

## TRIMETHOPRIM DETERMINATION WITH DRUG-SELECTIVE ELECTRODES

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## ABSTRACT

Developed ion-selective electrode has been industrialized for the determination of trimethoprim (TMP) in pure form and pharmaceutical preparations. The selective electrode was made from TMP with pairing agent methyl orange presence of o-nitrophenyl octyl ether (o-NPOE) and dibutyl phthalate as the plasticizing solvent mediator. Electrode 1 showed a Nernstian reply with a mean calibration graphs slope of 57.31 mv.decade<sup>-1</sup>, over the linear concentration range of 6.0×10<sup>-6</sup>–1.0×10<sup>-2</sup> M of the drug, with detection limits 3.0×10<sup>-6</sup> M. The electrode was effective at pH range between 2.0 and 5.5 for concentration of 10<sup>-2</sup> M, 1.5–4.5 for concentration of 10<sup>-2</sup> M, and 3.0–4.5 for concentration of 10<sup>-4</sup> M of TMP solutions. The electrode 2 gave a non-Nernstian slope equal to 41.20 mv.decade<sup>-1</sup>. The influence of interfering species such as inorganic cations was studied. Electrode 1 showed an effective response for a period of 27 days, without important variation in parameters of electrode. The suggested electrode was intended for the determination of TMP in pharmaceutical design and pure formula.

**Keywords:** Ion-selective electrodes, Trimethoprim, Nernstian slope, Potentiometric determination.

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## INTRODUCTION

Trimethoprim (TMP) is chemically 5-(3, 4, 5-trimethoxybenzyl) pyrimidine-2, 4-diamine which is a bacteriostatic agent against most common bacterial pathogens. TMP is active as single therapy in treating urinary and respiratory tract taints due to vulnerable organisms and for prophylaxis of urinary tract taints [1].

Literatures have shown some analytical methods for the determination of TMP such as spectrophotometric method by the reaction between TMP and bromocresol green and 2-4-dinitro-1-fluorobenzene [2]. The method depended on ultraviolet spectrophotometry to the determination of TMP [3].

Square wave voltammetry was founded to the validation of TMP [4] and determination of TMP by the first-order derivative spectrophotometer [5]. Characterization and formation of TMP cocrystal by milling process [6]. The interaction between dihydrofolate and TMP was obtained for determined TMP [7]. A new analytical method to monitor antibiotics such as TMP in wastewater is essential to evaluate new treatment technologies [8] and determined TMP resistance rates in the treatment of uncomplicated urinary tract infections [9]. TMP was synthesized of four membrane rings heterocyclic compounds [10]. Three TMP-selective electrodes were prepared to the determination of TMP [11]. Ion-selective electrode is one of the most effective, accurate, and economic analytical methods for the determination of organic and inorganic substances [12]. The target of this effort was to improve a subtle, selective, and certified ion-selective electrodes (ISEs) for the determination of TMP in pharmaceutical preparations and pure.

## SUBSTANCES AND PROCEDURES

## Apparatus

Electrochemical measurements were completed with microprocessor, pH/mV/C meter, pH211, and HANA with an Ag/AgCl electrode as an external reference electrode (lab. assembly), which contain 1 M KCl in the outer part. The pH measurements were made with pH electrode, H11131, HANA, and Made in Romania. Each potentiometric work was completed at 25±1°C with constant stirring by the use of hot plate magnetic stirrer type LMS-1003 (Daihan Labtech). Most of weights were taken up by analytical balance Sartorius Handy 4 digits.

## Reagent and materials

Analytical grade TMP (Samara IRAQ-SDI) the State Company of Medical Appliances and Drug Industries, Trimoks tablet (1 mg) made in Turkey. High-molecular-weight polyvinyl chloride (PVC) U.K. Ltd, type: Breon S110/10, dibutyl phthalate (DBP) 99%, o-nitrophenyl octyl ether (o-NPOE) 98%, HCl 98.9 NaOH 99.8%, KCl, tetrahydrofuran 97%, methyl orange (MO) 99.8 were from Fluka, Aldrich, and BDH in all chemical preparations distilled water were used.

## Preparation of solutions

About 0.01 M of MO was equipped by weighting with dissolving and 0.3273 g in distilled water and weighting with dissolving TMP by accurate weight equal to 0.2903 g in distilled water. Working solution ranging (10<sup>-1</sup>–10<sup>-6</sup> M) of TMP was prepared by sequential thinning of the stockpile solution. 0.1 M stock solutions of each interfering salts: NaCl, KCl, CaCl<sub>2</sub>, MgCl<sub>2</sub>, CuSO<sub>4</sub> and Fe<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>·3H<sub>2</sub>O, and AlCl<sub>3</sub>·6H<sub>2</sub>O were prepared in 50 ml of water, respectively.

## Preparation of ion pair (IP)

An IP of TMP -MO was equipped by mix equimolar amounts of 1×10<sup>-2</sup> mol/L TMP and anionic dye MO. The solution was stable for 2 days and the IP deposit was filtered (quantitative rapid filter paper). The deposit was become dry and used as an electrode effective material for make ready the TMP -sensitive electrode. The mostly accepted method of make ready a plasticized membrane is made of mixing of the electrode effective material with PVC dissolved in tetrahydrofuran after that in a glass ring was evaporation of the solvent. PVC needs plasticization and places a constraint on the select of mediator. Thus, plasticized PVC membranes were prepared by a method based on the references [13].

## Membrane of electrodes preparation

The sensing film was prepared by adding 0.17 g of PVC powder with 0.04 g from IP with 0.36 g of a plasticizer. The mix was moved until the PVC was well soaked, and then, the mix was distributed in 6–7 ml THF. The resultant mix was transported into a glass dish of 35 mm in diameter. The solvent was vaporized little by little at room temperature up until a solid membrane was made. A desired part of the membrane was cut and then was put to an end of polyethylene tube using sticky solution of PVC as a paste. The resultant sensor was then full with an internal solution of 1.0×10<sup>-1</sup> mol/L TMP.

### Construction of ISE

A cut membrane was attached to the tube made from polyethylene in an electrode formation and attached to the last part of glass tube; this electrode was full with inner solution which contains 0.1 M TMP. Ag/AgCl wire electrode was worked as an interior reference electrode and then this electrode attached to double junction Ag/AgCl electrode as external reference electrode [14].

### Effect of the type of plasticizer on the specific operation of the sensor

The influence of the plasticizer configuration was considered on the distinguishing operation of TMP-PVC membrane of electrode. PVC membrane sensors were equipped using various plasticizers

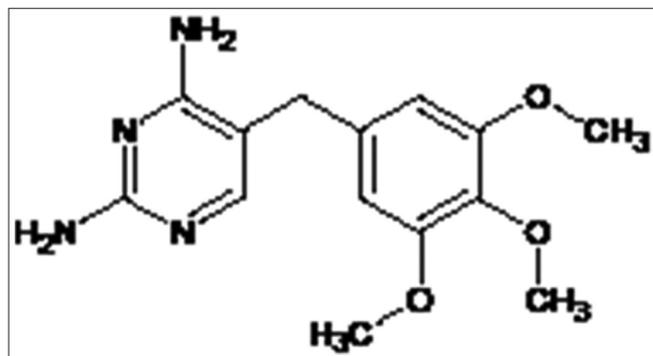


Fig. 1: Structure of trimethoprim

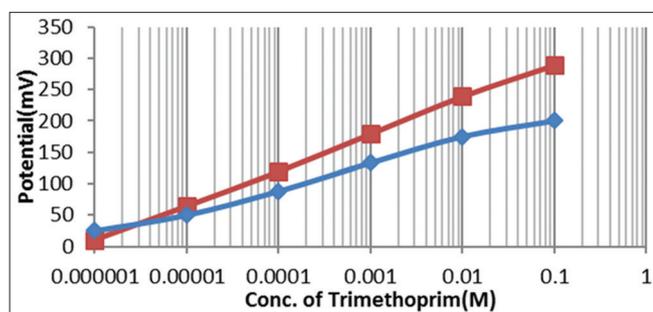


Chart 1: Response of trimethoprim electrodes

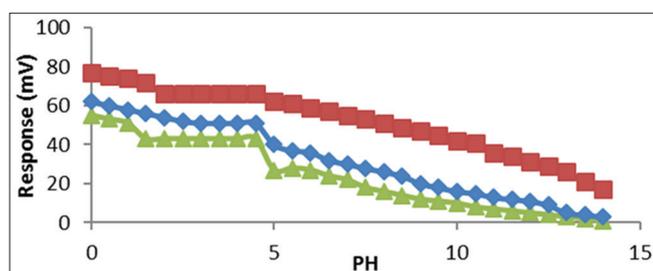


Chart 2: Different of PH for trimethoprim (TMP) solutions using TMP+PT+nitrophenyl octyl ether electrode

that are DBP and NPOE of various polarities which are commonly used for preparations of PVC membrane sensors. Sensors integrated membranes plasticized with DBP indicated fewer response (slope 42.00 mV/decade) for TMP maybe due to lowly solubility of IP in the plasticizer. The solubility of the composite was studied by its ability to formula translucent solution in the plasticizer. For electrode depended on NPOE as a plasticizer, clear translucent solution was made while in DBP muddled and suspended solutions were obtained. This point to that the complex solubility of NPOE>DBP. It looks that o-NPOE develops the membrane selectivity due to its height dielectric constant affects substantial dissolution of IP inside the membrane, accordingly improves its partition factor in the membrane and too providing apposite mechanical property of the membrane related with fewer permittivity of plasticizers [15].

### OUTCOMES AND ARGUMENT

For the determination of the potentiometric behavior of electrode against TMP, potentiometric measurements were taken in a series of standard solutions prepared by diluting a  $1.0 \times 10^{-1}$  M TMP stock solution with distilled water in appropriate quantities. The observed potential values depending on the varying TMP concentrations and the potential-concentration curve is shown as an inset in Chart 1. The developed TMP-selective electrode with o-NPOE as a plasticizer exhibited a linear response in a range of concentration was  $6.0 \times 10^{-6}$ – $1.0 \times 10^{-2}$  M TMP with a slope equal to 57.31 mV/decade. The detection limit of the electrode was near to  $3.0 \times 10^{-6}$  M which calculated from the intersection of two linear portions of the response curve as  $1.0 \times 10^{-6}$  M as recommended by the International Union of Pure and Applied Chemistry [16]. It is shown in Table 1 that the slope, linear working range, and detection limit values of the PVC membrane electrode prepared with the use of o-NPOE were better than of the membranes prepared with DBP as a plasticizer. Accordingly, it has been determined that o-NPOE is the most convenient plasticizer in terms of potentiometric performance characteristics. The response of a PVC membrane electrode, in most cases, depends on the concentration of lipophilic anionic salts incorporated in the membrane [17]. Lifetime of this electrode was 27 days, while TMP electrode which depended on DBP as a plasticizer was given a poor value of slope about 41.20 mV/decade with concentration range near to  $6.5 \times 10^{-6}$ – $1.0 \times 10^{-2}$  M detection limit was equal to  $2.5 \times 10^{-6}$  M and lifetime was 3 days. However, the lifetime of each electrode varies depending on the stability and type of the selective membrane, frequency of use, and the storage conditions. In PVC membrane electrodes, especially, the leakage of the electroactive component into the measurement media and swelling of the membrane in water in the long run causes a loss of performance in potentiometric properties. Table 1 shows the values of response for TMP-selective electrodes and Chart 1 shows the response of TMP electrodes.

### Impact of PH

The impact of the pH on the electrode response was investigated using the  $1.0 \times 10^{-2}$ ,  $1.0 \times 10^{-3}$  and  $1.0 \times 10^{-4}$  M aqueous TMP solutions at different pH levels. The pH of the solution was adjusted by adding the adequate amounts of HCl and NaOH solutions. The potential data of the electrochemical cell were collected depending on the pH change of the solutions and the plot shown in Chart 2 was constructed. As seen, the potentiometric response of the electrode depended on o-NPOE was

Table 1: Response of TMP electrodes

Type of membrane	TMP+NPOE+MO	TMP+DBP+MO
Linear range (M)	$6.0 \times 10^{-6}$ – $1.0 \times 10^{-2}$	$6.5 \times 10^{-6}$ – $1.0 \times 10^{-2}$
Correlation coefficient/R	0.9903	0.9990
Limit of detection (M)	$3.0 \times 10^{-6}$	$2.5 \times 10^{-6}$
Regre. Eq. $Y=mX+b$	$Y=24.885 \ln(x)+351.4$	$Y=18.24 \ln(x)+258.5$
Slope (mV/decade)	57.31	42.00
Lifetime (day)	27	3

TMP: Trimethoprim, NPOE: Nitrophenyl octyl ether, DBP: Dibutyl phthalate

**Table 2: Range of pH for TME+NPOE+MO electrode**

Number	Membrane composition	pH range		
		2-Oct	3-Oct	4-Oct
1	TMP+NPOE+MO	2.0-5.5	1.5-4.5	3.0-4.5
2	TMP+DBP+MO	4.5-5.2	3.0-4.0	1.0-3.5

NPOE: Nitrophenyl octyl ether, DBP: Dibutyl phthalate

2.0-5.5, 1.5-4.5, and 3.0-4.5 for concentration of TMP  $10^{-2}$ ,  $10^{-3}$ , and  $10^{-4}$ , respectively, and for benign prostatic hyperplasia electrode, value of pH was 4.5-5.2, 3.0-4.0, and 1.0-3.5 for concentration of TMP  $10^{-2}$ ,  $10^{-3}$ , and  $10^{-4}$ , respectively. However, at high pH values (>5.0), a big decrease in the potential values is observed. This can be explained by the increasing concentration of OH<sup>-</sup> ion in the medium. It is desired that an ion-selective electrode should be used continuously for a long period of time with no any significant variation in its potentiometric behavior.

**Table 3: Selectivity coefficients calculated for electrode 1 (TMP+NPOE+MO)**

Electrode 1 (TMP+NPOE+MO)					
Concentration of TMP					
Ion	$10^{-5}$	$10^{-4}$	$10^{-3}$	$10^{-2}$	$10^{-1}$
K <sup>1+</sup>	$2.4744 \times 10^{-5}$	$8.7056 \times 10^{-6}$	$4.1084 \times 10^{-7}$	$5.9721 \times 10^{-8}$	$3.0164 \times 10^{-8}$
Na <sup>1+</sup>	$1.4315 \times 10^{-3}$	$1.5089 \times 10^{-4}$	$5.8252 \times 10^{-6}$	$5.02256 \times 10^{-7}$	$4.3971 \times 10^{-6}$
Ca <sup>2+</sup>	$1.7476 \times 10^{-7}$	$6.8408 \times 10^{-8}$	$2.7874 \times 10^{-8}$	$8.9252 \times 10^{-9}$	$2.3087 \times 10^{-8}$
Mg <sup>2+</sup>	$8.1455 \times 10^{-8}$	$1.2156 \times 10^{-8}$	$1.9368 \times 10^{-8}$	$1.0910 \times 10^{-8}$	$1.91222 \times 10^{-9}$
Fe <sup>3+</sup>	$5.8457 \times 10^{-9}$	$9.5316 \times 10^{-10}$	$4.4727 \times 10^{-9}$	$2.7805 \times 10^{-9}$	$6.5087 \times 10^{-9}$
Al <sup>3+</sup>	$7.7443 \times 10^{-9}$	$1.1979 \times 10^{-7}$	$1.2212 \times 10^{-8}$	$5.6598 \times 10^{-8}$	$3.92004 \times 10^{-7}$

TMP: Trimethoprim, NPOE: Nitrophenyl octyl ether

**Table 4: Potentiometric techniques for analysis for TMP**

Electrode type	Concentration of TMP (M)				
	Sample	Response by potentiometric method			
		Direct	SAM	MSA	Titration
TMP+NPOE+MO	$1.0 \times 10^{-3}$	$0.9627 \times 10^{-3}$	$0.9682 \times 10^{-3}$	$0.9877 \times 10^{-3}$	$0.9728 \times 10^{-3}$
	RSD%	2.08	0.17	-	-
	Re%	96.27	96.82	98.77	97.28
	Er%	-3.73	-3.18	-1.23	-2.72
	$1.0 \times 10^{-4}$	$0.9829 \times 10^{-4}$	$0.9656 \times 10^{-4}$	$0.9922 \times 10^{-4}$	$0.9976 \times 10^{-4}$
	RSD%	1.29	0.98	-	-
	Re%	98.29	96.56	99.22	99.76
	Er%	1.71	-3.44	-0.78	-0.24

TMP: Trimethoprim, NPOE: Nitrophenyl octyl ether, RSD: Relative standard deviations, SAM: Standard addition method, MSA: Multi standard addition

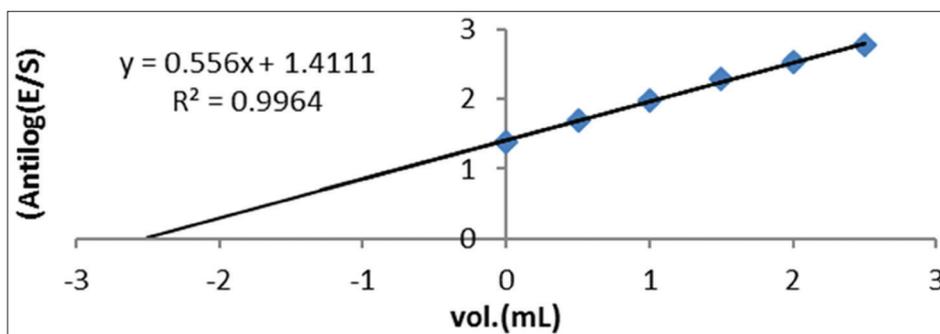


Chart 3: Antilog (E/S) versus volume of  $10^{-3}$  M added of trimethoprim (TMP) using electrode 1 (TMP+nitro phenyl octyl ether+MO)

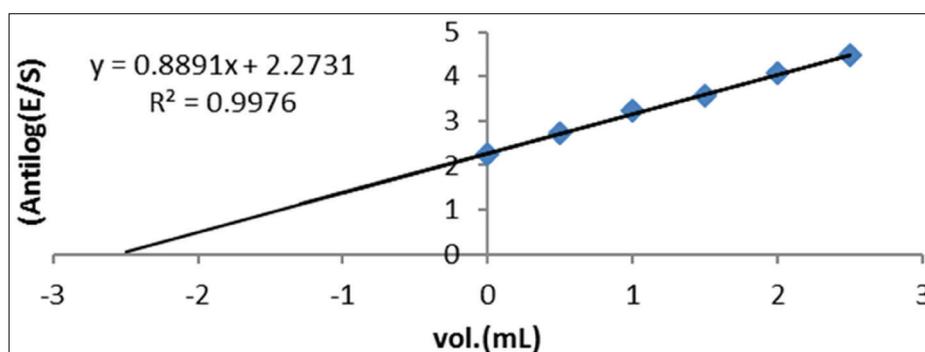


Chart 4: Antilog (E/S) versus volume of  $10^{-4}$  M added of trimethoprim (TMP) using electrode 1 (TMP+nitrophenyl octyl ether+MO)

**Selectivity**

A dynamic typical of an ISE is its selectivity to the primary ion in excess of interfering ions. Selectivity coefficients of the electrode, which are the

indicators of the interference degree of the corresponding interferents on the potentiometric response, were calculated according to the methods [18-20] for some commonly encountered cations species. The calculated selectivity coefficients are given in Table 4. According to the selectivity coefficient values, it is seen that the electrode exhibited a highly selective response against the measured species.

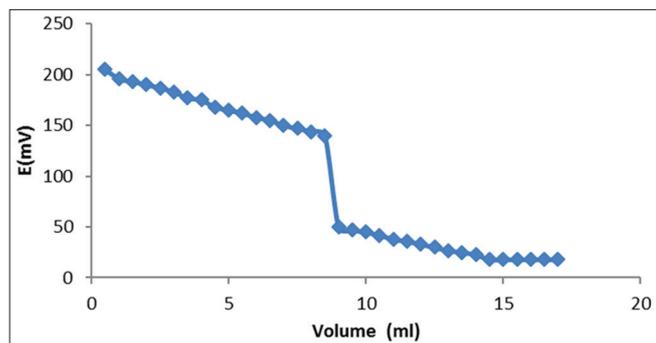


Chart 5: Titration curve for sample ( $1 \times 10^{-3} \text{M}$ ) trimethoprim (TMP) with ( $1 \times 10^{-3} \text{M}$ ) MP standard by electrode (TMP+MO+nitrophenyl octyl ether)

**Analytical applications**

The new examined electrode which depended on TMP+MO+NPOE was applied and was found to be suitable in the potentiometric measurements of TMP in tablets by direct, standard addition methods (SAM), or multi-SAM, potentiometric titration. Table 4 shows that the SAM was verified to be successful for the determination of the examined drug in its pure solutions using a similar electrode. The titration curves are indicated in Chart 5.

The values of mean recovery listed in Table 5 of determination tablet for TMP samples, and range by standard addition method was from 97.94% to 97.77% with lesser relative standard deviation (RSD) values were reaching from 0.28% to 1.44%. The standard addition method was showed to be successful for the determination of the investigated

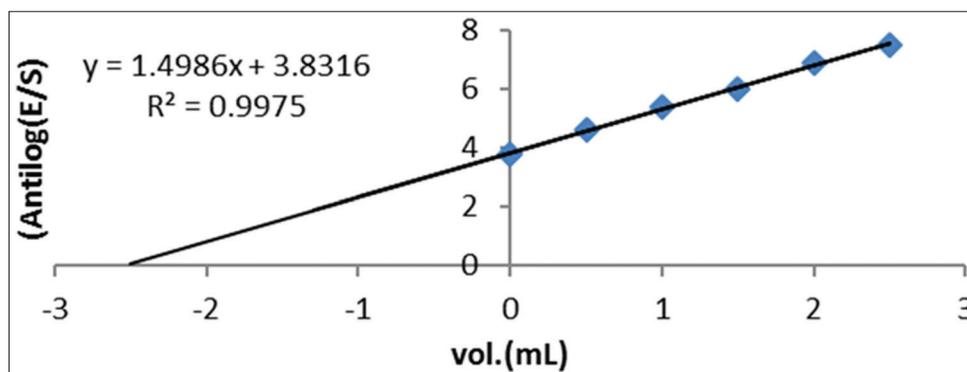


Chart 6: Antilog (E/S) versus volume of  $10^{-3} \text{M}$  added of trimethoprim (TMP) using electrode 1 (TMP+nitrophenyl octyl ether+MO)

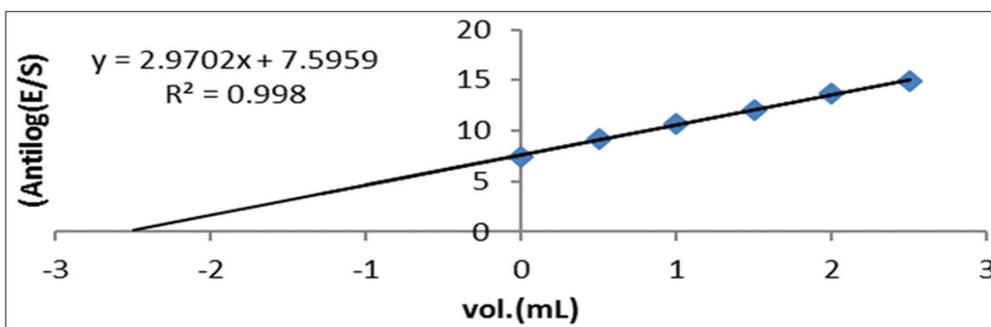


Chart 7: Antilog (E/S) versus volume of  $10^{-4} \text{M}$  added of trimethoprim (TMP) using electrode 1 (TMP+nitrophenyl octyl ether+MO)

Table 5: Potentiometric techniques for analysis of TMP samples (Trimoks tablet)

Electrode type	Concentration of TMP (M)				
	Sample	Response by potentiometric method			
		Direct	SAM	MSA	Titration
TMP+NPOE+MO	$1.0 \times 10^{-3}$	$0.9645 \times 10^{-3}$	$0.9794 \times 10^{-3}$	$0.9979 \times 10^{-3}$	$0.9922 \times 10^{-3}$
	RSD%	1.47	0.28	-	-
	Re%	96.45	97.94	99.79	99.22
	Er%	-3.55	-2.06	-0.21	-0.78
	$1.0 \times 10^{-4}$	$0.9738 \times 10^{-4}$	$0.9777 \times 10^{-4}$	$0.9992 \times 10^{-4}$	$0.9874 \times 10^{-4}$
	RSD%	1.03	1.44	-	-
	Re%	97.38	97.77	99.92	98.74
	Er%	-2.62	2.23	-0.08	-1.26

TMP: Trimethoprim, NPOE: Nitrophenyl octyl ether, RSD: Relative standard deviation

drug in its pure solutions. From the results shown in Table 4, it is clear that the obtained mean recovery values of the quantities taken of pure drug samples reached from 96.82% to 96.56% with lesser RSD values of 0.17–0.98%.

## CONCLUSIONS

New fabricated membrane was developing for TMP determination above a wide of concentration range, in pH range between 1.0 and 5.5. The electrode displayed a very suitable selectivity to TME in the presence of different common inorganic cation and unlike pharmacological-related mixtures. This electrode can be used as rapid, economic, easy, and sensitive analytical method for the determination of TMP in pharmaceutical formulations and pure powder.

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