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An Efficient Cloud Point Extraction for Doxycycline Pre-concentration in Pharmaceutical Samples prior to UV-Vis Spectrophotometric Analysis

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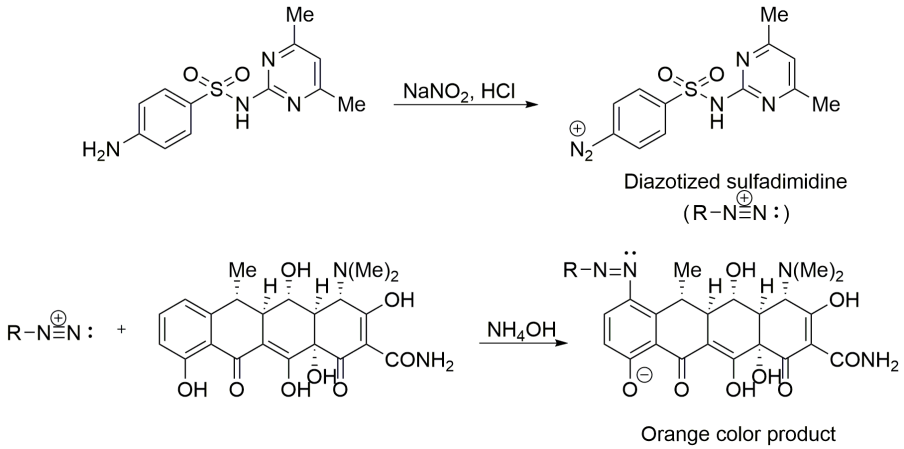
ABSTRACT

A sensitive and eco-friendly cloud point extraction (CPE) method was suggested for pre-concentration of micrograms amount of doxycycline hyclate (DOX) in pure and dosage forms. The method was based on formation a sensitive azo-dye produced by diazotization reaction of DOX with diazotized sulfadimidine (DSD) in a basic medium. The Triton X-114 rich phase containing the orange azo-dye was dissolved in ethanol after extraction and identified at the maximum wavelength at 430 nm using UV-Vis spectrophotometer. The proposed approach was investigated with and without extraction, and a straightforward comparison between the batch and CPE procedures was accomplished. The effects of several analytical factors on the CPE method, such as reagent and base concentrations, surfactant amounts, incubation temperature, and time were extensively examined. For the batch and CPE techniques, the linear ranges of calibration curves were 2-8 and 0.3-6 µg/mL with the detection limits of 1.0 and 0.041 µg/mL, respectively, under the selected optimum conditions. According to percentage recoveries ranged from 97.6 to 101.8% and relative standard deviation values of less than 3.5% for both procedures, the suggested methods were accurate and precise. Batch and CPE methods were employed successfully and with excellent accuracy in routine analyses of DOX in pharmaceutical formulations.

GRAPHICAL ABSTRACT



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Introduction

Doxycycline hyclate ($C_{24}H_{33}ClN_2O_{10}$), is a tetracycline derivative and has a wide range of antibacterial activity against gram-positive and gram-negative pathogenic bacteria as well as some protozoa [1, 2]. Due to their novel use as bacteriostatic and antibiotic medications, the research of determination of tetracycline and related compounds has been a notable task for drug and pharmaceutical studies [3]. Numerous techniques were described for estimation DOX in pharmaceutical and biological samples including spectrophotometry [4-8], high performance liquid chromatography (HPLC) [9-11], voltammetry [12-14], liquid chromatography [15, 16], flow injection spectrophotometry [17], and thin layer chromatography [18, 19]. Because of their simplicity and low cost as compared to other analytical techniques, spectrophotometric methods are still widely utilized for routine drug analysis. The cloud point extraction (CPE) approach is a green and fast extraction method based on the pre-concentration of small amounts of various chemical and inorganic substances using micelle systems. When surfactant molecules in aqueous solutions form micelles at a certain temperature known as "cloud point temperature," the solution turns turbid, and then turbid solution separates into an aqueous and surfactants rich phase above this temperature. The CPE method has various advantages over other extraction techniques, including high efficiency, sensitivity, and enrichment factor, as well as the use of safe aqueous medium rather than hazardous organic solvents. In the current study, the drug DOX was extracted and subsequently quantified using the safe and affordable reagent diazotized sulfadimidine (also a drug) in an alkaline medium. The azo dye product was extracted, dissolved, and spectrophotometrically quantified using the CPE procedure. To compare batch and CPE approaches, a straightforward comparison was done (with and without extraction).

Materials and Methods

A Shimadzu UV/Vis-spectrophotometer (1260/Japan) supplied with matching quartz cells (50 µL) was used to measure the absorbance of each sample as well as its absorption spectra. For the CPE process, a centrifuge (Hettich, EBA 21) equipped with standardised centrifuge tubes (50 mL) were utilized to separate the two phases. In addition, a thermostatic water bath (England) was used to demonstrate different ranges of temperature. The materials used in the current study were all of an analytical grade. DOX (purity 99.9%) was provided from the state company for drug industries (SDI/Iraq). The commercial pharmaceutical applications containing DOX (Doxycycline capsules ® 100 mg- Bamstaple, BNS,UK, Medomycin® 100 mg Madochemiie LTD-Cyprus) were obtained from local pharmacies.

Doxycycline solution (1000 µg/mL)

In 100 mL volumetric flask, stock solution of drug was prepared by weighing 0.1 g of standard DOX, dissolving in distilled water, and completed the flask to the mark with the same solvent.

Hydrochloric acid (0.5M)

Prepared by transferring 4.2 mL of concentrated HCl (36.4% w/w) to 100 mL volumetric flask, and diluted with distilled water, and then standardized.

Ammonium hydroxide (0.5M)

Prepared by transferring 9.4 mL of concentrated ammonium hydroxide (27.5% w/w) to 250 mL volumetric flask, and diluted with distilled water.
Diazotized sulfadimidine sodium (10 mM): Prepared in ice bath by transferred 0.9 mL of standard drug solution (333 mg/mL of sulfadimidine sodium) and 4 mL of hydrochloric acid solution (0.5 M) to 100 mL calibrated flask. After 5 minutes, 0.069 g of sodium nitrite was added then shaken and completed to the mark with distilled water.

Triton X-114 (10% v/v)

Prepared by diluted 10 mL of Triton X-114 (99.9%, Fluka) with 100 mL of distilled water.

Preparation solutions of DOX capsules

The contents of ten capsules covering active ingredient were weighed and the powder was mixed. An exactly weighed portion of the powder equivalent to 50 mg of DOX was dissolved in 30 mL of distilled water, and then filtered into a 50 mL volumetric flask. The residue was washed with distilled water and diluted to the mark with the same solvent to obtain 1000 µg/mL of DOX.

Procedure of batch and cloud point extraction methods

For batch method

3 mL of diazotized sulfadimidine sodium (0.01 M) was added in 10 mL volumetric flasks, and then increasing amounts of 100 µg/mL of doxycycline ranged from 0.2-0.8 mL and 1 mL of 0.5M ammonium hydroxide were added. The solutions of the flasks were shaken well and diluted with distilled water, after which they were left for 15 minutes for spectroscopic detection at 430 nm.

For CPE method

Into 10 mL calibrated flasks, 2 mL of diazotized sulfadimidine sodium (0.01M) was added, and then increasing amounts of 100 µg/mL of doxycycline (ranged 03-0.6 mL) and 2 mL of 0.5M ammonium hydroxide and 1 mL of Triton X-114 (10%) were added. Into 10 mL calibrated centrifuge tubes, the contents of flasks were transferred after mixing and diluting with distilled water. The tubes were then occupied at 60 °C for 30 min in thermostatic water bath. To separate the two phases, the tubes were centrifuged for 10 minutes (2500 rpm). After that, to speed up the separation process, the tubes were cooled (using an ice bath). The azo-dye-encircled micelles in the surfactant-rich phase (which was dissolved in 1 mL of ethanol) were spectrophoto-metrically measured at 430 nm while the aqueous phase was decanted.

Results and Discussion

Mechanism of reaction and absorption spectra

The absorption spectrum of the dye produced by coupling of DOX with DSD in a basic medium was displayed in Figure 1 to investigate the maximum wavelength for the coloured dye with and without extraction. Absorption spectra for the formation dye and the blank were recorded at a range of wavelengths between 400 and 600, with the maximum wavelength at 430 nm, indicating the complex formation between the DSD and medication. Using equimolar concentrations of both the reagent and the medication (2.25×10^{-4} M), the continuous variation approach (Job's method) was used to determine the molar ratio of the reactants (DSD and DOX). The results showed that the product (DSD: DOX) was generated in a 1:1 ratio. Sulfadimidine, a medicinal molecule, was diazotized utilizing sodium nitrite and hydrochloric acid as the first step in the reaction mechanism. The phenolic molecule (DOX), which had been transformed into the more reactive form "phenoxide," was then readily coupled with DSD to generate the azo-dye product, as depicted in Scheme 1.

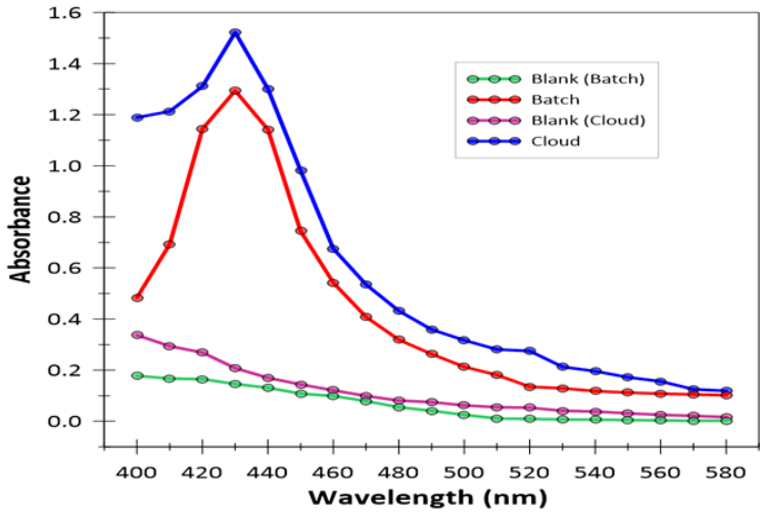
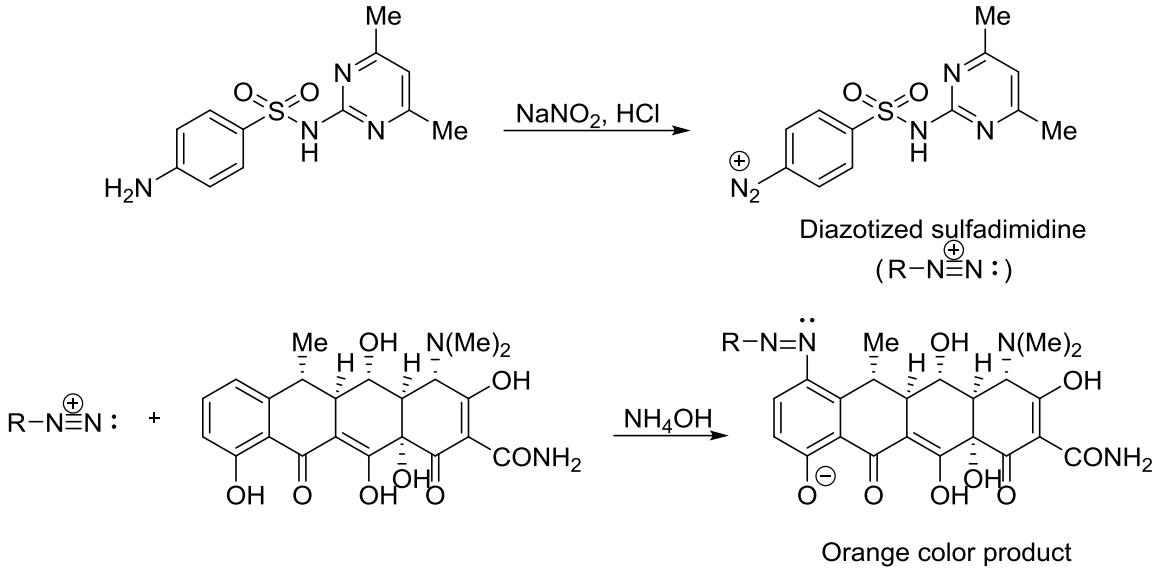


Figure 1: Absorption spectra of 10 ppm of DOX reacted with diazotized sulfadimidine with and without extraction



Scheme 1: The suggested reaction pathway

Study the experimental factors of batch and CPE approaches

The factors influencing the reaction product were carefully examined with and without CPE to increase the sensitivity of dye product. By varying one variable over time while holding the other constant, several chemical factors, including concentrations of ammonium hydroxide, DSD, and surfactants, as well as extraction conditions, including incubation temperature and time were investigated. In all optimization tests, 5 µg/mL of DOX was employed, and absorbance was measured for both methods at 430 nm against the blank.

Effect of the chemical conditions

Effect of DSD concentration

In diazotization coupling procedures, sulfadimidine drug is employed as a green colorimetric reagent. The effect of different DSD solution concentrations on the sensitivity of azo-dye product was examined. The concentrations evaluated varied from 1 to 5 mM (1 to 5 mL of 0.01 M DSD solution in 10 mL). The results reported in Figure 2 showed that for the batch and CPE procedures, respectively, the highest analytical signal was obtained using 3 and 2 mM of DSD (i.e. 3 and 2 mL of 0.01 M in 10 mL final volume). Therefore, these concentrations of DSD solution were chosen as the optimum values for further work.

Effect of base solution (type and concentration)

The initial studies revealed that alkaline medium is the most effective medium for the diazotization coupling reaction between the phenolic drug (DOX) and reagent. The coupling reaction is enhanced and promoted by alkaline medium by converting DOX into phenoxide species. Different types of bases included Na_2CO_3, NaOH, and NH_4OH were examined and the results showed that ammonium hydroxide solution provided maximum signal (Figure 3a). Different quantities of basic solution from 0.5 to 4 mL of 0.5M were used to create a range of NH_4OH concentrations, and their effects on azo-dye absorbance were studied. For the batch and CPE techniques, the maximum response was reached using 1 and 2 mL of NH_4OH, respectively (Figure 3b).

Study the CPE parameters

Effect of Triton x-114 concentration and extraction temperature

Triton-X114 is a type of surfactant used for extracting various organic and inorganic compounds, and is thought to be the most reactive and effective surfactant available. The surfactant plays a significant role in the extraction process by influencing the

effectiveness of the separation technique. Extraction of several samples containing 5 µg/mL of DOX was done using different amounts of 10% (v/v) surfactant to investigate this impact. High sensitivity was achieved with 1.0 mL of Triton, which was selected for advance use, as shown in Figure 4a. More surfactant would reduce the response; this may due to complete the separation process. Investigating the ideal incubation temperature is necessary to achieve complete separation. The extraction temperature was examined between 40 and 80 °C throughout a 30 min. The incubation temperature, 60 °C, was chosen as the ideal temperature since it produced the maximum analytical signal (Figure 4b).

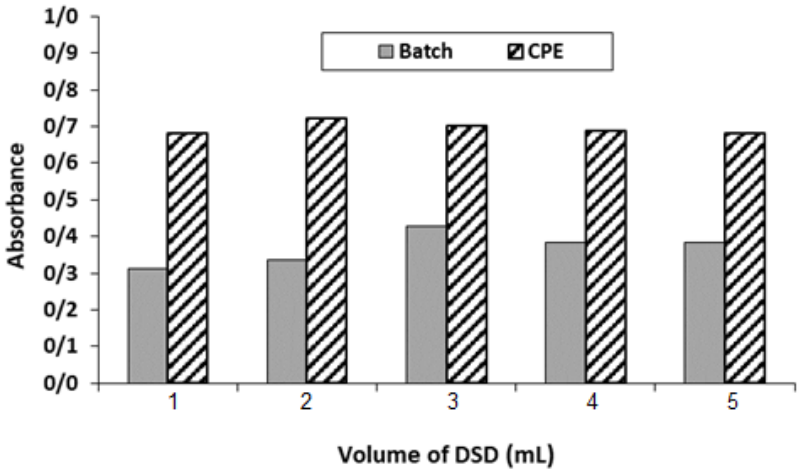


Figure 2: Influence of DSD concentration (Experimental conditions: 5 ppm of DOX; DSD, 2 mM; NH_4OH, 0.1 M; Triton X-114, 0.2% (v/v); Temp., 60 °C)

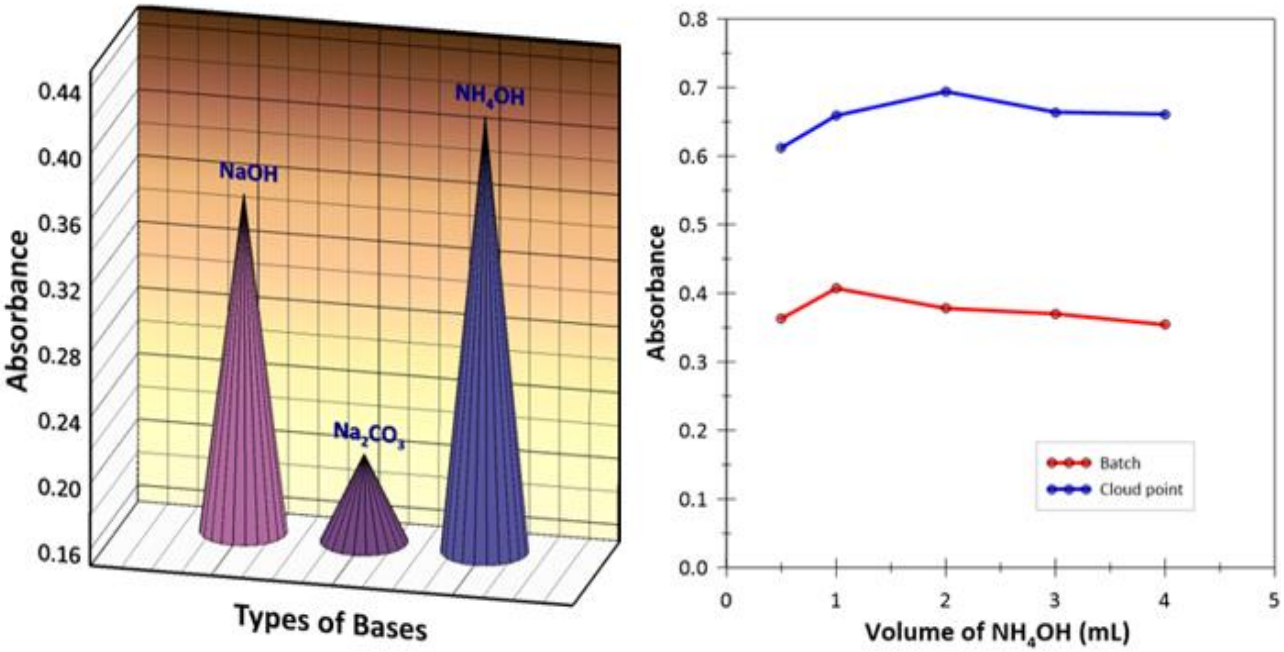


Figure 3: (a) Effect of type of base and (b) effect of the volume of ammonium hydroxide solution

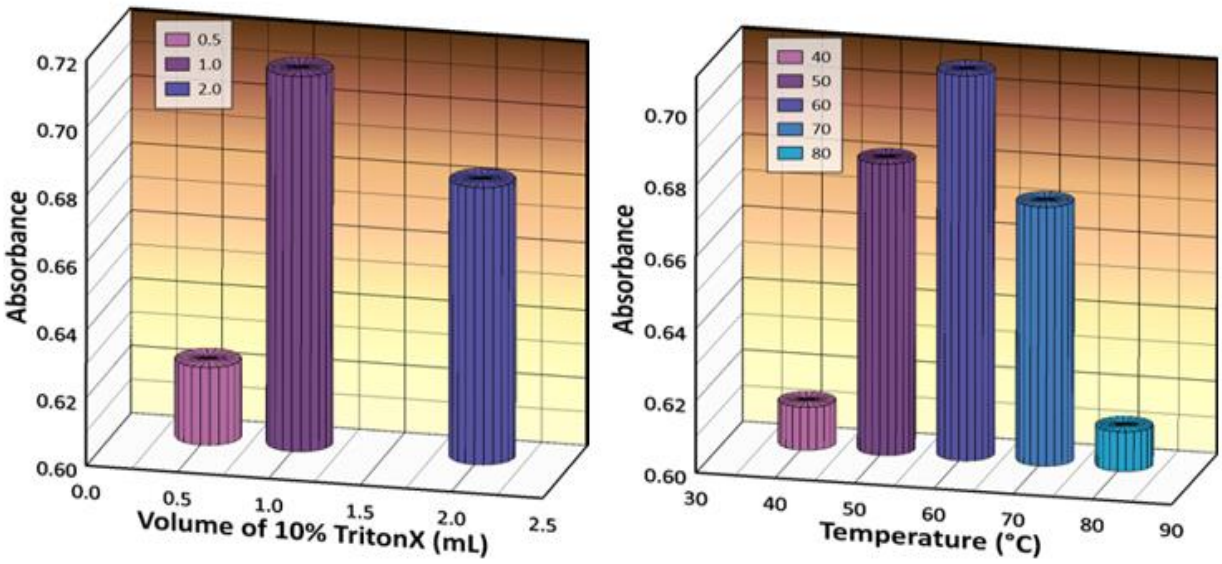


Figure 4: Effect of (a) volume of Triton X-114 and (b) incubation temperature

Study of the incubation time

The incubation period has a significant impact on the extraction effectiveness and the equilibrium between two phases. To study the required incubation time for full separations, 10 to 40 minutes at 60 °C were used. According to the results (Figure 5), increasing the incubation period up to 30 min increased the absorbance of extracted azo-dye before it slightly reduced. Therefore, 30 min was chosen as the ideal time to achieve quantitative extraction. A completed separation was achieved at 5.0 min of centrifugation duration, which was selected for continued use after being tested between 2 and 10 min.

Final optimum parameters

In Table 1 for batch and CPE techniques, all the evaluated chemical and physical parameters that can have an impact on the separation effectiveness and sensitivity of the formation dye are mentioned.

Methods validation

The calibration curves for both methods were created using the prior optimum variables for batch and CPE procedures, which were employed for estimate of DOX and are described in Table 1. For the batch and CPE techniques, the Beer's law ranges were 2-8 and 0.3-6 µg/mL with the detection limits of 1.0 and 0.041 µg/mL, respectively, under the selected optimum conditions. According to percentage recoveries ranged from 97.6 to 101.8% and relative standard deviation values of less than 3.5% for both procedures, the suggested methods were accurate and precise. The accuracy of the current approaches was also demonstrated by the small values of the analytical features, which included the standard deviation of the slope (Sb), intercept (Sa), and residual (Sy/x). In addition, the enrichment factor was considered to be ≈2.

Accuracy and precision

Both suggested methods' precision and repeatability were examined. Five replications of two different concentrations of DOX drug were assay using batch and CPE techniques. High accuracy and repeatability were shown by the low error values (excellent recoveries values) and acceptable relative standard deviation values recorded in Table 3, which supported the two suggested procedures.

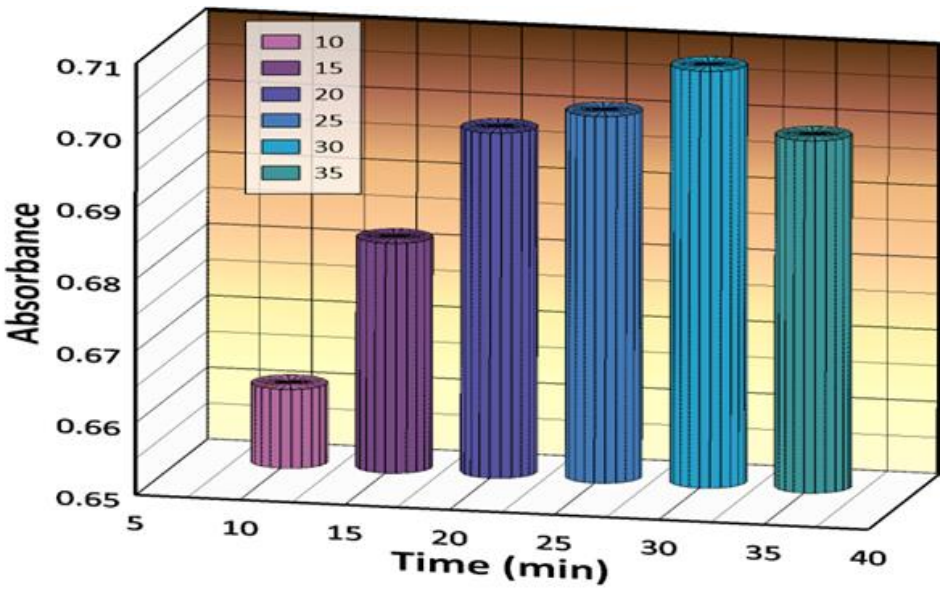


Figure 5: Effect time of the incubation (experimental conditions: 5 ppm of DOX; DSD, 2 mM; NH_4OH, 0.1 M; Triton, 0.2% (v/v); Temp., 60 °C)

Table 1: Selected values of the studied parameters for both suggested approaches

Parameter	Studied range	Selected value	
		Batch	CPE
Volume of 0.01M diazotized sulfadimidine (mL)	1-5	3	2
Volume of 0.5M NH_4OH (mL)	0.5-4	1	2
Volume of 10% (v/v) surfactant (mL)	0.5 –2	-	1.0
Incubation temperature (°C)	40-80	-	60
Incubation time (min)	10-40	-	30

Table 2: Analytical features for proposed batch and extraction approaches

Parameter	Value	
	Batch method	CPE method
λ_{max} (nm)	430	430
Regression equation	y=0.0863x + 0.0074	y = 0.133x + 0.0368
Correlation coefficient, R2	0.999	0.990
Dynamic linear range (µg/mL)	2-8	0.3-6
Limit of detection (µg/mL)	1.0	0.041
Molar absorptivity (L/mol cm)	38.354×10^3	59.109×10^3
Sandell's sensitivity (µg/cm^2)	0.0116	0.0075
Slope (mL/µg)	0.0863	0.133
Intercept	0.0074	0.0368
Enrichment factor	≈ 2	
$S_{y/x}$	0.0073	0.0231
S_b	0.0032	0.0011
S_a	0.0098	0.0103

Table 3: Accuracy and repeatability for batch and CPE approaches

Method	Conc. of DOX (µg/mL)		Recovery%	Erel%	RSD% (n=4)
	Present	Found			
Batch	3	3.04	101.33	1.33	1.00
	5	4.96	99.20	-0.8	3.68
	7	7.20	102.86	2.86	2.96
CPE	1	1.02	102.00	2.00	3.97
	3	2.93	97.67	-2.33	4.85
	5	4.91	98.20	-1.80	3.11

Table 4: Determination of DOX in capsules using both proposed methods

Pharmaceutical forms	Proposed methods									Official method
	Batch					CPE				
	Conc. DOX (µg/mL)		Rec. (%)	Mean Rec. (%)	RSD (%)	Conc. DOX (µg/mL)		Rec. (%)	Mean Rec. (%)	RSD (%)
	Taken	Found				Taken	Found			
Doxycycline® capsules/Bamstaple BNS,UK	3	3.03	101.00	100.80	1.89	3	3.02	100.67	101.24	2.59	100.55
	5	5.03	100.60		2.02	5	5.09	101.80		3.32	
Medomycin Madochemiie LTD-Cyprus	3	3.02	100.67	101.44	1.68	3	3.08	102.67	101.64	3.11	102.20
	5	5.11	102.20		1.69	5	5.03	100.60		2.16	
t (2.776)**	0.301					0.197					
F (19.000)**	12.797					1.206					

** n=4; ** theoretical value and RSD = relative standard deviation*

Assay of DOX in pharmaceutical samples

Batch and CPE methods were applied successfully for assay of DOX in a commercial pharmaceutical form (Table 4). The effectiveness and application of these methods in the routine analysis of DOX in pharmaceutical forms were demonstrated by the good obtained recoveries and RSD values of the present approaches. Recoveries values were also contrasted with those calculated using the HPLC standard method [1]. A statistical comparison between the methodologies was carried out using two standard tests (*t*-and *F*-tests at a confidence level of 95%) [20]. The measured t- and F-values were not acceding the tabulated values, indicating that there was no difference in the precision and accuracy between both procedures utilized for assay DOX in its pharmaceutical capsules.

Conclusion

For estimation and extraction of a microgram of DOX in pharmaceutical applications, the current work comprised fast, sensitive, and environmentally acceptable approaches. Using sulfadimidine as a green reagent in place of harmful and expensive reagents is the basis of a colorimetric/diazotization reaction. The azo-dye produced by the diazotization reaction was extracted with Triton X-114 and the cloud point extraction technique. Combining CPE with spectrophotometry offered a simple and affordable method for DOX measurement without the use of hazardous solvents or sophisticated methods. The DOX assay in pharmaceutical capsules has been carried out satisfactorily and with acceptable accuracy using the batch and CPE methods.

Disclosure Statement

No potential conflict of interest was reported by the authors.

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Authors' Contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

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References

[1]. British Pharmacopoeia on CD-ROM", Version 5, 3rd Ed., Vol. 1,Copyright by System Simulation Ltd, The Stationery Office Ltd., London, 2001 [Publisher]
[2]. Dollery C., *Therapeutic Drugs*, 1999, **2**:K25 [Google scholar]
[3]. Damaso D., MorenoLopez M., Daza R.M., Antibioticos y Quimioterapicos Antibacterianos, *Uso Clinico, Grutesa, Madrid*, 1984 [Google scholar]
[4]. Pavagada J.R., Kanakapura B., Mysore R.D., Nagaraju R., Kanakapura B.V., Hosakere D.R., Simple UV and Visible Spectrophotometric Methods for the Determination of Doxycycline Hyclate in Pharmaceuticals, *Journal of Analytical chemistry*, 2011, **66**:482 [Crossref], [Google scholar], [Publisher]
[5]. Al-Abachi M.Q., Al-Nedawi Z.A., Batch and Flow Injection Spectrophotometric Determination of Doxycycline Hyclate in Pharmaceutical Preparations, *Al-Nahrain Journal of Science,* 2015, **18**:24 [Google scholar], [Publisher]
[6]. Khalaf N.M., Othman N.S., Determination of Doxycycline as Pure and in Pharmaceutical Preparation (Capsule) Using 4-Aminoantipyrine in Presence of Potassium Periodate, *Rafidain Journal of Science***,** 2022, **31**:15 [Crossref], [Google scholar], [Publisher]
[7]. Awad F.H., Taki A.G., Spectrophotometric Determination of Doxycycline Via Oxidation Reduction Reactions, *Egyptian journal of chemistry*, 2021, **64**:6615 [Crossref], [Google scholar], [Publisher]
[8]. TalibHumeidy I., Determination of Doxycycline Hyclate in pharmaceutical samples via Oxidative Coupling Reaction using Spectrophotometric method, *Journal of University of Anbar for Pure Science,* 2018, **12**:29 [Crossref], [Google scholar], [Publisher]
[9]. Sunarić S.M., Denić M.S., Bojanić Z.Ž., Bojanić V.V., HPLC method development for determination of doxycycline in human seminal fluid, *Journal of Chromatography B,* 2013, **15**:17 [Crossref], [Google scholar], [Publisher]
[10]. Anca B., Maria-Alexandra R., Aranka I., Oana C., Validated HPLC Multichannel DAD Method for the Simultaneous Determination of Amoxicillin and Doxycycline in Pharmaceutical Formulations and Wastewater Samples, *Applied Sciences*, 2022, **12**:9789 [Crossref], [Google scholar], [Publisher]
[11]. Khafi M., Javadi A., Reza M., Mogaddam A., Combination of three-phase extraction with deep eutectic solvent-based dispersive liquid–liquid microextraction for the extraction of some antibiotics from egg samples prior to HPLC-DAD, *Microchemical Journal***,** 2023, **190**:108652 [Crossref], [Google scholar], [Publisher]
[12]. Gashu M., Kassa A., Tefera M., Amare M., Aragaw B.A., Sensitive and selective electrochemical determination of doxycycline in pharmaceutical formulations using poly (dipicrylamine) modified glassy carbon electrode, *Sensing and Bio-Sensing Research*, 2022, **37**:100507 [Crossref], [Google scholar], [Publisher]
[13]. Tian X., Fan Z., Novel ratiometric probe based on the use of rare earth-carbon dots nanocomposite for the visual determination of doxycycline, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2021, **260**:119925 [Crossref], [Google scholar], [Publisher]
[14]. Mulu G., Adane K., Molla T., Meareg A., Belete A.A., Sensitive and selective electrochemical determination of doxycycline in pharmaceutical formulations using poly(dipicrylamine) modified glassy carbon electrode, *Sensing and Bio-Sensing Research*, 2022, **37**:100507 [Crossref], [Google scholar], [Publisher]
[15]. Gajda A., Posyniak A., Zmudzki J., Tomczyk G., Determination of doxycycline in chicken fat by liquid chromatography with UV detection and liquid chromatography–tandem mass spectrometry, *Journal of Chromatography B,*

2013, **928**:113 [Crossref], [Google scholar], [Publisher]

[16]. Eugenia S., Fabio G.C., Elías B.C., Determination of streptomycin and doxycycline using LC/MS towards an effective treatment against an experimental Brucella abortus infection in mice, *Journal of Microbiological Methods*, 2022**,** **194**:106436 [Crossref], [Google scholar], [Publisher]

[17]. Palamy S., Ruengsitagoon W., A novel flow injection spectrophotometric method using plant extracts as green reagent for the determination of doxycycline, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2017, **171**:200 [Crossref], [Google scholar], [Publisher]

[18]. Sudavone P., Wirat R., The Determination of Doxycycline Using Ferrous Sulfate Contained in Vitamin Tablets as Reagent, *Isan Journal of Pharmaceutical Sciences*, 2020, **16**:79 [Crossref], [Google scholar], [Publisher]

[19]. Mohammad M.A.A., Zawilla N.H., Thin-layer and column-chromatographic methods for simultaneous analysis of ambroxol hydrochloride and doxycycline hyclate in a binary mixture, *JPC–Journal of Planar Chromatography–Modern TLC*, 2009, **22**:201 [Crossref], [Google scholar], [Publisher]

[20]. Miller J.C., Miller J.N., Statistics for Analytical Chemistry, Ellis Horwood, Chichester, UK, 1993, 153 [Google scholar]

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