

SPECTROPHOTOMETRIC DETERMINATION OF DOXORUBICIN HYDROCHLORIDE USING CHARGE TRANSFER COMPLEXES REACTION

Tabarak A. Abdulwahd, Omar A. Sheej Ahmad*

Chemistry Department of the College of Education for pure science. University of Mosul, Mosul- Iraq
*e-mail: dr.omar1979@uomosul.edu.iq

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Abstract: A new, simple, sensitive, and rapid spectrophotometric method has been developed for the determination of doxorubicin hydrochloride (DOX) via charge-transfer complex formation with *o*-chloranil in its pure form and pharmaceutical preparations (injection). The method is based on the formation of a stable 2:1 (Reagent: Drug) complex in an ethanol medium, showing maximum absorbance at 531 nm. In order to get the optimal conditions. All factors that might potentially affect the spectrophotometric absorption of the charge-transfer complex formed with doxorubicin were thoroughly examined. Beer's law was obeyed in the concentration range of 0.1–12 µg/mL with a molar absorptivity of 41148.60 L·mol⁻¹·cm⁻¹ and a detection limit value was of 0.0072 µg/mL while the quantification limit was 0.0220 µg/mL. The proposed method demonstrated excellent accuracy and precision, with average recoveries close to 100%. It was successfully applied to the quantification of doxorubicin in pharmaceutical formulations without interference from excipients. Compared to other methods such as HPLC and fluorometric, this technique offers a cost-effective, rapid, and accessible alternative for routine analysis in quality control.

Keywords: Spectrophotometric analysis, *o*-chloranil, doxorubicin, charge-transfer complex formation

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Introduction

Doxorubicin hydrochloride (DOX) is a chemical drug known as (8S,10S)-10[(3-Amino-2,3,6-trideoxy- α -L-lyxo-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-5,12-

naphthacenedione hydrochloride. It is officially documented in the British and United States Pharmacopoeia [1, 2]. Doxorubicin is a reddish-orange crystalline powder with the molecular formula C₂₇H₂₉NO₁₁ [2].

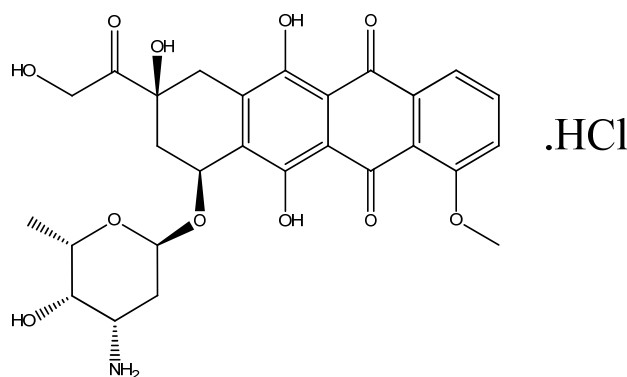


Fig. 1. Chemical structure of Doxorubicin hydrochloride

It is soluble in water and also in organic solvents. It is recommended to store it in light-resistant vials due to its high sensitivity to light [3]. Doxorubicin belongs to the anthracycline class it has a planar molecular structure

containing a naphthacenoquinone group attached to the amino sugar daunosamine via a glycosidic bond [4]. The aliphatic side chain is essential for its interaction with DNA, contributing to binding to the double helix. This compound is also

known for its high polarity and ability to distribute charges highly and is classified among good electron acceptors [5- 7]. Doxorubicin is commonly used as a chemotherapy drug to treat some cancers through intravenous injection such as leukemia, breast cancer, bronchial cancer, and thyroid cancer [4]. On the other side, Doxorubicin has cardiac toxicity, as it leads to cardiomyopathy once the permissible limit for the cumulative dose is exceeded, which has been approved so far as 550 mg/m²[8-9]. For that reason, effective monitoring of doxorubicin levels in biological fluids is essential. Several methods have been reported for determination of doxorubicin concentrations in body fluids and tissues. For example, capillary electrophoresis with UV absorption[10], Laser-induced fluorescence [11], Voltammetry [12-14]. High-

performance liquid chromatography (HPLC) [15-17], Liquid chromatography-based methods coupled with fluorescence detection[18], high-performance liquid chromatography with fluorescence detection have also been applied in the analysis of tumors and tissues [19], and Fluorescence spectroscopy [20-22]. However, only a limited number of papers have employed spectrophotometric techniques for the determination of doxorubicin in the visible and ultraviolet [23-24], which may be attributed to the bulk structure that could interfere with spectroscopic methods. This study targets to create a simple and reliable spectrophotometric method for the determination of the pharmaceutical compound (DOX) in its dosage form (injection), ensuring no interference from excipients.

Experimental Part

1. Analytical instrumentation. A Shimadzu UV-1800 PC connected with UV Probe 2.42 software was used to carry out spectrophotometric measurements.

2. Reagents and Chemicals: All chemicals were of analytical reagent grade and all solutions were prepared and distilled water - was used throughout the experiments.

3. Preparation of Solutions

3.1 Doxorubicin Solution: A stock solution of doxorubicin was prepared at a concentration of 100 µg/mL by precisely

weighing 0.0100 g of the drug and dissolving it in a 100 mL volumetric flask with distilled water. The resulting solution was transferred to a light-protected bottle and stored in a refrigerator, where it remained stable for over ten days.

3.2 3,4, 5,6 Tetrachloro-1,2-benzoquinone (o-Chloranil) Solution: A 1 × 10⁻² M solution of o-chloranil was prepared by dissolving 0.246 g of the compound in 100 mL of acetonitrile. The solution was stored in an amber bottle to maintain its stability for up to one week.

Result and Discussion

1. Optimization of Experimental Conditions

1.1. Effect of Solvent. Various solvents including methanol, ethanol, acetonitrile, acetone, and water were tested as reaction media to evaluate their influence on the absorbance intensity, sensitivity, and stability of the resulting complex. These findings are summarized in Table 1. It was observed that the complex

exhibited better solubility in organic solvents, whereas the drug (doxorubicin) showed preferential solubility in aqueous medium, as it is only soluble in water. Organic solvents are ideal for studying and forming chloranil–doxorubicin charge transfer complexes, due to superior solubility, interaction stabilization, and clearer spectroscopic behavior.

Table 1. The effect of the nature of the solvent used on the color intensity and λ_{\max} of the complex.

Doxorubicin Dissolved in	o-chloranil Dissolved in	Dissolved in	λ max (nm)	Absorbance
Water	Acetonitrile	Ethanol	531	0.542
Water	Ethanol	Water	500	0.033

Water	Ethanol	Ethanol	532	0.403
Water	Acetonitrile	Water	497	0.038
Water	Methanol	Water	476	0.049
Ethanol	Ethanol	Water	500	0.092
Ethanol	Ethanol	Ethanol	532	0.072
Ethanol	Acetone	Acetone	494	0.037
Ethanol	Acetonitrile	Acetonitrile	496	0.077
Methanol	Acetonitrile	Water	497	0.188
Methanol	Methanol	Water	500	0.126

1.2. Study of Acidic and Basic Effects.

The influence of the acidic and basic medium on the absorbance intensity was evaluated. A selection of strong and weak acids and bases (hydrochloric, sulfuric, nitric and acetic acid, sodium hydroxide, potassium hydroxide, sodium

carbonate, and sodium bicarbonate) were tested (0.01 M - 0.5 M). The experimental results showed that both acidic and basic media reduced the color intensity Table 2. Therefore, in the following experiments, no acids or bases have been utilized or added.

Table 2. Selection of a suitable medium for the reaction.

Type of bases and acid 0.01 M (0.5mL)	Absorbance	λ_{\max} (nm)	Final pH
NaOH	0.001	534	5.00
Na ₂ CO ₃	0.022	533	5.70
NaHCO ₃	0.007	534	4.47
KOH	0.020	534	4.10
HCl	0.242	531	2.70
HNO ₃	0.182	529	2.90
H ₂ SO ₄	0.195	527	2.85
CH ₃ COOH	0.272	534	3.31
Without	0.443	531	3.50

1.3. The effect of pH and buffer solutions.

After determining the pH of the formed complex, many buffer solutions were prepared within the pH range of the complex (1

mL). However, no significant improvement in absorbance was observed. Therefore, buffer solutions were excluded in the subsequent experiments Fig. 2.

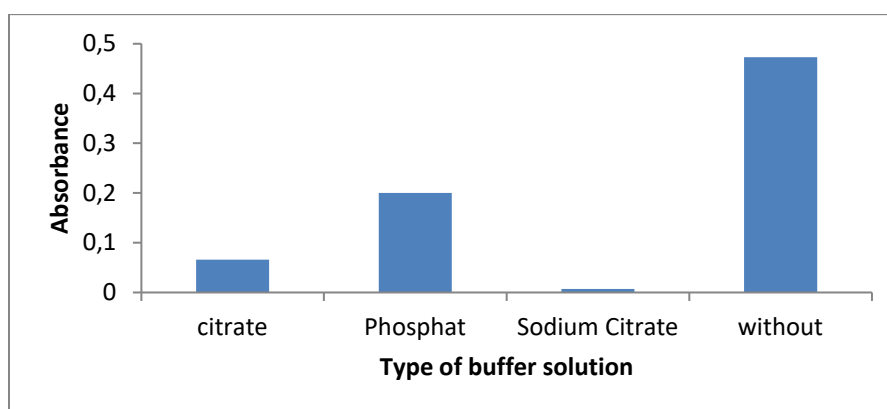


Fig. 2. The effect of pH and buffer solutions

1.4. Effect of o-Chloranil Reagent

Volume. To determine the ideal volume of the

reagent, increasing the volume of the reagent prepared at a concentration of (1×10^{-2} M) was

added. Consequently, the optimal amount (1 mL) was chosen as shown in Fig. 3.

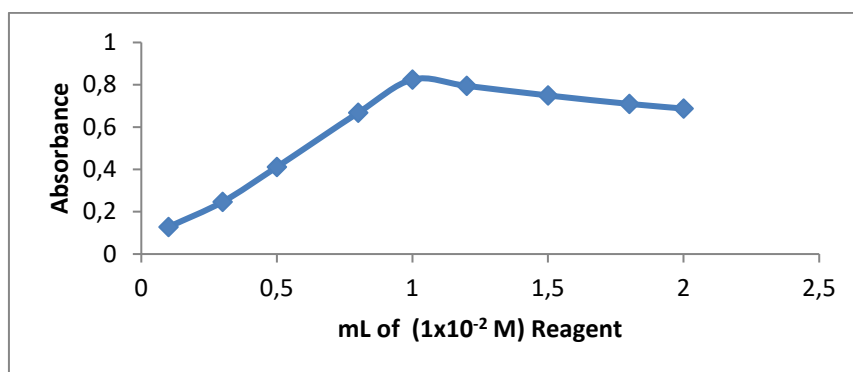


Fig. 3. The effect of the reagent volume

1.5. Effect of Temperature on the Complex Stability. To evaluate the impact of temperature on the stability and persistence of the complex formed from the reaction between the (DOX-o-chloranil), the stability of the complex was examined at three different temperatures

ranging from 0 to 30°C. The results revealed that the complex exhibited stability and persistence at 20°C, showing absorbance 5 minutes after dilution and remaining stable for approximately 90 minutes Fig. 4.

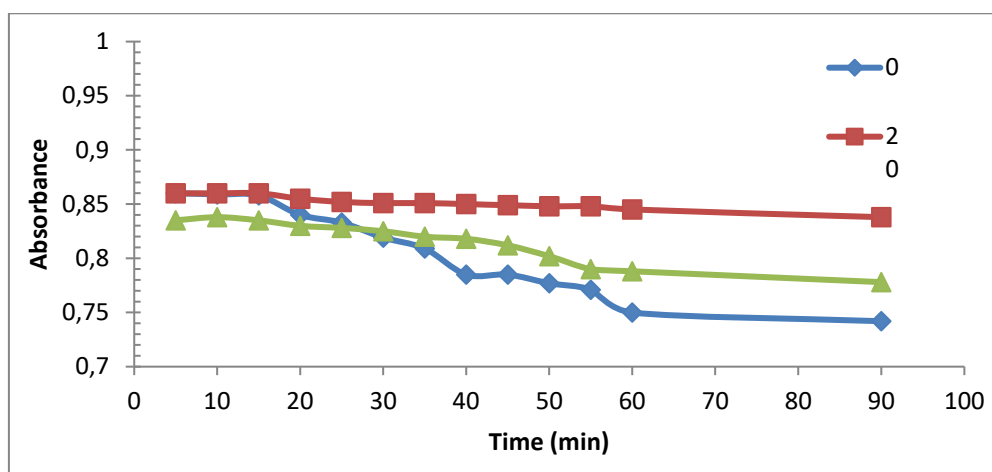


Fig. 4. Stability and Temperature Effect

After examining every variable that might enhance or reduce the absorbance value of the resulting complex, the optimum conditions were determined, as summarized in the table below.

With the reagent amount, pH, solvent type, stability time, and temperature now optimized, the final absorption spectrum was recorded and is presented in Fig. 5.

Table 3. Optimal conditions for the reaction

Experimental conditions	Doxorubicin
λ_{\max} (nm)	531
o-Chloranil $\times 10^{-2}$ M (mL)	1.0
Final pH	3.0
Temperature (C°)	20
Development time (min)	5
Stability period (min)	90
Solvent	Ethanol

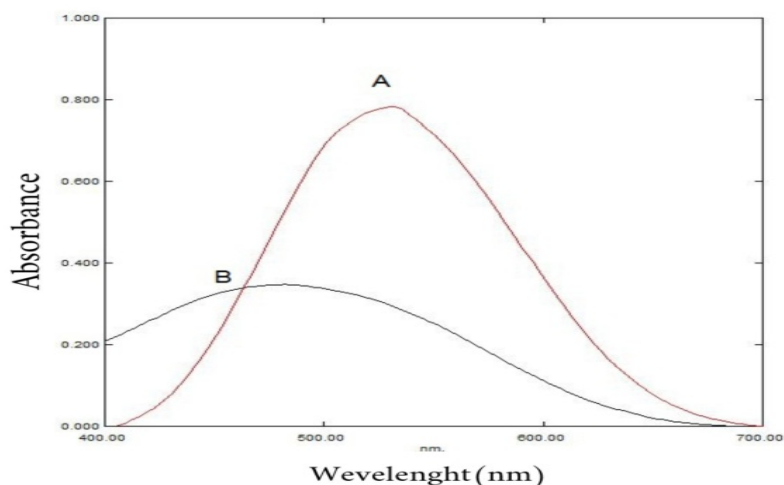


Fig. 5. Final absorption spectrum of the Doxorubicin Hydrochloride–ortho-Chloranil complex.

A: Absorption spectrum of the drug (10 µg/mL) versus the blank solution

B: Absorption spectrum of the blank solution versus absolute ethanol.

2. Calibration Curve. A precise spectrophotometric method was developed for the determination of doxorubicin within the concentration range of 0.1–12 µg/mL, based on its reaction with o-chloranil in an organic medium (ethanol). The method showed good agreement with Beer's law within this range,

with a molar absorptivity of 41148.60 L·mol⁻¹·cm⁻¹. A negative deviation was observed at higher concentrations, indicating the limits of linearity and confirming the method's capability for the quantitative determination of doxorubicin within the specified range Fig. 6.

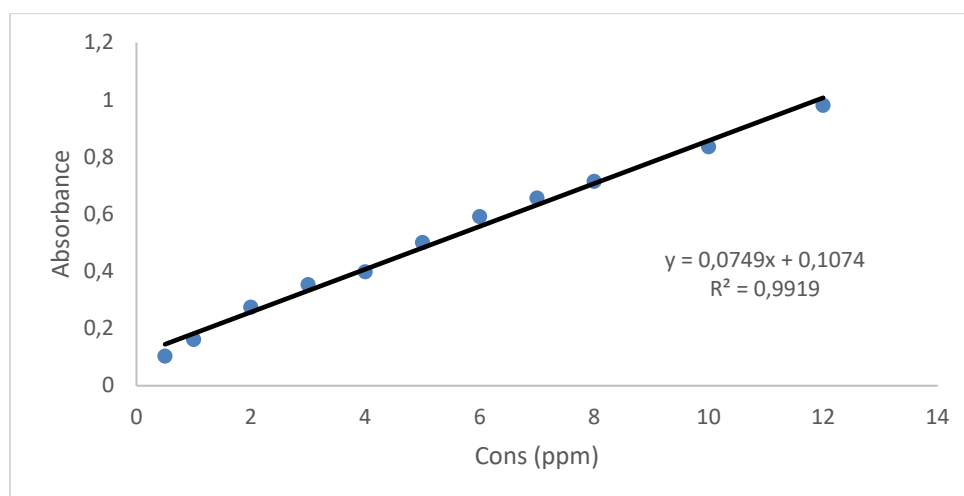


Fig. 6. Calibration Curve for Doxorubicin Hydrochloride

Table 4. Validation parameters

Parameter	Doxorubicin
Linearity range (µg/ml)	0.1-12
Molar absorptivity (l.mol ⁻¹ .cm ⁻¹)	41148.60
Sandell Sensitivity (µg/cm ²)	0.0720
LOD* (µg/ml)	0.0072
LOQ* (µg/ml)	0.0220
Intercept	0.1074
Slope	0.0749
Determination coefficient (R ²)	0.9919

3. Accuracy and Precision Evaluation. The method's accuracy and consistency were evaluated by analyzing six replicates of three different doxorubicin concentrations. The results showed that the relative standard deviation did not exceed 3.16%, while the average recovery rate was 100.1%, confirming the method's accuracy and reliability in quantitative measurements.

Table 5. Method Accuracy and Precision

Drug	Conc. of drug (µg/ml)		Recovery* (%)	Average recovery (%)	RSD* (%)
	Added	Found			
Doxorubicin	1	0.99	99	97.2	4.32
	3	2.87	95.6		4.19
	5	4.85	97		1.39

* Average of six determinations

4. Application of the proposed method to the pharmaceutical formulation

Analysis of Doxorubicin Injection. A standard solution of doxorubicin hydrochloride was prepared at a concentration of 500 µg/mL by dissolving 0.0500 g of the formulation in distilled

water in a 100 mL volumetric flask. From this, a 100 µg/mL solution was obtained by diluting 20 mL to volume in a 100 mL volumetric flask. Aliquots of 0.1, 0.3, and 0.5 mL were then used under the optimized conditions outlined in the table for spectrophotometric analysis.

Table 6. Determination of drug compound in pharmaceutical preparation by the proposed method

Company	Drug composition	Pharmaceutical preparation			
STADAPHARM GmbH Germany	50 mg of Doxorubicin hydrochloride	Injection			
Drug	Conc. of drug (µg/ml)		Recovery* (%)	Average recovery (%)	RSD* (%)
	Added	Found			
Doxorubicin	0.96	48	96	100.13	3.16
	3.12	52	104		2.90
	5.02	50.2	100.4		1.23

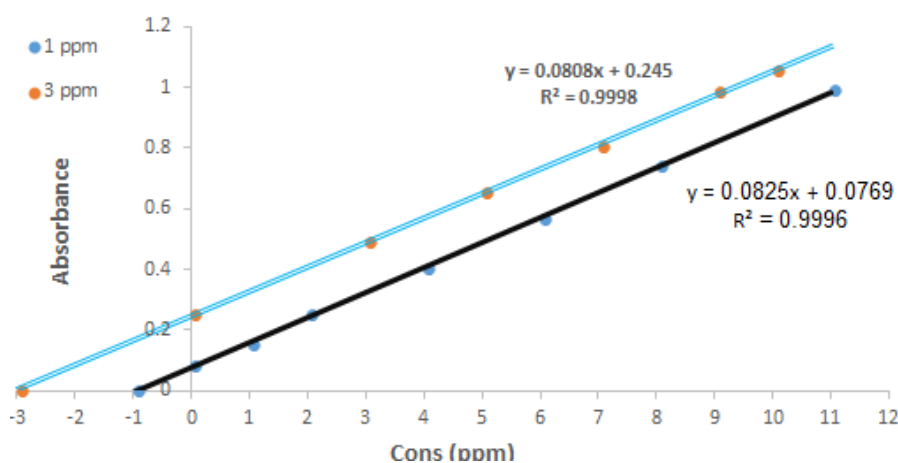


Fig. 7. Standard addition method

5. Application of the Standard Addition Method. The standard addition method was

practically applied to estimate the accuracy and selectivity of the suggested analytical method for

the determination of doxorubicin at different concentrations (1-3 ppm) of the pharmaceutical formulation, under the optimized conditions. The results, as presented in the table, demonstrated

that the method is free from interference by excipients and showed good agreement with the nominal content, confirming its efficiency and statistical selectivity as shown in Fig. 7.

Table 7. Comparison of the accuracy of the developed method for the determination of Doxorubicin in pharmaceutical formulation with the standard addition method

Pharmaceutical preparation	Certified value	Amount present ($\mu\text{g/ml}$)		Recovery (%)	Drug content found (mg)	
		Added	Found		Present method	Standard addition method
Doxorubicin hydrochloride	50 mg	1	0.99	99	48	49.5
		3	2.90	96.6	52	48.3

6. Methods of Analysis A determination of the stoichiometric ratio. Job's method was employed to determine the stoichiometric ratio of the complex formed between doxorubicin and o-chloranil [25]. Equimolar solutions of both compounds were mixed in varying volume ratios while keeping the total volume constant at 1 mL in 10 mL volumetric flasks. The final volume was adjusted with absolute ethanol under previously optimized conditions. Absorbance

was measured at 531 nm, and the results, as shown in Fig. 8, indicated a stoichiometric ratio of 2:1 (doxorubicin: o-chloranil). In addition, to validate the results obtained by the continuous variation method, the molar ratio method was employed to determine the stoichiometry of the doxorubicin:o-chloranil complex. Spectrophotometric measurements at 531 nm indicated a molar ratio of 2:1 (reagent:drug).

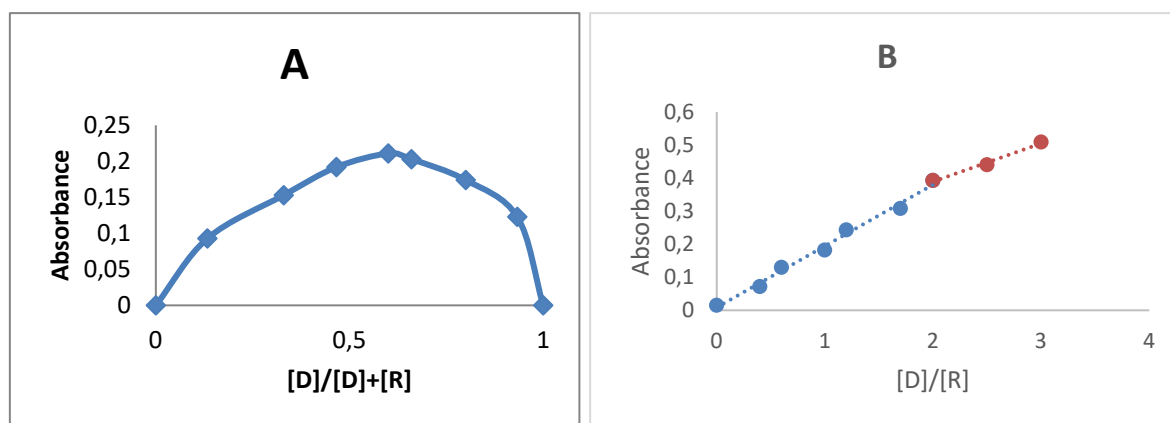


Fig. 8. Stoichiometric Reaction Ratio – A: Job's Method –B: Mole Ratio

7. Stability Constant of the Formed Complex. The stability constant of the 2:1 complex formed between doxorubicin and o-chloranil was determined using the molar ratio method. Solutions with equal and fixed concentrations of both components were prepared to measure the absorbance at

equilibrium (A_s), while additional solutions containing the same concentration of doxorubicin and an excess of o-chloranil were used to determine the maximum absorbance (A_m). The stability constant was calculated based on the measured values of A_s and A_m using the appropriate mathematics:

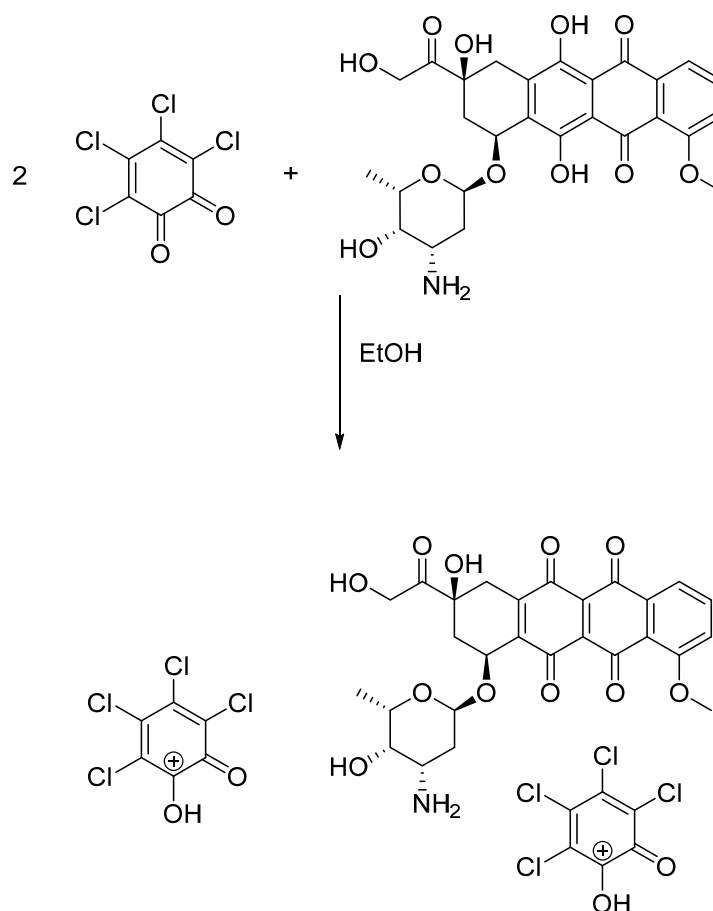
$$K = \frac{1-\alpha^2}{\alpha^3 \cdot C^2}$$

Table 8. Stability constant calculation for the resulting complex

Compound	Conc.(mol.l ⁻¹)	Absorbance		α	Average of $K_{st}(\text{l.mole}^{-1})$
		As	Am		
Doxorubicin hydrochloride	4.5×10^{-5}	0.128	0.620	0.794	6.46×10^7
	9×10^{-5}	0.281	0.812	0.653	
	1.35×10^{-4}	0.405	0.935	0.566	

8. Reaction mechanism. The results of the continuous variations and molar ratio methods revealed a 2:1 interaction ratio between o-chloranil and doxorubicin, confirming the formation of a coordination complex (**Fig. 9**).

The emergence of a new charge-transfer absorption band, distinct from the absorption patterns of the individual components, indicates a chemical interaction and complex formation.

**Fig. 9.** Suggested reaction mechanism

Conclusion

A simple and highly sensitive spectrophotometric method was developed for the determination of doxorubicin in its pure form and in its pharmaceutical formulation (injection). The method is based on the charge transfer complex formation between a π -acceptor (ortho-chloranil) and an n-donor (doxorubicin). The molar absorptivity was found to be $4.227 \times 10^4 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$, with a mean recovery of 101.23%

and a relative standard deviation of less than 3.16%, indicating good accuracy and precision of the method. The nature of the complex was investigated using the continuous variation and molar ratio methods, revealing a 2:1 (drug:reagent) stoichiometry. The stability constant of the complex was calculated to be $6.46 \times 10^7 \text{ L} \cdot \text{mol}^{-1}$. The method was successfully applied to the determination of doxorubicin

hydrochloride in injection form, and the standard addition method showed good agreement with the labeled content of the injection. This supports

the method's suitability for routine analysis of the drug compound.

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