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VISIBLE SPECTROPHOTOMETRIC ANALYSIS FOR THE MUTUAL DETERMINATION OF DOXYCYCLINE HYDROCHLORIDE AND IRON IN REAL SAMPLES AFTER CLOUD POINT EXTRACTION

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ABSTRACT

A new method is described for the mutual analysis of drug doxycycline hydrochloride (DOX. HCl) and iron(III) ions in pharmaceuticals, biological and river water samples by combined cloud-point extraction and UV-Vis spectrophotometry. The method is based on the reaction between iron(III) ions and the drug DOX. HCl in acidic medium forms a brown colored Fe-DOX complex, which is subsequently extracted by cloud point extraction (CPE) using Triton X-114 as an extracting medium followed the determination of both; DOX drug and Fe(III) ions individually, using visible spectrophotometry at same wavelength maximum 430 nm. The parameters impact on the extraction efficiency of CPE methodology was studied by using the classical optimization and the interferences effect of some metal ions on the determination of iron was also investigated. The pre-concentration and enrichment factors of the method were found to be of 55.5 and 24.35 fold, respectively, achieving the detection limit of 0.07 μg mL^{-1} with linear range of 0.2-8 μg mL^{-1} ($r = 0.9999$) for DOX and 9.57 ng mL^{-1} with linear range of 20-200 ng mL^{-1} ($r = 0.9984$) for Fe(III). The mean recovery percentage was 95.95 ± 1.09 in urine sample and 101.55 ± 0.77 in river water, and the precision (RSD%) ranged between 0.21-1.09% and 0.84-2.60 % for DOX and Fe(III), respectively. The proposed method was used for the determination of DOC in the urine samples of 10 subjects orally administered with DOX tablets like Medomycin 100 mg, in addition to drug formulations, While iron was determined in two selected supplements and in both cases, the experimental values agreed with the quoted values as stated by the manufacturer company.

Key words: Doxycycline hyderochloride, Iron (III) ions, Visible sepectrophotometry, Cloud point extraction.

INTRODUCTION

The advantages and importance of the mutual determination of two target analytes in the same reaction systems via using cloud point extraction (CPE) became clear in some

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detail in our recently published papers[1,2]. We think that this trend in the chemical analysis will take increasing interest in the near future because some of other researchers began to embrace this direction for their future works, which encouraged us to continue and deepen this topic to contribute in addition to other benefits; thus, enhancing the role of contemporary analytical chemistry. In this work, a complexometric reaction between the drug doxycycline hydrochloride (DOX. HCl) and iron (III) ions in acidic medium was selected, in an attempt to design a new CPE procedure for the mutual determination of the drug and metal ions at trace levels in various matrices by visible spectrophotometry.

Doxycycline hydrochloride belongs to the tetracycline class, which includes compounds containing four fused rings as well as containing double bonds. It is a broad-spectrum antibiotic used in many countries for the treatment of infectious diseases and as an additive in animal feed to facilitate the growth process[3]. It is chemically known (IUPAC name) as (4S, 4aR, 5S, 5aR, 6R, 12aS)-4-(dimethylamino)-3, 5, 10, 12, 12a-pentahydroxy-6-methyl-1, 11-dioxo 1, 4, 4a, 5, 5a, 6, 11, 12a-octahydro-2-tetracencarboxamid hydrochlorid (1:1) and its chemical structure is illustrated in Fig. 1[4].

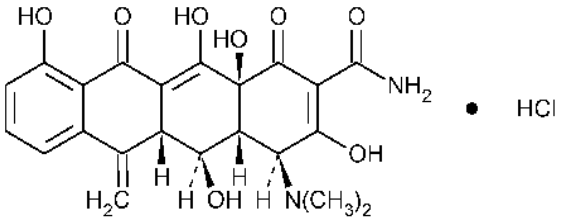


Fig. 1: The chemical structure of doxycycline hydrochloride ($C_{22}H_{25}ClN_2O_8$, 480.90 g mol^{-1})

Several methods are used in the determination of DOX in a pure form and pharmaceuticals, and a few in biological and food samples such as spectrophotometry[5-9], flow injection analysis (FIA)[10-14], high performance liquid chromatography(HPLC)[15-25] thin layer chromatography[26], capillary electrophoresis[27], polarography[28], optical fiber sensor[29] and ion selective potentiometry[30]. Also, HPLC is the choice of some pharmacopoeia for the determination of DOX in pure and dosage forms[31-33]. The use of HPLC technique has received considerable attention in estimating the trace levels of this drug in biological samples and food due to its high power detection. However, HPLC may not exist in most laboratories, needs an internal standard and sometimes involve more than one extraction step[34].

Iron, on the other hand, is known to be one of most important metal to humans, exists in all human body cells and has many vital functions. The disorders in the iron

metabolism are among the most common diseases in humans[35]. Iron deficiency causes anaemia and other pathological changes in the body, while iron overload also causes other diseases[36]. The former can be treated using some pharmaceutical products containing iron as dietary supplements taken orally or injection, while the latter can be mostly treated by iron chelation therapy using the drug deferoxamine[37,38]. Therefore, the U.S. Recommended Daily Allowance (USRDA) for iron is 18 mg for male and 11 mg for the female between the ages of 19-50 years[39]. Thus, in all cases, the determination of iron is very important from the point of view of biochemical and nutritional studies.

The presence of the drug or metal ions in biological, environmental and even in the dietary supplements at trace concentration levels require the pre-concentration step before measurement. It was reported that the cloud point extraction in combination with visible spectrophotometry comply with the above request as a routine method instead of using sophisticated and expensive instrumentations[40,41].

In this study, a new analytical method is established for the mutual determination of DOX drug and iron in the selected samples based on the spectrophotometric measurement after the cloud point extraction. The method is based on the reaction of Fe(III) ions with DOX.HCl in acidic medium to form Fe-DOX complex, which can extracted into Triton X-114 as extracting medium and determination of both target analytes by visible spectrophotometry at absoption maximum of 430 nm.

EXPERIMENTAL

Apparatus

A Shimadzu double-beam UV-Vis Spectrophotometer model UV-1800 (Kyoto, Japan) equipped with 5-mm optical path cell was used for the scanning of the absorption spectrum for the complex and all absorbance measurements of the two target analytes under study. A double-beam atomic absorption spectrometer AA400 (Analytic Jeana, Germany) equipped with flame atomizer was used for the determination of iron according to the instruction manual of the company. Thermostatic water bath model WNB7-45 Experts (England) was used thoughout the CPE experiments. For solution pH measurements, a portable pH/mV/C meter HI 83141 (HANNA, Romania) was used.

Reagents and solutions

The chemicals in this work were having high purity and used as received. Doubly distilled and/or deionzed water was used in the preparation of all solutions and for final

rinsing of glass wares. A pure grade (98%) of doxocycline hydrochloride was obtained from VAPCO Manufacturing CO. Ltd. (Amman). The stock solution of 1000 μg mL^{-1} (or 0.0020 M) for the drug doxycycline hydrochloride was prepared by dissolving 0.1000 g in a minimum amount of 1.0 x 10^{-3} M sulfuric acid (BDH) and diluted to mark with water in a 100 mL volumetric flask. This solution was stored in the refrigerator and working solutions were daily prepared by appropriate dilutions in water. A stock 1000 mg L^{-1} solution of Fe^{3+} ions (0.018 M) was prepared by dissolving 0.8634 g of pre-dried ammonium ferric sulfate (purity 99.5%, BDH) in 20 mL of 1.0 x 10^{-3} M sulfuric acid (BDH) and diluted to mark in 100 mL volumetric flask with the same solvent. Triton X-114 (purity >99.9%), was purchased from AMRESCO LLC (Solon, USA). A 10% (v/v) of Triton X-114 was prepared by diluting 10 mL in 100 mL water. A 1 g L^{-1} solution for the interfering ions such as Co^{2+}, Cu^{2+}, Zn^{2+}, Cr^{3+}, Ni^{2+} and Mg^{2+} was prepared from their chloride salts (purity 99%, and 98% BDH).

Recommended CPE for DOX.HCl

In a 10 mL volumetric flask, an amount of DOX.HCl standard or sample solution in the range of 0.2-8.0 μg mL^{-1}, and 0.4 mL of 1.8 x 10^{-3} M ferric ion solution was added. The solution pH was adjusted to 4 with 0.1 M H_2SO_4, then 0.2 mL of 10% Triton X-114 added, mixed well and diluted to mark with water. The content of each flask was transferred into a 10 mL centrifuging tube and kept in the thermostatic bath at 65°C for 15 min. Separation of the phases was conducted by centrifugation at 3500 rpm for 20 min. The aqueous phase was easily removed by pipette. The surfactant-rich phase that contains the complex was dissolved in 1.0 mL ethanol and the absorbance of the complex measured at 430 nm against a reagent blank prepared under similar conditions. The remaining DOX in aqueous solution was determined by traditional spectrophotometry at λ_{max} of 275 nm in order to determine the distribution ratio (D) and extraction efficiency (%E).

Recommended CPE for iron

In a 10 mL volumetric flask, an amount of Fe(III) standard or sample solution in the range of 20-200 ng mL^{-1} and 3.0 mL 2.0 x 10^{-4} M DOX standard solution was added. The solution pH was adjusted to 4 with 0.1 M H_2SO_4, then 0.2 mL of 10% Triton X-114 added, mixed well and diluted to mark with water. The content of each flask was transferred into a 10 mL centrifuging tube and then kept in the thermostatic bath at 65°C for 15 min. Separation of the phases was conducted by centrifugation at 3500 rpm for 20 min. The aqueous phase was easily removed by pipette. The surfactant-rich phase that contains the complex was dissolved in 1.0 mL ethanol and the absorbance of the complex was measured at 430 nm against a reagent blank prepared under similar conditions.

Preparation of samples

DOX tablets: 10 capsules of medomycin (Medochem Ltd, Cyprus) containing 100 mg of DOX.HCl were mixed well and homogenised. An equivalent amount to one capsule of active drug was dissolved in sufficient amount of water with continuous shaking and filtered. The filtrate was transferred into a 100 mL volumetric flask and diluted to mark with water. 10 mL of this solution was transferred to 100 mL volumetric flask and diluted to mark with water. Aliquots of 0.3, 0.5 and 0.7 mL of DOX sample solution were pipetted into three 10 mL centrifugal tubes and each solution followed the recommened CPE procedure for DOX. HCl and the content of drug was measured spectrohotometrically at λ_{max} of 430 nm for three repeated measurements.

Urine: 10 urine samples collected from volunteers (aged from 25-34 years) who were randomly designated to take orally a single tablet of Metomycin-containing 100 mg DOX.HCl. The samples were collected from the volunteers at three different times (2, 4 and 8 hrs) after administration and then kept in the refrigerator until analysis. The urine samples were thawed at ambient temperature, then 1.0 mL of each sample was pipetted into 10 mL centrifugal tubes and subjected to the recommended CPE procedure and the drug content was determined spectrophotometry at λ_{max} of 430 nm.

River water: About one liter of water sample was collected from Tigris (Dijla) river in the Al-Jadiryiah region of Baghdad /Iraq. The sample was first filtered off to remove any suspended materials. A 2.0 mL of sample was transferred into 10 mL centrifugal tube and spiked with 0.1, 0.3 and 0.5 mL of 100 μg mL^{-1} DOX standard solution, then followed the recommended CPE procedure for DOX. Each spiked sample was measured for three repeated time and content of the drug in river water was determined spectrophotometrically at λ_{max} 430 nm. Also, 1 mL of this sample was transferred into 10 mL centrifugal tube and spiked with 30, 60 and 100 ng ml^{-1} Fe(III) ion, then the general CPE procedure for iron was followed. The content Fe(III) ions in water was determined spectrophotometrically at λ_{max} 430 nm for three repeated measurements.

Iron tablets: Two pharmaceutical preparations as iron supplementation such Maltofer ® (*Sanofi-Synthélabo* Rt, Pharmaceutical, Budapest, Hungary) and Hemafer Fol (UNI-Pharma Kleon Tsetis Pharmaceutical Laboratories S.A., Greece) were purchased from local drug stores. Each supplement contains 100 mg iron as iron (III) hydroxide Polymaltos. 10 tablets of each sample were grinded in agate mortar, mixed well and homogenised. An equivalent amount to one tablet (100 mg) was dissolved in 3.0 mL of concentrated H_2SO_4 with gentle heating on hotplate until dry. Thereafter. 25 mL of water was added with continuous stirring, filtered and the residue washed several times with water. The filtrate was

transferred into 100 mL volumetric flask and completed to mark with water to obtain 1.0 mg mL^{-1} of iron. This solution was further diluted to 1.0 µg mL^{-1}. Aliquots of 0.5, 1.0 and 1.5 mL were pipetted into three 10 mL centrifugal tubes and each solution followed the recommened CPE procedure for iron and the content of Fe(III) was measured spectrophotometrically at λ_{max} of 430 nm for three repeated measurements.

Statistical analysis

Excel 2007 (Microsoft Office®) and Minitab version 14 (Minitab Inc., State College, PA, USA) were employed to carry out all statistical calculations such as regression and correlation analysis, ANOVA and significance tests.

RESULTS AND DISCUSSION

Absorption spectra

The UV-Vis spectra of Fe-DOX complex in dilute H_2SO_4 and in the presence of Triton X-114 show a substantial change in peak position compared to that of the free drug and iron salt due to the charge transfer between Fe(III) ion and drug ligand, which appeared in visible region with shoulder band at λ_{max} of 430 nm. whilst the free DOX drug displayed two absorption maxima at 275 and 380 nm because of π-π^* and n-π^* electronic transitions and Fe(III) ion solution gave one distinct absorption band at 300 nm (Fig. 2). Thus, the wavelength maximum at 430 nm for the Fe-DOX complex was used throughout this study.

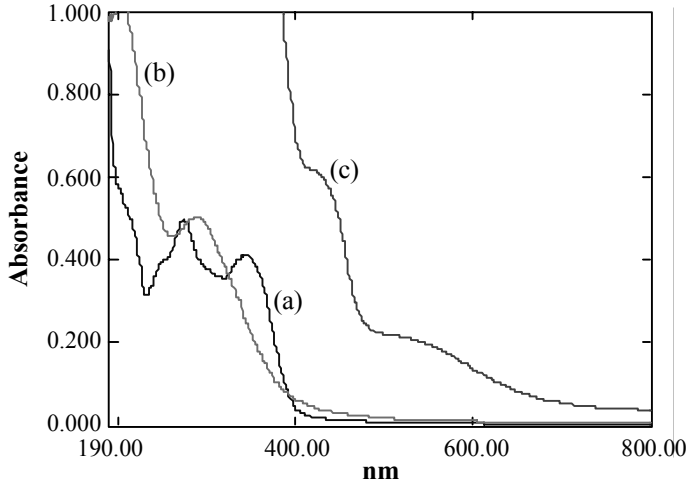


Fig. 2: Absorption spectra of (a) 2.0 x 10^{-5} M DOX.HCl (b) 1.0 x 10^{-5} M Fe(III) solution and (c) brown Fe(III)-DOX complex in micelle-mediated extraction

Optimization of CPE procedure

The use of complexation reaction between Fe(III) ions and DOX. HCl drug in the mutual determination of both target analyte by the proposed method almost requires to study and select the optimum experimental conditions. All parameters described below that impacts on the extraction efficiency of CPE methodology were investigated by the classical optimization via changing one factor, while keeping other factors constant.

Effect of pH

The solution pH is an important parameter that affects strongly the binding between the drug DOX and Fe(III) ion to form a stable hydrophobic complex, which can be easily extracted by CPE. It was found that H_2SO_4 at certain concentration is most suitable medium for this purpose[5]. Therefore, the effect of pH on the Fe(III)-DOX complex formation was investigated by change the pH from 1.0-6.0 using 0.1 M H_2SO_4. The experiments were carried out for 10 mL solution containing 0.5 mL of 100 μg mL^{-1} DOX, 0.1 mL of 1.8 x 10^{-3}M Fe(III) ion and 0.5 mL of 10% Triton X-114 followed by heating the solution at 70ºC for 20 min. The results are depicted in Fig. 3. It was observed that the absorbance increases with increasing pH and reached maximum at pH 4 followed by a decrease in the absorbance thereafter. This might be due to the hydrolysis of Fe(III) and there is no chance for complex formation stoichiometrically. Thus, the solution pH of 4.0 was adopted in the further experiments.

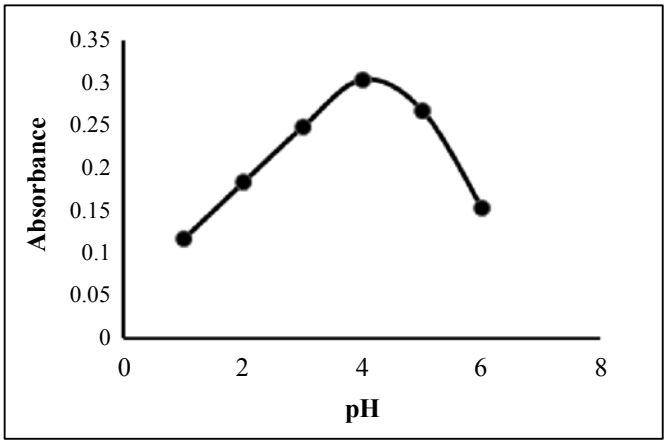


Fig. 3: Effect of pH on the formation of Fe(III)-DOX complex by CPE

Effect of Fe(III) ions concentration

The influence of Fe(III) concentration also plays a significant role for complete

complex formation with DOX, and in the thermodynamic equilibrium for the formation and stability of the complex in acidic medium extracted by CPE. This effect was examined by changing the volume of 1.8 x 10^{-3} M Fe(III) from 0.05 to 1.00 mL and a fixed amount of drug. The results are shown in Fig. 4. It revealed that maximum absorbance was fulfilled, when concentration of Fe(III) reaches to 7.2 x 10^{-5} M (0.4 mL of 1.8 x 10^{-3} M Fe(III) in 10 mL final aqueous solution). Beyond this concentration, a decrease in absorbance for the complex was obvious due to the deviation of the equilibrium toward the backward reaction because of a law of mass action. Whilst at low concentration of Fe(III) ion than optimal, there is no chance for complex formation completion resulting in less amount of the complex extracted into the CPE. Thus the concentration of to 7.2 x 10^{-5} M was selected in following experiments.

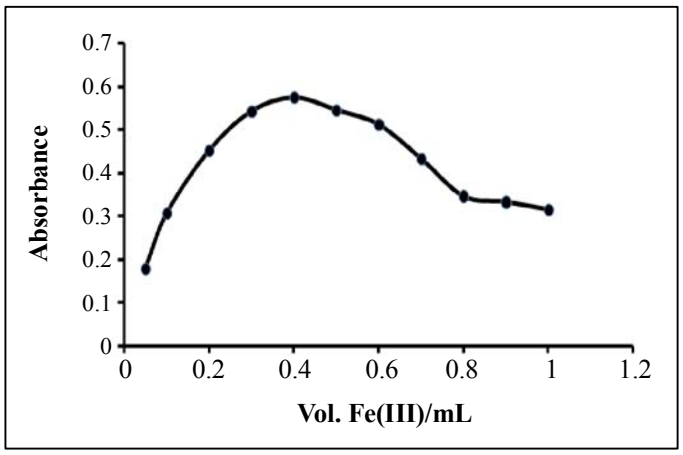


Fig. 4: Effect of iron(III) concentration in the formation of Fe(III)-DOX complex by CPE [condition: DOX, 5.0 µg mL^{-1}; pH,4.0 ; TX-114, 0.5 mL of 10%; Temp., 70ºC; Time, 20 min]

Effect of surfactant amount

The amount of surfactant (Triton X-114) is very influential in maximizing the efficiency of extraction as well as raising the pre-concentration factor. So, the variation of the absorbance with Triton X-114 amount on the extraction of Fe(III)-DOX complex was studied within volume range 0.1-2.0 mL of 10% Triton X-114. As shown in Fig. 5, at a low amount of surfactant, the absorbance was low but the maximum remarkable extraction was achieved when Triton X-114 amount was 0.2 % (i.e. 0.2 mL of 10% TX-114 in the final 10 mL of aqueous solution) which gave the best pre-concentration factor. Therefore, this value were adopted in the recommended CPE procedure.

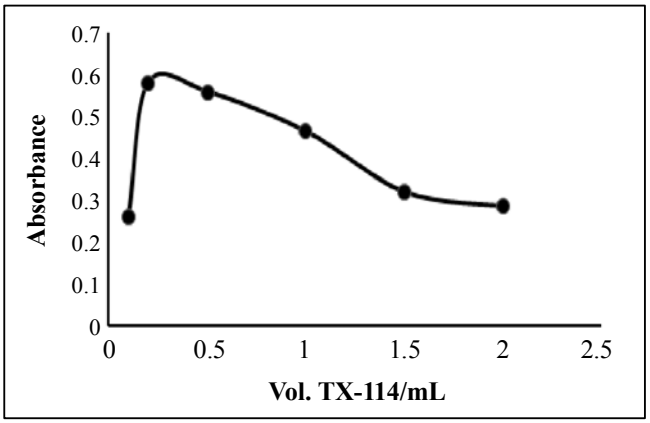


Fig. 5: Effect of Triton X-114 amount in the formation of Fe(III)-DOX complex by CPE [condition: DOX, 5.0 µg mL^{-1}; Fe(III), 7.2 x 10^{-5}M; pH,4.0; Temp., 70ºC; Time, 20 min]

Effect of temperature and time

Fig. 6 displays the effect of equilibration temperature on the absorption signal of the Fe(III)-DOC complex, by varying the temperature from 25 to 80ºC in incubation time of 20 min and keeping the other factors at optimal. It appeared that the maximum absorption signal of both target analysts was achieved at 65°C; thereafter a significant decrease of the absorbance response was observed, probably due to the instability or dissociation of the dye products. Thus 65°C was used as in the recommended CPE procedures. The incubation time was also studied in the range of 10-40 min as showed in Fig. 7.

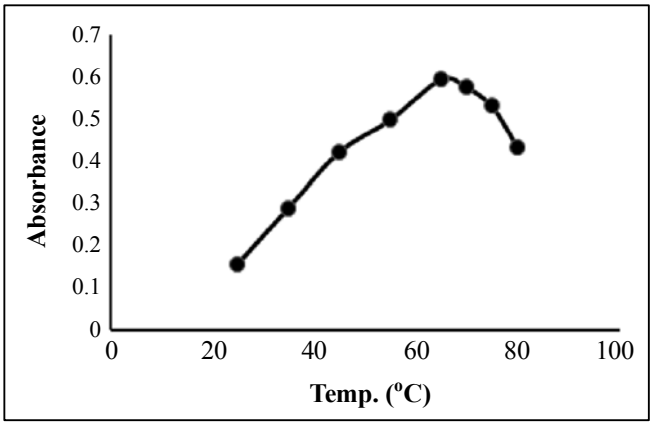


Fig. 6: Effect temperature in formation of Fe(III)-DOX complex by CPE [condition: DOX, 5.0 µg mL^{-1}; Fe(III), 7.2 x 10^{-5} M; pH,4.0; TX-114 , 0.2%; Time, 20 min]

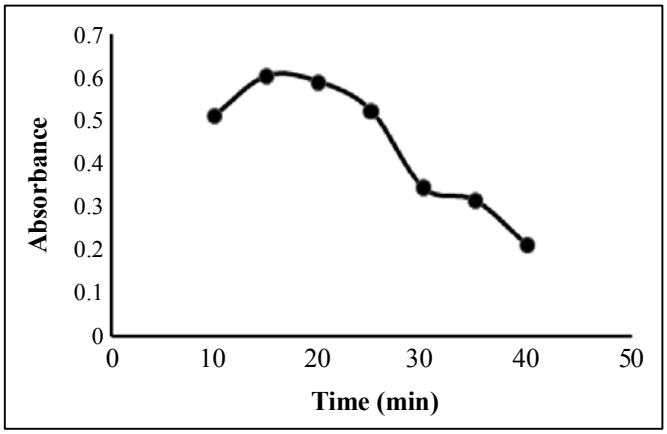


Fig. 7: Effect of time in the formation of Fe(III)-DOX complex by CPE [condition: DOX, 5.0 µg mL^{-1}; Fe(III), $7.2x10^{-5}$ M; pH,4.0; TX-114 , 0.2%; Temp.65 ºC]

It was found that the incubation time of 15 min was sufficient for the maximum absorption signal of Fe(III)-DOX complex extraction. A centrifuging time of 20 min at 3500 rpm was selected for the entire general CPE procedures as being optimum and beyond this time, no confirmation was observed for improving analytical response.

Optimization parameters for Fe(III) using DOX drug

The same parameters as with DOX drug determination were optimized for Fe(III) ions, but a discrete variable here is the concentration of DOX used as chelating agent.

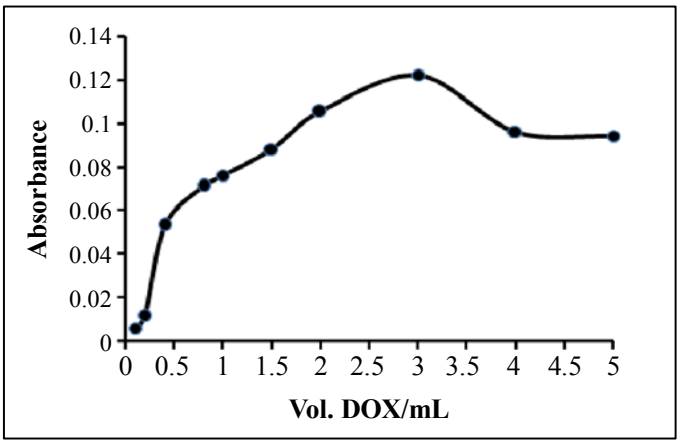


Fig. 8: Effect of DOX concentration for the formation of Fe(III)-DOX complex by CPE

At constant and trace concentration of Fe(III) of 50 ng mL^{-1}, the optimum conditions of most parameters were similar to that obtained in the case DOX optimization, but here it needs 6.0 x 10^{-5} M of DOX (3.0 mL of 2.0 x 10^{-4} M in 10 mL final solution) as optimal at nanogram amount of Fe(III) for the formation of Fe(III)-DOX complex (Fig. 8).

Composition of Fe(III)-DOX Complex

It was previously observed that a brown colour is instantly formed, when Fe(III) ions solution is added to a certain amounts of DOX drug in the presence of a wide concentration of H_2SO_4, indicating the complex formation. But, in this study, the optimum pH of 4.0 (~5.1 x 10^{-3}M H_2SO_4) was found to be enough for the stable colored complex formation, which appeared in visible region with maximum absorbance at 430 nm (Fig. 2). Therefore, the composition of Fe(III)-DOX complex was studied using mole ratio and Job's continuous variation methods. In mole ratio method, 1 .0 mL of 2 x 10^{-3} M Fe(III) solution was added to a series of 10 mL volumetric flask containing varying volume (0.2-2.0 mL) of 2 x 10^{-3} M DOX, each flask was diluted to mark after adjusting the pH to 4.0 and each solution is taken in a 10 mm cell to measure the absorbance at 430 nm. The results are shown in Fig. 9.

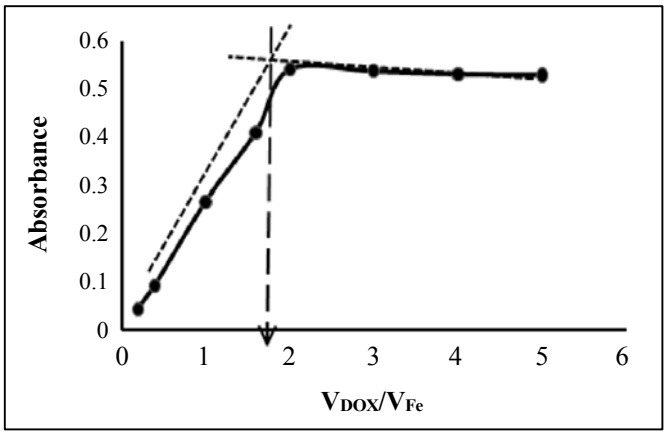


Fig. 9: Mole ratio method for Fe(III)-DOX complex

The plotted curve exhibits a maximum volume of DOX added via the point of intersection of the two lines which equals to 1.0 mL and is corresponding to 2.0 x 10^{-6} mole of DOX versus 1.0 x 10^{-6} mole of Fe(III), indicating that the expected ratio of Fe(III): DOX in the complex was about 1:2. The Job plot (continuous variation method) also confirmed similar result to molar ratio method, which exhibited an intersection at 0.3, which corresponding to 2.0 L/M ratio (Fig.10), indicating again the formation of a 1:2 (Fe(III)-DOX) complex.

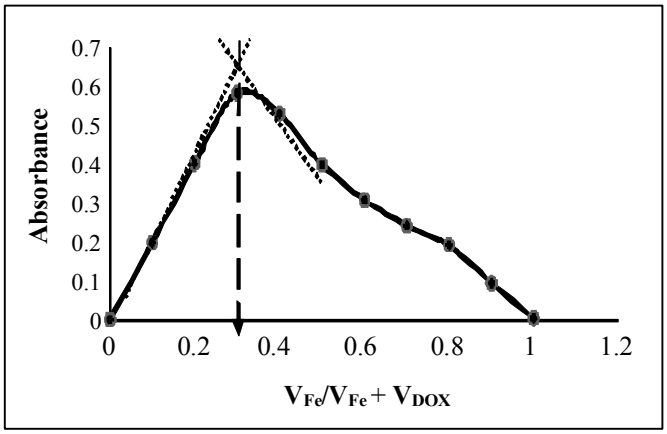
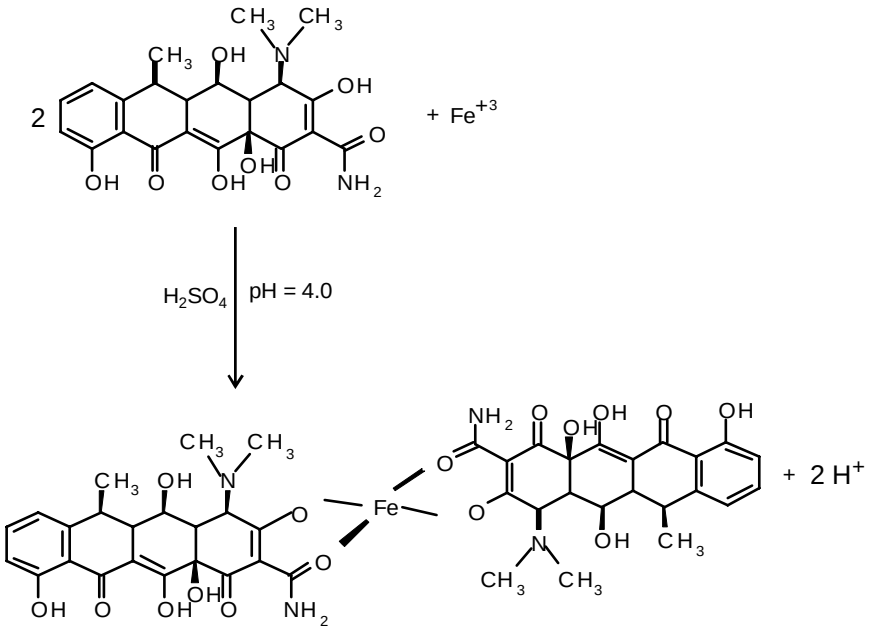


Fig. 10: Job's method for Fe(III)-DOX complex

This ratio has also been reported elsewhere by Ramesh et al.[5] and Sultan et al.[42] By assuming that only a single complex is present, the formation constant (K_f) of the Fe(III)-DOX complex was calculated according to the procedure reported[43] by using the above data, taking into account the concentration of drug at maximum absorbance i.e. 5.33 x 10^8 at 432 nm. On the basis of above results, the most probable structure of the complex formed between Fe(III) and DOX in dilute acidic medium is displayed in **Scheme 1**.



Scheme 1: The probable reaction path between DOX and Fe(III)

For the purpose of verification of complexation reaction between Fe(III) ions and DOX drug from thermodynamic point of view, the standard free energy (ΔG°) can be calculated from the following relationship [44];

$$\Delta Go = -2.303\ RT \log K \quad \ldots(1)$$

where R is the gas constant (1.987 cal mol^{-1} $degree^{-1}$), T is the optimum temperature in Kelvin (338 K), and K is the formation constant of Fe (III)-DOX complex. It found that the standard free energy (ΔG°) for the complexation reaction between Fe(III) ions and DOX drug at optimum cloud point temperature has a negative value equal to (-13.5 kcal mol^{-1}), indicating that the complexation reaction is a spontaneous.

Method validation

Under the established optimum conditions, a series of solutions containing increased amounts of DOX or Fe(III) ions were measured against the corresponding reagent blank at λ_{max} of 430 nm. A linear calibration graphs for the spectrophotometric detection of DOX and Fe(III) were constructed as depicted in Figs. 11 and 12. The representative statistical data for the analytical figures. of merits of DOX determination using Fe(III) ions and iron determination using DOX as chelating agent by CPE-spectrophotometry are summarized in Table 1 and 2.

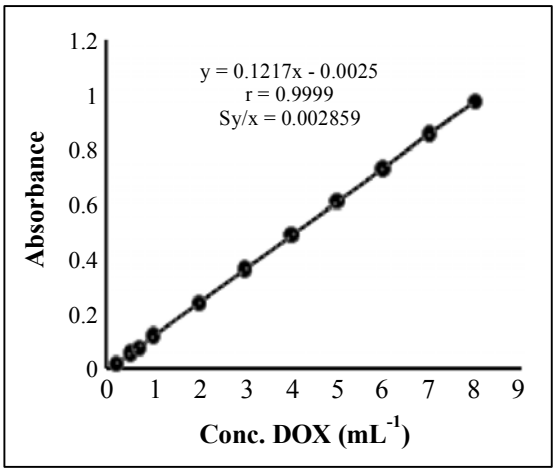


Fig. 11: Calibration curve for DOX by the proposed method

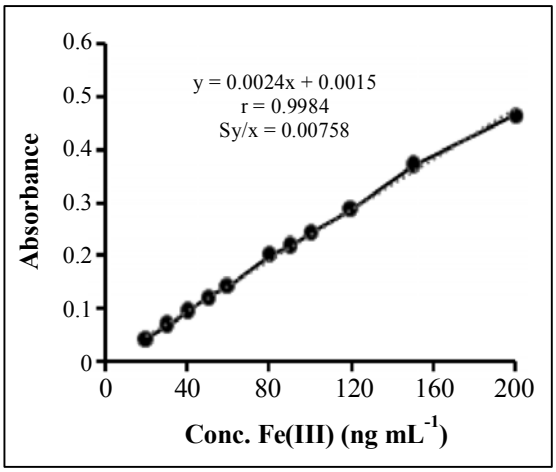


Fig. 12: Calibration curve for iron(III) by the proposed method

Beer's law is obeyed up to 8 μg mL^{-1} (r = 0.9999) for the drug DOX and 200 ng mL^{-1} (r = 0.9984) for iron ions at 11 calibration points. The proposed method gave a preconcentation and enrichment factor of 55.5 and 24.35 fold which led to achieve limit of detection (LOD) of 0.07 8 μg mL^{-1} and 9.57 ng mL^{-1} for DOX and Fe(III), respectively.

Table 1: The statistical data and analytical figures of merits for DOX determination using Fe(III) ions by CPE-spectrophotometry

Parameter	**DOX**
Colour of complex	Brown
λ_{max} (nm)	430
Regression equation	y = 0.01217x - 0.0025
Standard deviation of regression line	0.002859
Correlation coefficient (r)	0.9999
Coefficient of determination (R^2)	99.99%
C.L. for the slope (b± ts_b) at 95%	0.1217 ± 0.00243
C.L. for the intercept (a± ts_a) at 95%	-0.0025 ± 0.0105
Concentration range (μg mL^{-1})	0.2-8
Limit of detection (μg mL^{-1})	0.07
Limit of quantitation (μg mL^{-1})	0.24
Sandell's sensitivity (mg cm^{-2}/ 0.001A.U)	0.0082
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	5.85×10^4
Compsition of complex (Fe-DOX)*	1:2
RSD% (n = 3) at 0.5 μg mL^{-1}	5.78
RSD% (n = 3) at 1.0 μg mL^{-1}	3.12
RSD% (n = 3) at 3.0 μg mL^{-1}	0.41
Preconcentration factor	55.5
Enrichment factor	24.35
Recovery% (n = 3)	98.33 ± 0.56
Distribution ratio (D)	24
Extraction efficiency (%E)	96

Table 2: The statistical data and analytical figures of merits for Fe(III) ions determination using DOX chelating agent by CPE- spectrophotometry

Parameter	Fe(III) ion
Colour of complex	Brown
λ_{max} (nm)	430
Regression equation	y = 0.0024x + 0.0015
Standard deviation of regression line	0.00758
Correlation coefficient (r)	0.9984
Coefficient of determination (R^2)	99.69%
C.L. for the slope ($b \pm ts_b$) at 95%	0.0024 ± 0.00327
C.L. for the intercept ($a \pm ts_a$) at 95%	0.0015 ± 0.03285
Concentration range (ng mL^{-1})	20-200
Limit of detection (ng mL^{-1})	9.57
Limit of quantitation (ng mL^{-1})	31.90
Sandell's sensitivity (μg cm^{-2}/ 0.001A.U)x10^{-3}	0.421
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	1.33 x 10^5
Compsition of complex (Fe-DOX)*	1:2
RSD% (n = 3) 40 ng mL^{-1}	3.88
RSD% (n = 3) 90 ng mL^{-1}	1.84
RSD% (n = 3) 120 ng mL^{-1}	1.60
Recovery %	97.00 ± 3.26

Accuracy and precision

The trueness of the developed method was evaluated with accuracy test in the terms of percent recovery by spiking 0.7, 2.0 and 7.0 μg mL^{-1} of DOX standard solution into three divided portions of a blank urine sample taken from normal volunteer. Also, three portions of river water taken from Tigris river in Baghdad were spiked with 30, 60, 100 ng mL^{-1} iron standard solution. All the spiked samples were subjected to the recommended CPE procedure and each target analyte was determined spectrophotometry at 430 nm. The results are tabulated in Tables 3 and 4. The data indicated that the accuracies of the proposed

method were within acceptable level for the determination of both DOX drug and Fe(III) ions, indicating that the established method is unbiased. This can confirm that the proposed method is relatively free from matrix interferences.

Table 3: The accuracy and precision of the proposed method for the determination of DOX using Fe(III) in urine sample

Amount of DOX added (µg mL^{-1})	Amount of DOX found (µg mL^{-1})	Recovery (%)	Recovery ± Sd (%)	Erel. (%)	RSD (%)
0.7	0.67	95.71		-4.29	1.36
2.0	1.90	95.00	95.95±1.09	-5.00	0.34
7.0	6.80	97.14		-2.86	0.21

Table 4: The accuracy and precision of the proposed method for the determination of Fe(III) ions using DOX chelating agent in river water

Amount of Fe(III) added (ng mL^{-1})	Amount of Fe(III) Found (ng mL^{-1})	Recovery (%)	Recovery ± Sd (%)	Erel. (%)	RSD (%)
0	71.0	-		-	1.16
30	101.8	100.79	101.55 ± 0.77	0.79	2.60
60	133.0	101.52		1.52	0.63
100	175.0	102.33		2.33	0.84

Meanwhile, each spiked sample was repeated five times for precision testing in term of %RSD and found in the range of 0.21-1.36% for DOX and 0.84-2.60% for Fe(III) determination indicative of a good repeatability.

Interference study

To test the interference effect for the Fe(III) determination using DOX drug as the chelating agent, the study was conducted by the addition of 100, 200 and 300 fold more of each ions to 50 ng mL^{-1} Fe(III) standard solution. The results are listed in Table 5. It can be seen that there is no appreciable effect of most metal ions in the determination of iron using DOX drug as chelating agent (%E_{rel} less than ± 5%), except of Cu (II) ions, which caused

an appreciable interference in the measurement of iron with this chelating agent. Therefore, it should be removed or masked before determination of iron, or added to standard Fe(III) solutions before the construction of the calibration curve.

Table 5: Effect of diverse ions on the percent recovery of Fe(III) using DOX chelating agent

Foreign species	Amount fold / Recovery %			E_{rel}%	Recovery % mean±s_d
	100	200	300		
Co(II)	101.63	101.63	104.09	2.45±1.42	102.45±1.42
Zn(II)	100.81	100.81	102.45	1.36±0.95	101.36±0.95
Cr(III)	101.61	101.56	104.09	2.44±1.45	102.42±1.45
Ni(II)	99.18	97.54	95.08	2.76±2.06	97.24±2.06
Cu(II)	104.91	105.73	109.83	6.82±2.64	106.82±2.64
Mg(II)	100.00	101.63	101.56	1.09±0.95	101.09±0.95
Na(I)	98.63	96.72	95.08	3.19±1.78	96.81±1.78

Applications

In the light of the above findings obtained from the proposed method with the standard solutions for both target analytes, which gave satisfying analytical features, the method was applied for the determination of the medicament DOX and iron ions content in different matrices to test its eligibility and reliability in routine chemical analysis.

Determination of DOX

The pharmaceutical dosage (Medomycin, 100 mg as DOX.HCl) was selected for this purpose to determine its DOX content in tablets and serum samples by the proposed method. The results in Table 6 show that the content of DOX in tablet was an insignificant compared to the alleged value stated by manufacturer. This is because the statistical treatment proved that the calculated t-value of 3.13 for DOX determination using Fe(III) as mediating metal ion was less than the t-critical (4.303) at $\alpha = 0.05$ level and (n-1) degrees of freedom, indicating the acceptance of null hypothesis (Ho) and concluding that there is no evidence for systematic and random errors at the 95% confidence level; thus, manufacturer's claim can be acceptable.

Table 6: Determination of DOX in tablet by the proposed method and statistical comparison with quoted values

Commercial name, and content	Practical content (proposed method)	$t=(x-\mu)\sqrt{n}/s$ proposed method Vs. Claimed value at 95% C.I.	$\%E_{rel}$	%RSD (n=3)
Medomycin* 100 mg as DOX.HCl	99.0 97.9 98.9 98.5 ± 0.83	$t_{cal} = 3.13$ 3.13 < 4.303	-1.5	0.3

*Medochem Ltd., Limassol –Cyprus Euripe

It was reported that one of excretion paths of the MOX drug after its absorption in the body occur in kidneys and ultimately to the urine and faeces[45]. Thus, the application of the method was directed toward the determination the drug DOX in urine samples for ten volunteers to whom a single tablet of Medomycin tablet containing 100 mg of DOX was orally administrated as described in the experimental section. The results are presented in Table 5 and Fig. 13. From these results, it is believed that the differences in the concentration of DOX excreted between volunteers over the time (as shown in Table 4) may be due to the different amounts of DOX absorbed from one person to another (i.e. depends on the nature of the metabolism between each volunteer) or to how much of DOX excreted by kidneys? This is the reason that there is a difference in the proportion of DOX excreted in the urine because of the difference in kidney function among the volunteers. It is also evident that the concentration of the DOX decreases significantly over time through excretion in the urine via the kidneys**.**

Table 7: Determination of DOX in urine by the proposed method

Sample No.	Conc. DOX ($\mu g\ mL^{-1}$) After 2 h	Conc. DOX ($\mu g\ mL^{-1}$) After 4 h	Conc. DOX ($\mu g\ mL^{-1}$) After 8 h
1	15.0	9.7	6.7
2	12.6	9.3	6.4
3	12.9	9.9	6.8
4	18.3	11.0	7.0
5	14.3	9.0	6.6

Cont…

Sample No.	Conc. DOX ($\mu g\ mL^{-1}$) After 2 h	Conc. DOX ($\mu g\ mL^{-1}$) After 4 h	Conc. DOX ($\mu g\ mL^{-1}$) After 8 h
6	18.7	10.0	7.6
7	15.9	9.7	6.5
8	15.2	9.1	7.1
9	16.1	9.6	6.9
10	20.1	12.0	7.9
mean	15.91	9.93	6.96
SE mean	0.156	0.291	0.782
C.L at 95% $t_9 = 2.262$	±0.35	±0.66	±1.77

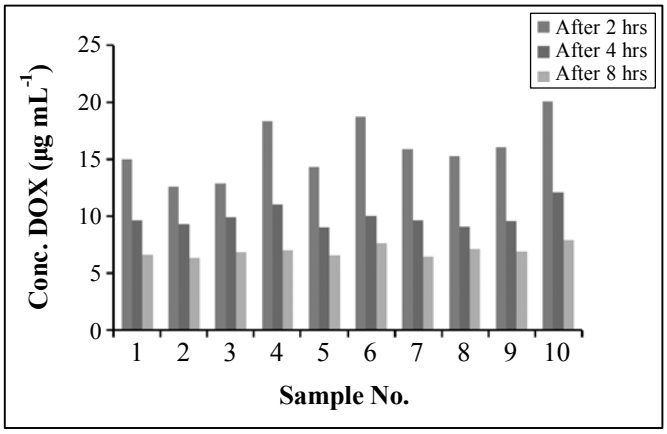


Fig. 13: The concentration of MOX in urine samples by the proposed method

Moreover, the proposed method was also applied for the detection of MOX in river water to check its validity in environmental samples through expectations that most of the pharmaceutical industries disposes their wastes into the water bodies. Because of the sample that withdrawn from Tigris river was devoid with DOX drug, it was spiked with 1, 3 and 5 $\mu g\ mL^{-1}$ of standard DOX solution followed the recommended CPE procedure. The results are presented in Table 8. It can be seen that the recovery percentage was acceptable and within the range of 101.36 ± 0.603%.

Determination of iron (III) in pharmaceuticals

Two pharmaceutical preparations as iron supplementation were selected for the determination of iron(III) by the developed method using DOX drug as chelating agent. The

tablets were digested with H_2SO_4 as described in the experimental section. The results of the established method are displayed in Table 9. The statistical computations of both iron supplements revealed that the calculated *t*-values for iron determination using DOX drug as the chelating agent are less than t-tabulated (4.303) at 95% confidence interval and (n-1) degrees of freedom, indicative of the acceptance of the null hypothesis Ho and hence, there is no evidence for systematic and random errors at the 95% confidence level. Accordingly, the results of the proposed method are fully consistent with the value declared by manufacturers.

Table 8: DOX in spiked river water sample by the proposed method

Amount of DOX added (µg mL^{-1})	Amount of DOX found (µg mL^{-1})	Recovery (%)	Recovery ± Sd (%)	Erel. (%)	RSD (%)
1	1.01	101.0	101.36 ± 0.603	1.0	0.83
3	3.05	101.7		1.7	0.42
5	5.11	102.2		2.2	0.21

Table 9: Determination of iron in pharmaceutical formulation by the proposed method and statistical comparison with quoted values

Commercial name, and content	Practical content (proposed method) (mg)	$t=(x-\mu)\sqrt{n}/s$ proposed method Vs. Claimed value at 95% C.I.	$\%E_{rel}$	%RSD (n=3)
Maltofer ®*contains 100 mg Fe as iron (III) hydroxide polymaltose	97.0 96.0 98.7 97.23 ± 3.39	tcal = 3.51 3.51< 4.303	-2.77	1.40
Hemafer Fol** contains 100 mg Fe as Iron (III) Hydroxide Polymaltose	95.00 100.75 98.00 97.92 ± 7.14	tcal = 1.25 1.25 < 4.303	-2.08	2.94

*Sanofi-Synthélabo Rt , pharmaceutical, Budapest , Hungary

**UNI-Pharma Kleon Tsetis Pharmaceutical Laboratories S.A., Greece

CONCLUSION

In this study, a new eco-friendly method by using cloud point extraction coupled with visible spectrophotometry was developed and successfully used in the mutual estimation of the DOX. HCl and Fe(III) ions in real samples. As we reported before, the potential advantages of the established method are also the time-saving, reducing the amount of reagents used and analyst effort as well as with adequate sensitivity and high accuracy.

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