Analytical Methods

PAPER

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Fabrication of multi-walled carbon nanotubes/oxide reinforced hollow fibers by layer-by-layer self-assembly for rapid determination of metronidazole in milk

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Three types of multi-walled carbon nanotubes (MWCNTs)/oxide reinforced hollow fibers, *i.e.* MWCNTs/SiO_2, MWCNTs/TiO_2, MWCNTs/ZrO_2, based on sol–gel techniques were fabricated, compared and applied to extract metronidazole in milk samples by solid phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS). The factors influencing the extraction and desorption process were optimized, and the adsorption mechanism of MWCNTs/SiO_2 reinforced hollow fibers was briefly discussed. MWCNTs/SiO_2 reinforced hollow fiber might be selective to some organic compounds due to specific and non-specific adsorption of MWCNTs and SiO_2 nanoparticles. It was found that the method provided linear range from 0.01–1000 mg L^{-1} ($R = 0.9985$), low detection limit of 0.01 mg L^{-1}, preferable recoveries (69–96%) at three different concentrations. The obtained results demonstrated that MWCNTs/SiO_2 hollow fiber solid phase microextraction could become a potential tool for quality control to monitor the amount of metronidazole residues in milk products.

1. Introduction

Metronidazole (2-methyl-5-nitroimidazole-1-ethanol) is a nitroimidazole derivative that is commonly used to treat protozoal diseases including trichomoniasis and giardiasis.[1,2] It is one of the most commonly used drugs all over the world, one of the top 100 most prescribed drugs in United States and one of the 10 most used drugs during pregnancy.[3,4] It is also widely used in China as veterinary antibiotics to treat mastitis, anaerobic infections of the gastrointestinal canal and preventive infection of obstetric operations in dairy cattle.[5] Therefore, the possible presence of metronidazole residues will influence the quality of raw milk due to the risk of direct toxic effects to consumers, allergic reaction in hypersensitive individuals, and the development of antibiotic-resistant pathogens.[4,6,7] Since a spate of scandals about dairy safety have occurred in China, undoubtedly, rapid, accurate, environmental-friendly analytical methods for the determination of veterinary drug residues and other contaminants in raw milk are urgently needed for process monitoring and control in dairy plants.

Biological and environmental sample analysis is often complicated by low analyte concentrations, complex sample matrices and the limited sample volumes available for the determinations.[8–10] The most common sample preparation technique is solid-phase microextraction (SPME), which still presents some problems such as fiber consubstantial properties and reduced possibility of carry-over.[11–14] Among the different approaches to stationary phase development for SPME fibers, Xu introduced a novel SPME technique that uses ZrO_2 hollow fiber as a sorbent opening the possibility to fabricate numerous interesting inorganic hollow fiber structures with a homogeneous controllable wall and porous substructure,[15] and introducing a novel SPME technique: hollow fiber solid phase microextraction (HF-SPME). This method has the advantages of simplicity, good accuracy and precision, relatively short extraction time, low cost and is friendly to the environment.

Recently, a silica-based, organic-inorganic polymer containing carbon nanotubes that was prepared based on sol–gel techniques was injected into a piece of polypropylene hollow fiber and the process of *in situ* gelation commenced in the fiber,[14,16–21] which was subsequently used to perform the HF-SPME procedure. Inspired by this *in situ* gelation procedure, our team has prepared oxide (ZrO_2, TiO_2 and SiO_2) hollow fibers and studied their application to different kinds of complex sample matrices.[22–25] The results were satisfactory; however, these oxide hollow fibers still have disadvantages, such as relatively low specific surface areas and absorption capacity. The advantages of multi-walled carbon nanotubes (MWCNTs) are their extraordinary adequacy of structural, mechanical and electronic properties which make them potentially useful for

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nanotube-reinforced materials, such as sorbents for SPME.[26–30] MWCNTs incorporated into the extractor phase occurs more homogenously, the number of active adsorbent sites is high, and the chemical bonds between analyte and extractor are stronger.[16]

The idea therefore was to obtain a device based on MWCNTs, which act as an analyte trap, resulting in higher selectivity and enrichment due to the specific and non-specific adsorption of MWCNTs. We prepared novel composite hollow fibers with multi-walled carbon nanotubes and mesoporous nanoparticles of inorganic oxide (ZrO_2, TiO_2 and SiO_2) co-deposited on the porous surface of polypropylene (PP) hollow fiber by sol–gel techniques. Not only do these novel composite materials help to controllably enhance surface area and pore-size distribution of the PP hollow fiber, but they also avoid the centrifugation process requirement by using powder as the adsorbent. Besides, the composite hollow fibers might be selective to some organic compounds due to electrostatic attraction, π–π stacking, hydrophobic interaction and hydrogen bonding. These MWCNTs/oxide reinforced hollow fibers are designed to pre-concentrate organic compounds directly from complicated matrices.

Conventionally, routine methods for analysis of low level of contaminants in dairy products involved complicated and time-consuming purification, even derivation, and required quantities of organic solvents. In this work, the reusable composite hollow fibers are prepared and applied for the microextraction and concentration of metronidazole residues in raw milk, and then desorbed in microliter elute reagent, followed by GC-MS. The factors influencing the extraction and desorption process are optimized and the adsorption mechanisms of MWCNTs/SiO_2 reinforced hollow fibers are briefly discussed. The results are promising with respect to HF-SPME. Therefore, this approach could potentially develop wider application in the fields of food safety, the environment, drug analysis and catalysis.

2. Experimental

2.1 Reagents and materials

Zirconyl chloride octahydrate ($ZrOCl_2 \cdot 8H_2O$), tetrabutyl titanates (TBT), tetraethyl orthosilicate (TEOS), hydrochloric acid, sulfuric acid, acetic acid, hydrogen peroxide (30%), *n*-hexane, chloroform, ethyl acetate, acetone, methanol, ethanol, sodium chloride and anhydrous sodium sulfate, all of analytical grade, were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai), China. High purity deionized water was used in the experiments.

The Accurel Q3/2 polypropylene (PP) hollow fiber membrane (600 μm i.d., 200 μm wall thickness, and 0.2 μm pore size) was purchased from Membrana GmbH (Wuppertal, Germany). Metronidazole was purchased from the National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). Hydroxylated multi-walled carbon nanotubes (OD, 30–50 nm; –OH content, 1.06 wt%; purity > 95%) were purchased from Chengdu Organic Chemical Co. Ltd., Chinese Academy of Sciences. The raw milk was bought directly from a pasture rancher and four batches of milk products from two manufacturers were bought in a local supermarket.

2.2 Apparatus

GC-MS, Thermo Scientific DSQ™ II (Thermo Fisher Scientific) assembled in China was equipped with a splitless injector and was employed for metronidazole . A Thermo TR-5 MS column, 6 30 m × 0.25 mm i.d., 0.25 μm film thicknesses (Thermo, USA) was applied to analyze the extracted analytes. Helium (99.999%) was used as the carrier gas and kept at a flow rate of 1.0 mL min^{-1}. The MS transfer line heater temperature was 280 °C; ion source temperature was 250 °C; inlet temperature was 220 °C; EI was 70 eV. The GC-MS temperature program used was as follows: initial temperature 60 °C, first increased to 160 °C in 20 °C min^{-1} intervals and held for 3 min, then raised to 300 °C in 20 °C min^{-1} intervals and held for 6 min. Standards and samples were analyzed in full scan mode with a scan range of *m*/*z* 50–500. Samples were injected in splitless mode.

A JSM-5600 (Jeol, Tokyo, Japan) SEM system was used for the SEM experiments. To prepare samples for SEM, composite hollow fibers were fixed on the stub by a double-sided sticky tape and then coated with Aurum by a JFC-1600 auto fine coater (Jeol, Tokyo, Japan) for 30 s.

2.3 Preparation of standard solutions

The standard stock solution was prepared by accurately weighing 100.0 mg of metronidazole into a 100 mL volumetric flask and dissolving in methanol under sonication. The stock solution was diluted with methanol to obtain an eight-point calibration standard solution, 0.01, 0.05, 0.1, 1, 10, 100, 200, and 1000 mg L^{-1}, respectively.

2.4 Preparation of MWCNTs/oxide reinforced hollow fiber

2.4.1 Preparation of oxide sol solutions. Zirconia sol: 0.2 mol (6.4464 g) $ZrOCl_2 \cdot 8H_2O$ was dissolved in 90 mL absolute ethanol under sonication at room temperature. 30% Hydrogen peroxide (8 mL) was added to the above solution dropwise under vigorous stirring. At first, the solution turned opaque milky white and after about one and a half hours a transparent zirconia sol was finally obtained. The sol was finally transferred to a closed glass container and aged for another 3 h at 70 °C.

Titania sol: tetrabutyl titanate (TBT) was dissolved at room temperature under vigorous stirring in a mixture solvent containing ethanol and acetic acid. Deionized water was added to the above solution dropwise under vigorous stirring for about 10 min. The final volume ratios were TBT: CH_3COOH: ethanol: H_2O = 1 : 0.3 : 4.5 : 0.15.

Silica sol: 30 mL TEOS was added into the mixed solvent of 30 mL absolute ethanol and 9 mL deionized water at room temperature. 38% Hydrochloric acid (1 mL) was added to the above solution dropwise under vigorous stirring. The sol was finally transferred to a closed glass container and aged at room temperature for 24 h.

2.4.2 Preparation of MWCNTs-oxide composite sols. 50 mg of hydroxylated MWCNTs was added to 5 mL of the above oxide

sol *via* stirring for 30 min. MWCNTs/oxide composite sol solutions were formed.

2.4.3 Preparation of MWCNTs/oxide-reinforced hollow fibers. The schematic illustration of the preparation of MWCNTs/SiO_2-reinforced hollow fibers was shown in Fig. 1. Polypropylene hollow fibers were cut into small segments with a length of 1 cm and pre-rinsed in acetone for 5 min to remove any possible impurities in the fiber. The polypropylene hollow fiber was entirely immersed in the above-prepared MWCNTs/oxide composite sols for 30 min under sonication at room temperature to allow MWCNTs and mesoporous oxide nanoparticles to be co-deposited on the porous surface of PP hollow fiber by sol–gel technique, followed by a temperature-controlled drying procedure at 393 K maintained for 60 min. Subsequently, the above immersion and drying processes were performed repeatedly, and three types of MWCNTs/oxide-reinforced hollow fibers (MWCNTS/ZrO_2, MWCNTS/TiO_2, and MWCNTS/SiO_2) about 8 mm length were obtained. Fig. 2 shows the SEM image of the three types of MWCNTs/oxide reinforced hollow fibers at different view angles, which clearly represent the presence of MWCNTs in the outer surface and wall pores of the composite hollow fibers.

2.5 Extraction and desorption procedure

Prior to use, MWCNTs/oxide reinforced hollow fiber was pre-rinsed with acetone and dried in order to remove impurities. Extraction was carried out as follows: the composite hollow fibers were first immersed in the milk sample (1.0 mL) in a small glass vial under sonication for a prescribed time. After extraction, the fiber was taken out from the milk carefully, gently dried with a filter paper and put in an oven to dry at 40 °C for 10 min. Finally, the fiber was directly placed in a centrifuge tube (1.5 mL) to desorb by elute reagent (0.30 mL) under sonication for another 10 min. Of the 0.30 mL final elute solution, 1 μL was directly injected into the GC-MS system for analysis. After desorption, the extraction fiber was washed with sulfuric acid and absolute ethanol repeatedly so as to remove any possible residual analytes or substances in raw milk. And then it was dried in the oven at 393 K for 2 h to avoid carry-over effects. Thus, the extraction fiber can be repeatedly used without reduction for efficient extraction.

3. Results and discussion

In the study, agitation of the sample solution enhances the mass transfer in the aqueous phase; but when stirring, the stirrer could potentially damage and break the MWCNTs/oxide hollow fiber by colliding with it repeatedly. Instead of a stirrer, an ultrasonic bath was used in our study to facilitate the extraction process. In order to evaluate HF-SPME procedure, consideration was given to such factors as the types of hollow fibers, extraction time and temperature, desorption solvents, and salt addition that influenced extraction efficiency.

3.1 Comparisons of extraction performance of three types of composite hollow fibers

As a comparison, HF-SPME with the MWCNTs/SiO_2, MWCNTs/TiO_2, MWCNTs/ZrO_2 reinforced hollow fibers were evaluated for metronidazole extraction. Fig. 3 shows the comparison of extraction performance of three types of composite hollow fibers at identical spiked concentrations (0.5 mg L^{-1}). It clearly shows the extraction capacity of the MWCNTs/SiO_2 reinforced hollow fiber is much higher than those of MWCNTs/ZrO_2 and MWCNTs/TiO_2. Though there was not much difference in morphology in the three types of composite hollow fibers, the extraction capacities differ dramatically. The possible reasons were as follows: zirconia and titania are an amphoteric metal oxide, which exhibits both anion- and cation-exchange properties depending on the solution pH and the nature of the buffer.[31] A large number of strong Lewis acid sites on the surfaces of zirconia and titania can interact with Lewis bases as $R–SO^{3-}$, $R–PO^{3-}$, $R–COO^{-}$ groups, *etc.*[32,33] This chemo-affinity sorbent has found widespread use for specific enrichment of phosphorylated peptides prior to analysis.[34] The protein existing in raw milk is mainly casein, most of which is phosphorylated to form the binding sites for calcium. Thus, there is a great chance that once MWCNTs/ZrO_2 and MWCNTs/TiO_2 reinforced hollow fibers were first immersed in raw milk, the adsorption sites were taken up by casein immediately due to specific enrichment of phosphorylated peptides. Accordingly, the biomacromolecules blocked up the structural channels in MWCNTs which are located on the surface of ZrO_2 and TiO_2 reinforced hollow fibers, causing the amounts of metronidazole extracted to decrease dramatically. Therefore, the MWCNTs/

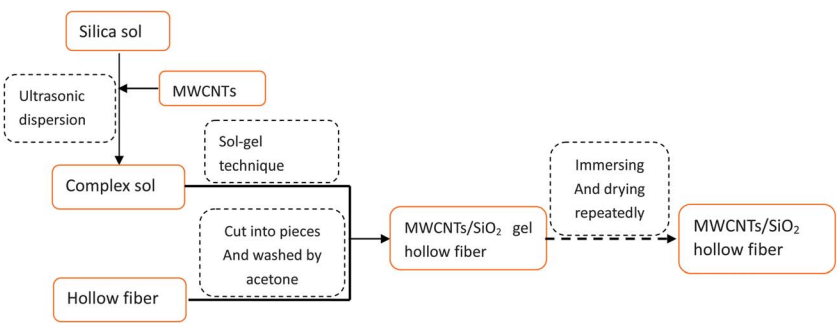

Fig. 1 Schematic illustration of preparation of MWCNTs/SiO_2 reinforced hollow fiber.

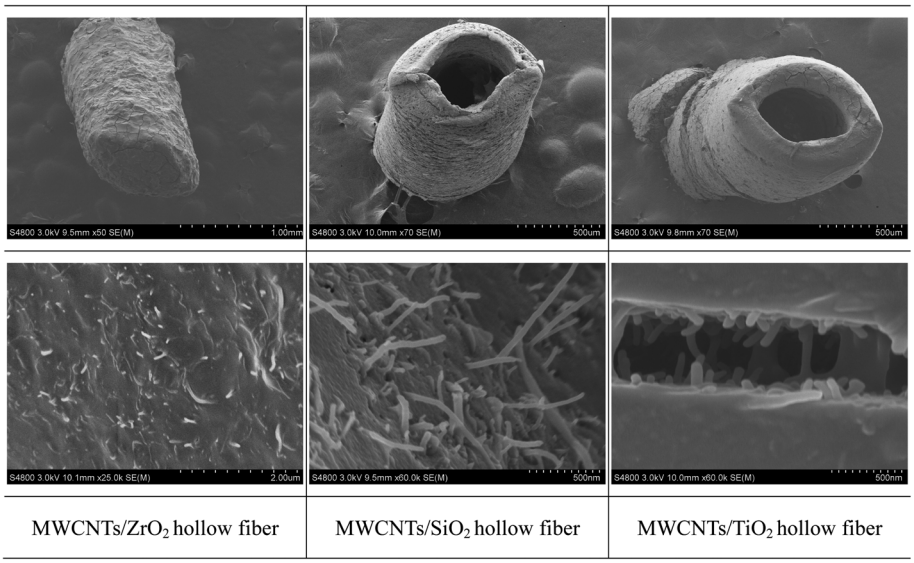

Fig. 2 SEM images of MWCNTs/ZrO_2, MWCNTs/SiO_2 and MWCNTs/TiO_2 reinforced hollow fibers.

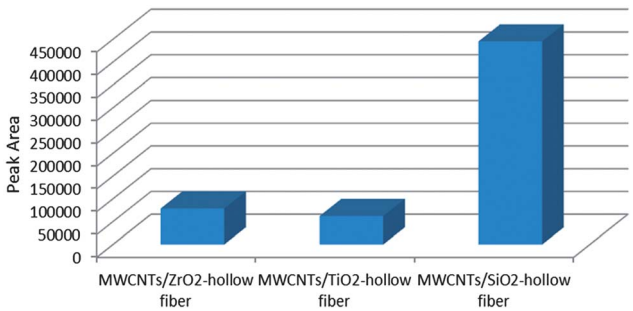

Fig. 3 Comparison of extraction performance of MWCNTs/SiO_2, MWCNTs/TiO_2, and MWCNTs/ZrO_2 reinforced hollow fibers for metronidazole extraction in milk spiked at concentration of 0.5 mg L^{-1}.

SiO_2 reinforced hollow fiber gave the most satisfactory results and was used for further experiments. Due to the specific enrichment of the phosphorylated protein, the other two fibers may have wider adsorption applications in the biological field.

3.2 Mechanism of metronidazole extracted by MWCNTs/SiO_2 reinforced hollow fiber

The amount of analyte and the extraction efficiency of HF-SPME depended on the interactions between metronidazole and MWCNTs/SiO_2 reinforced hollow fiber, which include π–π stacking, hydrophobic and electrostatic interactions between the analyte and MWCNTs, as well as hydrogen bonding of hydroxylated functional MWCNTs and silica. Due to the hydrolysis of SiO_2 on the outer surface with ambient water, the Si–OH groups are expected to be located on the coating surface of composite hollow fiber. While metronidazole started to contact with the composite hollow fiber, firm hydrogen bonds probably formed among MWCNTs-OH, Si–OH and O_2N– of metronidazole, increasing the amount of adsorption of the targeted analyte onto the composite hollow fiber.

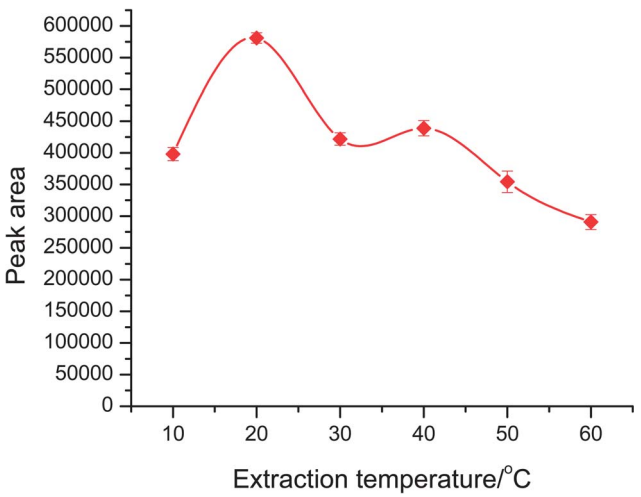

Fig. 4 Effect of extraction temperature on the extraction efficiency. *Conditions*: 8 mm MWCNTs/SiO_2 hollow fiber, analyte concentration: 0.5 mg L^{-1}, sample volume: 1.0 mL, extraction time 30 min, desorption solvent: methanol, desorption time: 10 min, no salt addition.

3.3 Effects of extraction temperature

Extraction temperature was also an important variable with a significant effect for target analyte. HF-SPME is a sample pretreatment and enrichment technique that is based on equilibrium between the concentration of the analyte in the sample and that on the device. Fig. 4 shows extraction efficiency of MWCNTs/SiO_2 reinforced hollow fiber for HF-SPME at different extraction temperatures. The extraction temperatures from 10 to 60 °C were studied. Usually, the amount of extracted analyte increased diffusion and adsorption with the

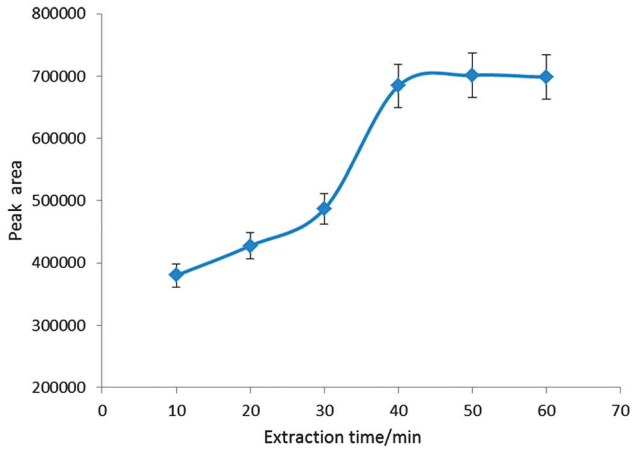

Fig. 5 Effect of extraction time on the extraction efficiency. *Conditions*: 8 mm MWCNTs/SiO_2 hollow fiber, analyte concentration: 0.5 mg L^{-1}, sample volume, 1.0 mL, extraction temperature 20 °C, desorption solvent: methanol, desorption time: 10 min, no salt addition.

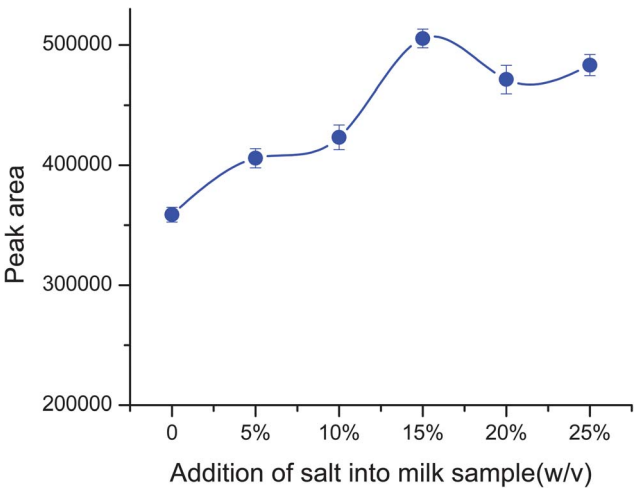

Fig. 6 Effect of salt addition on the extraction efficiency. *Conditions*: 8 mm MWCNTs/SiO_2 hollow fiber, analyte concentration: 0.5 mg L^{-1}, sample volume: 1.0 mL, extraction temperature 20 °C, extraction time: 40 min, desorption solvent: methanol, desorption time: 10 min.

temperature as shown in other analytes by HF-SPME,[25,26] but in the actual situation this is not the case. It can be seen that the amount of metronidazole increased with temperature before 20 °C and then decreased afterwards. As the temperature becomes higher, the possibility of protein denaturation also increased; the resulting protein coagulation might also block the diffusion and adsorption sites for metronidazole. Not only did an increase of temperature increase diffusion of the analyte from milk sample to the surface of the device but also helped other substances migrate freely, which were massive in the milk and might block-up the structural channels and occupy the adsorption sites in nano-size MWCNTs. Consequently, the adsorption of metronidazole on the MWCNTs/SiO_2 hollow fiber declined on the contrary to expectations from 20 to 60 °C.

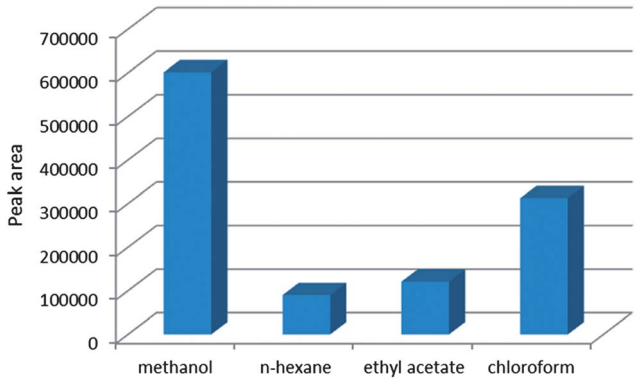

Fig. 7 Comparison of desorption solvent on the extraction efficiency. *Conditions*: 8 mm MWCNTs/SiO_2 hollow fiber, analyte concentration: 0.5 mg L^{-1}, sample volume: 1.0 mL, extraction temperature 20 °C, extraction time: 40 min, salt addition: 15% w/v, desorption time: 10 min.

Therefore, 20 °C was selected as the optimal condition and used in further work.

3.4 Effect of extraction time

Since HF-SPME is an equilibrium extraction mode, the maximum amount of analyte that can be extracted by the sorbent is achieved at equilibrium. HF-SPME involves dynamic partitioning of metronidazole between the MWCNTs/SiO_2 and the sample solution. Since mass transfer is a time-dependent process, the extraction time was examined to give the highest microextraction efficiency. The extraction time was attributed to both the thickness and porosity of the sorbent chosen. For a thicker sorbent of MWCNTs/SiO_2 reinforced hollow fiber illustrated in Fig. 2, the time needed for analyte diffusion into the sorbent was longer than for the thinner materials. However, a thicker sorbent provided better sensitivity because the kinetics of microextraction is dependent on diffusion of the analyte in the bulk solution and the sorbent. Extraction was performed from 10 to 60 min to determine the effect of extraction time. Fig. 5 shows the peak area *versus* extraction time profiles for the analyte. It can be seen that equilibrium is attained after 40 min. Therefore, 40 min was chosen as optimum extraction time.

3.5 Effect of salt amount in sample solution

Addition of salt into the sample solution may have several effects upon extraction. It was assumed that apart from the salting-out effect, salt addition causes a second effect named, salting-in effect. Usually, depending on the solubility of the target analytes, adding salt to the sample enhances extraction of the more polar analytes. In the case of metronidazole, salt addition generally helped to decrease the solubility of metronidazole in milk and increased the extraction of analytes by MWCNTs/SiO_2. For the purpose of the present experiments, the influence of salt on the studied system was investigated by adding various amounts of NaCl in a series of concentrations (0%, 5%, 10%, 15%, 20%, 25%, w/v), and results in the Fig. 6

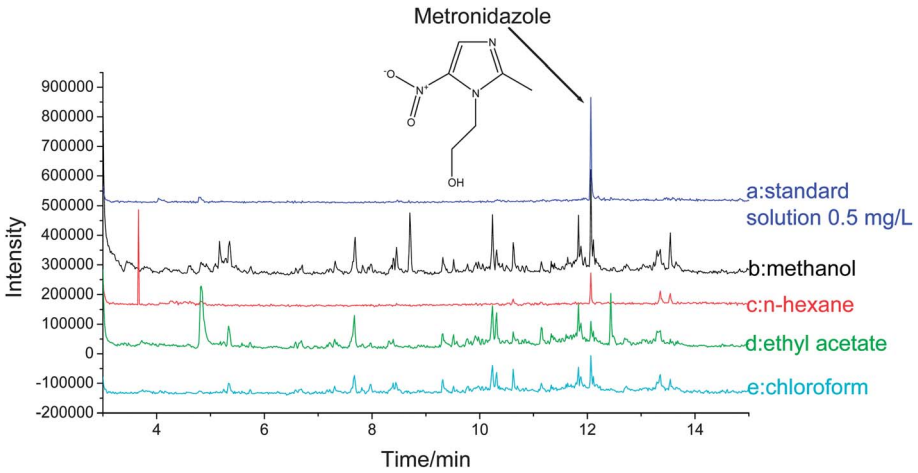

Fig. 8 GC-MS chromatograms of standard solution of 0.5 mg L^{-1} (a) and fresh milk spiked at 0.5 mg L^{-1} of metronidazole extracted by MWCNTs/SiO_2 hollow fiber-SPME under optimum conditions and eluted by different solvents (b): methanol; (c): *n*-hexane; (d): ethyl acetate; (e): chloroform.

show that the peak area increased with increasing salt addition in the aqueous sample. Above 15% of salt addition, no considerable increase in extraction efficiency was observed. Besides, as the salt concentration becomes higher, the possibility of protein denaturation also increased; the resulting protein coagulation might block the enrichment and adsorption of metronidazole onto the hollow fiber. Therefore, 15% (w/v) salt addition was added to the sample solution in further extractions.

3.6 Effect of elute reagents

In this case, the analyte was desorbed using an organic solvent from the MWCNTs under sonication after extraction. The analytes considered here are relatively more polar molecularly and may not be easily desorbed. Thus, a suitable organic solvent is needed for this process and the desorption temperature was set as 40 °C. Both the PP membrane and MWCNTs/SiO_2 are insoluble in most common organic solvents such as hexane, ethyl acetate, chloroform, and methanol; these typical solvents with different polarity were therefore investigated. Fig. 7 shows the influence of desorption solvent on HF-SPME performance. The more polar solvents (methanol) gave better desorption efficiency than other relatively less polar solvents. Fig. 8 illustrated GC-MS chromatograms of a standard solution of 0.5 mg L^{-1} (a) and fresh milk spiked at 0.5 mg L^{-1} of metronidazole extracted by MWCNTs/SiO_2 hollow fiber and eluted by different reagents (b): methanol; (c): *n*-hexane; (d): ethyl acetate; (e): chloroform. Though *n*-hexane extracted fewer impurities than other solvents, it gave the lowest desorption efficiency. Chloroform and ethyl acetate obtained much the same results: some impurities and moderate quantities of analyte. Methanol obtained the highest extraction efficiency, but it also extracted many more impurities from milk; however, none of these impurities interfered with the peak of metronidazole and did not affect the quantitative analysis of the target. Therefore, methanol was selected as the optimal elute reagent and used for the further work.

Usually, pH value of the sample solution is also one of the major factors that progress the transfer of analyte from the sample to the HF-SPME device. A suitable pH can improve the extraction efficiency and reduce matrix interferences. However, we consider that adjusting the pH value of milk might cause coagulation and precipitation of protein, changing the composition of raw milk. Thus, the pH value of sample solution was not considered in this case.

After first desorption, the extraction device was further desorbed in order to test carryover effects. No peak of metronidazole was detected in the second desorption, and no observable damage to the device was observed for up to 20 analyses.

Table 2 Recoveries at three spiked levels by MWCNTs/SiO_2 HF-SPME

Spiked level (mg L^{-1})	Recovery (%)	RSD (%)
0.05	96.30	5.80
0.5	81.47	7.30
5	68.90	8.40

Table 1 Method validation of MWCNTs/SiO_2 HF-SPME for metronidazole determination

Analyte	Linear range (mg L^{-1})	Calibration curve	*R*	LOD (mg L^{-1})	LOQ (mg L^{-1})	Precision (n = 5) RSD (%)	Repeatability (single fiber, n = 3) RSD (%)	Repeatability (fiber to fiber, n = 3) RSD (%)
Metronidazole	0.01–1000	$y = 108\,717x - 9893$	0.9985	0.003	0.01	3.79	6.44	8.23

Table 3 Analytical results of metronidazole from four milk products

Samples	Manufacture	Batch number	Specification	Production place	Metronidazole content (mg L^{-1})
Pure milk	A	20121128011	250 mL	Huhehaote, China	Not found
Pure milk with high calcium	A	20121203011	200 mL	Huhehaote, China	Not found
Pure milk	B	20121130G4	250 mL	Lanzhou, China	Not found
Skimmed milk with high calcium	B	20121018D42	250 mL	Lanzhou, China	Found[a]

[a] Found, could not be quantified.

Additionally, the extraction efficiency was not compromised when the device was used repeatedly. The device was generally robust with only the consideration of its longevity being dependent on the durability of the protective PP membrane envelope.

3.7 Method validation

The linearity of metronidazole calibration plot was investigated over a concentration range of 0.01–1000 mg L^{-1}. The correlation coefficient (R) for the analyte was 0.9985, indicating good linearity. The limits of detection (LOD) and limits of quantification (LOQ) (see in Table 1) were 0.01 and 0.003 mg L^{-1}, respectively, calculated at $S/N = 3$ and $S/N = 10$ from the chromatograms of standards at low concentration levels.

Five replicate measurements of the calibration solution of 1.0 mg L^{-1} were applied to calculation of relative RSD value. The repeatability was studied for three replicate experiments using the same MWCNTs/SiO_2 reinforced hollow fiber of 8 mm. Blank milk (1.0 mL) spiked with standard solution of 1.0 mg L^{-1} metronidazole (1.0 mL) was extracted by MWCNTs/SiO_2 reinforced hollow fiber at the above optimum conditions. Fiber to fiber repeatability was studied for three replicate experiments using three MWCNTs/SiO_2 hollow fibers of 8 mm prepared in the same batch. The repeatability and relative standard deviations (RSDs) are shown in Table 1.

Under the optimized conditions, the accuracy of the method was confirmed by recovery test by standard addition methods. Recoveries of metronidazole extracted from blank milk were investigated as follows: fresh milk spiked with metronidazole at 0.05, 0.5, and 5 mg L^{-1} was extracted by MWCNTs/SiO_2 hollow fiber under the optimized conditions. A summary of the procedural recovery data obtained with HF-SPME was shown in Table 2. The result indicates that the method enables the relatively accurate determination of metronidazole in milk products. This is ascribed to the more efficient adsorption of metronidazole by MWCNTs and the greater capacity of this material. However, the recovery at 5 mg L^{-1} was found to be lower, a possible reason could be related to adsorption capacity. When applied in real milk samples, other substances in the milk may also occupy the adsorption sites of the MWCNTs/SiO_2 hollow fiber, which originally would have been occupied by metronidazole. Thus, the extraction amount of metronidazole was decreased, causing the recovery at 5 mg L^{-1} to be lower. Though the recoveries of MWCNTs/SiO_2 HF-SPME applied in milk were not very satisfactory, still the advantages of HF-SPME were obvious, which was its simplicity, no need for sample pretreatment, fast collection of analyte from the milk matrix, and a decrease in the volumne of organic solvent used.

3.8 Application to the milk samples

The MWCNTs/SiO_2–HF-SPME procedure was applied to the four real milk samples. As shown in Table 3, metronidazole was found in one of the analyzed samples, but it could not be quantified. This demonstrated that the method is very promising for the rapid detection of low levels of metronidazole in milk products.

4. Conclusions

Novel types of hollow fibers, MWCNTs/SiO_2, MWCNTs/TiO_2, MWCNTs/ZrO_2 based on sol–gel techniques were fabricated and demonstrated the effective application of HF-SPME combined with GC-MS in the determination of metronidazole. The MWCNTs in the wall pores of the hollow fiber can absorb target molecules, thus effectively and selectively extracting analytes from complex sample matrix. This method allows combining of extraction, enrichment and clean-up in one step and has advantages such as simplicity, good accuracy and precision, relatively short extraction time, low cost, and minimum organic solvent consumption.

Three types of composite hollow fibers were compared and MWCNTs/SiO_2 was chosen for metronidazole extraction and determination in the milk samples. After the optimization of the extraction and desorption conditions for metronidazole, this technique provided good linearity and acceptable precision. The approach could potentially be explored further for applications in the fields of food safety, the environment, drug analysis and catalysis.

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