RESEARCH PAPER

$CoFe_2O_4$ nanoparticles grafted multi-walled carbon nanotubes coupled with surfactant-enhanced spectrofluorimetry for determination of ofloxacin in human plasma

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ABSTRACT

An efficient and simple magnetic solid phase extraction based on the use of $CoFe_3O_4$ nanoparticles grafted multi-walled carbon nanotubes (MWCNTs) as adsorbent coupled with surfactant enhanced spectrofluorimetric detection was developed for determination of ofloxacin from biological samples. The adsorbent uses the advantages of both magnetic nanoparticles (i.e., magnetic separation) and MWCNTs (i.e., high adsorption capacity). The main factors affecting the quantitative recovery including SDS concentration, pH, extraction and desorption times, adsorbent amount, and desorption conditions were investigated in detail. Under the optimized conditions, the calibration curves were linear over a wide concentration range of 100-750 ng mL^{-1} with detection limit (LOD) of 23 ng mL^{-1}. The relative standard deviation (RSD %) of 3.3% for concentration of 250 ng mL^{-1}, n = 5 and the preconcentration factor of 93 were obtained. Finally, the proposed method was successfully applied to the extraction and preconcentration of ofloxacin in human plasma samples.

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INTRODUCTION

Recently, mixed transition metal oxides with spinel structure have received much attention due to their diverse advantages such as strong super paramagnetic property, good biocompatibility, high electronic conductivity, abundant resources, low cost and environmental friendliness [1, 2]. Among the transition metal oxides, the spinel-type compounds with the general formula of AB_2X_4 which A and B are metal and X is chalcogen, have shown as promising sorbent activity. These types of oxides are usually synthesized through solid state method, hydrothermal technique, co-precipitation, micro-emulsion route and sol-gel process [3-5]. Among other spinel ferrites, $CoFe_2O_4$ nanoparticles (NPs) have received much attention in the field of magnetic NPs due to their high chemical and chemical stability, moderate saturation magnetization, easy preparation, and rapid separation [3, 6]. $CoFe_2O_4$ NP is a partially inverted mixed spinel ferrite, which has large cubic magnetocrystalline anisotropy, responsible for its large H_C manufacturing by cheap techniques [7]. All these properties, together with many other outstanding characteristics make $CoFe_2O_4$ NPs extremely interesting for application in several technological fields including magnetic hyperthermia, spintronics, supercapacitors and catalysis [8].

In the last decades, carbon nanotubes (CNTs) have become attractive materials in analytical science because of their novel structural, electronic, semiconductor, mechanical, chemical and physical

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properties as well as their extremely large surface area [9-11]. CNTs are hollow graphitic material composed of one (single-walled CNTs, SWCNTs) or multiple (multi-walled CNTs, MWCNTs) layers of graphene sheets. The walls are not reactive but their fullerene-like tips are known to be more reactive. Hence, its end functionalization is used relatively common to generate some functional groups such as COOH, OH and C=O on the surface of the nanotubes. One another approach is giving magnetization property by use of magnetic nanoparticles as modifier causing a convenient separation in addition to higher adsorption capacity.

Ofloxacin is a synthetic second-generation fluoroquinolone antibiotic drug with a broad spectrum of activity against gram-positive and gram-negative bacteria [12]. There are several analytical methods for determination of this drug in pharmaceuticals and biological samples such as spectrofluorimetry [13], chromatography [14-16], electrophoresis [17], electrochemical analysis [18, 19], chemiluminescence [20] and bioassay [21, 22]. However, it has conjugated π-electrons and rigid planar structure which leads to having good fluorescent signal. it excites at 340 nm and emits at of 480 nm. Hence, fluorescence spectrophotometery is a simple and efficient method for ofloxacin measurement but lacking enough sensitivity based on fluorescent itself which led to the narrow linear range. Thus, finding a specific and sensitive detection method for trace ofloxacin is necessary.

In this paper, an efficient magnetic adsorbent, namely MWCNT, decorated with $CoFe_2O_4$ NPs is synthesized and used in a magnetic solid phase extraction (MSPE) procedure coupled with micelle enhanced spectrofluorimetric method for determination of ofloxacin in biological samples. The factors affecting the extraction efficiency and signal enhancement of the analyte are investigated and optimized and the method is successfully applied to the determination of ofloxacin in human plasma samples.

EXPERIMENTALS

Reagents and chemicals

Pristine MWCNTs with 40 nm diameter and 1-25 μm length were purchased from Research Institute of the Petroleum Industry (Tehran, Iran). Standard ofloxacin was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Cobalt nitrate hexahydrate ($Co(NO_3)_2.4H_2O$), ferric nitrate ninehydrate ($Fe(NO_3)_3.9H_2O$), sodium hydroxide, sodium acetate, sodium dodecyl sulfate (SDS), acetic acid, sulfuric acid, nitric acid, methanol, ethanol, propanol, dichloromethane, dichloroethane, acetone, dioxane, chloroform, acetonitrile, and ammonia solution (25% w/w) were of analytical grade and were purchased from Merck company (Darmstadt, Germany).

Instrumentation

Fluorescence spectra were taken on a Varian Cary Eclipse fluorescence spectrophotometer (Palo Alto, CA, USA). A Phillips EM2085 transmission electron microscope (Amsterdam, Netherlands) with an accelerating voltage of 100 kV was used to characterize the morphology of the adsorbent. Phase characterization was performed by an Ital Structures (Riva Del Garda, Italy) APD 2000 X-ray diffractometer (XRD) using Cu K_α radiation source with the wavelength of 0.154059 nm. A Metrohm 827 pH/mV meter (Herisau, Switzerland) with a combined glass electrode was used for pH measurements. An ElmaS60H Elmasonic ultrasonicator (Singen, Germany) with ultrasonic frequency of 37 kHz and power effective of 150 W was used for dispersion of the adsorbent.

Synthesis of $CoFe_2O_4$-MWCNTs adsorbent

Before modification of MWCNTs with $CoFe_2O_4$ nanoparticles, pristine MWCNTs were oxidized with strong acid treatment. Briefly, 0.5 g of MWCNTs was added to 30 mL of 6 M H_2SO_4:HNO_3 (3:1, v/v) solution and the suspension was refluxed at 70 °C for 6 h. The oxidized MWCNTs were washed five times with 200 mL of deionized water and dried in vacuum oven at 50 °C. In other experiment, 100 mL of 0.2 M cobalt nitrate hexahydrate and 100 mL of 0.4 M ferric nitrate ninehydrate were dissolved in deionized water. The mixture was heated at 80 °C for 5 min and 0.4 g of oxidized MWCNTs was added to the solution under nitrogen atmosphere. After that, 100 mL of 3.0 M sodium hydroxide were added and the mixture was stirred for 30 min. The precipitate was isolated from the solution by applying an external magnet, washed three times with 200 mL deionized water and finally dried in a vacuum oven at 50 °C for 24 h.

MSPE procedure using $CoFe_2O_4$-MWCNTs adsorbent

Ofloxacin solution with the concentration of 100 ng mL^{-1} was transferred to a 250 mL flask and its pH was adjusted to 6.0 by addition of 0.1 M

HCl/0.1 M NH_3 solution. The volume was adjusted to 100 mL with deionized water and 100 mg of $CoFe_2O_4$-MWCNTs was added. The solution was stirred for 10 min and the adsorbent was collected at the bottom of the flask using an external magnetic field. The supernatant was separated by decantation and the supermagnet was removed. Then, 1.0 mL (2×0.5 mL) of methanol was added and the suspension was stirred for 10 min (2×5 min). After desorption, the eluent was separated by magnetic decantation and evaporated to dryness under nitrogen gas flow at room temperature. The dry residue was dissolved in solution containing 1.0 mL of 20 mM of SDS dissolved in 2 mL of 0.5 M acetate buffer with pH 6. The solution was stirred for 10 min and used for taking fluorescence spectra. A blank sample containing all the reagents except the drugs was also prepared.

Real sample analysis

Human plasma samples of three healthy male volunteers aged between 30-35 years old were obtained from Iranian Blood Transfusion Organization (Tehran, Iran) and stored at -18 °C. In analysis time, the samples were placed in an oven at 37 °C for 5 h to thaw. Then, 150 mL of each sample was added to 20 mL of acetonitrile for deproteinization through vortex-mixed and centrifuged at 3000 rpm for 30 min. For recovery tests, appropriate amount of the ofloxacin was spiked to the samples. The recommended MSPE procedure was performed under the optimum conditions.

RESULT AND DISCUSSION

Preparation of the adsorbent

Lower adsorption capacity of naked $CoFe_2O_4$ NPs compared to MWCNTs [23] brings the idea of coupling of these adsorbents to produce an efficient and high capacity adsorbent with magnetic property. Thus, two modification procedures were performed; oxidation with concentrated acids which produces oxygen-containing functional groups and grafting to $CoFe_2O_4$ nanoparticles. The morphology of $CoFe_2O_4$-MWCNTs was characterized by TEM images. As shown in Fig. 1, grafting of $CoFe_2O_4$ nanoparticles to the MWCNTs can be easily observed. The magnetic nanoparticles, which look like nodes growing from the tubes, were wrapped by the MWCNTs bundles [24]. Fig. 2 shows the XRD patterns of MWCNTs and $CoFe_2O_4$-MWCNTs. The diffraction peak at 2θ=26.8 corresponds to the diffraction of MWCNTs and several relatively intense peaks at position (2θ) of 29.6, 34.9, 42.2, 52.7, and 56.3 were matched well with those from the Joint Committee on Powder Diffraction Standards for magnetic NPs.

Effect of SDS concentration

High intensity of a signal guarantees a sensitive and accurate analytical measurement. Since, water is a good quencher for many fluorescent compounds, it can be protected by formation of micelles around fluorophores. In this research, SDS surfactant was used to enhance ofloxacin fluorescence intensity resulting in increase of the sensitivity determined [25]. The effect of SDS concentration on the fluorescence signal of

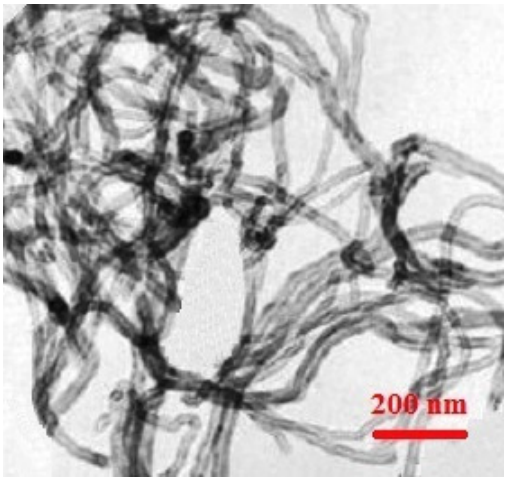

Fig. 1: TEM image of the $CoFe_2O_4$ nanoparticles grafted MWCNTs.

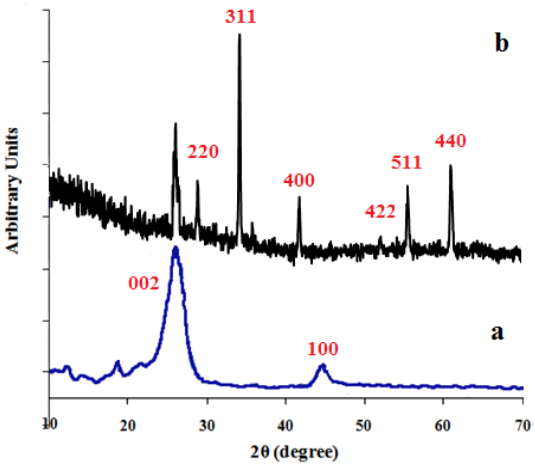

Fig. 2: The XRD pattern of the (a) MWCNTs and (b) $CoFe_2O_4$ nanoparticles grafted MWCNTs.

ofloxacin was investigated by adding different amounts of the surfactant in the range of 1-50 mM. The experimental results are shown in Fig. 3 which reveals a significant fluorescence enhancement with increasing in surfactant concentration. This signal reaches the maximum in 20 mM which is above the critical micellar concentration value of SDS (i.e. 8.2 mM). The reason is that ofloxacin will stabilize in micro-environment created by SDS micelles and therefore, its quantum efficiency increases. In order to achieve a micellar media, SDS concentration must be above the critical micelle concentration [26, 27]. Increase of SDS concentrations does not change the fluorescence intensity, and 10 mM of SDS was selected as the optimum value for the subsequent experiments.

Effect of pH

Batch experiments were performed using 100 ng mL^{-1} of ofloxacin, and fluorescence signal was used to evaluate the effect pH on the extraction efficiency. This parameter has a critical role in any MSPE procedure and determines the analyte structure and surface charge of the adsorbent. Thus, pH of the sample solutions was changed in the range of 3-8. Fig. 4 represents the results of these evaluations indicating that the maximum recovery of the analyte is observed at pH 6.0-8.0. Ofloxacin has two ionizable groups in its structure and therefore has two pK_a values of 6.05 and 8.22 corresponding at the carboxylic group and at the piperazinyl group, respectively [28]. At pH values between pK_{a1} and pK_{a2}, they are in zwitterionic form and the highest adsorption was observed in this range. Therefore, pH 6.0 was selected for the subsequent MSPE experiments.

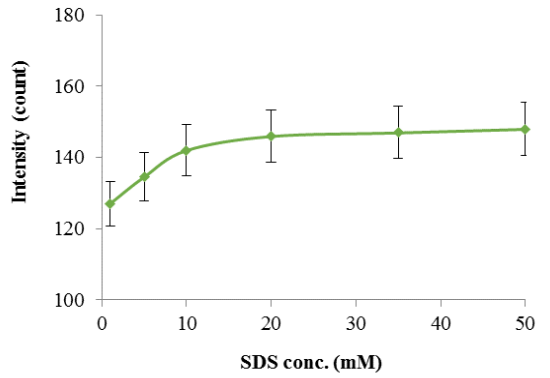

Fig. 3: Effect of SDS concentration on the fluorescence signal of ofloxacin.

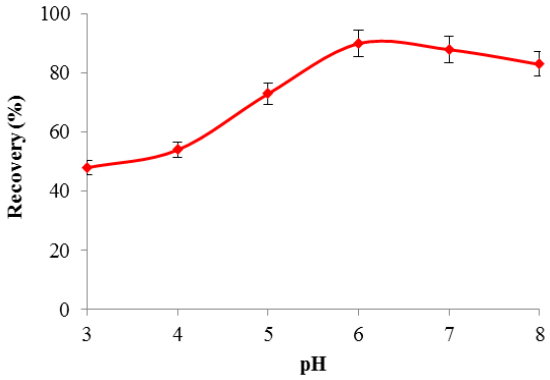

Fig. 4: Effect of pH on the fluorescence signal of ofloxacin. Drug concentration, 100 ng mL^{-1}; adsorbent amount, 100 mg; extraction and desorption times, 10 min; sample volume, 100 mL.

Effect of adsorbent amount

In order to investigate the minimum adsorbent amount which is necessary for the extraction of ofloxacin, the amount of $CoFe_2O_4$-MWCNTs adsorbent was investigated in the range of 50-200 mg. The results revealed that the extraction efficiency is increased by increasing the adsorbent amount and reaches maximum in 100 mg. It was known that nano-sized adsorbents have higher surface areas compared to the ordinary sorbents, therefore, satisfactory results can be obtained by lower amounts of these sorbents. More adsorbent amount does not lead to an obvious change in the recovery of target analyte. Hence, 100 mg was used as the optimum value for all the subsequent experiments.

Effect of desorption conditions

In these experiments, the effects of desorption solvent type and volume were evaluated. To obtain maximum recovery, different organic solvents including methanol, ethanol, dichloromethane, acetone, chloroform, and acetonitrile were examined. From Fig. 5, it was found that ofloxacin can be quantitatively desorbed from the adsorbent by elution with methanol as eluent. As can be seen, polar solvents have more elution capability which may probably due to more interactions with polar groups on the drug.

In another experiments, volume of the eluent was investigated in the range of 0.1-10 mL. Based on the results, the minimum volume of eluent required for quantitative desorption was found to be 2×0.5 mL. Hence, 1.0 mL was selected as the optimum value for the subsequent experiments.

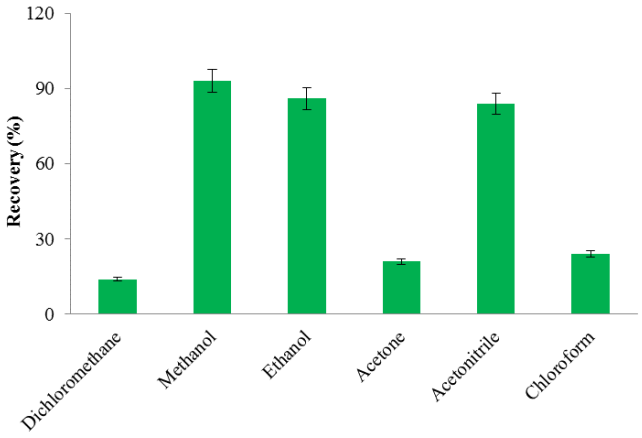

Fig. 5: Effect of eluent type on the extraction efficiency of ofloxacin. Drug concentration, 100 ng mL^{-1}; adsorbent amount, 100 mg; extraction and desorption times, 10 min; pH, 6.0.

Effect of extraction and desorption times

The effects of extraction and desorption time on the recovery of ofloxacin were investigated. The experimental results revealed that 10 min was sufficient for achieving complete recovery for both extraction and desorption. Since $CoFe_2O_4$-MWCNTs can be easily and rapidly collected from the solution using an external magnetic field, the whole MSPE procedure can be performed in less than one hour and the analysis time greatly reduces compared to the conventional extraction methods.

Analytical characteristics

The analytical characteristics of the optimized MSPE, coupled with SDS enhanced fluorescence, were investigated in the optimum conditions. A wide linear range of 100-750 ng mL^{-1} (six-point calibration) with correlation coefficient (R^2) of 0.992 was obtained. The limit of detection (LOD) was defined as three times of blank standard deviation per slope of the calibration curve, and it was found to be 23 ng mL^{-1}. Method precision (as relative standard deviation, RSD%) was investigated by applying five replicate determinations of 250 ng mL^{-1} of ofloxacin and it was found to be 3.3% indicating a good precision of the proposed method. The preconcentration factor (PF) of the analytes was calculated using $PF=V_s/V_e \times R\%$, where V_s is sample volume, V_e is elution volume, and R% is percent recovery. By extracting 100 mL of sample solution containing analyte and collecting into the final volume of 1.0 mL, PE of 93 was obtained for ofloxacin.

Application of method

Efficiency and feasibility of the proposed method was evaluated by applying it to the analysis of real plasma samples. The samples obtained from three healthy male volunteers were analyzed by the proposed method. The applicability was determined by calculating recovery, and the standard solutions were added at three different concentration levels (i.e. low, middle and high quantification concentrations of the linear range). Three-replicate analyses were performed and the mean value it was expressed. Table 1 summarizes the results. As seen in this table, satisfactory recoveries (89-94%) are obtained using the proposed method. These data clearly reveal that the combination of MSPE procedure and enhanced spectrofluorimetry is capable of achieving a high reproducibility with excellent sensitivity for the analysis of synthetic ofloxacin in biological samples.

Table1: Determination of ofloxacin in plasma samples (mean ±RSD, n=5).

Sample	Ofloxacin (ng mL^{-1})		
	Amount added	Amount found ±RSD	Recovery (%)
Plasma	0	-	-
	200	178 ± 4.1	89
	400	368 ± 3.9	92
	700	658 ± 3.2	94

CONCLUSION

An efficient MSPE method, based on the use of $CoFe_2O_4$ nanoparticles grafted onto the oxidized MWCNTs, was proposed and applied to the extraction and preconcentration of ofloxacin in human plasma samples. High adsorbent capacity of $CoFe_2O_4$-MWCNTs causes to need small amounts of the adsorbent and organic solvent. In addition, sensitive spectrofluorimetric detection through fluorescence enhancement of the analyte with SDS micelles is the major advantage of the proposed method. The results revealed that the method has high analytical potential for the preconcentration of ultra-trace amounts of ofloxacin in different matrices.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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