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ISSN No: 2319-5886

International Journal of Medical Research & Health Sciences, 2016, 5, 8:332-339

Rapid magnetic solid phase extraction–spectrophotometric determination of doxycycline and erythromycin in real samples with nanoparticles

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ABSTRACT

In recent years, pharmaceutical and personal care products (PPCPs) have been detected in diverse environments (including groundwater, river water, and municipal wastewater).This work characterizes removal of trace antibiotics (doxycycline (DC), erythromycin (ERY)) from water samples. For this purpose a matrix solid-phase dispersion (MSPD) microextraction method was developed for extraction of doxycycline and erythromycin. Nanoparticles were used as sorbent. Spectrophotometric determine was developed for isolation and pre-concentration of doxycycline and erythromycinform aqueous samples. In this experiment several parameters such as pH, nanoparticle amount, effect of the salt, extraction time and extraction temperature were optimized for the extraction with two nanoparticles. Under the optimized conditions, a linear range of 10 -50000 $\mu g L^{-1}$ with R^2= 0.994, 0.971and detection limit of 0.05 and 0.037 $\mu g\ L^{-1}$ were obtained for doxycycline and rythromycin with Fe_3O_4 nanoparticles, respectively .Results were revealed that the Fe_3O_4 nanoparticles were generally able to extraction of two antibiotics.

Keywords: Erythromycin, doxycycline, spectrophotometry, nanoparticles, antibiotic

INTRODUCTION

The issue of antibiotics and other pharmaceuticals as emerging environmental contaminants has been an increasing focus of recent environmental research [1–3]. Significant and continual inputs of antibiotics to the environment occur via disposal of expired or unused drugs and by human and animal excretion. Some of the major concerns about this continual input to the environment include possible chronic effects to nontarget organisms [4], and the development of antibiotic-resistant microbes [5, 6]. Research aiming to understand the occurrence, fate, and effects of these compounds in the environment is under way, but presently knowledge in these areas is incomplete [3]. To understand the behavior of antibiotics in the environment, reliable and sensitive measurement methods must first be established. Antibiotics have frequently been detected in the ng/L to g/L concentration range in wastewaters and at ng/L concentrations in surface waters [7–13].The most common techniques used for the determination of residues of antibiotics are solid-phase extraction (SPE) [14-17], solid phase microextraction (SPME) [18] and stir bar sorptive extraction (SBSE) [19]. For aqueous samples, solid phase extraction (SPE) is nowadays the most common technique [20–22]. Processing by SPE allows simultaneous extraction of multiple samples and generally gives good recovery of target compounds [7].The use of solid phase microextraction (SPME) for the extraction of pharmaceuticals from aqueous sample is of interest, as it may offer benefits over traditional SPE techniques. The SPME apparatus consists of a sorbent. Upon exposure to a sample, sorption of compounds to the phase occurs, resulting in simultaneous extraction, clean-up, and concentration. Thus, required processing time and labor is greatly reduced [23]. In addition, SPME is potentially more cost-effective than SPE since an individual fiber can be used for multiple extractions [23] and very little solvent is required for the overall process. In contrast, SPE supplies are one-time use onlyand significantly more solvent is consumed. Matrix effects may also be overcome, because unlike SPE, relatively little of the sample matrix components are transferred to final extracts, resulting in less interference during analysis [24]. While SPME has found wide application, its use for the extraction of pharmaceuticals has primarily been in biological matrices such as milk, urine, and plasma [25]. Its application to environmental matrices is much

less frequent. Most such applications used gas chromatography (GC) for analysis, requiring derivatization of polar, non-volatile drugs such as selective serotonin reuptake inhibitors in river and wastewater [26], and non-steroidal anti-inflammatory drugs [27]. Derivatization not only increases sample preparation times, but also can produce variable and/or incomplete derivatization of analyses [28]. One of the important factors in SPME apparatus is a suitable sorbent. One of the most interesting and promising fields is the research on metal-oxide nanoparticles. In this work Fe_3O_4 nanoparticles (NPs) were used as sorbent. These nano-adsorbents have a high surface area and a small particle size. Its nanoparticles are capable of, for example, catalyzing the oxidation of CO at mild temperatures [29] and improving the thermal stability of phenolic resins [30]. Magnetite nanoparticles have shown great potential for many nanotechnology applications, including effective adsorbents for removal of undesirable contaminants in water treatment [37]. There are many methods to prepare Fe_3O_4 nanoparticles such as energy milling [38], reducing [39], ultrasonic assisted impregnation [40], Co-precipitation [41] method and using Tridaxprocumbens leaf extract [42].The objective of this study was to develop a simple and reliable µ-SPE method based on Fe_3O_4 nanoparticles for the preconcentration and determination of trace amounts of DC and ERY in aqueous samples, then developed a comparison between two nanoparticles for extraction of DC and ERY.

MATERIALS AND METHODS

A shimadzu UV 160 spectrophotometer equipped with matched 1-cm quartz cells was used for recording all absorption spectra. Ultrasound mixing was carried out by a model UP-100Hultrasound cleaner (Hielscher-Germany). The FT-IR spectrogram was recorded by M-500 Fast-Scan infrared spectrometer (Buck Scientific,East Norwalk, CT 06855, USA). The morphology and size of nanoparticles were observed by Jeol 2010 transmission electron microscope (TEM) (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 $0.05°s^{-1}$ in the 2h range from 10 to 80° with high-intensity CuK_α radiation (µ=0.154178 nm).

Reagents and solutions

Analytical reagent grade chemicals and deionized water were used. Two antibiotics(erythromycin and doxycycline) were of 98% purity . Standard stock solutions were prepared by dissolving each medicine in methanol at a concentration of 1000 $mg.L^{-1}$and kept at a temperature below 4 ◦C. Working solutions of pharmaceutics was prepared by diluting solution in deionized water.

Synthesis of Fe_3O_4 nanoparticles

The magnetic Fe_3O_4 NPs were prepared by the chemical co-precipitation method. Briefly, 20 g of $FeCl_3$-6H2O, 8 g $FeCl_2$-$4H_2O$, and 4 mL HCl (conc.) were dissolved in 50 mL water under a N2 stream. This solution was added drop-wise into 300 mL of sodium hydroxide (1.8 M) under a nitrogen atmosphere and vigorously stirred for 40 min. The resulting black precipitate was separated with a magnet and washed several times with degassed water; it was stored in 500 mL degassed water under a nitrogen atmosphere.

µ-SPE procedures

10 mg magnetic nanoparticles were added to 10 ml solution of 5 mg L^{-1} DC and ERY. Then was mixed on a shaker by a definite rate. The mixture was shacked and allowed to complete the extraction process for 4 min. Subsequently, an Nd-Fe-B strong magnet (10 cm × 5 cm × 4 cm, 1.4 T) was placed in the bottom of the beaker, and the Fe_3O_4 NPs were isolated from the solution. After about 0.5 min, the solution became limpid and supernatant solution was decanted. Then 0.5 ml of methanol was added to 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 and ERY were measured.

Pre-concentrate factor=A_2/A_1
A_1 and A_2 are the adsorption before and after extraction respectively.
Other time all process were carried out with Fe_3O_4 nanoparticles.

RESULTS AND DISCUSSION

Absorption and FT-IR spectrums of doxycycline and erythromycin

Fig. 1 shows the FT-IR spectrum of (a) doxycycline(aromatic ring at 1600–1500 cm^{-1}; $CONH_2$ at 1650 cm^{-1}; and COOH at 1700 cm^{-1}) and (b)erythromycin(OH at 3473.97 cm–1; and C=O at 1732.56 cm^{-1}). Fig 2 shows UV- VIS spectrum of (a) doxycycline (λ_{max}=280nm)and (b) erythromycin (λ_{max}=228nm)

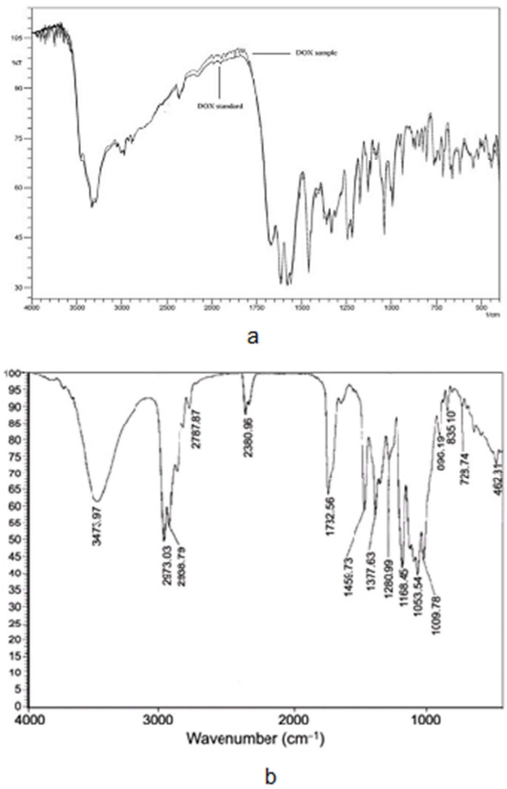

Figure 1: FT-IR spectrums of (a) doxycycline (b) erythromycin

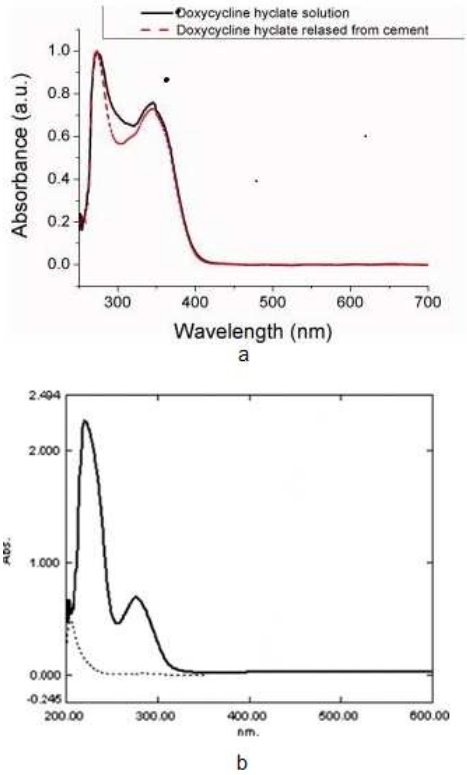

Figure 2: UV-VIS spectrum of (a) doxycycline (b) erythromycin

FT-IR spectrum and characterization of Fe_3O_4 nanoparticles

Fig.3 has shown the FT-IR spectra ofFe_3O_4 nanoparticles. The spectrum of Fe_3O_4 nanoparticles shows an intense and broad band appeared in the region 3200–3600 cm^{-1} region is corresponding to the O–H stretching vibration. Note that the iron oxide surfaces are easily covered with hydroxylgroups in an aqueous environment [44].

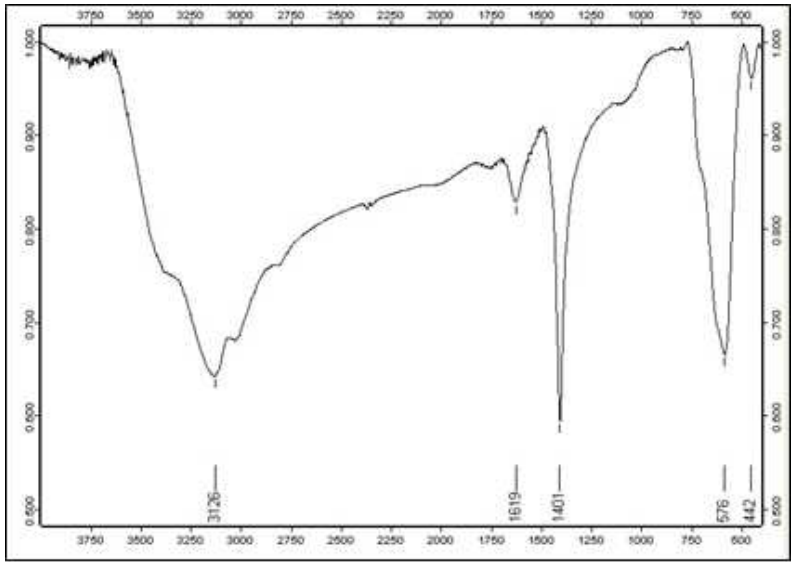

Figure 3: FT-IR spectrum of Fe_3O_4 nanoparticles

The morphology and size of nanoparticles is approved by transmission electron microscopy (TEM) image of Fe_3O_4 nanoparticles (see fig. 4), the average particle size of two products are about 20 nm, according with the calculated value.XRD patterns of the nanostructures have shown in fig 5.

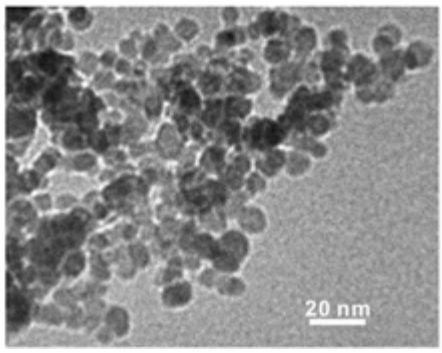

Figure 4. TEM image of the (a) CuO (b) Fe_3O_4 nanoparticles

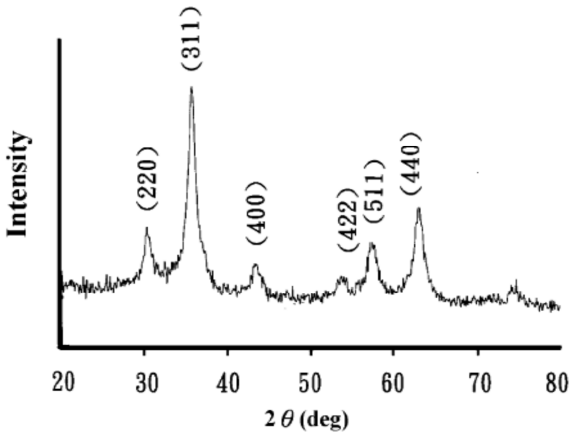

Figure 5. XRD pattern of (a)CuO (b) Fe_3O_4 nanoparticles

Effect of extraction time

In order to come off complete extraction, the effect of extraction time on the absorption was investigated. The results are shown in Fig. 6, which corroborates that the extraction efficiency of the ERY and DC increased with the

increased extraction time from 2 to 10minwithFe_3O_4 nanoparticles, after this time the extraction efficiency was. Therefore, 10 min was selected for extraction time with Fe_3O_4nanoparticles.

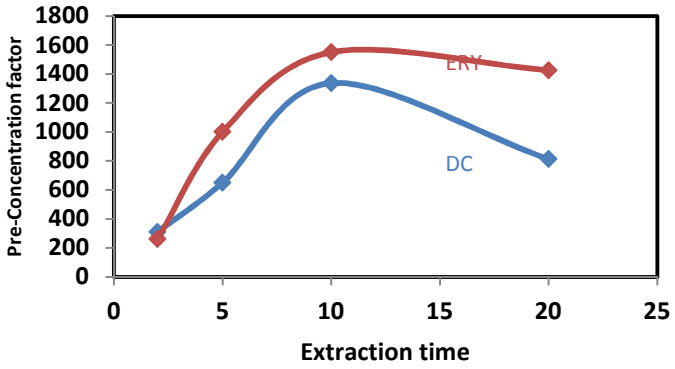

Figure 6.Effect of extraction time on extraction efficiency of DC and ERY with Fe_3O_4 nanoparticles, Experimental conditions: Fe_3O_4amount of 10.0 mg; initial DC and ERY concentrations of 5.0 mg L^{-1}; pH 8.0, percent of NaCl 10% w/v

Effect of nanoparticle amount

Fewer amounts of nano-adsorbents may be achieved more satisfactory results than micro-adsorbents because of their greater surface are as[45]. To attain good recovery, the adsorbent amount was evaluated. The different amounts of then noparticles (5, 10, 15, 20 mg) were tested. As shown in Fig. 7, the recovery of ERY and DC increased with increasing sorbent doses to 10 mg of Fe_3O_4nanoparticles, and then addition of the adsorbent did not show any significant change in concentration factor of two antibiotics. Thus 10 mg was employed used in the next experiments with Fe_3O_4 nanoparticles.

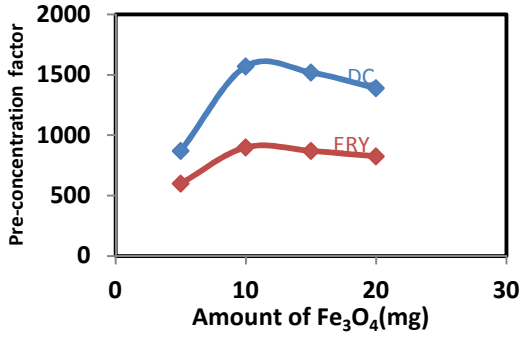

Figure 7. Effect of amount of Fe_3O_4on extraction efficiency of DC and ERY with Fe_3O_4 nanoparticles, Experimental conditions: extraction time of 10 min; initial DC and ERY concentrations of 5.0 mg L^{-1}; pH 8.0, percent of NaCl 10% w/v

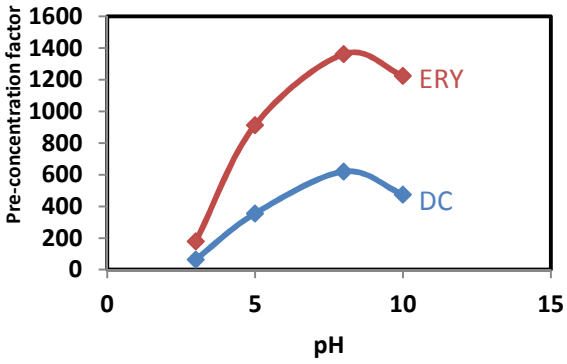

Figure 8. Effect of pH on extraction eficiency of DC and ERY with, with Fe_3O_4 nanoparticles, Experimental conditions: extraction time of 10 min; initial DC and ERY concentrations of 5.0 mg L^{-1}; Fe_3O_4 amount of 10.0 mg, percent of NaCl 10% w/v

Effect of pH of aqueous sample

The pH of the sample solution is an important factor on the adsorption of the analytes to the sorbents. The pH not only alters the structure of the analytes, but also changes the interaction between the sorbents and the analytes. In this experiment, the pH optimization was performed by adding the appropriate hydrochloric acid or sodium

hydroxide solution to sample solutions over the pH range from 3.0 to 10.0. As seen from Fig. 8, the highest extraction performance for these antibiotics is obtained at pH 8.0 with two nanoparticles. The adsorption recovery of DC and ERY decreased when the pH increased greatly from 8.0 to 10.0. Although the satisfactory recovery was attained at pH 8.0.

Effect of solution temperature

The effect of temperature on the extraction of solution that containing DC and ERY was interrogated at pH 8.0 while a extraction time of 5.0 and 10 min for extraction with Fe_3O_4 nanoparticles were performed, respectively. The results showed that the extraction efficiency of the ERY and DC as a function of temperature in the range of 25-60° C was almost stabilized.

Effect of salt into aqueous sample

To evaluate the effect of salt(Ionic strengths) on SPME performance, extractions of 5 mgL^{-1}analyte solutions with adding varying from 0 to 20% (w/v) sodium chloride were performed. The addition of sodium chloride increased significantly the extraction of two compounds (Fig. 9), in agreement with prior studies that found greater extraction efficiency with increasing ionic strength [46–48,49]. The increased extraction of compounds from aqueous sample in the presence of salt can be explicated by the "salting out" phenomenon, by which the addition of a salt shifts the sorption equilibrium to favour sorption of the target analyte to the sorbent. Based on our results, an Ionic streng the of 10% (w/v) NaCl for extraction with Fe_3O_4 nanoparticles was chosen for further experiments. Ionic strengths above these amounts approached solution saturation and were hence not considered.

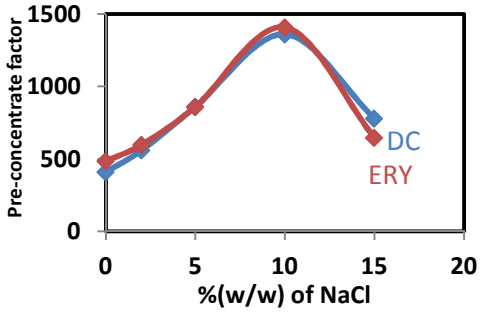

Figure 9. Effect of NaCl on extraction efficiency of DC and ERY with Fe_3O_4 nanoparticles, Experimental conditions: extraction time of 10 min; initial DC and ERY concentrations of 5.0 mg L^{-1}; pH 8.0, Fe_3O_4amount of 10.0 mg

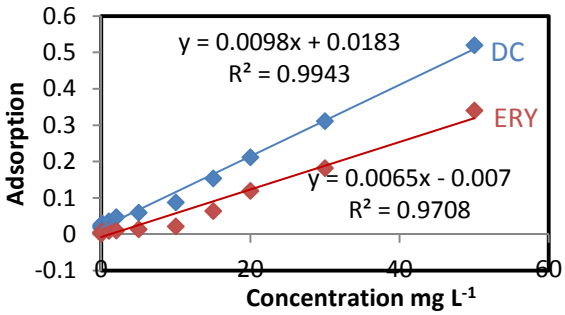

Figure10. Calibration curve constructed with Fe_3O_4 nanoparticles

Table 1. Percent recovery results for spiked DC and ERY in water samples with Fe_3O_4 nanoparticles (n = 3)

Sample		Added (mg/l)	Found (mg/l)	Recovery %	RSD % (n = 3)
Tapwater	DC	0	ND[1]	-	-
		3	3.03	101.02	1.24
		25	24.66	98.6	1.11
	ERY	0	ND[1]	-	-
		3	3.04	101.53	1.18
		25	24.3	97.23	1.61

[1] *not detected*

Calibration curve and real samples analysis

For constructing the calibration curve (Fig. 10), standard solutions contain of DC and ERY with different concentrations were prepared and their absorbance were measured by UV–Vis spectrometer at 280 **(** $\lambda_{max}$of DC) and

228 nm (**λ_{max}** of ERY). After the method was established, the proposed method was applied to the determination of doxycycline and erythromycin in tap water (dezful city, Iran). The results are outlined in Table 1.

CONCLUSION

The suggested methodology showing good analytical function for the analysis trace of doxycycline and erythromycin in water samples. This method provided good recoveries, low detection limits, satisfactory precision and suitable linear dynamic ranges, under optimized experimental conditions. In this research we used of Fe_3O_4 nanoparticles as sorbent for extraction and determination of two antibiotics from complex sample matrices**.** A comparison between efficiency extraction with Fe_3O_4nanoparticles indicated that Fe_3O_4 nanoparticles had higher extraction ability, therefore it illustrated these nanoparticles had better extraction efficiency.

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