Pre-concentration and Determination of Fluoxetine in Hospital Wastewater and Human Hair Samples using Solid-phase µ-Extraction by Silver Nanoparticles Followed by Spectrofluorimetric

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ABSTRACT
Fluoxetine (N-methyl-c-[4-phenoxy] benzene epropanamine) (FLU), the main drug in the antidepressant class is serotonin reuptake inhibitor (SSRI), which has emerged as a therapeutic advancement in psychiatry. It has been shown to be effective in treating depression worldwide and has also been shown to be much more effective in treating other syndromes, such as bulimia nervosa, panic attacks, and obsessive-compulsive disorder. An instrumental setting including off-line solid phase microextraction with spectrophotometry has been developed to improve the sensitivity of fluoxetine quantification in real samples. This method was used to analysis wastewater specimens with 89.9% recovery. This research is a review of new developments in substances and format technology that lead to the extraction of semi-polar compounds in various extraction methods. This mainly consists of a solid phase µ-extraction, using a simple silver nanoparticle. The use of µ-extraction conditions such as pH, salt effect, yield and desorption conditions are examined. This procedure has a high enrichment factor and excellent sample selection cleaning. Reasonable relative improvement was also obtained. Linear calibration curves were obtained in the range of 0.1-35 mg L⁻¹. We have used this method to clean up and pre-concentration of fluoxetine from factual samples.

KEYWORDS
Fluoxetine
Silver nanoparticles
Solid-phase micro-extraction
FLU
**Introduction**

Lately, a new extraction, solid phase microextraction (SPME) is being advanced and has interested a lot of attention due to its sensitivity, speed, simplicity and free of solvent. SPME can extract small amounts of organic chemicals from environmental test [1-3], biological [4, 5] and food specimen [6, 7]. Nanomaterials, large surface area, mechanically resistant and chemically inert [8-20], have added new opportunities to improve the performance of SPME fibres [21].

Water treatment is one of the most important technologies in all of the world [22-37]. Nanomaterials and especially metal-based nanostructure showed many advantages in removal, photo-catalysis, and sensing and other related water treatment fields [38-47]. This ability is relative to complex structure and unique properties of nanomaterials [48-60]. Silver nanoparticles have specific physical and chemical properties and biological activities [61].

Following extensive research, the use of silver nanoparticles, particularly in the area of health widely expanded [62]. For example, in the medical field covering on wound, surgical instruments and bone prostheses are coated by silver nanoparticles. Therefore, various methods such as physical and chemical precipitation, reverse micelles, hydrothermal and chemical vapour deposition is innovated to produce this nanoparticles [62]. Particularly the colloidal silver due to its distinctive features, such as good conductivity, chemical stability, catalytic and antibacterial is under more attention [63]. The UV/Visible spectroscopy method is an inexpensive and accessible and reliable method [64]. The fluorescence spectroscopy method is high-sensitivity and due to used colloidal silver nanoparticles the fluorescence spectroscopy method is not appropriate and don't clearly show the emission intensity. In this study, we report the volume in extent of milligrams per litter of fluoxetine drug by using pre-concentration by silver nanoparticle with the UV/Visible spectroscopy method. The drug and nanoparticle structure have been singly investigated by fluorescence spectroscopy method until the lowest concentration of drug shown by peak intensity [65]. In addition, the optimal conditions and production of nanoparticle is also mentioned for this study until in this conditions the accuracy and correlation coefficient increases. Various methods for measuring fluoxetine is reported [66-70]. This drug were analysed by the UV/Visible spectroscopy method by using AgZnO and MWCNT-Fe3O4 nanoparticles that it's resulting spectra showed that the revived silver nanoparticles by hydroxylamine is more appropriate to its separation.

**Material and methods**

All absorption spectra were carried out using Shimadzu recording spectrophotometer UV 160 equipped with matched 1-cm quartz cells. The ultrasonic processor apparatus model UP-100H (Hielscher-Germany) was used. The FT-IR instrument that was used for inscribing the infrared spectrum was M-500 Fast-Scan IR spectrometer (Buck, East Norwalk, CT 06855, USA). The transmission electron microscopic analysis that used was a Jeol 2010 instrument with a voltage of 200 kV. Fluoxetine (N-methyl-c-[4-phenoxy] benzene epropanamine) (FLU), AgNO3, ethanol H2SO4, HNO3, NaOH, n-hexane, n- heptane, ethanol and acetonitrile were obtained from Merck (Darmstadt, Germany). The standard mixtures were arranged by dissipating 50.0 mg of fluoxetine in 50.0 mL of methanol. All chemicals used were of analytical make level and purified water by Milli-Q system was used throughout the tests. The fluorescence spectra were recorded using a Varian. Cary Eclipse spectrofluorrometer (Springvale, Victoria, Australia) supplied with a xenon lamp and quartz cell. Spectra inscribing were done in fluorescence scan mode with the slit widths of 5 nm and the excitation and emission wavelengths of 293 and 582 nm, respectively.
Hospital wastewater and human hair sample collection

The hospital wastewater sample assembled from the Sina hospital (Mashhad, Iran). The hair sample of one individual human who used up the drug (fluoxetine). These real samples were all filtered through a 0.45µm filter and supplied at 4 °C. Fresh solutions were prepared daily by diluting the stock solution in distilled water. All experiments carried out at room temperature, 22±0.5 °C.

The person who introduced me to a male barbershop in Mashhad, Iran, gave us most of the gray hair that is essential to verifying method development. The absence of was confirmed using spectrofluorescence analysis of real hair samples as environmental and biological samples suspected of being contaminated with fluoxetine samples that had a length of 2–4 cm chosen for analysis. Fat and other external part or layer spreading of impurities on the hair should eliminate. Thus, the hair cleaned by following solvents on a hierarchy basis: Washing managed by 15 mL methanol, 20 mL acetone and 20 mL dichloromethane, respectively and then they were dried. The treatment hair finally cut into nearly 1 mm pieces and assimilated by the following steps: 2.0 mL methanol as an organic solvent added to 50 mg of hair in a 10 mL screw cap cylindrical tube. The pH regulated to 7.2 by phosphate buffer. The samples incubated at 55 °C. Then residuary solid hair sample filtered and cleaned with 0.5 mL ethanol [29].

Fabrication of the method
Preparation of the silver nanoparticle

300 mg of AgNO₃ and 120 mL of ethanol added to a glass jar with 2 temperatures, 60 and 65 °C, under constant agitating. Fabrication of the HF-SPME fiber, beforehand dissolved in ethanol, broadcast in AgNO₃ alcohol solution order to get AgNO₃ and triethylenetetramin 1:8 ratio with the label M1. The UV-VIS absorption spectra of samples then subtracted obtained with Shimatzu PC160A plus UV-VIS. Spectrophotometer using methanol as reference. The solution precipitated by evaporation of ethanol at 75 °C for 70 min. The solution is prepared at 60 °C and treated at 90 °C (it was labelled A). The prepared system treated at 70 °C and 90 °C with the label B. The latter showed precipitation before evaporation of alcohol.

As a result, the amine solution is precipitated with nanoparticles were obtained.

The FT-IR Spectrum of silver nanoparticles shows in figure 1. The FT-IR spectra mainly used to identify the presence or absence of functional groups.

![Figure 1: The FT-IR Spectrum of silver nanoparticles](image.png)

The peak related to 600-800 cm⁻¹ the C-Cl bond is observed, the 1500-1600 cm⁻¹ region is related to the stretching C=C and C=O, the 2000 cm⁻¹ region and before 3000 cm⁻¹ C-H is observed and
3200-3500 cm\(^{-1}\) region stretching vibration of N-H and O-H group was observed.

\textit{μ-SPE} procedures

For this purpose, 10 mg silver nanoparticles added to 15 mL solution of 2 mg L\(^{-1}\) fluoxetine and mixed on a shaker by a definite rate. The solution centrifuged for 10 minutes in 3000 rpm. In this step, the nanoparticles deposited and the tap solution removed. Then 0.4 mL of methanol added to silver nanoparticles, after agitating and centrifuging (400 rpm, 6 min), the nanoparticles was settled and the enriched methanol of analysis were conveyed to the spectrophotometer cell. Then absorbance of the fluoxetine were evaluate.

**Result and Dissection**

\textit{Optimization of SPME}

The silver nanoparticles coupled with spectrophotometer used for extraction of fluoxetine from samples. To perform the most successfully extraction efficiency, the effects of extracting parameters, such as the adsorption time, agitating rate, solution volume, pH and desorption conditions were studied. The absorbance used to estimate the most extraction efficiency under different conditions.

\textit{Effect of extraction time and desorption solvent selection}

Therefore, individual desorption solvents like ethanol, methanol, acetonitrile, n-heptane and n-hexane probed. Based on the obtained results, ethanol that has been found to have the best extraction efficiency. Therefore, ethanol was selected as the desorption solvent.

Micro-extraction is a process of equilibrium and in terms of equilibrium affects the extraction time. When extracting solute molecules have a high chance of transferring from the donor phase to the interface between the aqueous solution and the nanoparticles. Therefore, extraction time is an important factor affects the efficiency of the method. Extraction was performed from 5 to 40 minutes. All the analyses demonstrated the highest increase at intensity in the period of 20 min. Subsequently the intensity declined with expanding of extraction time. Thereafter extraction efficiency decreased with increasing time. Maybe its organic causes the solvent to evaporate. Therefore, a 20 min period used for subsequent experiments.

\textit{Effect of volume aqueous solution and volume desorption solvent}

In this method, the analytic excreted using it proper desorption organic solvent as this research has shown, ethanol is often the most excellent solvent to disperse. The volume of organic solvent is consequential in the disposal capacity of efficiency. The scheme as well as the total time demanded by micro-extraction to achieve equilibrium. Five distinctive volumes of desorb solvent (0.1-0.4 mL) for target analytic was evaluated. Most extraction productivity observed to be a 1 mL desorption solvent. When the volume of desorption solvent used more in the extraction, a decrease in intensity was observed. This phenomenon may be due to the analytical effects of the target dilution. Repeatability decreased in the volume of excretion solvent less than 0.1 mL so 0.1 mL has used as the optimal volume.

\textbf{Figure 2}: Scanning electron microscopy the of the silver nanoparticles
The volume used was a decrease in the response observed when larger volume of solvent excretion applied in the extraction. This may be due to dilution target analyte the effects of reproducibility on the desorption solvent decreased when volume of less than 0.1 mL.

The effect of extraction time and stirring rate
Extraction was done from 5 to 30 min to evaluate the effect of extraction time on the method. The results that shows target analyte have the comparable trend. Afterwards the efficiency declined with increasing of time. Evaporation and dissolution of organic solvent in aqueous solution may cause this accident. Therefore, a 10 min period has used for subsequent experiments. Stirring increases mass transfer and declines the time required to achieve thermodynamic equilibrium. The response of the device was recorded for several rates from 150 to 1000 rpm for an extraction time of 10 minutes from a 5 mL aqueous phase at a concentration of 100 μg / mL. The results showed that stimulation of the sample greatly enlarged the efficiency extraction. However, severe agitation (> 700 rpm) leads to massive air bubbles and a decline in pre-concentration factors. Therefore, stirring rate of 700 rpm has selected in later experiments.

Effect addition of salt
It was known that adding salt (NaCl) to the solution can have two opposite results. Due to the competitive adsorption of Na⁺ and Cl⁻, it may aid in extraction by the "desalination" effect. Extraction efficiencies were studied as a function of salt concentration from 0-10% (w/w). When 3% (w/w) sodium chloride was added to the aqueous phase, the highest values were obtained for extraction efficiency of sample. Further addition, sodium chloride did not increase the extraction efficiency. Therefore, experiments was down by adding 3% salt.

The effect of desorption solvent
Desorption solvent is another factor in extraction. We used various solvent, including polar and non-polar solvent. We used two non-polar solvent (cyclohexane, dichloromethane) and two polar solutions (methanol, acetonitrile) for desorption. The best desorption solvent was methanol, that was shown in Figure 3.

![Figure 3: The effect of desorption solvent on efficiency of extraction](image)

Figures of merit
To evaluate the practice application of micro extraction technique, Figures of merit for this method including pre-concentration factor, Relative recovery, regression equation, this method including pre-concentration factor, Relative recovery, regression equation, correlation coefficient (r²), limit of detection (LOD) and linear dynamic range (LDR) was calculated. It was evaluated under the best conditions. The calibration curves Hospital wastewater and human hair samples were evaluated. For each level, three duplicate extraction was performed. The results are tabulated in Tables 2.
The micro-extraction method used to determine the content of the fluoxetine in real samples. The relative recovery of the target analytic was determined in two real samples at two concentration levels.

**Conclusion**

In the present study, silver nanoparticle synthesized. This method has excellent pre-concentration factors selective cleaning of fluoxetine in aqueous and hair human matrices based μ-extraction. The matrix also had good linearity and reasonable relative improvement gained. The experimental operations involved in the extraction are many simple. In addition, this method has particular benefits over it. Customary extraction methods such as reducing extraction time, it is also easy to use and economical. In this method, we have introduced a credible and qualitative method to determine the low level of drugs concentrations in fact samples and applied to two real samples, achieving satisfactory results.

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**Conflict of Interest**

We have no conflicts of interest to disclose.

**References**


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