

*Full Paper*

## **A Green Potentiometric Application for Selective Monitoring of Doxylamine Succinate Dissolution Profile in Combined Dosage Form**

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*Received: 22 June 2020 / Received in revised form: 20 July 2020 /*

*Accepted: 21 July 2020 / Published online: 31 July 2020*

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**Abstract-** "Green analytical chemistry" (GAC) succeeded to become an eco-friendly environmental crucial area in the field of analytical chemistry targeting at the chemical processes' and products' optimization regarding to material consumption, generation of waste and intrinsic safety, toxicity and environmental burdens. For an expressive comparison, an electro-analytical in-line potentiometric selective determination of Doxylamine succinate (DOX) in a multi- component pharmaceutical dosage form containing both Caffeine (CAF) and Paracetamol (PAR) has been successfully developed and validated. A real-time monitoring of the dissolution profile of DOX from its pharmaceutical formulation was achieved by the proposed sensor without any interference from paracetamol or caffeine even without pretreating neither the sample nor its derivatization. A cationic exchanger; Potassium tetrakis (4-chlorophenyl) borate (KTCPB), polyvinyl chloride (PVC) based membrane and a plasticizer; 2-nitrophenyl-octyl-ether (2-NPOE) were employed for the fabrication DOX-selective sensor. The proposed sensor showed Nernstian response slope of 29.8 mV/concentration decades from  $10^{-6}$  to  $10^{-2}$  mol L<sup>-1</sup>. ICH guidelines' validation parameters; linearity, accuracy, precision and robustness were performed on the proposed green eco-friendly potentiometric method.

**Keywords-** Green analytical Chemistry; In-line potentiometry; Doxylamine succinate; Caffeine; Paracetamol clofenac

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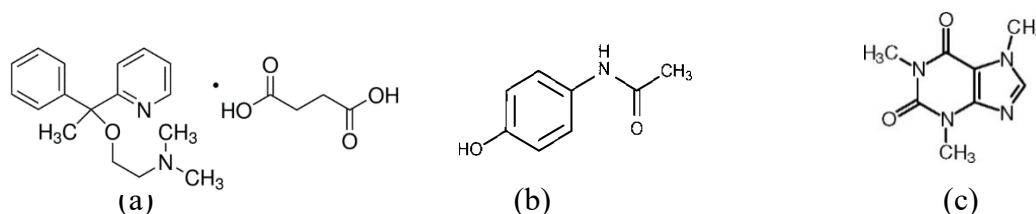
## 1. INTRODUCTION

Emission of chemical pollutants has become a major threat that is not only intimidating human health but the whole environment as well. Out of this problem, chemists' skills, knowledge and talents were intertwined to bring green chemistry to light in order to avoid these threats with a low, if not zero, pollutants' emission [1]. Recently, the term "green analytical chemistry (GAC) was spawned from green chemistry principles and gained a mounting acceptance and wide responsiveness in both industry and academia [2].

**Doxylamine succinate (DOX)**, Fig. 1(a), is N, N-dimethyl-2-[ $\alpha$ -methyl- $\alpha$ -(2-pyridyl)benzyloxy] ethylamine hydrogen succinate with a noticeable tranquillizing and antimuscarinic effect [3]. It is U.S.P official drug [4], used solely as a short term tranquilizer or grouped with other drugs preventing nocturnal allergy and providing cold relief. DOX can be also given with vitamin B6 avoiding pregnant women's morning sickness or with analgesics as codeine and paracetamol in analgesic/calmativ e preparations [5,6].

**Paracetamol (PAR)**, Fig. 1(b), is N-(4- hydroxyphenyl) acetamide possessing analgesic and antipyretic activity for fever, headaches, pain and other minor aches' relief and also can be used for treatment of cold if combined with other drugs [7].

**Caffeine (CAF)**, Fig. 1(c), as a naturally occurring alkaloid is (1, 3, 7-trimethylxanthine), found in kola nuts, tea leaves, cocoa, coffee beans and other plants. Bronchial muscle relaxation, central nervous system stimulation, gastric acid secretion and diuresis are the most common physiological effects caused by caffeine [8].



**Fig. 1.** Structural formula for (a) Doxylamine succinate, (b) Paracetamol and (c) Caffeine

Numerous analytical methods have been conveyed for DOX, PAR or CAF determination either as sole components or in their combination with other drugs viz titrimetric [9,10], spectrophotometric [3, 11-17], HPTLC [18-21] and HPLC [13, 21-26] methods.

These above stated analytical techniques compromise a high degree of specificity; however, they faced some challenges as tedious sample preparation, instrumentation limitations, prolonged time of analysis and the consumption of harmful and costly organic solvents, thus, opposing their use in routine analysis.

On the contrary, an interesting and appealing environmental friendly potentiometric analytical method becomes a proficient alternative in biomedical analysis of pharmaceutical dosage forms [27-29].

Studying the usefulness of potentiometry in the in-line dissolution monitoring and assessing the opportunities offered by ion-selective electrodes (ISEs) for the continuous tracking of the pharmaceuticals' dissolution profiles were the chief goals of this work. The innovation of the current potentiometric technique arises from the great selectivity of the developed ISE to solely respond, determine and monitor the dissolution curve of DOX in a tertiary formulated dosage form containing paracetamol and caffeine without revealing any interference from the other co-formulated drugs taking into consideration that the cited drug's potentiometric determination hasn't been reported in any previous literature.

## 2. EXPERIMENTAL

### 2.1. Apparatus

Double-junction Ag/AgCl reference electrode (Aldrich Chemical Co., Germany) and pH glass electrode (Jenway, UK) for pH adjustment. Digital ion analyzer (Jenway, UK). Magnetic stirrer, BandelinSonorox, Rx510S (Budapest, Hungary) were employed for potentiometric measurements.

### 2.2. Materials

#### 2.2.1. Pure samples

Doxylamine Succinate (DOX) pure sample was supplied by the National Organization for Drug Control and Research (NODCAR), Giza, Egypt, where, its purity was checked and found to be  $99.15 \pm 1.57$  according to the USP [4].

Paracetamol (PAR) and Caffeine (CAF) were supplied by Al-Amriya Pharmaceutical Industries, Al-Amriya, Alexandria, where, their purity was checked and found to be  $98.76 \pm 1.02$  and  $99.33 \pm 1.40$ , respectively, according to the reported method [30].

#### 2.2.2. Pharmaceutical dosage form

Cafamol<sup>®</sup> tablets dosage form, batch number (150760) was manufactured by Al-Amriya Pharmaceuticals and Chemical Industries, Alexandria, Egypt and containing 3 mg of DOX, 450 mg of PAR and 30 mg of CAF.

### 2.3. Chemicals and reagents

Chemicals of analytical grade, solvents and bi-distilled water were used.

- Potassium tetrakis (4-chlorophenyl) borate (KTCPB), 2-nitrophenyl-octyl-ether (2-NPOE), Polyvinyl chloride (PVC) and tetrahydrofuran (THF); Aldrich, Germany.
- Sodium hydroxide (96%), concentrated hydrochloric acid, sodium chloride (97%), Potassium chloride (98%), and sodium dihydrogenphosphate (98%); El Nasr pharmaceutical

Co., Cairo, Egypt.

- 0.02 mol L<sup>-1</sup> sodium dihydrogenphosphate was used for preparing phosphate buffer (pH 6.8) using NaOH for pH adjustment, 0.1M of both hydrochloric acid and sodium hydroxide were also prepared.

## 2.4. Standard solutions

### 2.4.1. DOX stock standard solution ( $1 \times 10^{-2}$ mol L<sup>-1</sup>)

0.097 g of DOX was transferred into a 25-mL volumetric flask, dissolved in a sufficient amount of 0.1 N HCl then bringing the volume up to the mark using the same solvent.

### 2.4.2. DOX working standard solution ( $1 \times 10^{-6}$ - $1 \times 10^{-2}$ mol L<sup>-1</sup>)

From the stock solution, serial dilutions were taken to prepare solutions of various strengths ( $1 \times 10^{-6}$ -  $1 \times 10^{-2}$  mol L<sup>-1</sup>) using 0.1 N HCl.

## 2.5. Procedures

### 2.5.1. Fabrication of PVC master membrane sensor

PVC membrane was fabricated in a petri dish (5-cm diameter) via mixing PVC (190.0 mg), KTCPB (10.0 mg) and 2-NPOE (400.0 mg), totaling 600.0 mg to construct DOX sensing membrane, then the mixture was dissolved by stirring with 6.0 mL of THF. Whatman filter paper No. 3 was used for covering the petri dishes and left overnight for evaporating the solvent obtaining master membrane with 0.1 mm thickness.

### 2.5.2. Electrochemical assembly

From the prepared PVC membrane and via a cork borer, a disk (approximately 8-mm diameter) was cut, firmed to a PVC tip by THF and clipped into the electrode glass part's end. An internal reference solution containing equal volumes of  $1 \times 10^{-3}$  mol L<sup>-1</sup> DOX and  $1 \times 10^{-3}$  mol L<sup>-1</sup> potassium chloride was prepared. An internal reference electrode; Ag/AgCl wire (1-mm diameter) was dipped in the internal reference solution. The sensor was conditioned via soaking it for 24 hours in a  $10^{-3}$  molL<sup>-1</sup> DOX stock standard solution and preserved in the same solution.

### 2.5.3. Sensor calibration

Calibration of DOX sensor was achieved by its conjugation with a double-junction Ag/AgCl reference electrode then immersing in DOX's respective drug solutions ( $1 \times 10^{-6}$ - $1 \times 10^{-2}$  mol L<sup>-1</sup>) to obtain a constant potentiometer reading by continuous stirring till equilibration occurs followed by recording the electromotive forces (e.m.f). Calibration graph

was constructed between the resulted electrode potentials and the log molar corresponding concentrations of the cited drug. Before and after each run, washing DOX sensor was done using 0.1 N HCl until reaching a constant potential.

#### 2.5.4. Effect of pH on electrode response

Variations occurring in the studied response of the electrode potential were inspected by adding 0.1 N NaOH and 0.1 N HCl to  $10^{-3}$  mol L<sup>-1</sup> solution of DOX to produce a gradual increase and decrease in the pH ranging from 2.0 to 10.0.

#### 2.5.5. Effect of interfering substances on the electrode selectivity

The degree of interference of the foreign substances with the response of the electrode to its primary ion (DOX) was estimated by calculating the potentiometric selectivity coefficient [ $\log (K^{\text{Pot}}_{\text{Primary ion, interferent}})$ ] by the separate solutions method (SSM) [31] through applying the following equation:

$$-\log K^{\text{Pot}}_{\text{Primary ion, interferent}} = (E_1 - E_2) / S$$

where;  $E_1$  is the primary ion solution's potential,  $E_2$  is the interferent solution's potential and  $S$  is the investigated sensor's slope.

#### 2.5.6. Determination of laboratory prepared mixtures containing diverse DOX, PAR and CAF ratios

Into a series of 25-mL volumetric flasks and by the usage of 0.1 N HCl as a solvent, precise volumes of DOX ( $1 \times 10^{-2}$  mol L<sup>-1</sup>), PAR ( $1 \times 10^{-2}$  mol L<sup>-1</sup>) and CAF ( $1 \times 10^{-2}$  mol L<sup>-1</sup>) were transferred accurately for DOX determination.

#### 2.5.7. Determination of DOX in Cafamol<sup>®</sup> tablet

Ten Cafamol<sup>®</sup> tablets were accurately weighed then an accurate weighed portion of the tablet powder was transferred to 25-mL volumetric flask and diluted with 0.1 N HCl to the mark producing DOX solution with a concentration claimed to be  $1 \times 10^{-4}$  mol L<sup>-1</sup>. The prepared electrode with the reference electrode was concurrently immersed in the equipped solution and then recording the resulting potential. The concentration of DOX was calculated from the equivalent regression equation.

#### 2.5.8. Dissolution curve by potentiometric method

The studied tablet was inserted in a dissolution apparatus containing 900 mL of 0.1 N HCl controlled at  $37 \pm 0.5$  °C, where the prepared sensor together with the reference electrode were introduced, then stirring the content of the vessel at a rate of 75 rpm [32].

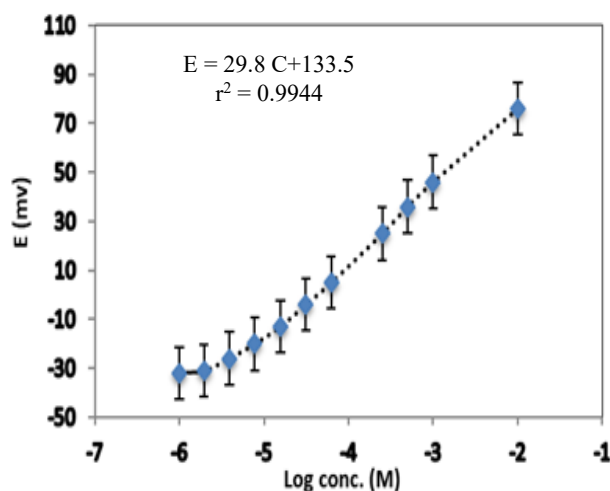
For DOX, the potentiometric signals produced from the potentiometer's reading were recorded then converted to dissolution percentages using the obtained concentration from the equivalent calibration curve.

### 3. RESULTS AND DISCUSSION

#### 3.1. ISE characteristics

Doxylamine sensor's equipment arises from the fact that DOX is a cation which necessitates fabrication of ion selective electrode with cation exchange ability and this was accomplished by soaking potassium tetrakis (4-chlorophenyl) borate (KTCPB) as a cation exchanger in  $1 \times 10^{-3}$  mol L<sup>-1</sup> DOX solution to exchange K<sup>+</sup> as the main replaceable counter ion with DOX showing a good linear response in 0.1 N HCl. Moreover; a plasticizer (NPOE) possessing a good solvent mediator's ability was used thus allowing DOX ions owing a lipophilic nature to be transferred to the membrane from the aqueous solution, thus maximum sensitivity and selectivity are being successfully offered.

The results obtained from DOX proposed sensor's electrochemical response are displayed in Table 1. Calibration plot is shown in Fig. 2 showing a slope of 29.8 mV/concentration decades. Moreover, 20 seconds was the desired time for DOX sensor to attain stable values within  $\pm 1$  mV from day to day measurements.



**Fig. 2.** Profile of the potential (mV) against DOX log concentration (mol L<sup>-1</sup>) attained from the proposed sensor in 0.1 N HCl

#### 3.2. Effect of pH and temperature

The proposed sensor's response was inspected over neutral pH range (6.5–7.0) by using phosphate buffer and acidic pH range (2.5–3.5) by using 0.1 N HCl.

Studying the pH effect at different values arises from the fact that DOX owes two electrically charged groups within its structure. The first is the tertiary amino group with pKa

value of 8.87 while the second one is the amino group located in the pyridine ring with pKa value of 3.23.

**Table 1.** Electrochemical response characteristics for DOX sensor

Parameter	DOX Sensor
Slope (mV/decade) <sup>a</sup>	29.8
Intercept (mV)	133.5
Correlation coefficient	0.9944
Response time (s)	20
Working pH range	2-10
Concentration range(mol/L)	$1 \times 10^{-6}$ - $1 \times 10^{-2}$
Average recovery(%) $\pm$ SD <sup>a</sup>	$98.36 \pm 0.63$
Precision (RSD %)	
• Repeatability <sup>b</sup>	0.602
• Intermediate precision <sup>c</sup>	1.210
Robutness <sup>d</sup>	0.925
LOD (mol/L) <sup>e</sup>	$1.25 \times 10^{-5}$

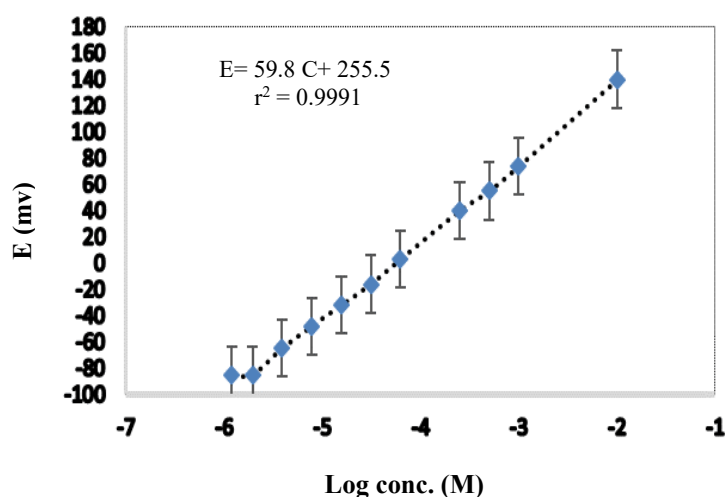
<sup>a</sup> Average of three determinations

<sup>b</sup>The intraday (3 $\times$ 3), average of three concentrations being analysed three times within the day

<sup>c</sup>The interday (3 $\times$ 3), average of three concentrations being analysed three times in three different days

<sup>d</sup>Robustness, RSD% for the previously determined concentration under deliberate variation in dissolution parameters (pH, temperature, volume and agitation rate)

<sup>e</sup>Limit of detection, showing the interception of the extrapolated arms of Fig. 2



**Fig. 3.** Profile of the potential (mV) against DOX log concentration (mol/L) attained from the proposed sensor in phosphate buffer pH=6.8

For that reason, a substantial difference was noticed in the Nernstian slopes in acidic

rather than neutral conditions. In acidic conditions, DOX molecule is doubly charged (divalent ion), where the tertiary amino group as well as the amino group in the pyridine ring are completely ionized and sensed, hence, a Nernstian slope of almost 30 mV was obtained. While at neutral conditions (pH 6.8), the DOX molecule is almost singly charged (due to the presence of the ionizable tertiary amino group), and therefore it was almost sensed as monovalent ion resulting in 60 mV/decade Nernstian slope as displayed in Fig. 3.

### 3.3. Sensor selectivity

The interfering substances' effect upon the performance of the sensor was studied.

From the results of the calculated selectivity coefficients which determine the proposed sensor's response in the existence of susceptible excipients, organic and inorganic related substances, it was observed that DOX sensor shows high selectivity with no significant interference from the susceptible interfering species Table 2.

**Table 2.** Potentiometric selectivity coefficients ( $-K_{pot}^{ion}$ ) of the proposed sensor

Interferent ( $10^{-3} \text{ mol L}^{-1}$ ) <sup>a</sup>	DOX Sensor
<b>NaCl</b>	0.40
<b>KCl</b>	0.40
<b>Glycine</b>	0.33
<b>Moxifloxacin</b>	0.77
<b>Paracetamol</b>	0.90
<b>Caffeine</b>	0.0

<sup>a</sup> Average of three determinations

Clearly, the accreditation of the high sensor selectivity towards DOX compared to other inorganic cations ( $\text{Na}^+$  and  $\text{K}^+$ ) can be attributed to the hydrophilic nature of  $\text{Na}^+$  and  $\text{K}^+$  which face a difficulty in their ion exchange into the lipophilic membrane permitting only the exchange of the lipophilic DOX. Additionally, it is important to point out that the co-formulated drugs; Paracetamol and Caffeine have practical water insoluble nature with no electroactive groups in their chemical structure thus showing a marked non-Nernstian response. Moreover, this remarkably high discrimination facilitates the development of a DOX-ISE due to its higher lipophilic nature (Log P= 2.5) compared to the lipophilic nature of CAF (Log P= -0.1) thus allowing the selective and sole determination of DOX in its combined dosage form; Cafamol<sup>®</sup> tablets.



### 3.4. Determination of laboratory prepared mixtures containing diverse DOX, PAR and CAF ratios

Laboratory-prepared mixtures' analysis covering various ratios of DOX, PAR and CAF showed the unique selectivity of the proposed sensor towards DOX's determination in the presence of the other two drugs without any remarkable interference and with no need for prior separation, Table 3.

**Table 3.** Potentiometric determination of laboratory prepared mixtures comprising various ratios of DOX, PAR and CAF

Ratio DOX: CAF: PAR	DOX Conc. (mol L <sup>-1</sup> )	Recovery (%)
1:10:150*	$3 \times 10^{-4}$	98
1:2:1	$1.2 \times 10^{-4}$	102
1:1:2	$4.8 \times 10000^{-5}$	101.99
1:3:2	$1.92 \times 10^{-5}$	101.91
	<b>Mean</b>	<b>100.97</b>
	<b>SD</b>	<b>1.98</b>
	<b>RSD%</b>	<b>1.960</b>

\* Ratio found in pharmaceutical dosage form

### 3.5. Potentiometric determination of DOX in Cafamol<sup>®</sup> tablets

Using the proposed sensor and through the obtained percentage recoveries, a successful determination of DOX in Cafamol<sup>®</sup> tablet was accomplished. A statistical comparison was performed between the obtained results and the USP monograph displaying insignificant difference between both methods as shown in Table 4.

**Table 4.** Determination of DOX in Cafamol<sup>®</sup> tablet by the suggested potentiometric method

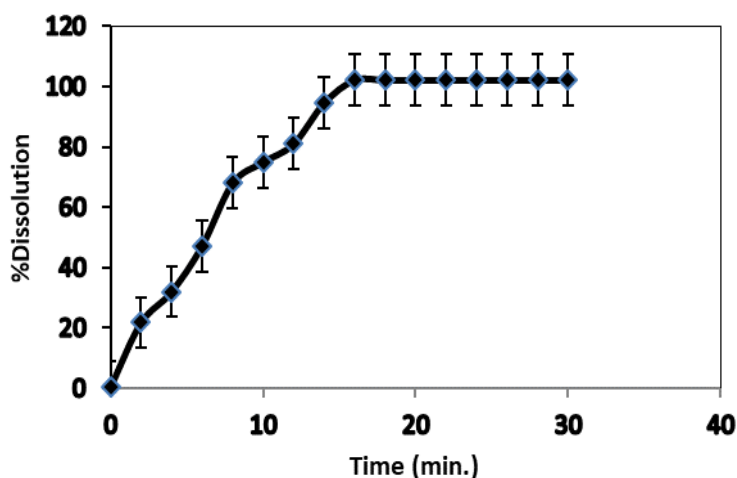
Proposed sensor	Found <sup>a</sup> % ± SD
<b>KTCPB</b>	<b>97.66 ± 0.97</b>

<sup>a</sup> Average of three determinations

### 3.6. Dissolution curves

One Cafamol<sup>®</sup> tablet was immersed in the dissolution medium, where the proposed DOX sensor together with the reference electrode being dipped during the whole experiment were employed for measuring the potential without taking a sample each time interval from the dissolution media and measuring it by any other complicated instrumental means. Moreover, ISE's being unaffected by sample turbidity produced from the excipients during the experiment eliminated the need of sample centrifugation. From the e.m.f. measured and through the plotted calibration curve, calculation of the dissolved amount was performed and

then the dissolution profile was constructed as shown in Fig. 4. For DOX, more than 90% of active ingredient exhibited a quick release and entered plateau within 16 min and remained constant till 30 min.



**Fig. 4.** Dissolution profile for Cafamol<sup>®</sup> tablet comprising 3 mg DOX by in-line potentiometric method using the proposed sensor

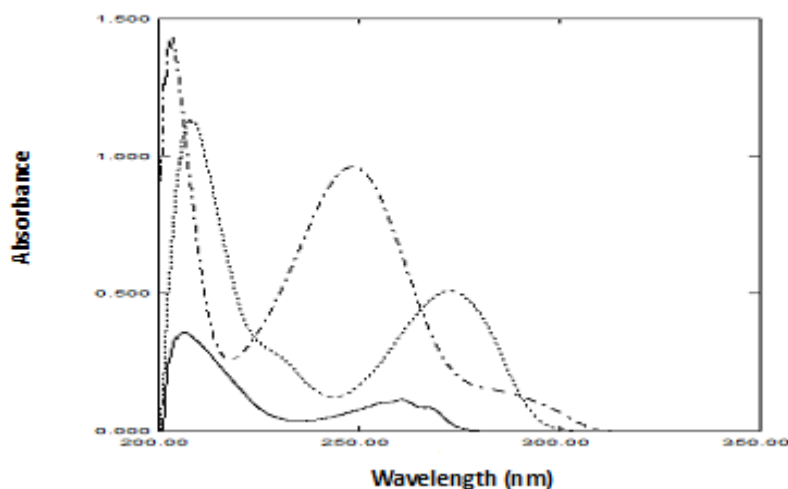
**Table 5.** Statistical comparison between the results obtained by the proposed potentiometric method and an official method for DOX in its pure powdered form

Item	Potentiometric method	Official method [4]
Mean	99.42	99.15
S. D	1.65	1.57
RSD%	1.659	1.583
Variance	2.72	2.46
n	5	5
<i>t</i> - test <sup>a</sup> (2.447)	1.699	
F value <sup>a</sup> (5.91)	1.10	

<sup>a</sup> The values in parenthesis are the corresponding theoretical values at P = 0.05

### 3.7. Validation of the proposed method

Linearity range, accuracy, precision and robustness were the validation parameters resulted from the proposed potentiometric method as presented in Table 1. The specificity of the proposed method was ensured from the results obtained from the analysis of laboratory prepared mixtures containing various ratios of the drugs, Table 3.



**Fig. 5.** Zero-order spectra of 10  $\mu\text{g/mL}$  of DOX (—), CAF (....) and PAR (- - -) separately in methanol

The studied method was also employed for the determination of the DOX in Cafamol<sup>®</sup> tablets obtaining satisfactory results and a statistical comparison between the proposed method's results and the USP monograph's analysis was performed showing lower calculated  $t$  and  $F$  values than the theoretical ones which proves that there was no considerable difference between the proposed and USP monograph method regarding both accuracy and precision as shown in Table 5.

Studying other analytical methods, dissolution monitoring via in-line potentiometric method may be considered a motivating analytical task where UV spectrophotometry shows a great monitoring difficulty for the dissolution processes due to the very small amount comprised by DOX in the dosage form and the complete overlap (superimposition) of the absorption spectra of DOX, PAR and CAF involving numerous manipulation steps, as shown in Fig. 5.

#### 4. CONCLUSION

The response of the fabricated sensor is sufficiently precise, accurate and proves the great selectivity of the sensor for DOX sole and selective quantitative determination in its pure form and in pharmaceutical dosage form even in the presence of other co-formulated drugs. Moreover, the need for pretreating the drug or any employed separation steps were completely eliminated upon using the proposed sensor, thus, can therefore be hired for DOX routine analysis in quality control laboratories. In general, the ISEs proposed in this study offered a unique design's simplicity and a very low limit of detection as well as being rapid, simple, inexpensive and environmentally friendly thus could compete with the many sophisticated methods currently available.

## **Funding**

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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