

*Full Paper*

## **Differential Pulse Voltammetric Assay of Antibacterial Drug Doxycycline Hyclate**

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**Abstract-** The electrochemical oxidation of Doxycycline Hyclate has been carried out in Britton - Robinson buffer at carbon paste and glassy carbon electrodes. Doxycycline Hyclate exhibits a well-defined irreversible oxidation peak in a broad pH range (2-11). Differential pulse voltammetry was used to determine Doxycycline Hyclate in pure form. The peak current varied linearly in the following ranges:  $2.0 \times 10^{-7}$ -  $3.0 \times 10^{-6}$  mol L<sup>-1</sup> and  $2.0 \times 10^{-7}$  -  $2.6 \times 10^{-6}$  mol L<sup>-1</sup> in case of carbon paste electrode and glassy carbon electrode, respectively. In case of carbon paste electrode the limits of detection (LOD) and quantification (LOQ) were  $6.56 \times 10^{-8}$  mol L<sup>-1</sup> and  $2.19 \times 10^{-7}$  mol L<sup>-1</sup>, respectively. For glassy carbon electrode the LOD and LOQ were  $9.55 \times 10^{-8}$  mol L<sup>-1</sup> and  $3.18 \times 10^{-7}$  mol L<sup>-1</sup>, respectively. The percentage recoveries were found in the following ranges: 99.58-101.16% and 99.46-100.98% for carbon paste electrode and glassy carbon electrode, respectively. The relative standard deviations were found in the following ranges: 0.53-1.79% and 0.48-1.96% in case of carbon paste electrode and glassy carbon electrode, respectively. Differential pulse voltammetry was successfully applied for the determination of Doxycycline Hyclate in pharmaceutical form.

**Keywords-** Doxycycline hyclate, Oxidation, Differential pulse Voltammetry, Pharmaceutical Form

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### **1. INTRODUCTION**

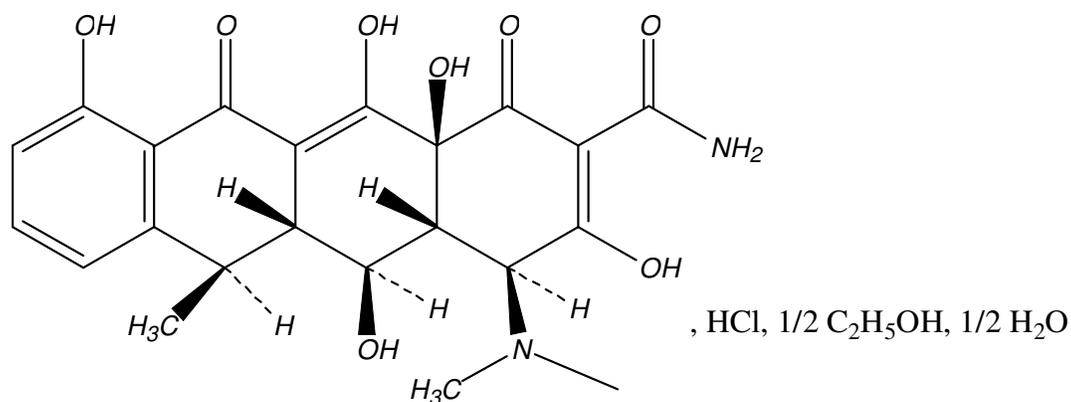
Doxycycline hyclate (DOX) is a tetracycline derivative with uses similar to those of tetracycline. It may sometimes be preferred to other tetracyclines in the treatment of

susceptible infections because its fairly reliable absorption and its long half life that permits less frequent (often once daily) dosing. It also has the advantage that it can be given (with care) to patients with renal impairment. However, relatively high doses may need to be given for urinary tract infections because of its low renal excretion. DOX has antiprotozoal actions and may be given in conjunction with quinine in the management of falciparum malaria resistant to chloroquine [1]. DOX is hydrochloride hemiethanol hemihydrate of (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-octahydrotetracene-2-carboxamide [2]. Several methods have been described for the determination of DOX, such as spectrophotometry [3-6], fluorimetry [7,8], thin-layer chromatography [9-11], liquid chromatography [12-25], flow injection analysis [26-28], capillary electrophoresis [29,30], fluorescence [31,32], and electrochemistry [33,34]. DOX selective membrane electrodes have been also reported [35, 36].

All reported spectroscopic methods suffer from low sensitivity. On the other hand, HPLC methods while having the advantage of requiring minimal sample preparation are relatively slow and expensive because they require filtration, degassing, and expensive reagents and equipments.

Carbon based electrodes are currently in widespread use in electroanalytical chemistry, because of their broad potential window, low cost, rich surface chemistry, low background current and chemical inertness. Carbon paste electrode (CPE) has some special characteristics and benefits such as the ease of surface renewal, individual polarizability, and easy to apply modifications. The disadvantage of CPE is the tendency of the organic binder to dissolve in solutions containing an appreciable fraction of organic solvent. Glassy carbon electrode (GCE) is a class of nongraphitizing carbon that is widely used as an electrode material in electrochemistry. It is also known as vitreous carbon. Glassy carbon electrode is used very commonly because of its excellent mechanical and electrical properties, impermeability to gases, and extremely low porosity [37].

The literature survey revealed that few attempts have been made to study the voltammetric behavior of DOX as an oxidation process and thus in continuation to our previous work [38-43]. The aim of this study is to establish and to optimize the experimental conditions for the determination of DOX in pure and dosage forms by using cyclic voltammetry and differential pulse voltammetry (DPV) techniques.



**Fig. 1.** The molecular structure of DOX

## **2. EXPERIMENTAL**

### **2.1. Apparatus**

Voltammetric measurements were carried out using a computer-driven AEW2 analytical electrochemical workstation with ECP3 electrochemistry software (Sycopel, England) in combination with a C-2 stand with a three-electrode configuration: a glassy carbon disc electrode (BAS model MF-2012) or a carbon paste electrode (BAS model MF-2010) working electrode, a Ag/AgCl/3 M NaCl (BAS model MF-2063) reference electrode, and a platinum wire (BAS model MW-1032) counter electrode. Origin 7.0 software was used for the transformation of the initial signal. A cyberscan 500 (EUTECH Instruments, USA) digital pH-meter with a glass combination electrode served to carry out the pH measurements.

### **2.2. Reagents**

DOX was supplied from Unipharma Company, Egypt. Its pharmaceutical form (Vibramycin capsules) was manufactured by Pfizer Company, Egypt. Stock solution of DOX  $1.0 \times 10^{-3} \text{ mol L}^{-1}$  was prepared by dissolving an appropriate amount of DOX in methanol which was obtained from El-Nasr Pharmaceutical Company, Egypt. The stock solution was stored in a refrigerator. Britton-Robinson (BR) buffer was prepared by mixing the acid mixture containing phosphoric acid ( $0.04 \text{ mol L}^{-1}$ ), acetic acid ( $0.04 \text{ mol L}^{-1}$ ) and boric acid ( $0.04 \text{ mol L}^{-1}$ ). Buffer solutions were adjusted by adding the necessary amount of  $2.0 \text{ mol L}^{-1}$  NaOH solution in order to obtain the appropriate pH. Graphite powder and paraffin oil were supplied from Aldrich and Sigma, respectively.

### **2.3. Preparation of the working electrodes**

The paste was prepared by mixing 0.5 g of graphite powder with 0.3 mL of paraffin oil in a mortar with a pestle. The carbon paste was packed into the hole of the electrode body and smoothed on a filter paper until it had a shiny appearance.

To improve the sensitivity and resolution of the voltammetric peaks, the glassy carbon electrode was polished manually with 0.5  $\mu\text{m}$  alumina powder on a smooth polishing cloth prior to each electrochemical measurement. Then, it was thoroughly rinsed with methanol and double distilled water, and gently dried with a tissue paper.

### **2.4. Assignment of the optimum conditions for the determination of DOX**

To obtain the optimum pH, an appropriate amount of DOX working standard solution  $1.0 \times 10^{-3} \text{ mol L}^{-1}$  was placed in the electrolytic cell containing 5 mL of BR buffer and the cyclic voltammogram was recorded. The experiment was repeated by using buffer solutions of different pH values and the optimum pH was obtained.

To study the effect of scan rate ( $\nu$ ) on the peak current ( $I_p$ ) of DOX, the working electrode was immersed in the optimum buffer solution containing an appropriate amount of DOX standard solution  $1.0 \times 10^{-3} \text{ mol L}^{-1}$  and the cyclic voltammograms were recorded at different scan rates over the scan range 10-250  $\text{mV s}^{-1}$ . Plot  $\log I_p$  versus  $\log \nu$  to know the nature of the process, diffusion controlled process or adsorption controlled process.

The optimum instrumental conditions for the determination of DOX by using DPV method were chosen from the study of the variation of the peak current with pulse amplitude, pulse width and scan rate. During the study, each parameter was changed while the others were kept constant: pulse amplitude over the range of 25-100 mV, pulse width 30-90 ms, and scan rate 10-50  $\text{mV s}^{-1}$ .

### **2.5. Determination of DOX in pure form**

Voltammetric analyses were performed in 5 mL of BR buffer. Aliquots of the drug solution ( $1.0 \times 10^{-3} \text{ mol L}^{-1}$ ) were introduced into the electrolytic cell and the procedures were repeated. The voltammograms were recorded. The peak current was evaluated as the difference between each voltammogram and the background electrolyte voltammogram. All measurements were carried out at the room temperature.

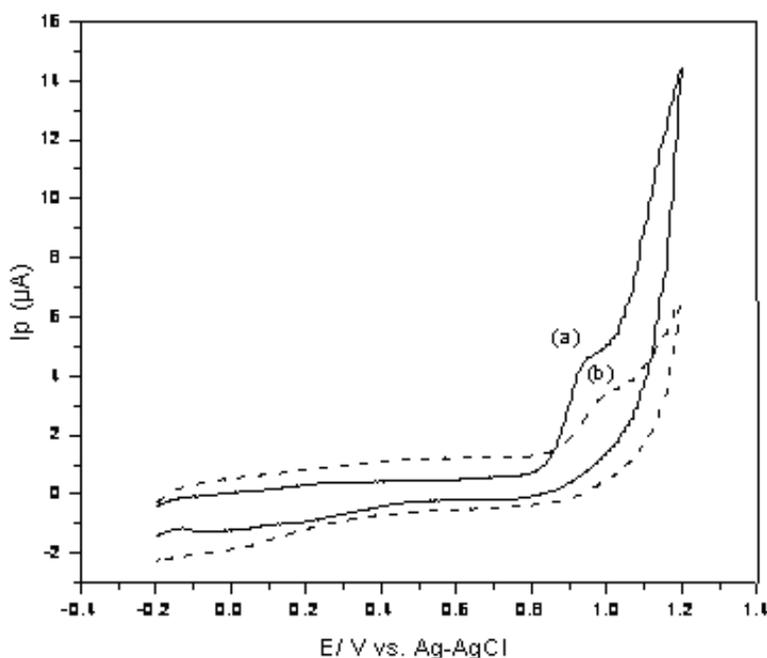
### **2.6. Determination of DOX in Vibramycin capsules**

Five capsules of Vibramycin were weighed and the average mass per capsule was determined. A portion of the powder needed to obtain  $1.0 \times 10^{-3} \text{ mol L}^{-1}$  drug solution was accurately weighed and transferred into a 100 mL volumetric flask which contains 70 mL of methanol. The content of the flask was sonicated for about 20 min. and then made up to the volume with methanol. The solution was filtered to separate the insoluble excipients.

Aliquots of the drug solution were introduced into the electrolytic cell and the general procedure was carried out.

### 3. RESULTS AND DISCUSSION

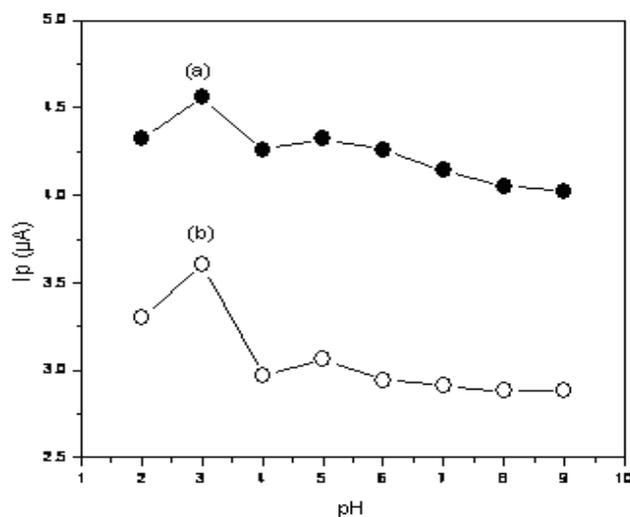
To elucidate the electrode reaction of DOX, the cyclic voltammograms at carbon paste and glassy carbon electrodes were recorded at different pH values and at different scan rates. As an example, Fig. 2 shows the cyclic voltammograms of  $4.0 \times 10^{-5} \text{ mol L}^{-1}$  DOX solution in BR buffer of pH 3.0 in case of CPE and GCE at a scan rate of  $100 \text{ mV s}^{-1}$ . Each voltammogram exhibits one well-defined anodic peak, with no peak on the reverse scan, suggesting the irreversible nature of the electrode reaction.



**Fig. 2.** Cyclic voltammograms of  $4.0 \times 10^{-5} \text{ mol L}^{-1}$  DOX solution in BR buffer of pH 3.0 for CPE (a) and GCE (b). Scan rate  $100 \text{ mV s}^{-1}$

#### 3.1. Effect of pH

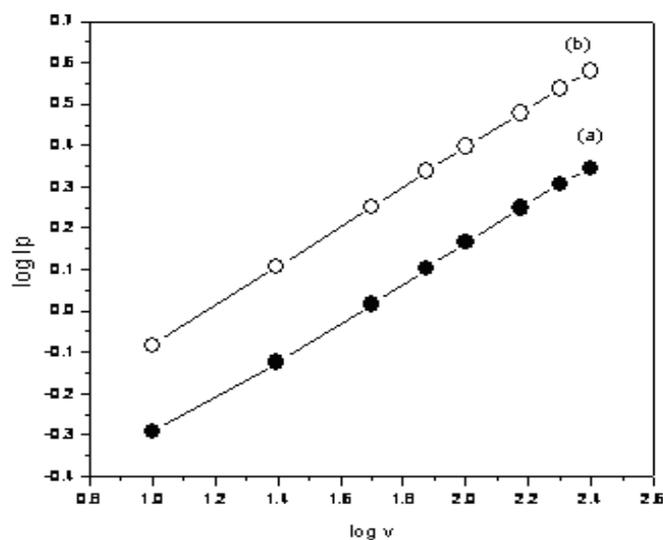
The influence of pH on DOX at carbon paste and glassy carbon electrodes was studied. Fig. 3 shows the plot of peak current ( $I_p$ ) vs. pH. It is obvious from the figure that the peak current reaches its maximum value at pH 3.0 in case of CPE and GCE, i.e; acidic medium is the suitable medium for the determination of DOX by using DPV technique.



**Fig. 3.** Effect of pH on peak current of  $4.0 \times 10^{-5}$  mol L<sup>-1</sup> DOX solution in BR buffer at CPE (a) and GCE (b). Scan Rate 100 mV s<sup>-1</sup>

### 3.2. Effect of scan rate

The effect of scan rate ( $\nu$ ) on the peak current ( $I_p$ ) of DOX was shown in Fig. 4. Linear relationships were observed between  $\log I_p$  and  $\log \nu$  over the scan range 10-250 mV s<sup>-1</sup> and correspond to the following equations:  $\log I_p = -0.77 + 0.46 \log \nu$  and  $\log I_p = -0.56 + 0.48 \log \nu$  in case of CPE and GCE, respectively. The slopes of 0.46 and 0.48 are close to the theoretically expected value of 0.50 for a diffusion controlled process [44].



**Fig. 4.** Anodic peak current response of  $4.0 \times 10^{-5}$  mol L<sup>-1</sup> DOX solution as a function of scan rate ( $\nu$ ) in BR buffer of pH 3.0 for CPE (a) and GCE (b)

### 3.4. Effect of instrumental parameters

It was found that the peak current was increased with the increasing pulse amplitude and scan rate, while it decreased with the increasing pulse width. To obtain relatively high and narrow peaks the values of 50 mV, 30 ms and 20 mV s<sup>-1</sup> were finally chosen for pulse amplitude, pulse width and scan rate, respectively.

### 3.5. Determination of DOX in pure form

On the basis of the electrochemical oxidation of DOX at CPE and GCE, DPV method was developed for the determination of the drug under investigation. Linear relations between the peak current (Ip) and DOX concentration (C) were found in the following ranges: 2.0×10<sup>-7</sup>-3.0×10<sup>-6</sup> mol L<sup>-1</sup> and 2.0×10<sup>-7</sup>-2.6×10<sup>-6</sup> mol L<sup>-1</sup> in case of CPE and GCE, respectively. The calibration plots were described by the following equations:

$$I_p (\mu A) = 0.460C(\mu M) + 0.735r^2 \text{ (Correlation coefficient)} = 0.9995 \text{ for CPE} \quad (1)$$

$$I_p (\mu A) = 0.382C(\mu M) + 0.780 r^2 \text{ (Correlation coefficient)} = 0.9999 \text{ for GCE} \quad (2)$$

Three replicate calibration curves were obtained over the concentration ranges 2.0×10<sup>-7</sup>-3.0×10<sup>-6</sup> mol L<sup>-1</sup> in case of CPE and 2.0×10<sup>-7</sup>-2.6×10<sup>-6</sup> mol L<sup>-1</sup> in case of GCE. The LOD and LOQ were calculated by using the following equations: LOD=3 S.D./m and LOQ=10 S.D./m, where "S.D." is the standard deviation of the intercept of the calibration curve and "m" is the slope of the calibration curve [45]. The LOD and LOQ were 6.56×10<sup>-8</sup> mol L<sup>-1</sup> and 2.19×10<sup>-7</sup> mol L<sup>-1</sup>, respectively in case of CPE. For GCE, the LOD and LOQ were 9.55×10<sup>-8</sup> mol L<sup>-1</sup> and 3.18×10<sup>-7</sup> mol L<sup>-1</sup>, respectively.

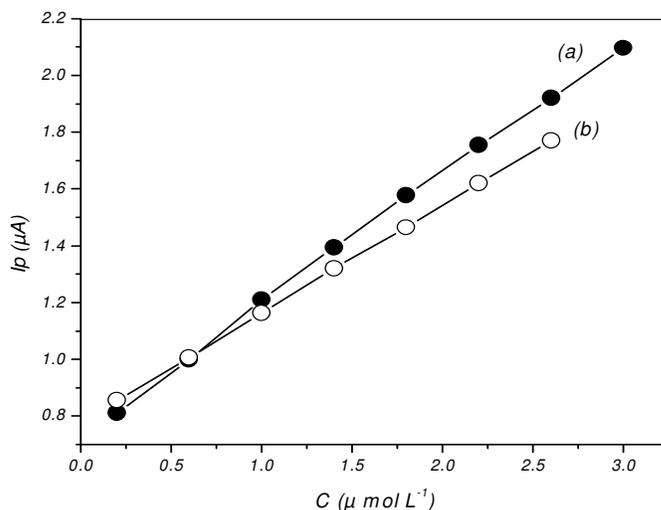
Accuracy and precision of the proposed method were determined by replicate analyses of five different concentrations of DOX; the results were given as shown in Table 1. The recovery was in the range of 99.58-101.16% and the relative standard deviation (RSD) was in the range of 0.53-1.79% in case of CPE.

For GCE, the recovery was in the range of 99.46-100.98% and the relative standard deviation was in the range of 0.48-1.96%. The values of the recovery and the relative standard deviations indicate to adequate accuracy and precision of the proposed method.

The proposed method is more sensitive than that of fluorimetric method: 3.9×10<sup>-7</sup>-2.73×10<sup>-6</sup> mol L<sup>-1</sup> [8], thin-layer chromatographic method: 19.5×10<sup>-5</sup>-19.5×10<sup>-4</sup> mol L<sup>-1</sup> [11], chromatographic methods: 5.85×10<sup>-6</sup>-11.70×10<sup>-5</sup> mol L<sup>-1</sup> [18] and 9.75×10<sup>-7</sup>-17.8×10<sup>-5</sup> mol L<sup>-1</sup> [25], capillary electrophoresis method: 1.95×10<sup>-5</sup>-19.5×10<sup>-5</sup> mol L<sup>-1</sup> [30], fluorescence method: 4.05×10<sup>-7</sup>-2.03×10<sup>-4</sup> mol L<sup>-1</sup> [32] and ion selective membrane electrode method: 7.9×10<sup>-5</sup>-1.9×10<sup>-3</sup> mol L<sup>-1</sup> [35].

Also our method is more sensitive than chromatographic, fluorescence, electrochemical and ion selective membrane electrode methods which have higher detection limits: 1.81×10<sup>-7</sup>

mol L<sup>-1</sup> [24], 2.0×10<sup>-7</sup> mol L<sup>-1</sup> [31], 4.4×10<sup>-7</sup> mol L<sup>-1</sup> [34] and 4.0×10<sup>-6</sup> mol L<sup>-1</sup> [36], respectively.



**Fig. 5.** Calibration curve of DOX at CPE (a) and GCE (b) by using DPV method, pulse amplitude 50 mV and scan rate 20 mV s<sup>-1</sup>

**Table 1.** Analytical parameters of the calibration plots for the determination of DOX

Parameter	CPE	GCE
Linearity range (mol L <sup>-1</sup> )	2.0×10 <sup>-7</sup> -3.0×10 <sup>-6</sup>	2.0×10 <sup>-7</sup> -2.6×10 <sup>-6</sup>
Slope	0.460	0.382
Intercept	0.735	0.780
Correlation coefficient (r <sup>2</sup> )	0.9995	0.9999
LOD (mol L <sup>-1</sup> )	6.56×10 <sup>-8</sup>	9.55×10 <sup>-8</sup>
LOQ (mol L <sup>-1</sup> )	2.19×10 <sup>-7</sup>	3.18×10 <sup>-7</sup>
RSD* (%)	0.53-1.79	0.48-1.96
Recovery (%)	99.58-101.16	99.46-100.98

\* Five different concentration of DOX; number of replicates (n) = 5.

### 3.6. Determination of DOX in capsules

The proposed method was successfully applied to determine DOX in dosage form (Vibramycin capsules) without interference from some common excipients used in

pharmaceutical preparations such as starch, magnesium stearate and microcrystalline cellulose. Replicate analyses of standard solutions have been carried out to obtain the accuracy and precision of the proposed method; the results were given as shown in Table 2. The linearity range was  $2.0 \times 10^{-7}$ -  $3.0 \times 10^{-6}$  mol L<sup>-1</sup> with mean recovery of 99.86% and mean relative standard deviation of 1.68% in case of carbon paste electrode. In case of glassy carbon electrode the linearity range was  $2.0 \times 10^{-7}$ -  $2.6 \times 10^{-6}$  mol L<sup>-1</sup> with mean recovery of 100.13% and mean relative standard deviation of 1.12%.

The proposed method is more sensitive than that of spectrophotometric methods:  $1.56 \times 10^{-5}$ -  $7.02 \times 10^{-5}$  mol L<sup>-1</sup> [5] and  $5.8 \times 10^{-6}$ -  $34.66 \times 10^{-6}$  mol L<sup>-1</sup> [6], and flow injection analysis method:  $9.75 \times 10^{-7}$ -  $13.65 \times 10^{-6}$  mol L<sup>-1</sup> [27]. Also our method is more sensitive than chromatographic and flow injection methods which have higher detection limits:  $3.9 \times 10^{-6}$  mol L<sup>-1</sup> [21] and  $1.0 \times 10^{-6}$  mol L<sup>-1</sup> [28], respectively.

DOX was determined by using the reported fluorimetric method with a linear range from  $1.7 \times 10^{-7}$  to  $1.7 \times 10^{-6}$  mol L<sup>-1</sup> [7]. By comparing the results obtained by using the proposed method we found that our method shows a wider range than this method.

**Table 2.** Determination of DOX in Vibramycin capsules

Parameter	Linearity range (mol L <sup>-1</sup> )	RSD (%)	
		Recovery (%)	
CPE	$2.0 \times 10^{-7}$ - $3.0 \times 10^{-6}$	0.71-2.12	99.27-100.95%
GCE	$2.0 \times 10^{-7}$ - $2.6 \times 10^{-6}$	0.53-2.07	99.20-100.80%

#### 4. CONCLUSION

The proposed DPV method could be used successfully to determine DOX in pure and pharmaceutical forms. It compares reasonably with the reported methods. It is a good alternative for the analytical determination of DOX due to its sensitivity, accuracy and simplicity. The proposed procedure showed clear advantages such as high percentage of recovery, wide application range and low relative standard deviation.

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