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FUNCTIONALIZED MAGNETIC NANOPARTICLE WITH MULTI-WALLED CARBON NANOTUBE -BASED NANOCOMPOSITE FOR MICRO SOLID PHASE EXTRACTION OF CIPROFLOXACIN

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Abstract

Due to the increase in consuming medicines and increase in environmental pollution through this, controlling consumption of medicines and ability of measuring low amount of them is an important issue in the current age. The present study investigates measurement of low amounts of ciprofloxacin (antibiotic) based on micro solid phase extraction method followed by spectrophotometry. In this study, absorbent would be made based on Magnetic nanoparticle supported Multi-walled carbon nanotube. Then, optimization would be performed. Different factors such as pH, volume of adsorption organic solvent, absorption time, desorption time, stirring speed and type of solvent were investigated separately for optimization purpose. For this purpose, spectrophotometer device has been applied for obtaining maximum wavelength of ciprofloxacin (275 nm) and also obtaining optimized conditions. Dynamic range has been obtained 0.05-0.2 mg/l. In addition, values of pre-concentration factors, LOD and %RSD have been also obtained respectively to 169.28, 0.003 mg/l and 1.588

Keyword: **ciprofloxacin, Multi-walled carbon nanotube, micro solid phase extraction, magnetic nanoparticle**

1. Introduction

Quinolones are a group of antibiotics that can disable assimilation of bacteria [1, 2]. Derivatives of quinolones are fluoroquinolones that can be considered as animportant group of antibiotics and are being widely applied for purpose of treating different diseases such as infections of uric tract, infections of respiratory system and infections of digestive system. They can be also applied in food products of animals [3-6].Ciprofloxacin is a kind of fluoroquinolone (FQ) that can be widely applied for different purposes [7]. Recently, studies have found that FQs can be detected in sirface of underground and drink waters [8-10]Due to the increase in consuming medicines and increase in environmental pollution through this, controlling consumption of medicines and ability of measuring low amount of them is an important issue in the current age.

Different methods have been reported for purpose of measuring amount of the medicine such as water chromatography with high performance [11-15]; electrophoresis of capillary pipe [16] biosensor [17]; and chemiluminescence [18, 19]; and deionizedwater chromatography [20].

Nanocomposites can be applied as strong absorbents in some cases in mixing with magnetic particles to facilitate isolation of particles.

At the present study, an absorbent has been made based on using carbon nanotube factorized with magnetic nanoparticles, in which nanoparticle of Fe_3O_4 encompasses inner part of MWCNT [21].

In this method, Fe_3O_4@MWCNT nanocomposites can be dispersed in sample solution to perform extraction. As a result, the joint between nanocomposites and analyte would be rapidly increased and this can be suitable for enhancing efficiency of extraction and facilitates transferring density of analytes. However, absorbent nanocomposites can be collected through a magnet easily and be transited to the organic phase.

In this study,after transferring nanocomposites to organic phase, nanocomposites were placed under ultrasonic waves, so that absorbed medicine by nanocomposites in aqueous solution can be desorbed in organic solvent.

spectrophotometry or chromatography methods have most application among qualitative and quantitative methods

since they provide wide range of information compared to other methods. Measuring absorption can provide suitable method for analyzing numbers of organic and mineral species [22].

At the present study, micro solid phase extraction method and spectrophotometry have been applied for purpose of investigating pre-condensation and measurement of ciprofloxacin in water sample.

2. Experimental

2.1. Apparatus and instruments

Digital scale stareaous-Cp124s with accuracy of 0.00001 was applied.

Ultrasonic device model H100 made in Hielscher Germany was applied for desorption of analyte in organic phase.

pH meter device model Metrohm 780 made in Swiss has been applied for purpose of settin g pH ratio.

Single-radiation Spectrophotometry device UV-VIS model Corry Eclipse made in Australia with wavelength range of 200-800nm has been also applied for purpose of measuring analyte absorption.

2.2. Reagents

Multi-walled carbon nanotubes (purity= 99.8%) were purchased from Tehran's Research Center for Oil Industry. ciprofloxacin was purchased from Fluka. Acid nitric, acid phosphoric, NaH_2PO_4, borax, hydrazine and methanol HPLC grade purchased from Merck.

2.3. Preparation and production of standard and daily solutions

Fresh working solutions were prepared daily by diluting the stock solution in distilled water. Stock solutions of pesticides (2000 µg/mL) were prepared by dissolving calculated amounts of them in methanol. All experiments were carried out at room temperature, 22 ± 0.5 ◦C. The absorbance of the extract was measured at 275 nm in micro cells against the reagent blank run through the entire procedure.

2.4. Synthesis of encapsulating Fe_3O_4 particles in multi-walled carbon nanotubes

Firstly, 0.5 g of MWCNT was added to acid nitric 70% (W/W) and then it was refluxed for 30 min. oxidized MWCNT was filtered and was washed using deionized water. The fibers were left, to dry, in ambient condition. 0.25g of acidized MWCNT was taken and 0. 6g $FeCl_3$, $5H_2O$ solved in 20 ml deionized water and hydrate hydrazine solution was

added to it (volume ratio of 1:3). A green-colored solution was gained and it was sonicated for half an hour. The pH was adjusted between 11 and 13. Then, the solution was refluxed for 2hrs under temperature of 100°C. 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 120 ◦C for 3 h.

2.5. Procedure

1mg/l of the solution was produced and its absorption was also measured using spectrophotometer. pH of the solution was reached to 7 by buffer phosphate. 2 mg of Fe_3O_4/ MWCNT nanocomposites was added to it. The solution was stirred for 10 min. the magnetic sorbent was collected using an external magnet and supernatant water was decanted. Finally, the extracted analyte was desorbed with 0.7 mL methanol for 10 min and its spectrum was recorded.

Finally, due to high absorption, density of the medicine was reduced to 0.2mg/l and all mentioned steps were iterated. After optimization of absorption rate of ciprofloxacin was reached to 0.9 after desorption operation using spectrophotometer device.

3. Results and discussion

3.1. Effect of pH

One of the most important factors in the process is surface absorption ciprofloxacin on magnetic nanocomposite and enhancement of efficiency of pH factor micro-extraction. Ciprofloxacin includes two ionized groups including one carboxylic acid group (pK1= 5.90) and an alkali pyrazole (pK2= 8.89) [23, 24]. Different values from 3-10 pH were selected and investigated to obtain optimized pH ratio. The most output in micro-extraction was observed in pH=7 (fig.1)

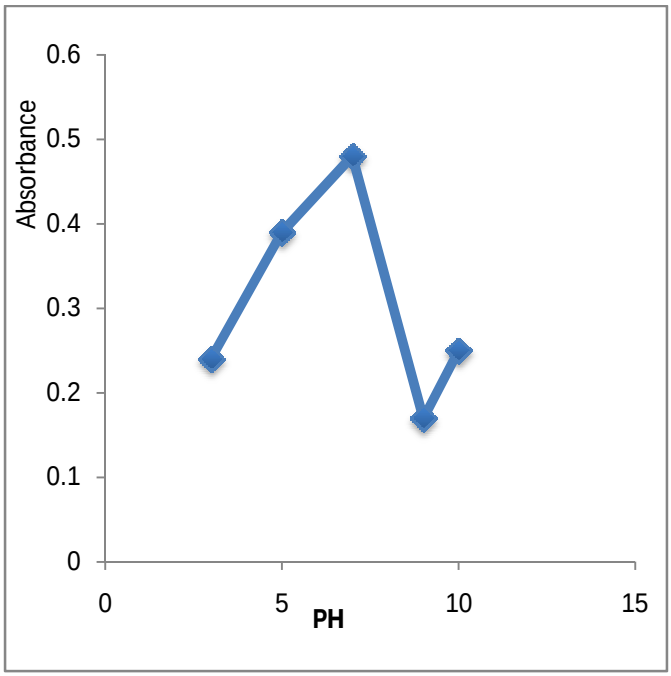

Fig. 1. The effect of pH of aqueous feed on the extraction The effect of pH on the extraction efficiency of ciprofloxacin using nanocomposites SPME with

methanol as the desorption solvent. Other extraction conditions: analyte concentration 0.2 mg/l; stirring rate 200 rpm; desorption time 10 min.

3.2. Effect of stirring rate

Stirring rate the solution can cause increase in speed of mass transfer and can also decrease time of reaching thermodynamic balance. Different stirring rate from 50 to 200 rpm were investigated. According to figure 2, obtained results indicate that the maximum output of extraction was observed in stirring rate of 200 rpm and also transferring the medicine from water phase to nanocomposite was performed properly. the increase on the extraction efficiency after 200 rpm can be considered as not significant

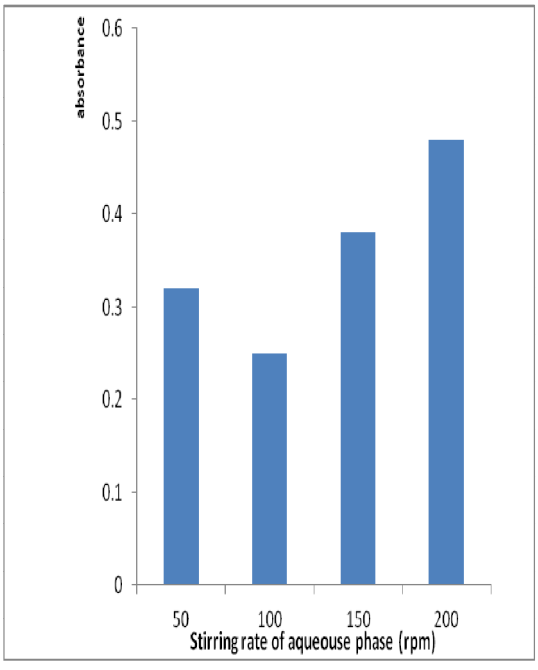

Fig. 2. The effect of stirring rate on the extraction efficiency of ciprofloxacin using nanocomposites SPME with methanol as the desorption solvent. Other extraction conditions: analyte concentration 0.2 mg/l; pH aqueous phase 7; desorption time 10 min.

3.3. Effect of extraction time

The extraction recovery is highly dependent on the mass transfer of analyte from sample solution to the sorbent. As a result, the extraction was performed for various times in the range of 5–20 min. Analyte molecules existed in water solution should have required time for reaching surface of nanocomposite (mass transfer), so that absorption operation can be. According to figure 3, the most output for extraction was achieved in 5 min. In 15 min; the absorption would be increased; although it would be decreased after that. Then, the absorption would be increased after 15 min and it would be decreased again after 20 min. this can be attributed to absorption of molecules of medicine from water solution by nanocomposite and can be also attributed to increase in return time of molecules to the water solution. Therefore, the extraction time was fixed in 5 min.

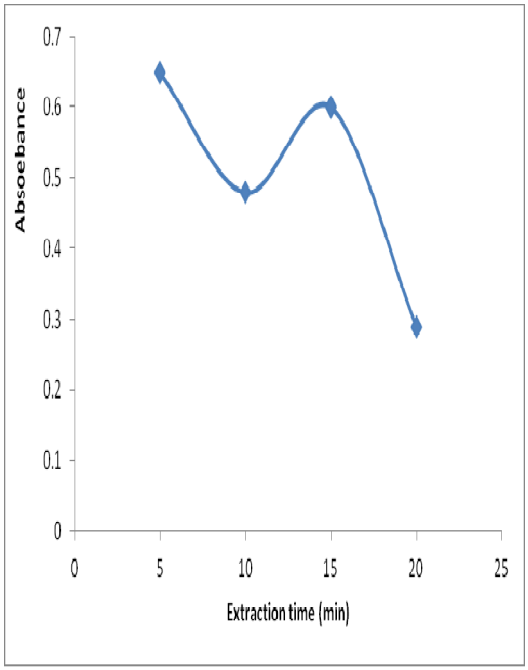

Fig. 3. The effect of extraction time on the extraction efficiency of ciprofloxacin using nanocomposites SPME with methanol as the desorption solvent. Other extraction conditions: analyte concentration 0.2 mg/l; pH aqueous phase 7; desorption time 10 min, stirring rate 200 rpm.

3.4. Effect of the donor phase volume

As the analytes are extracted from relatively large sample volumes into a very small volume of acceptor phase, most SPME applications provide substantial analyte enrichment. As the volume of the sample increases, the pre-concentration factor also increases [24–26]. In SPME, extraction is an equilibration process and, therefore the amount of target analyte partitioning into the acceptor phase becomes independent of the sample volume when this volume is much higher than the product of the partition constant and the volume of the acceptor phase. Here, different volumes of sample volume of 5-20 ml were tested through fixing other parameters. Obtained results from figure 4 indicate that in volume of 5 ml, was found to get the best extraction efficiency.

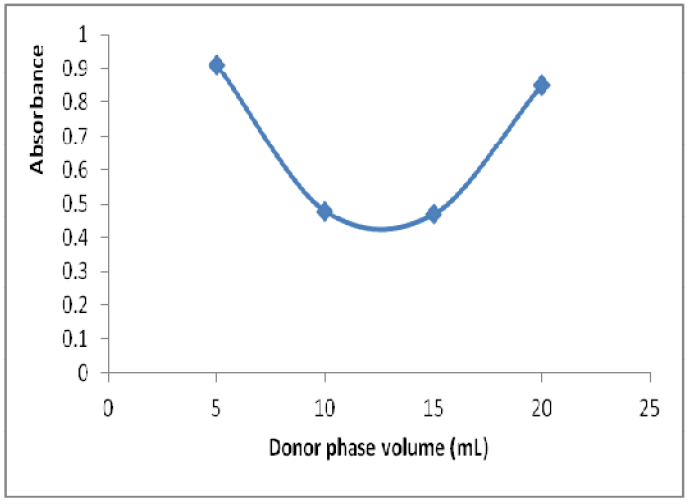

Fig. 4. The effect of donor phase volume on the extraction efficiency of ciprofloxacin using nanocomposites SPME with methanol as the desorption solvent. Other extraction conditions: analyte concentration 0.2 mg/l; pH aqueous phase 7; extraction time 5 min, stirring rate 200 rpm

3.5. Effect of desorption solvent

Accordingly, several desorption solvents such as ethanol, acetonitrile, chloroform and methanol were investigated as desorption solvent. Also because of its low vapor pressure atthe extraction conditions the extract was stable at the extraction period. Therefore, methanol was selected as the desorption solvent (see fig. 5).

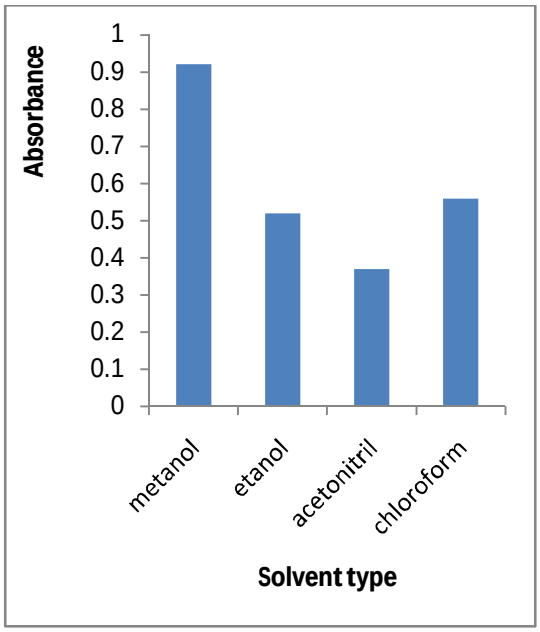

Fig. 5. The effect of solvent type on the extraction efficiency of ciprofloxacin using nanocomposites SPME. Other extraction conditions: analyte concentration 0.2 mg/l; pH aqueous phase 7; desorption time 10 min, stirring rate 200 rpm, volume of donor solvent 5 mL.

3.6. Effect of the desorption time

To reach the highest sensitivity, the desorption time was also evaluated to ensure that the analyte was completely desorbed from the nanocomposites. Experiments showed that for all the studied four analytes, desorption was almost complete after 10 min (figure 6). Thus, these conditions were chosen for routine analysis.

After isolation and pre-concentration in the nanocomposites, the analyte is directly move to the solvent desorption where they are desorbed from the nanocomposites. But to be sure, that desorption was complete the nanocomposites were checking-cleaned after each desorption.

desorption.

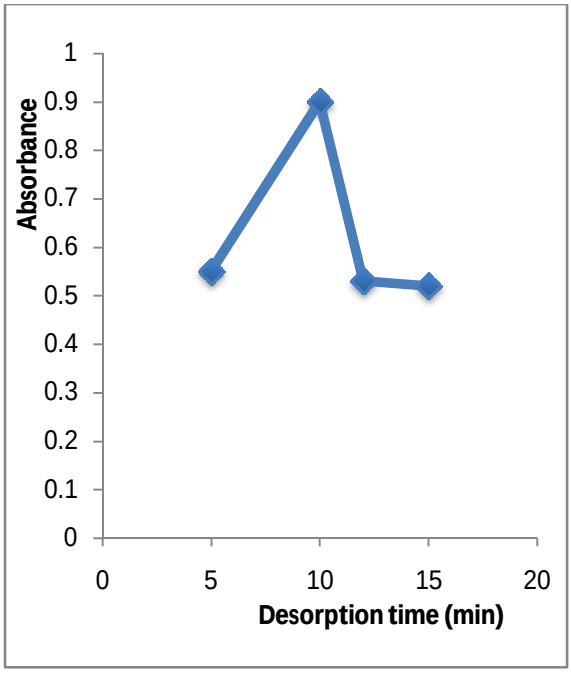

Fig.6. The effect of desorption time on the extraction efficiency of ciprofloxacin

using nanoparticles SPME with methanol as the desorption solvent. Other extraction conditions: analyte concentration 0.2mg/l; pH aqueous phase 7; extraction time 5 min, stirring rate 200 rpm.

3.7. Effect of desorption solvent volume

As it was mentioned in section previously, methanol was the best desorbtion solvent. Volume of methanol is important on the desorption of extraction device. So, desorption volumes ranging from 0.7 to 1 mL for target analyte was examined. The highest extraction efficiency was observed when 0.7 mL desorption solvent volume was used. A decrease in peak areas was observed in the larger ones. This might be due to the dilution target analytes effect. Repeatability decreased in the desorption solvent volume less than 0.7 mL, thus 0.7 mL was used as the optimal volume.

4. Evaluation of the method performance

4.1. Figures of merit

To evaluate the practical applicability of SPME technique, the figures of merit of this method including pre-concentration factor, the corresponding regression equation, correlation coefficient (r^2), limit of detection (LOD) and linear dynamic range (LDR) were investigated under the best conditions (table 1). For each level, three replicate extractions were performed. The results are tabulated in Table 1.

Pre-concentration factor was also alculated as follows:

Pre-concentration factor=

$A_{final}V_{a2}/A_{initial}V_{in}$

Table1.Figures of merit of the proposed method in the determination of the ciprofloxacin in aqueous matrices.

Analyte	LDR [a] (ng/mL)	R [b]	LOD [c] (ng/mL)	P.F [d]	RSD%(n=4) [e]	Equation [f]
Ciprofloxacin	0.2-0.05	**0.9941**	0.003	**169.28**	**1.588**	y = 4.7107x - 0.0505

[a] Linear dynamic range

[b] Correlation coefficient

[c] Limit of detection

[d] Pre-concentration factor

[e] Relative standard deviation

[f] Y and x are peak area and concentration of the analytes (ng mL-1), respectively

5. Real sample analysis

5.1. Real water analysis

Applicability of the extraction method to extract the ciprofloxacin from waste water of Sina Hospital in Mashhad was investigated. No ciprofloxacin concentration was found in it. In order to determine accuracy of the method in real water matrix, solution with density of 0.15mg/l of ciprofloxacin in sample matrix of waste water was extracted under optimized conditions. The obtained results showed the R.S.D% about 2.80, which indicates that the proposed method is repeatable. It can be seen that the relative recovery for
spiked samples was in 95.6.

5.2. Purity percent of ciprofloxacin

A known number of tablets were weighed and ground into a powder. A weighed amount of powder was mixed with about 20mL of deionized water, stirred for about 10 min and filtered (Whatman No. 42 filter paper) into a 25mL calibrated flask. The residue was washed with 5–10mL of water and the washings were combined with filtrate. After dilution and extraction under optimized conditions, purity percent of ciprofloxacin in tablet was obtained to 36.6.

6. Conclusion

The method applied in this study is rapid, easy and including high efficiency for different types of analytes. Over the years, presence of pharmaceutical compounds has been reported in the environment. As ciprofloxacin has abundant uses in the society, investigation of presence of these compounds in environmental waters is so important. The present study has applied a micro solid phase extraction absorbent using functionalized carbon nanotube with Fe_3O_4.

After completing extraction and before pre-concentration, spectrophotometry method was applied for purpose of investigating and measuring ciprofloxacin in real water sample and for determining purity percent of ciprofloxacin in pills. Presented method indicated good pre-concentration factor and high selecting ability. Obtained linear limit and relative recovery were also desirable. In general, applied method has been rapid, easy and cost effective. The discussed method can be introduced as a trustable and valid technique for measuring medicines in the environment and other real samples.

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