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Extraction of Fluoxetine from Urine and Aqueous Samples Using Solid-phase Microextraction Silica Fiber Coated by Functionalized Multi-walled Carbon Nanotubes Followed by Spectrofluorimetric Determination

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ABSTRACT

Fluoxetine (N-methyl-c-[4-phenoxy] benzenepropanamine) (FLU) is the parent drug of the selective serotonin reuptake inhibitor (SSRI) antidepressant class, which has emerged as a major therapeutic advance in psychopharmacology. It has been approved worldwide in the therapy of major depression and has also demonstrated to be effective in the treatment of other syndromes, such as bulimia nervosa, panic fits and obsessive–compulsive disorder. An instrumental setup including off-line solid phase microextraction coupled to fluorescence spectrometry has been constructed to improve the sensitivity for quantification of fluoxetine in real samples. The method was applied to analysis of spiked wastewater samples with the recovery 90.17%. This research provides an overview of the new developments in material and format technology that improve the extraction of semi-polar compounds in several extraction techniques. It mainly includes a solid-phase microextraction, that uses a simple carbon nanotube bonded silica fiber. The influences of microextraction conditions such as pH, ageing time, salt effect, performance and desorption conditions were investigated. It is a promising pre-treatment method for the fast, trace analysis in many complicated matrixes such as aqueous and biological samples. The method has a high enrichment factor and excellent selective cleanup of sample. Reasonable relative recovery was also obtained. The linear calibration curves was obtained in the range of0.1-10 μg.L^{-1}.We used the method to pre-concentration and clean up fluoxetine from real samples.

Keywords: Fluoxetine, Multi-Walled Carbon Nanotube, Solid-Phase Microextraction, Silica Fiber

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INTRODUCTION

Recently, a new extraction technology, solid phase microextraction (SPME), is being developed and has attracted considerable attentions due to its high sensitivity, rapidity, simplicity and free of solvents. SPME can extract trace amount of organic chemicals from environmental [1-3], biologic [4,5] and food samples [6-8]. The nanomaterials, having large surface area and being mechanically robust and chemically inert, have added new opportunities for improving the performance of SPME fibers [9,10]. Carbon nanotubes (CNTs), as a kind of effective sorbent nanomaterials [11–16], have been successfully used as the SPME fiber coating for analysis of organic compounds. In this study, we reported a chemical bonding method for fabricating multiwalled carbon nanotubes (MWCNTs)/fused-silica fiber based on the surface modification of both MWCNTs materials and fused-silica fibers. Then the fiber coupled with spectro fluorescence (FLU) was used for solid phase microextraction of fluoxetine from water and biological samples, which are largely used in our daily lives and

industrial productions, and also are polluting our environment. Analysis of fluoxetine in two different sources (water and urine samples) gave satisfactory results.

MATERIALS AND METHODS

Apparatus and materials

Fluoxetine(N-methyl-*c*-[4-phenoxy] benzenepropanamine)(FLU), Triton x-100, H_2SO_4, HNO_3, NaOH, 3-aminopropyltriethoxysilane (APTE**)** and Acetonitrilewere obtained from Merck(Darmstadt, Germany). The standard mixtures of the seven phenols were prepared by dissolving 10.0mg of fluoxetine in 10.0mL of methanol and toluene. All chemicals used were of analytical grade and purified water byMilli-Q system was used throughout the experiments. The fluorescence spectra were recorded using a Varian CaryEclipse fluorescence spectrophotometer (Springvale, Victoria, Australia) equipped with quartz cell and a xenon lamp. Spectra recording were carried out in fluorescence scan mode with the slit widths of 5 nm and the excitation and emission wavelengths of 293 and 582 nm, respectively. The PMT detector was used for recording the emission lines and set on 600 V.

Environmental sample collection

The sanitary wastewater sample was collected from the sina hospital (Mashhad, Iran). The urine sample of one person who consumed the drug (fluoxetine). These samples were all filtered through a 0.45µm filter and stored at 4 ◦C.

Procedures

Oxidation of MWCNTs

In this work, MWCNTs were treated with acid as mentioned below; MWCNTs were dispersed into a flask containing mixed acid (concentrated H2SO4/HNO3 = 3/1) solution and refluxed at 130 ◦C for 3 h. After cooling, the MWCNTs were washed with the deionised water until the pH of the solution reached approximately 7. Then the solution was filtered and dried at 110 ◦C for 5 h to obtain the acidified MWCNTs.

Preparation of MWCNTs coated fiber for SPME

Prior to coating, in order to remove the polyimide layer from a 1cm segment of the fiber at one of a 2-cm-long fused silica fiber ends, this layer was burnt off using a naked flame. The burnt section of the fiber was cleaned with methanol, dried, and dipped into 1mol L^{-1} sodium hydroxide solution for 1h to expose the maximum number of silanol groups on the silica surface of the fibers, and then into 0.1 mol L^{-1} HCl for 30 min to neutralize the excess sodium hydroxide. The activated fibers were rinsed with distilled water, dried at 120 ◦C for 1h and kept dried in desiccator before use. The hydroxylated part of the fiber was dipped in an APTES solution for 16h, and then it was put in an oven at 120◦C for 30min to react with APTES. These two operations were repeated for six cycles to form a silanized layer on the fiber. Finally, it was rinsed with toluene and ethanol, and dried at room temperature.

Chemical bonding of MWCNT layer to fiber

10mg oxidized MWCNTs were dispersed in a 3 mL triton x-100 solution (1%) for 30min ultrasonically bath to prepare the MWCNTs suspension. The pretreated fiber section was put into the MWCNTs suspension for 4 h in a hot water bath (70 ◦C), and then it was heated in a 130 ◦C oven for 40 min. This procedure was repeated until the coating reached a required thickness (20µm). Finally, the coated fiber was aged at 270 ◦C for 14h under the protection of dry nitrogen before the SPME experiments.

Solid-phase microextraction procedure

A 15mL glass vial was used as a sample container. 10mL of water sample was placed into the vial and stirring by a shaker. The ion strength and pH of the sample were adjusted with NaCl and 0.1M HCl or 0.1M NaOH, respectively. The section of coated fiber was completely immersed into the sample solution. The extraction was performed at room temperature and with stirring rate of 2000rpm. After extraction, analyte on the fiber were desorbed by 0.4 ml methanol then placed into to a quartz cell and the fluorescence emission spectrum was recorded**.**

RESULTS

Preparation of MWCNTs/SPME fibers

Fig. 1 shows the preparation process of MWCNTs/SPME fibers. The MWCNTs were oxidized by mixed acids (H_2SO4/HNO_3 = 3/1) to create –COOH groups at the sidewall of the MWCNTs. This activation process was verified by IR spectrum as shown in Fig. 2, in which an extra peak at 1720cm^{-1} corresponding to C= O appeared for the oxidized MWCNTs while absent in pristine MWCNTs. The silica fibers were firstly hydroxylated by NaOH solution to break the Si–O–

Si bonds to form Si–OH groups which were then transformed to –NH2 group by reacting with silanizing agents. MWCNTs/SPME fibers were formed by reaction between –COOH and –NH2 groupsupon heat treatment. The generation of amide was confirmed by the peak at 1530cm^{-1} in the IR spectrum (Fig. 2). Apart from covalent bonding, strong van der Waals interactions are also present in MWCNTs [20], which would promote the MWCNTs in the dispersed solution adsorbing spontaneously on the MWCNTs on the fiber. Thus a multilayer-MWCNTs coating can be finally formed on the fiber surface.

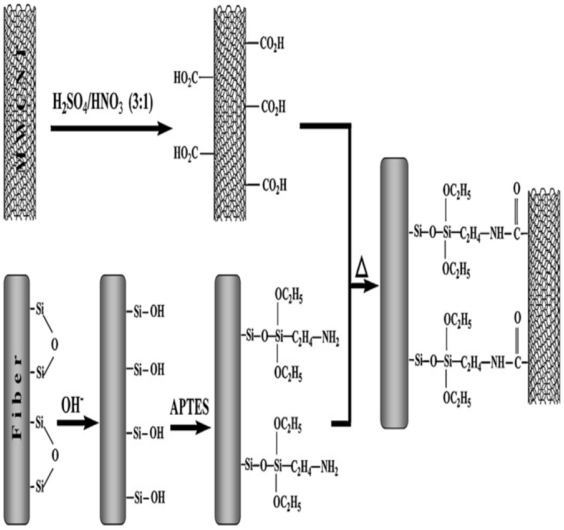

Figure1:Schematic illustration of the preparation process of the MWCNTs/fiber

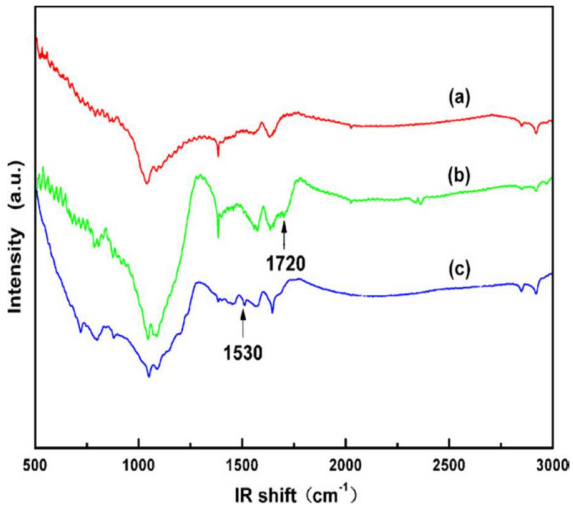

Figure 2: FT-IR spectra of the MWCNTs: (a) pristine, (b) after oxidation, and (c) after chemical bonding with the fiber.

Fig. 3a shows the SEM micrograph of the as-prepared MWCNTs/ SPME fiber. It can be observed that a coating uniformly covered on the fiber surface, implying success of the synthetic strategy. Magnified image in Fig. 3b indicates that the coating is made up ofcross-linked CNTs.

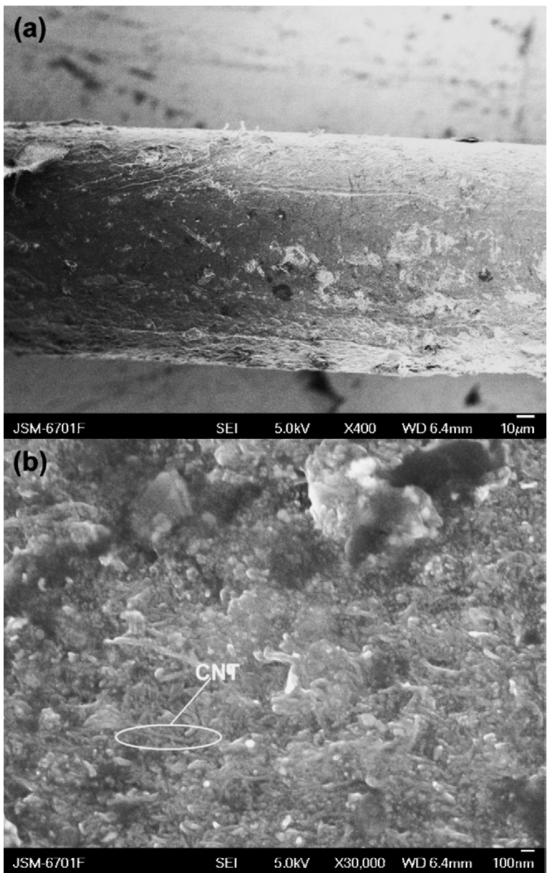

Figure 3:SEM images of the MWCNTs-bonded SPME fiber at a magnification

Optimization of SPME

The as-prepared MWCNTs/fibers coupled with spectro fluorescence were used for solid phase microextraction of fluoxetine from real samples. To achieve the best extraction efficiency, the effects of extracting parameters, such as the adsorption time, Effect of ionic strength, stirring rate, solution volume, pH and desorption conditions were systematically studied.

Effect of extraction parameters

Effect of extraction time

The exaction time is a very important parameter in a SPME procedure because it influences the partition of the target analytes between sample

solution and fiber coating. four different extraction times were tested and the corresponding results are demonstrated in Fig. 4.The exaction efficiency of the MWCNT fiber increased whenthe extraction time increased from 5 to 20min then decreased as the time increased future. So the best adsorption time for this experiment was 20 min.

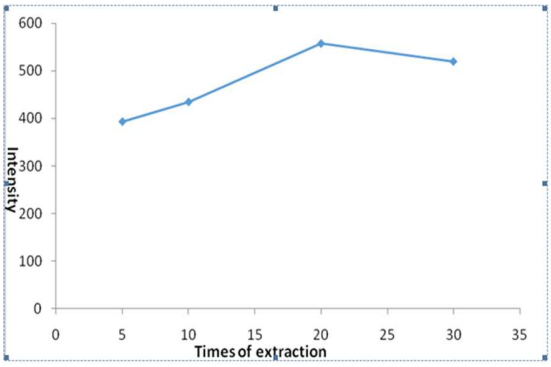

Figure 4: Extraction time for fluoxetine

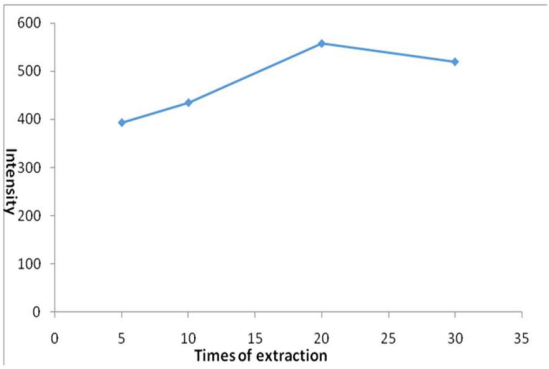

Figure 5: Effect of pH on peak area of phenols extracted by the as-prepared MWCNTs/fiber

Effect of pH

The pH value of the solution is another important factor for extracting pH sensitive compounds. It did not only influence the dissociation of functional groups in analytes, but also influence the surface charge density of MWCNTs [21]. The effects of the pHvalue of the solution on the extraction efficiency for fluoxetine was studied for 4 different pH (2, 4, 7, 11). As shown in Fig. 5, the chromatographic peak areas decreased from 2 to 4 and then increased from 4 to 7 and then increased from 7 to 11 and then decreased as the pH value increased future. So this result was mainly ascribed to the different surface charge density between the internal and external surfaces. Whenthe pH value was 2, the external surface of CNTs was negatively charged, and the internal surface was positively charged. Such a condition might offer a preferential environment to adsorb fluoxetine compounds on the surface. So PH=2 was chosen in this experiment.

Effect of ionic strength

It is known that adding a salt (NaCl) into the solution can have two contrary outcomes. It may help with the extraction by the 'salt out effect' or deteriorate extraction due to the competitive adsorption of Na^+ and Cl^-. The extraction efficiency as a function of saltconcentration from 0% to 5% was studied. A concentration of 1% (w/v) was selected as the optimized salt concentration as its shown in Fig.6.

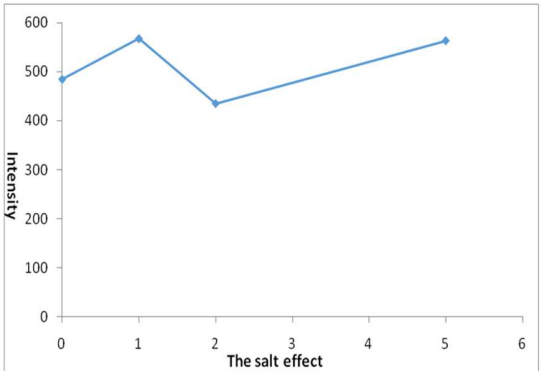

Figure 6: Effect of ionic strength on peak area of fluoxetine

Effect of desorption solvent

The desorption solution is another important factor of extraction parameters.We used different solution for analytes desorption including polar and non-polar solutions. We used two non-polar solutions (cyclohexane, dichloromethane) and two polar solutions (methanol, acetonitrile) for the desorption solutions. The bestdesorption solution was methanol as shown in Fig.7.

The Magnetic stirring rate

Magnetic stirring is most commonly used in SPME experiments to accelerate the extraction. It was found that the chromatographic peak areas for fluoxetine increased monotonously when increasing stirring rate. The range of stirring rate was from 0-2000 rpm. So the 2000rpm stirring rate was chosen. In short, the optimized conditions for the extraction of fluoxetine in real samples with prepared SPME fiber were: extraction time,20 min; desorption solution,

Methanol (desorption time, 15 min; pH= 2; stirring rate , 2000rpm; Effect of ionic strength 1% .

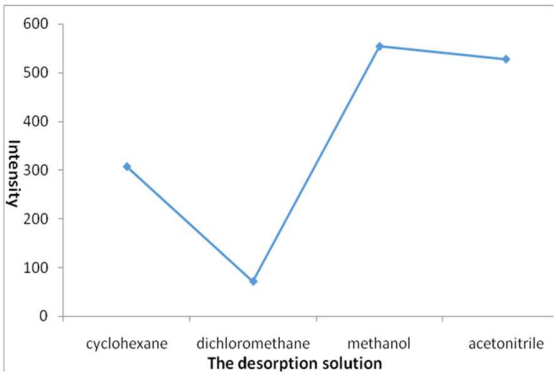

Figure7:The desorption solution on peak area of fluoxetine

Detection limit, precision and accuracy

The analytical parameters including the linearity, accuracy and precision, and detection limits for the extraction of fluoxetine in ultrapure water with the prepared MWCNTs/fiber were listed in Table 1.

Table1:Characteristic data of the established MWCNTs/fiber-SPME–Fluorescence method for determination of fluoxetine

Sample	Linear range (µg.L-1)	Linearity	LOD (µg.L-1)	R.S.D. (%)
Fluoxetine	0.1-10	0.971	~ 0.0001	2.63

As shown in Table 1, good linearities were achieved in the range of 0.1-10 µg.L-1 for fluoxetine. The detection limit for fluoxetine was 0.0001 in this work.

Real samples

Fresh human urine samples were obtained from different volunteers who had or had not taken fluoxetine and stored at −4 ◦C until analysis. The urine samples were centrifuged at 2500 rpm for 15 min at room temperature and the supernatant was triple dilutedwith distilled water before extraction. Sanitary wastewater (Mashhad, Iran) water samples were collected and filtered with millipore filter before extraction. The established SPME–spectro fluorescence method was used to determine the content of the fluoxetine in sanitary wastewater and urine samples. The recovery of the target compound was determined in two real samples at two concentration levels. For sanitary wastewater we spiked at 7µg.L^{-1} of solution and for urine sample without any spike we got the suspended concentration of real sample.

The recoveries and RSD % were shown in Table 2.

Table 2: Recoveries and determination precisions of fluoxetine in spiked sanitary wastewater and urine sample

Sample	Sanitary waste water		Urine sample	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Fluoxetine	93.14	1.1	95	2.91

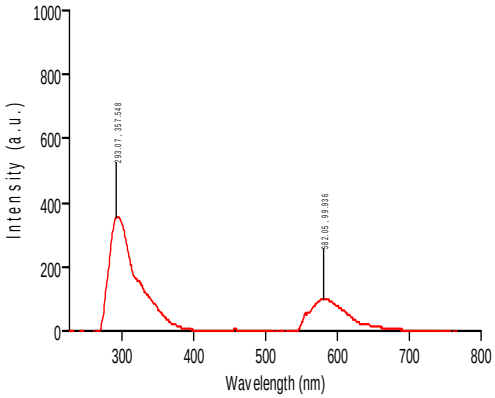

Figure 8: Fluorescence spectrum of fluoxetine extracted from sample Urine

CONCLUSION

MWCNTs-covered SPME fibers were produced by covalent bonding based on the surface modification of both multi-walled carbon nanotube and the fused-silica fibers. This SPME fiber was then used to extract fluoxetine from wastewater and urine samples coupled with Spectro Fluorescence, and applied to two real samples, achieving satisfactory results. Having an incompact structure, which brought forth by the chemical bonding design, and combining with the inherent stability of MWCNTs, the MWCNT/SPME fiber possesses some special properties such as good stability at high temperature, in organic solvent (polar and non-polar), acid and alkali solutions, wide linearity range and low LODs for extracting fluoxetine coupled with Spectro Fluorescence.

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