Measurement of doxycycline in pharmaceutical Samples with SnO₂ nanoparticles by spectrophotometric method

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ABSTRACT

In this work, The SnO₂ nanoparticles were characterized which developed a novel SnO2 nanoparticle-assisted matrix solid-phase extraction (SnO₂ -SPE) method for extraction of doxycycline from pharmaceutical and environmental samples. A simple, rapid and sensitive method termed as solid phase extraction (SPE) combined with spectrophotometric method has been proposed for the determination of trace amounts of doxycycline (DOX) in pharmaceutical samples. The influences of effective parameters on the extraction efficiency were studied. The effect of the different parameters such as pH, extraction time, effect of the salt, nanoparticle amount and extraction temperature has been studied. Under optimized condition, the method was successfully applied to the extraction of DOX from aqueous samples and relative recovery amount of 89%, detection limit of 25μg L-1 and a relative standard deviation (RSD) of 2.3% were obtained. The method linear response was over a range of 10–50000μgL–1 with R² = 0.991. The whole procedure showed to be conveniently fast and high extraction efficiency of DOX from real samples.

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 was the most commonly detected antibiotic, being present in 80 % of the wastewater in fluent and sewage. DOX is one of the kinds of antibiotics of the TC family containing the ß diketonate 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.

Here SnO2 nanoparticles were synthesized from the sol of tin chloride dehydrate with polyethylene glycol (PEG; MW 300). In this process, PEG was used as both the gelling medium and the stabilizing reagent. The

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introduction of organic polymer, such as PEG 300 to the sol derived precursor solution was a novel chemically modified alkoxide method [16,17]. The microstructure and photocatalyst properties of the sol–gel derived TiO2 films were successfully improved by the addition of PEG to the precursor solution. In this study, the influence of PEG addition on the microstructure characteristics of SnO2 investigated has a positive impact on the size of nanoparticle. Prior to toxicological analysis, the step of sample preparation is crucial [18]. Some separation/pre-concentration procedures including liquid–liquid extraction (LLE) [19] and solidphase extraction (SPE) [20] have been applied for the extraction of psychotropic drugs from biological samples. Although LLE offers high reproducibility and high sample capacity, it is a time-consuming and labor intensive procedure with a tendency for emulsion formation and with a poor potential for automation. In addition, LLE requires large amounts of high-purity solvents which are expensive and toxic resulting in the production of hazardous laboratory wastes; while SPE requires a specific device loaded with certain adsorption material as well as a high-pressure delivery system that can be relatively expensive.

Recently, nanometer-sized materials have attracted substantial interest in the scientific community because of their special properties. These materials have been used in various scientific fields such as biotechnology, engineering, biomedical, environmental, and material science [21.

Compared with micrometer-sized particles, nanoparticles (NPs) offer a significantly higher surface area-to-volume ratio and a short diffusion route, resulting in high extraction capacity, rapid extraction dynamics, and high extraction efficiencies [22]. Another advantage of NPs is that NPs' surface functionality can be easily modified to achieve selective sample extraction [23].

Here, SnO2 nanoparticles were used as sorbents in the solid phase extraction (SPE) of ultra trace amounts of doxycycline from water and pharmaceutical samples.

MATERIALS AND METHODS

Reagents and Standards

Acetyl acetone, $SnCl_2.2H_2O$, sodium hydroxide, sodium dodecyl sulphate (SDS), Polyethylene glycol, NaCl, HCl, NaOH and methanol were purchased from Merck (Darmstadt, Germany). DOX was purchased from Sigma-Aldrich (Milwaukee, WI, USA). Standard stock solution of doxycycline at 2000 mg L-1 was prepared in methanol. The working standard solutions were prepared at various concentrations by diluting the stock solution as required in deionized water and stored at 4 °C.

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. Shaking of the solutions was carried out by a Rotator Pars Azma Co. The solutions were stirred by Heidolph MR3001 magnetic stirrer (Schwabach, Germany) and a 8 9 1.5 mm magnetic stirring bar.

Synthesis and characterization of SnO2 nanoparticles

The tin oxide nanoparticles were prepared by the sol–gel method. In a typical procedure, 17 g hydrated tin chloride ($SnCl_2.2H_2O$ 98% purity, QRec) was dissolved in pure ethanol. The solution was stirred with a magnetic stirrer for 50 min in a closed three-necked flask vessel. 19 ml of acetyl acetone (AcAc), a completing agent, was added drop wise for the hydrolysis of SnO_2. After 40 min, the solution was continuously refluxed at 90 °C for 6 h to form the SnO_2 solution. Polyethylene glycol PEG (M.W. 6000) was applied as a chemical modifier reagent, by adding 2 ml in the SnO_2 solution and was aged for 72 h at 45 °C. The sol was dried at 100 °C for 40 min before calcinations process at 450 °C, obtaining the SnO_2 nanoparticles (see Fig 1).
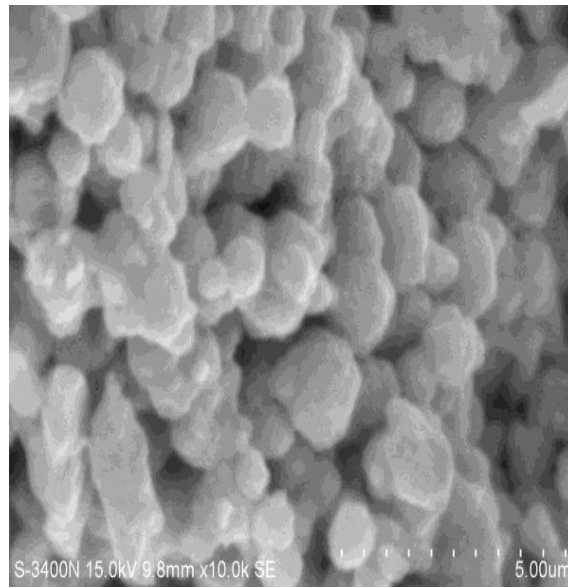
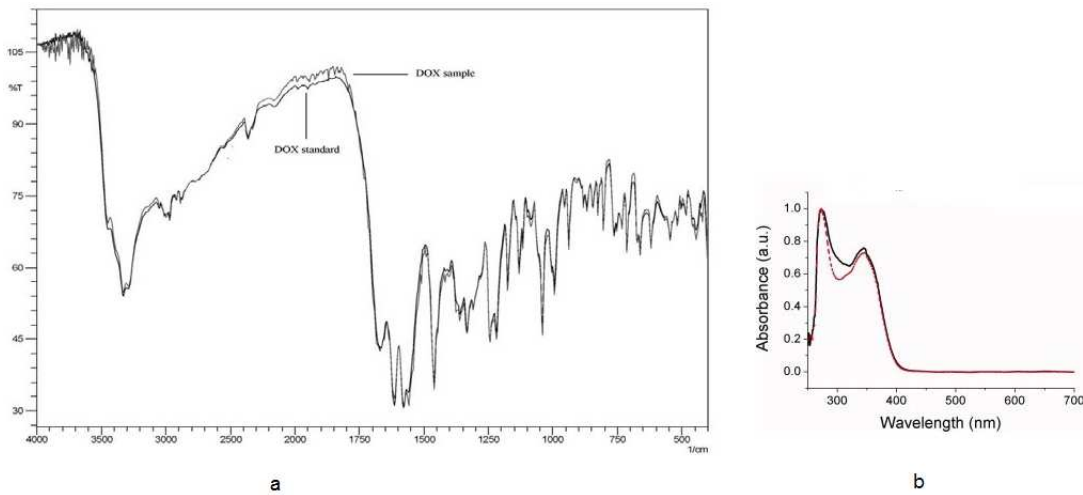**Figure 1: SEM image of the SnO$_2$nanoparticles.**

a

b

Figure 2: FT-IR (a) and UV-VIS (b) spectrums of DOX
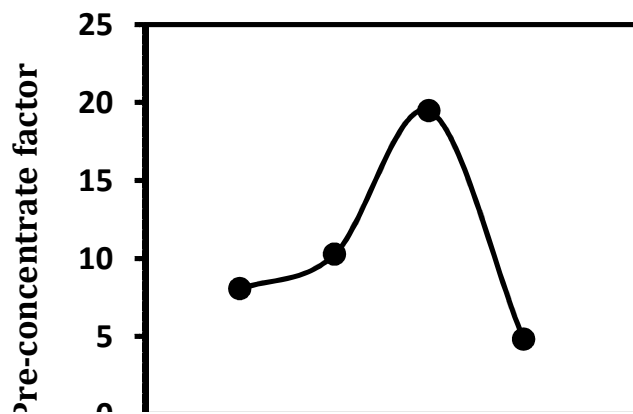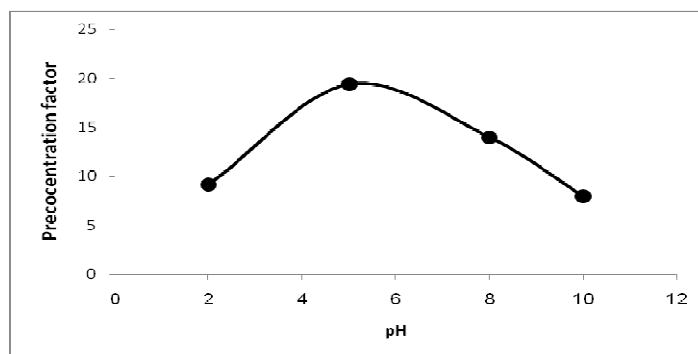

Fig.3. Effect of pH of aqueous sample

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Fig. 4: Effect of pH of doxycycline solutions on extraction efficiency.
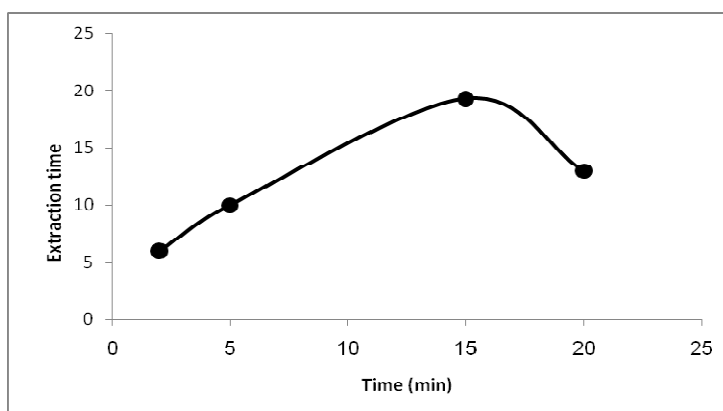

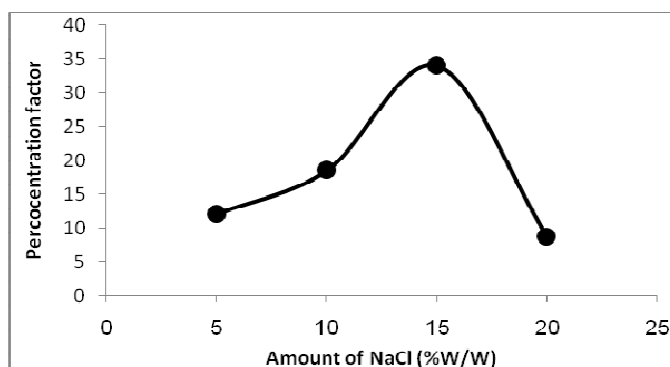**Fig. 5: Effect of extraction time on extraction efficiency of doxycycline**

Fig. 6: Effect of NaCl percent on concentrate factor

RESULTS AND DISCUSSION

Absorption and FT-IR spectrums of DOX
Fig. 2.a shows the FT-IR spectrum of (a) DOX DOX (aromatic ring at 1600–1500 cm^{-1}; CONH2 at 1650 cm^{-1}; and COOH at 1700 cm^{-1}). Fig 2.b shows UV- VIS spectrum of (a) DOX ($\lambda max=280nm$)

Solid phase extraction procedure
50 milliliter of aqueous sample containing DOX with concentration of 300 ng/mL (pH= 5.0) was transferred to 100 mL glassware beaker. Then, 2.0 mL of the suspension of SnO_2 NPs and 0.8

mL of the SDS solution (5 mg/mL) were sequentially added together and completely mixed with the sample solution. The mixture was shacked and allowed to complete the extraction process for 3 min. the solution became limpid and supernatant solution was decanted. Then 0.5 ml of methanol was added to nanoparticles, after mixing and centrifuging (6000 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 DOX was measured.

Pre-concentrate factor=A2/A1
A1 and A2 are the adsorption before and after extraction respectively.

Effect of nanoparticle amount

The pre-concentration factor of DOX on SnO_2 nanoprticles was studied at different adsorbent amount (2.0–20 mg), DOX concentration of 2 mg L^{-1}, pH 5.0 and agitation time of 15 min. Figure 3 shows that by increasing the amount of adsorbent up to 15.0 mg, the adsorption of DOX increased. This could be explained by this fact that more adsorbent became available for adsorbing the DOX molecules. Further addition of the adsorbent did not show any significant change in Concentrate factor of DOX. Thus, 15.0 mg of the nanoparticles was chosen as the optimum amount for performing the following steps of the optimization procedure.

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 DOX. 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 DOX 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. 4).

Effect of extraction time

Extraction was performed from 2 to 30 min to determine the effect of extraction time on the method efficiency (see fig 5). The effect of extraction time on the extraction efficiency of DC is shown in Fig. 5. The analyses the highest increase in the extraction efficiency in the period of 15 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.

Effect of salt into aqueous sample

It is well known that the addition of salt can increase or decrease the extraction of analyses [24]. The effect of salt on extraction was studied by varying the amount of NaCl within the range of 0–20% (w/v) (see fig6). The extraction efficiency increased for doxycycline at salt concentration below 10% (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 analyses, thus increasing the diffusion rates of the analyses into the sorbent. The extraction efficiency decreased for solution with 10% (w/v) salt. Somewhat surprisingly, extraction efficiency decreased with increasing salt concentration for doxycycline. The effect of NaCl on the extraction of target analyses is probably due to salting-out effect, which decreases the solubility of the analyses and thus increases their partition [25] into absorbent. So, further extractions were carried out at addition of 10% (w/v) salt.

Effect of solution temperature

The effect of temperature on the extraction of target analyses solution was investigated at pH 5.0 while a shaking time of 15.0 min was performed. The results showed that the extraction efficiency of the doxycycline as a function of temperature in the range of 25-45° C was not significantly affected by temperature.

Method validation

Thirty tablets were accurately weighed, crushed into a fine powder and mixed using a mortar and pestle. A quantity of tablet powder equivalent to 80 mg of doxycycline was weighed accurately into a 150 mL calibrated flask; 50 mL of diluent solution was added and the mixture was sonicated for 10 min to complete dissolution of the target analyses; the mixture was then diluted to the mark with the diluent and mixed well. A small portion of the resulting mixture was withdrawn and filtered through a 0.45 μm filter to ensure the absence of particulate matter. The filtrate was appropriately diluted with the distilled water.

Linearity was evaluated by regression analysis. Twenty microliters of working standard solutions (50–150 μg/mL doxycycline) were manually added onto the spetrophotometr cell in triplicate. The equation of the line was determined by linear regression analysis by the method of least squares. The equation of the line, determined by the method of least squares,

is $y = 348.9x - 561$, with a correlation coefficient (r) 0.991.

The accuracy of the method was determined by measuring the reference standard recovery in triplicate at three levels from 70 to 120% of the method concentration (80 µg/mL), according to ICH recommendations. A standard stock solution containing 300 µg/mL of doxycycline was prepared in 1×10^{-2} mol/L of hydrochloric acid. In volumetric flasks of 10 mL, aliquots of 300, 1,500 and 2,300 µL of this standard solution (concentrations of 2.5, 7.0 and 12.5 µg/mL, respectively) were individually added to 3000 µL of sample solutions at 100 µg/mL (concentration of 50 µg/mL). The final concentrations were 64, 80 and 96 µg/mL, which correspond to 70, 100 and 120% of the target concentration, respectively. The mean recoveries, expressed in terms of percent recovery of the tablets (Doxitrat 80 mg) of the assay and the relative standard deviation (RSD), were determined. Precision was evaluated with respect to both repeatability and intermediate precision. Repeatability was evaluated by analyzing doxycycline working standard solutions at the same concentration and during the same day. Intermediate precision was studied by repetition of the assays on two different days by two analysts. Five replicates at a concentration of 70 µg/mL were prepared and assayed. The data were analyzed at 280 nm. The RSD percentages of the analytical responses were calculated.

The limit of detection (LOD) of the method was obtained. The result was 3.63 for 80 mg DOX tablets. The values are low, which indicates the sensitivity of the method.

Assay of pharmaceutical products

The validated method was applied for the quantitation of DOX in tablets (80 mg Doxitrat tablets). The results were obtained by comparing the measurements of the sample solutions ($n = 5$) with those obtained from DOX standard solutions ($n = 5$) at the same concentration levels.

CONCLUSION

In this research, SnO_2 nanoparticles were used in water sample in order to extract and determination of doxycycline. In this method, the extraction can be done faster and easier by using SnO_2 nanoparticles. Moreover, using small amount of solvent not much wastewater is produced and meanwhile it is a cheep and affordable method. This method can be used in environmental pollution control organizations, pharmacy companies and clinical diagnosis laboratories.

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