

## Microextraction and determination trace of doxycycline in aqueous samples with CuO nanoparticles by spectrophotometry

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### ABSTRACT

*In this work, The CuO nanoparticles were characterized which developed a novel CuO nanoparticle-assisted matrix solid-phase dispersion (CuO-MSPD) method for extraction of doxycycline (DC) from environmental samples. Spectrophotometric determination was developed for isolation and pre-concentration of doxycycline form aquatic and CuO nanoparticles (NPs) was used as a sorbent. The unique properties of CuO NPs including high surface area was utilized effectively in the MSPE process. The effect of the different parameters such as pH, extraction time, effect of the salt, nanoparticle amount and extraction temperature have been studied. Under optimized condition, the method was successfully applied to the extraction of DC from aqueous samples and relative recovery amount of 87%, detection limit of  $25\mu\text{g L}^{-1}$  and a relative standard deviation (RSD) of 2.3% were obtained. The method linear response was over a range of  $10\text{--}50000\mu\text{g L}^{-1}$  with  $R^2 = 0.9968$ . The relative recovery in aqueous matrices was investigated and values of 89% were obtained. The whole procedure showed to be conveniently fast and high extraction efficiency of DC from real samples.*

**Keywords:** Doxycycline, spectrophotometry, nanoparticles, antibiotic;

### INTRODUCTION

Antibiotics are an important group of pharmaceuticals that are released to the aquatic environment in different pathways and have been detected in various aquatic environments such as ground water, agricultural wastewater and in drinking water [1–3]. The animal excreta are the substance send up in manure that are either stored or applied as fertilizers. They are generally weakly absorbed by the human body and thus defecated either unchanged or altered, viaurine or feces [4].

There is an increasing interest about their existence, persistence and fate in the environment because low levels of antibiotics can help the proliferation of antibiotic-resistant bacteria. Tetracyclines (TCs) constitute one of the most important antibiotic families, ranking second in production and usage worldwide [5]. Among 25 antibiotics, tetracycline (see fig 1) was the most commonly detected antibiotic, being present in 80 % of the wastewater in fluent and sewage. Doxycycline (DC) is one of the kinds of antibiotics of the TC family containing the  $\beta$  diketone configuration. Its curative effect is superior to that of TC. It is critical to develop reliable, rapid, precise and economical analytical procedures for monitoring TCs that are harmless to the environment as well. In this regard, a tremendous technique for separation and removal of TCs has been reported [6–9]. Some researchers have proposed the adsorption of TCs on oxide minerals [10, 11], Fe–Mn binary oxide [12], sediments [13], humic substances [14] and clay minerals [15]. But the problems for application of these adsorbents are their reusability, removal efficiency, adsorption capacity and/or long equilibrium time; therefore, a reliable and well-defined method is still in need despite a few methods currently available.

Substances in the nanometer range are expected to exhibit new properties for a wide range of applications, including catalysis, optics, microelectronics, and sorbent [16–18]. One of the most interesting and promising fields is the research on metal-oxide nanoparticles. Copper oxide (CuO) is one of the relatively few metal oxides that tends to be p-type. Its nanoparticles are capable of, for example, catalyzing the oxidation of CO at mild temperatures [19] and

improving the thermal stability of phenolic resins [20]. Therefore, many methods have been attempted to synthesize nanostructured copper oxide, such as the sonochemical method, sol-gel technique [21-22], one-step solid-state reaction method at room temperature [23], electrochemical method [24], thermal decomposition of precursors [25], and co-implantation of metal and oxygen ions [26]. The aim of the present study was to develop a simple and reliable  $\mu$ -SPE method based on CuO nanoparticles for the preconcentration and determination of trace amounts of DC in aqueous sample. Affecting factors of the extraction efficiency of DC were investigated. This methodology has not been employed previously for the determination of trace amounts of DC in aqueous sample.

## MATERIALS AND METHODS

### Apparatus

A Shimadzu UV 160 spectrophotometer with using 1-cm quartz cells was used. The ultrasonic processor apparatus model UP-100H (Hielscher-Germany) was used in this study. The FT-IR instrument that was used for recording the infrared spectrum was M-500 Fast-Scan IR Spectrometer (Buck Scientific, East Norwalk, CT 06855, USA). The transmission electron microscopic analysis used a Jeol 2010 instrument with an accelerating voltage of 200 kV.

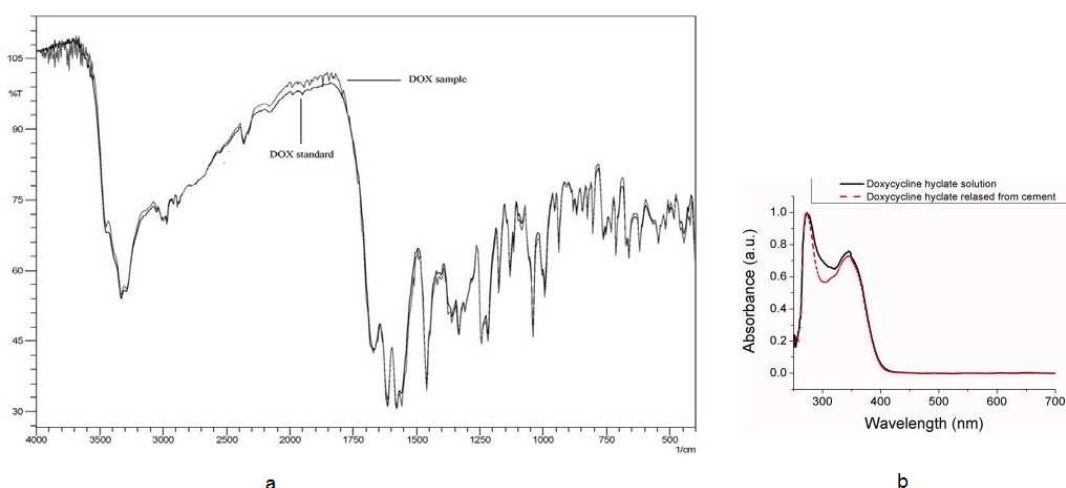
X-ray powder diffraction patterns of the products were recorded on a Shimadzu XRD-6000 x-ray diffractometer at a scanning rate of  $0.05^\circ\text{s}^{-1}$  in the  $2\theta$  range from  $10$  to  $80^\circ$  with high-intensity  $\text{CuK}\alpha$  radiation ( $\lambda=0.154178$  nm).

### Reagents and solutions

All reagents used were of analytical reagent grade and used without further purification. Deionized water was used for all solution preparation throughout the experiments. Standard stock solutions of DC  $1000$  mg.L $^{-1}$  was prepared by accurately weighing and dissolving appropriate amounts of doxycycline in methanol and was kept in a refrigerator at a temperature below  $4^\circ\text{C}$ . The working solutions were prepared daily by appropriate dilution of the stock solution in deionized water.

### Synthesis of CuO nanoparticles

$300$  mL aliquot of  $0.02$  mol.L $^{-1}$  copper acetate aqueous solution was mixed with  $1.5$  mL of glacial acetic acid in a round-bottomed flask equipped with a refluxing device. Acetic acid was added to prevent hydroxylation of  $\text{Cu}^{2+}$  ions. The solution was heated to  $90^\circ\text{C}$  with stirring. Subsequently,  $0.96$  g of sodium hydroxide as reducing agent was added into the above solution and stirred for  $15$  min until the value of the mixture reached pH  $5.5$ – $7$ , where a large amount of black precipitate was formed. After being cooled to room temperature, the resultant suspension was stirred with ultrasonic horn for  $70$  min. Finally the precipitate was centrifuged, washed once with double distilled water and three times with ethanol, respectively, and dried at room temperature for  $24$  h. The obtained dry black powder sample was used for further analysis.



**Fig. 1.** FT-IR (a) and UV-VIS (b) spectrums of doxycycline  
The absorption spectra of the doxycycline have maximum absorbance at  $280$  nm.

### $\mu$ -SPE procedures

$10$  mg CuO nanoparticles were added to  $10$  ml solution of  $2$  mg L $^{-1}$  DC and were mixed on a shaker by a definite rate. The solution was centrifuged for  $5$  minutes in  $5000$  rpm. In this step, the nanoparticles were deposited and the tap solution was removed. Then  $0.5$  ml of methanol was added to CuO nanoparticles, after mixing and centrifuging

(5000 rpm, 5 min), the nanoparticles was deposited and the enriched methanol of analysis was transferred to the spectrophotometric cell. The absorbance before and after adsorption of the DC were measured. Pre-concentrate factor =  $A_2/A_1$ .  $A_1$  and  $A_2$  are the adsorption before and after extraction respectively.

## RESULTS AND DISCUSSION

### Absorption and FT-IR spectrums of doxycycline

Fig. 1 shows the FT-IR UV-VIS spectrums of doxycycline ( $30 \text{ mg.L}^{-1}$  in HCl 0.1 N). The following characteristic bands can be observed: aromatic ring at  $1600\text{--}1500 \text{ cm}^{-1}$ ;  $\text{CONH}_2$  at  $1650 \text{ cm}^{-1}$ ; and  $\text{COOH}$  at  $1700 \text{ cm}^{-1}$ .

### FT-IR spectrum and characterization of CuO nanoparticles

Fig. 2 has shown the FT-IR spectra of CuO nanoparticles, which indicates three adsorption peaks observed at  $437 \text{ cm}^{-1}$ ,  $532 \text{ cm}^{-1}$  and  $584 \text{ cm}^{-1}$ . These frequencies modes are due to  $\text{Cu(II)-O}$  of CuO nanoparticles which are in good agreement with the previous reports [27].

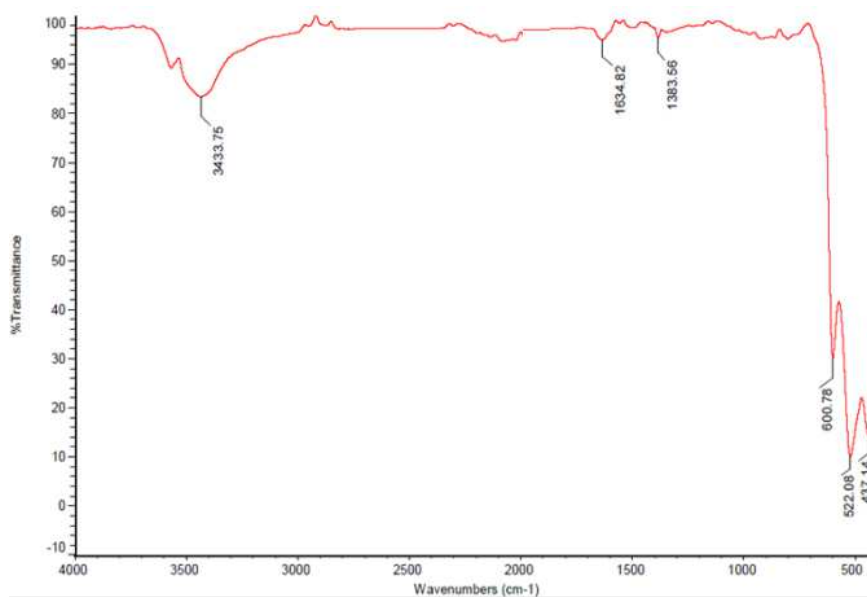


Fig. 2. FT-IR spectrum of CuO nanoparticles

The morphology of the product is examined by transmission electron microscopy (TEM) images of CuO nanostructures (see fig. 3), the average particle size is about 20 nm, according with the calculated value. XRD patterns (Fig. 4) of the products obtained are alike to the single-phase CuO with a monoclinic structure and the diffraction data were in good agreement with JCPDS card of CuO (JCPDS 80-1268). The peak broadening clearly indicates the small size of the products. The average size of the CuO nanoparticles is estimated to be 18-23 nm according to the Scherrer equation [28].

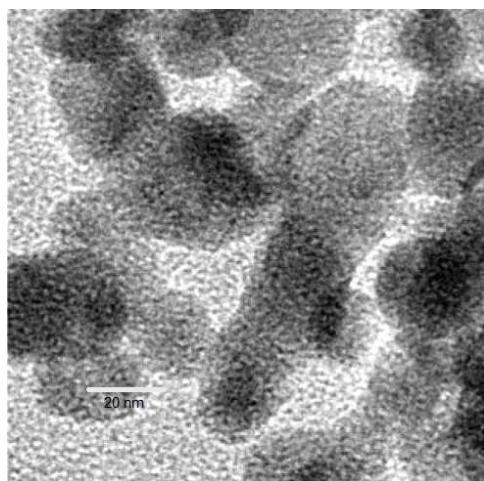


Fig. 3. TEM image of the CuO nanoparticles

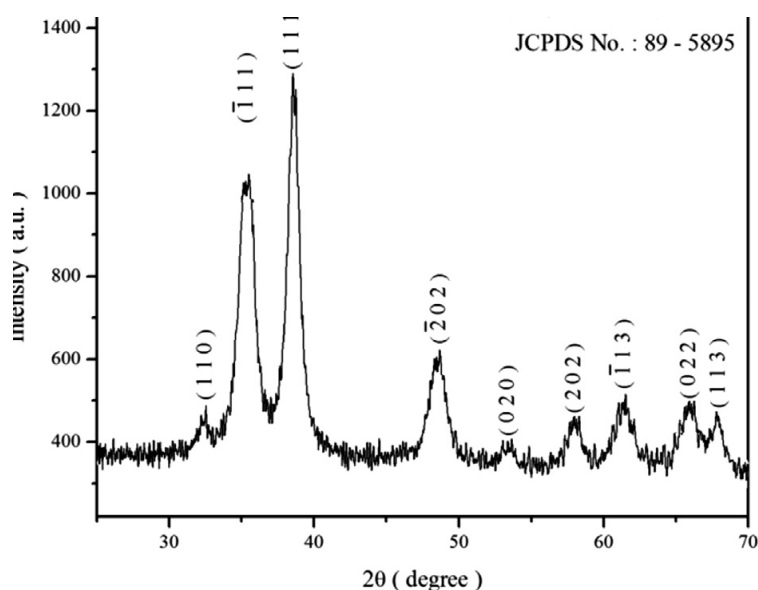


Fig. 4. XRD pattern of CuO nanoparticles

#### Effect of nanoparticle amount

The pre-concentration factor of DC on CuO nanoparticles was studied at different adsorbent amount (5.0–20 mg), DC concentration of  $2 \text{ mg L}^{-1}$ , pH 5.0 and agitation time of 10 min. Figure 5 shows that by increasing the amount of adsorbent up to 10.0 mg, the adsorption of DC increased. This could be explained by this fact that more adsorbent became available for adsorbing the DC molecules. Further addition of the adsorbent did not show any significant change in Concentrate factor of DC. Thus, 15.0 mg of the nanoparticles was chosen as the optimum amount for performing the following steps of the optimization procedure.

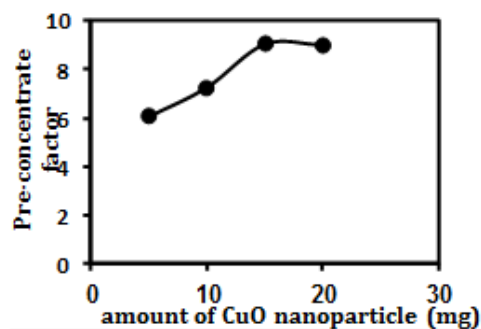


Fig.5. Effect of the amount of CuO nanoparticles on extraction efficiency of DC. Experimental conditions: pH 5.0; DC concentrations of  $2.0 \text{ mg L}^{-1}$ ; extraction time of 10 min and amount of NaCl 15% w/v.

#### Effect of pH of aqueous sample

As is well known, compounds will be present as different states in the different pH environments, and the sample pre-concentration is related directly to the present state of the compounds in most cases. In the proposed new extraction procedure, pH of sample solution would throw an important impact on the pre-concentration of DC. Therefore, the effect of sample pH was optimized over the range of 2–10 by adding the appropriate hydrochloric acid or sodium hydroxide solution to water samples. The results confirmed that the DC extraction performance reaches a better level at pH 5. In the pH above or below than 5, the extraction efficiency decreased rapidly. It is supposed that, it is due to the occurrence of degradation under high alkaline condition. Based on thorough consideration, pH 5 was selected for further experiments (see Fig. 6).

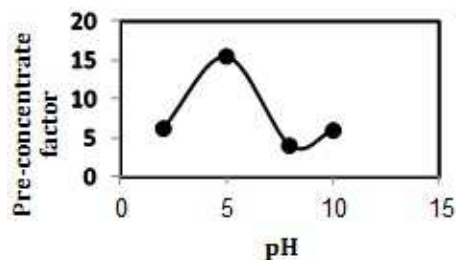


Fig. 6. Effect of pH of DC solutions on extraction efficiency of DC, Experimental conditions: CuO amount of 15.0 mg; initial DC concentrations of 2.0 mg L<sup>-1</sup>; shaking time of 10 min and amount of NaCl 15% w/v.

#### Effect of extraction time

Extraction was performed from 2 to 20 min to determine the effect of extraction time on the method efficiency (see fig 7). The effect of extraction time on the extraction efficiency of DC is shown in Fig. 7. the analyte the highest increase in the extraction efficiency in the period of 10 min. Afterwards the extraction efficiency were decreased with increasing of extraction time. Perhaps dissolution in water solution causes this incident. So, a period of 10 min was used for the subsequent experiments.

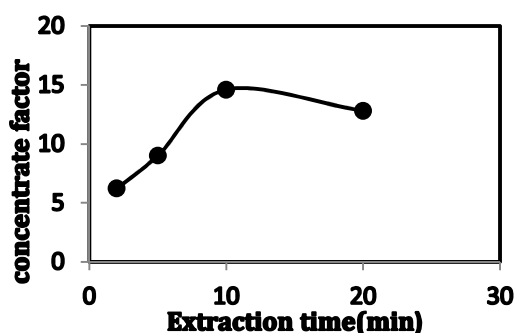


Fig. 7. Effect of extraction time on extraction efficiency of DC, Experimental conditions: CuO amount of 15.0 mg; initial DC concentrations of 2.0 mg L<sup>-1</sup>; pH 5.0 and percent of NaCl 15% w/v.

#### Effect of solution temperature

The effect of temperature on the extraction of DC solution was investigated at pH 5.0 while a shaking time of 10.0 min was performed. The results showed that the extraction efficiency of the DC as a function of temperature in the range of 25-50° C was not significantly affected by temperature.

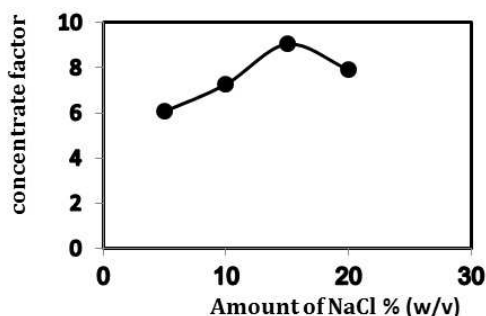


Fig. 8. Effect of NaCl percent on concentrate factor of DC, Experimental conditions: CuO amount of 15.0 mg; initial DC concentrations of 2.0 mg L<sup>-1</sup>; pH 5.0 and shaking time of 10 min.

#### Effect of salt into aqueous sample

It is well known that the addition of salt can increase or decrease the extraction of analyte [29]. The effect of salt on extraction was studied by varying the amount of NaCl within the range of 0–20% (w/v) (see fig8). The extraction efficiency increased for DC at salt concentration below 15% (w/v), resulting, presumably, from the salting-out effect. However, apart from the salting-out effect, the presence of salt can change the physical properties of the extraction film in the interface of aqueous feed solution and sorbent. It may lead to change of the aqueous activity coefficient of analytes, thus increasing the diffusion rates of the analytes into the sorbent. The extraction efficiency decreased slightly for solution with 20% (w/v) salt. Somewhat surprisingly, extraction efficiency decreased with increasing salt concentration for DC. The effect of NaCl on the extraction of DC is probably due to salting-out

effect, which decreases the solubility of the analytes and thus increases their partition [30] into absorbent. So, further extractions were carried out at addition of 15% (w/v) salt.

#### Calibration curve and real samples analysis

For constructing the calibration curve (Fig. 9), standard DC solutions with different concentrations were prepared and their absorbances were measured by UV-Vis spectrometer at 280 nm. Applicability of the proposed method to real samples was evaluated by extraction and determination of spiked DC in tap water (dezfoul city, Iran) as reported in Table 1.

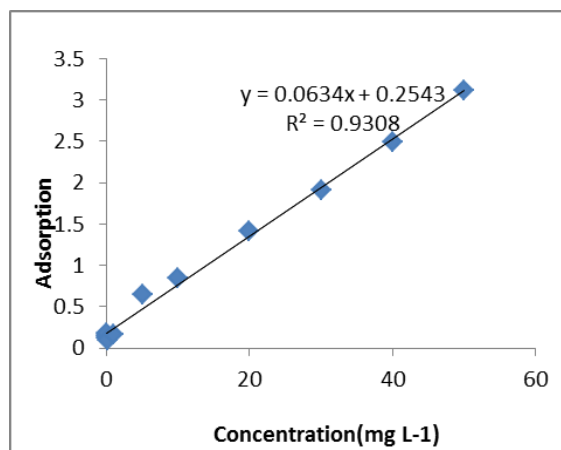


Fig. 9. Calibration curve constructed at 280 nm

Table 1. Percent recovery results for spiked DC in water samples (n = 3)

Sample	Added (mg/l)	Found (mg/l)	Recovery %	RSD % (n = 3)
Tap water	0	ND*	-	-
	15	14.50	96	2.04
	25	25.96	103.0	1.31

\*Not Detected

#### CONCLUSION

In this research, CuO nanoparticles were used in water sample in order to extract and determination of DC. In this method, the extraction can be done faster and easier by using CuO nanoparticles. Moreover, using small amount of solvent not much wastewater is produced and meanwhile it is a cheap and affordable method. This method can be used in environmental pollution control organizations, pharmacy companies and clinical diagnosis laboratories.

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