [23]. Furthermore, for brucine, higher EF (132-fold) was obtained by CNTs with smaller inner diameter which was in contrast to 99-fold by the ones with larger inner diameter. However, the extraction efficiencies for strychnine provided by the two specifications of CNTs were at the same level. So in this experiment, the CNTs with the diameter of 8–15 nm were chosen. Usually, the extraction efficiency becomes better accompanied by an increase in CNTs doping level [24]. Therefore, the CNTs–HF was prepared with CNTs doping level from 2.50 to 5.80 mg mL^{-1}. The results illustrated that the CNTs doping levels had an important role in the extraction ability of sorbent. Increasing the amount of CNTs would make an improvement in extraction efficiency, while the doping levels above 4.0 mg mL^{-1} had a negative influence as agglomeration of CNTs bundles would form [25]. According to these results, the CNTs doping level of 4.0 mg mL^{-1} was chosen as the optimal value.

3.1.2. Organic solvent conditioning

Though CNTs act as the main sorbent in this SPME mode, the organic solvent has its special functions. Both CNTs and polypropylene membrane are hydrophobic in nature, so CNTs–HF was hard to be wetted when exposed directly to a sample solution, which leads to slow extraction rates and low extraction efficiencies [26]. 1-octanol is a commonly-used wetting solvent which was not only compatible with the fiber and immiscible in water but also stable enough over relatively long extraction time [27]. In our previous experiments, 1-octanol performed perfectly its wetting function via the higher extraction efficiency of the conditioned CNTs–HF than the one without solvent conditioning [19,20]. To eliminate the possible extraction effect of trace 1-octanol, CNTs–HF was cleaned with purified water and then wiped up with filter paper after extraction.

3.1.3. Selection of extraction time

The absorption and desorption of analytes on CNTs–HF is a dynamic equilibrium. So extraction efficiency usually varies with

extraction time and reaches the highest point when arriving at extraction equilibrium [28]. Thus the function of extraction time was examined in this study. As the corresponding results in Fig. 3 illustrated, an increase in the extraction time would result in higher enrichments of both strychnine and brucine. The improvement of extraction efficiency was most significant from 30 min to 70 min, and longer extraction time led to a slight decrease in peak area. 1-octanol would flow away during the extraction, so the contact between the analyte and the MWCNTs became poor [29,30]. Although the extraction time was relatively long, a large number of samples may be extracted simultaneously due to the simple setup and low cost of the CNTs–HF.

3.1.4. Desorption condition

As in the case of SPME, analytes were desorbed by an organic solvent from the MWCNTs after extraction. The analytes considered here are relatively hydrophobic and may not be easily desorbed. Therefore, a suitable organic solvent and ultrasonic-assisted desorption time were needed in the process [26]. Both the polypropylene membrane and MWCNTs are insoluble in most common organic solvents such as acetone, methanol, dichloromethane and hexane. Among them, acetonitrile and methanol as widely-used mobile phases would not demonstrate strong and clear interferential peaks. So extraction efficiencies of the two desorption solvents were investigated. The results showed that methanol gave higher peak area responses (Fig. 4) and sharper chromatographic peaks for the target analytes. So, in this experiment, methanol served as the desorption solvent. The effect of desorption time over the range of 10–35 min was studied subsequently. Fig. 5 indicated that peak area responses of analytes obtained the highest values at desorption time of 25 min. An obvious decrease in peak area was observed when longer desorption time was applied since desorbed analytes would be absorbed by CNTs again and would diffuse to the pores or lumen of hollow fiber with the help of concentration difference and ultrasonication [20].

3.1.5. Effect of pH value of the sample solution

In SPME, mass transfer is promoted by optimal pH conditions in sample solution. Since analytes are absorbed by MWCNTs via the main interactions like hydrophobic and π–π interactions, the pH value of the sample solution should suppress analytes ionization to keep them in molecular form. Considering that strychnine and brucine are basic compounds, a base like sodium hydroxide should

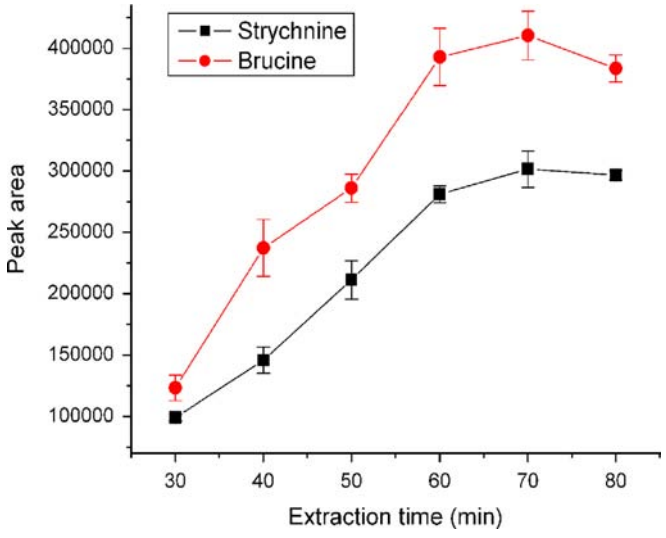

Fig. 3. Effect of extraction time on the peak areas of strychnine and brucine extracted with CNTs–HF-SPME. Extraction conditions: organic solvent, 1-octanol; sample volume, 10 mL; sample pH, 14; stirring rate, 1050 rpm; desorption solvent, methanol; and desorption time, 25 min.

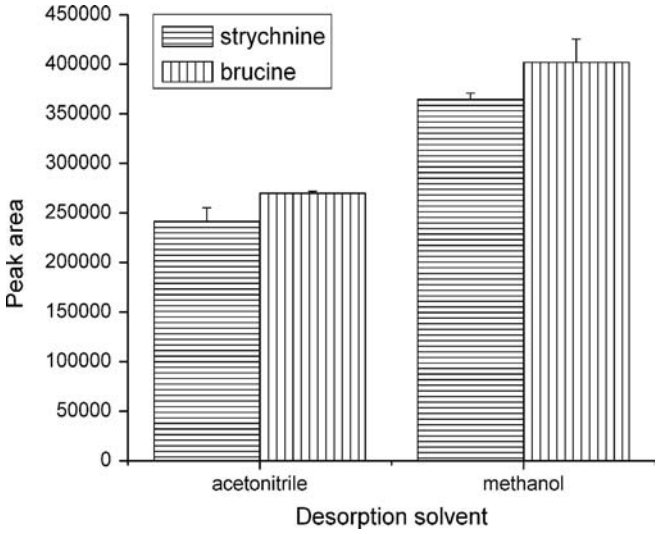

Fig. 4. Effect of desorption solvent on the peak areas of strychnine and brucine extracted with CNTs–HF-SPME. Extraction conditions: extraction time, 70 min, and the others were the same as those of Fig. 3.

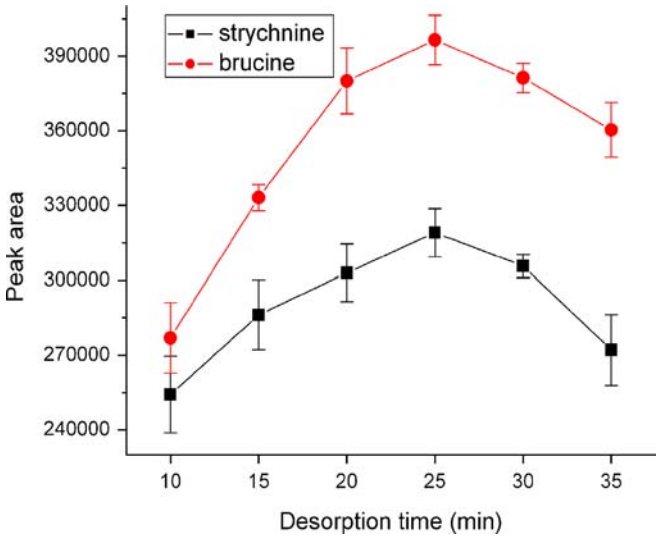

Fig. 5. Effect of desorption time on the peak areas of strychnine and brucine extracted with CNTs–HF-SPME. Extraction conditions were the same as those of Fig. 4.

be added to make the pH of sample solution higher than 7. In the present study, the extraction was performed under different pH conditions ranging from 10 to 14 (Fig. 6). An increase in peak area was observed when the pH value increased. Therefore, the optimum pH value of the sample solution was selected as 14.

3.1.6. Effect of donor phase volume

The sample volume is important in determining the loading capacity of MWCNTs. Meanwhile, it affects the overall time required to reach equilibrium [31]. What is more, EFs can be improved by increasing the volume ratio of donor phase to acceptor phase. So five different sample volumes from 6 to 14 mL at the interval of 2 mL were studied (Fig. 7). The results shown in Fig. 7 indicated that the peak areas of the two target analytes increased with sample volume increasing from 6 to 10 mL, but decreased with further increasing. This phenomenon might be attributed to the fact that lower EFs were obtained in smaller volume ratios, while the MWCNTs capacity would be

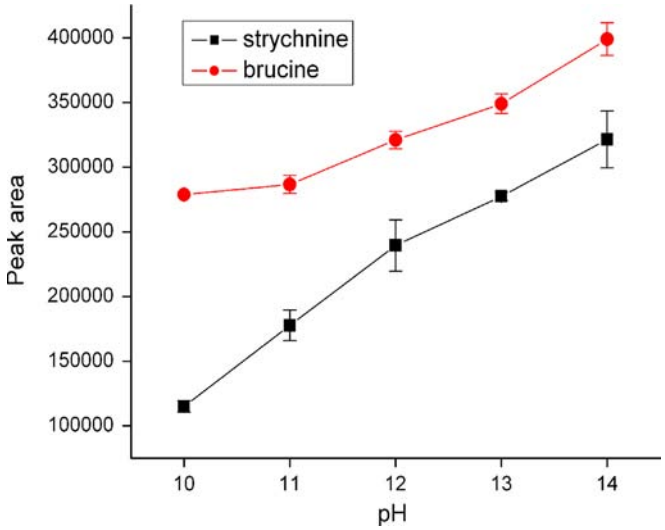

Fig. 6. Effect of sample pH on the peak areas of strychnine and brucine extracted with CNTs–HF-SPME. Extraction conditions were the same as those of Fig. 4.

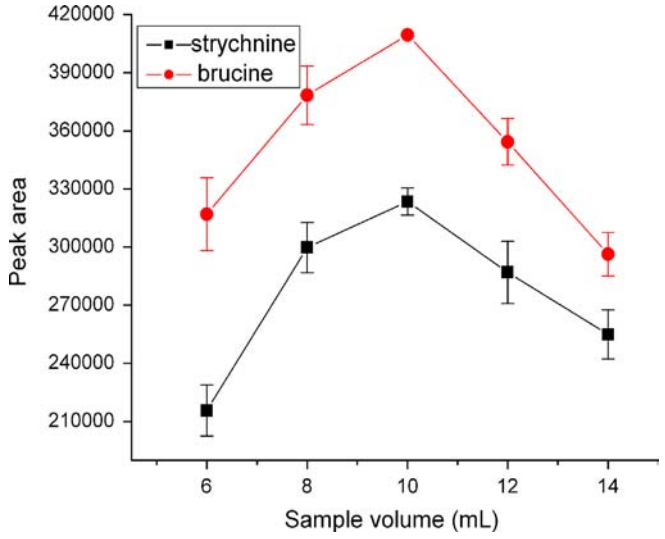

Fig. 7. Effect of sample volume on the peak areas of strychnine and brucine extracted with CNTs–HF-SPME. Extraction conditions were the same as those of Fig. 4.

saturated and poorer mass-transfer kinetics occurred for a larger sample volume [31,32].

3.1.7. *Effect of stirring rate*

Agitation of the sample solution increases mass transfer and accelerates the extraction kinetics, which would reduce the time needed to reach equilibrium [33]. So different stirring rates between 750 and 1150 rpm were tested to determine their effects on the pre-concentration of the analytes. According to the experimental results in Fig. 8, 1050 rpm was selected as the optimum agitation speed which could yield the highest extraction efficiency. At higher stirring rates, vigorous stirring may increase shearing strength, which poses a negative effect on the adsorption process owing to a mesoporous coating on the pores of the hollow fiber [33].

3.1.8. *Effect of salt*

Depending on the nature of the target analytes, addition of salt to the sample solution can affect the solubility of the analytes and

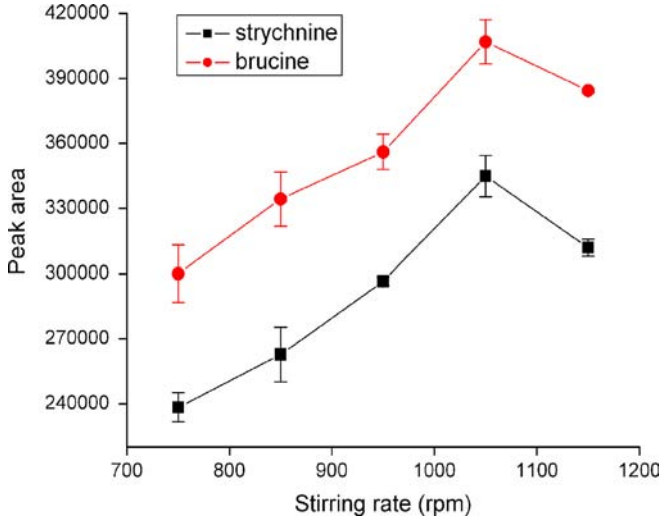

Fig. 8. Effect of stirring rate on the peak areas of strychnine and brucine extracted with CNTs–HF-SPME. Extraction conditions were the same as those of Fig. 4.

therefore extraction efficiencies would change. The amount of extracted analytes decreased with the increase of salt concentration in the sample, which was demonstrated in previous experiments [7,34]. So in our experiment, extraction was conducted without addition of salt.

3.2. *Method evaluation*

The performance parameters of the CNTs–HF-SPME technique, such as linearity, limits of determination (LODs) and quantification (LOQs), EF, repeatability and batch-to-batch reproducibility are listed in Table 1. Blank urine sample spiked with 100 μg L^{-1} of analytes and 50 μg L^{-1} of I.S. were used to evaluate the above parameters except the determination of LODs and LOQs. As surfactant-assisted dispersion is a non-destructive method, CNTs would perform its effective sorption and extraction property. Compared with the produced CNTs–HF, hollow fiber with only surfactant immobilized in its wall pores had negligible extraction ability, so CNTs played irreplaceable roles in this novel microextraction mode. The satisfactory EF was mainly attributed to the Π–Π and hydrophobic interactions. The calibration curves in this study adopted the internal standard method in which linearities were constructed by plotting the peak area ratio of analytes to I.S. against their concentration ratio at six different concentrations and each concentration level was carried out in four parallel experiments. As shown in Table 1, both of the target analytes had wide linearity ranges and satisfactory correlation coefficients. Moreover, the linear regression equations for strychnine and brucine were $y=2.846x-0.076$ and $y=1.908x+2.550$, respectively. The LODs were 0.7 μg L^{-1} for strychnine and 0.9 μg L^{-1} for brucine which are comparable with other methods shown in Table 2. Meanwhile the LOQs of 2.0 μg L^{-1} and 3.0 μg L^{-1} were obtained for strychnine and brucine, respectively.

The intra-day and inter-day precisions of the instrument were evaluated by injecting standard solution repeatedly. Intra-assay precision was measured for five continuous injections during the same day whereas inter-assay precision was measured on three consecutive days. The RSDs of peak areas in intra-assay precision were 1.71% and 1.74% for strychnine and brucine while 3.12% and 3.64% for strychnine and brucine, respectively, in inter-assay precision.

Five replicate experiments of the urine samples containing 100 μg L^{-1} of analytes and 50 μg L^{-1} of I.S. were performed with the same batch fiber under optimum conditions to give RSD values

Table 1
Performance parameters of the CNTs–HF-SPME.

Analytes	EF[a]	LOD[b] ($\mu g\ L^{-1}$)	LOQ[c] ($\mu g\ L^{-1}$)	Linear range ($\mu g\ L^{-1}$)	R	RSD (%) ($n=5$)	
						Repeatability	Batch-to-batch reproducibility
Strychnine	112	0.7	2.0	5–1500	0.9995	4.35	5.47
Brucine	132	0.9	3.0	5–2500	0.9990	4.58	3.28

[a] EF was the concentration ratio of analyte presented in the injection solution to that originally presented in the sample.
[b] LOD was defined as the concentration for which the signal-to-noise ratio was 3.
[c] LOQ was defined as the concentration for which the signal-to-noise ratio was 10.

Table 2
Comparison of some methods used for determination of strychnine and brucine.

No.	Matrix	Target compounds	Extraction method	Detection	EF	LOD($\mu g\ L^{-1}$)	LOQ($\mu g\ L^{-1}$)	Recovery (%)	Ref.
1	Urine	Strychnine	HF-LPME	CE-PDA	50	1	–	–	[3]
		Brucine			35	2	–	–	
2	Urine	Strychnine	HF-LPME	CE-PDA	50	1	–	–	[7]
		Brucine			35	2	–	–	
4	Serum	Brucine	SWNTs-MIP	HPLC-PDA	–	2.1×10^{-7} M	–	99.5–103.2	[34]
3	Blood	Strychnine	SPME	GC–MS	–	6.83	8.91	–	[35]
5	Semen Strychni	Strychnine	GNPs-CPE	DPV	–	4.6×10^{-7} M	–	–	[36]
6	Serum	Strychnine	SPE	HPLC-PDA	–	–	9.1	62.4 ± 10.0	[37]
		Brucine			–		4.1	69.2 ± 1.2	
	Urine	Strychnine			–		7.5	63.1 ± 11.5	
		Brucine			–		4.1	62.6 ± 1.0	
7	Urine	Strychnine	SPME	HPLC-PDA	112	0.7	2.0	83.81–91.84	This one
		Brucine			132	0.9	3.0	102.45–116.14	

HF-LPME: hollow fiber liquid phase microextraction; MIP: molecular imprinted polymer; SPME: solid-phase microextraction; GNPs-CPE: gold nanoparticles modified carbon paste electrode; SPE: solid phase extraction; GC–MS: gas chromatography–mass spectrometry; and DPV: differential pulse voltammetry.

of 4.35% and 4.58%. Four different batch fibers were prepared by the same procedure and used for evaluating batch-to-batch reproducibility. The results demonstrated that the RSD values of peak areas were 5.47% and 3.28% for strychnine and brucine, respectively, which proved that this preparation method of CNTs–HF via surfactant and sonification was quite stable and acceptable.

3.3. Analysis of real urine samples using CNTs–HF-SLPME

3.3.1. Matrix effect

The accuracy of the method was confirmed by the spiked recovery test. Firstly, 40 $\mu g\ L^{-1}$ of analytes and 50 $\mu g\ L^{-1}$ of I.S. were added to the urine samples as original amount. Then, three different quantities, i.e. 80%, 100% and 120%, which stood for low, medium and high levels, respectively, of the above concentration were added. Finally, the three sets of spiked urine samples were extracted and analyzed. The recoveries were in the range of 83.81–91.84% for strychnine and 102.45–116.14% for brucine with the largest RSD values less than 8.12%, which proved that this method was sensitive and applicable.

The relative recovery, the ratio of peak area of the extracted analytes in urine and ultrapure water spiked at the same concentration level, was investigated to study the matrix effect. The CNTs–HF-SPME gave mean relative recoveries of 94.28% and 91.30% for strychnine and brucine, respectively. These indicated that the matrix effects on CNTs–HF-SPME method were negligible owing to the small pore size of hollow fiber and selective absorption of CNTs.

3.3.2. Determination of analytes in real samples

The proposed CNTs–HF-SPME method was employed to extract strychnine and brucine in real human urine samples in order to investigate its applicability. A 5.0 mL aliquot of urine sample was diluted to a total volume of 10 mL and then the pH value was adjusted to 14.0 with sodium hydroxide. Since the amount of residual strychnine and brucine in urine was about 40 $\mu g\ L^{-1}$ after taking medicines containing Semen Strychni [3,4,7], 40 $\mu g\ L^{-1}$ of analytes and 50 $\mu g\ L^{-1}$ of I.S. were added into the urine sample and then extracted and analyzed as the proposed procedure. The typical chromatogram of blank and spiked human urine sample extracted with CNTs–HF-SPME is illustrated in Fig. 9.

3.3.3. Method comparison

3.3.3.1. Comparison of CNTs–HF preparing procedures. In this experiment, CNTs–HF was prepared via surfactant assistance procedure instead of sol–gel technology which was developed in our previous study [20]. Some aspects of the CNTs–HF such as extraction efficiency, thermal stability, batch-to-batch repeatability and the amount of CNTs loaded per HF were compared between the two preparing procedures. The CNTs–HF prepared by the two methods was separately used to extract the two analytes at spiked level of 100 $\mu g\ L^{-1}$ in urine under the same extraction conditions. The sol–gel method provided 102- and 91-fold EFs for strychnine and brucine, respectively, which are lower than that of surfactant assistance procedure. In terms of thermal stability, the prepared CNTs–HF was heated at a certain temperature in the range of 80–200 °C for 3 h, and then used to extract the analytes in urine. The results showed that for CNTs–HF made by surfactant-aided procedure, the extraction efficiency remained nearly the same with increasing temperature from 80 to 150 °C, but decreased with further increasing of temperature. What is more, the CNTs–HF would shrink when the temperature exceeded 170 °C; consequently, it could not be used for extraction. Whereas, the CNTs–HF prepared by sol–gel method would retain the extraction efficiency till 135 °C and the appearance would also change when the temperature exceeded 170 °C. For batch-to-batch repeatability, sol–gel displayed a disadvantage with an RSD of 9.32% for strychnine and 7.17% for brucine, which could be attributed to the fact that the formation of homogenous sol–gel dispersion was affected by too many factors such as pH,

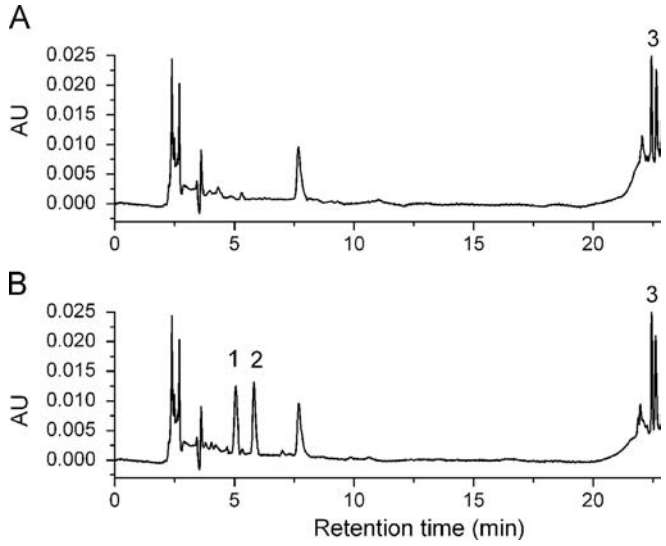

Fig. 9. Chromatograms of (a) blank urine spiked with 50 μg L^{-1} of I.S. and (b) urine spiked with 40 μg L^{-1} of analytes and 50 μg L^{-1} of I.S. extracted with CNTs–HF-SLPME. Peak 1: brucine, peak 2: strychnine, peak 3: I.S. and detection wavelength: 255.4 nm.

temperature, stirring time, ratios of precursors, etc. The amount of CNTs loaded per HF was also compared between the two preparation methods. Twenty HFs with the length of 4 cm were weighted before and after loading the CNTs. The results showed that 0.722 and 0.573 mg of CNTs were loaded per HF through surfactant-aided and sol–gel preparing methods, respectively, which are in accordance with the subsequent result that higher extraction efficiency was provided by CNTs–HF prepared by the former method.

3.3.3.2. Comparison of microextraction modes. In our previous study, CNTs–HF prepared with sol–gel technology could be used not only in conventional solid phase microextraction mode (SPME) [19], but also in solid/liquid phase microextraction mode (SLPME) [20]. In this study, the two microextraction mode was also compared by applying CNTs–HF prepared by surfactant assistance procedure. For SLPME, 1-octanol was filled with the pores and lumen of CNTs–HF, and then the whole assembly was used for the extraction following the same procedure for SPME. The results suggested that SLPME provided slightly higher EFs (130- and 147-fold for strychnine and brucine, respectively), lower LODs (0.5 and 0.7 μg L^{-1}) and LOQs (1.7 and 2.6 μg L^{-1}) than those of SPME. Considering that the advantages of SLPME over SPME were not obvious, the more widely accepted extraction mode SPME was chosen in the present work.

3.3.3.3. Comparison of published methods to determine the analytes. Table 2 describes the methods used to determine strychnine and brucine in several matrices in recent years. In comparison, this novel method called CNTs–HF-SPME has the advantages of improved simplicity, low cost, relatively low LODs and feasible conversion into green analytical techniques.

4. Conclusion

A successful development and application of a new SPME method for the measurement of strychnine and brucine in urine sample are described in this experiment. The experimental setup is very simple and highly affordable. Under ultrasonic-assisted effect, CNTs dispersing in surfactant aqueous solution are immobilized in the wall pores of hollow fiber to develop a novel mixed matrix membrane, carbon nanotubes reinforced hollow fiber. Then solid phase microextraction mode was applied to measure analytes in urine sample. The hollow fiber is disposable, so the single use of hollow fiber would reduce the risk of cross-contamination and carry-over problems. Moreover, the cleanup function of hollow fibers and selective absorption ability of CNTs make matrix effect negligible, so this mode can be applied in relatively complicated matrices directly. In conclusion, this method is simple, highly affordable and sensitive and is a novel and simple application of SPME using the unique features of CNTs.

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