New Polymeric Ion Selective Electrode for Determination of Sulfamethoxazole in Pure and Pharmaceutical Samples

Sarra A. Abrahem and Khaleda H. Al-Saidi
Department of Chemistry, College of Science, Al-Nahrain University, Al-Jaderia, Baghdad-Iraq.

Abstract

Sulfamethoxazole ion selective electrodes were constructed based on sulfamethoxazole - tengestophosphoric acid as ion pair complex in a polyvinylchloride matrix and plasticized by four plasticizers, Di-octyl phthalate (DOPH); Tri-butyl phosphate (TBP); Acetophenone (AP) and Nitrobenzene (NB). Sulfamethoxazole electrodes (E1, E2, E3 and E4) gave slopes (52.008, 58.381, 56.909 and 50.309 mV/decade) and linear ranges from (1×10^{-5}-1×10^{-2},1×10^{-7}-1×10^{-2},1×10^{-5}-1×10^{-2} and 1×10^{-5}-1×10^{-2} M) respectively. The best electrode (E2) was based on TBP plasticizer which gave a slope 58.381mV/decade, correlation coefficient 0.9997, detection limit of 9×10^{-8} M, lifetime 27 day. pH and life time of the electrodes were also studied and the proposed electrode displayed a good stability and reproducibility and were used to determine the Sulfamethoxazole in pharmaceutical samples. The interferences measurements in the presence of (Na^{+}, K^{+}, Cu^{+2}, Mn^{+2}, Fe^{+3}, Al^{+3}, trimethoprim, starch, sucrose and gelatin) were studied using the separated method and mixed method for selectivity coefficient determination.

Keywords: Sulfamethoxazole, Tengestophosphoric acid, Ion selective electrode (ISE).

Introduction

Sulfamethoxazole (SMZ), 4-Amino-*N*-(5-methylisoxazol-3-yl)- benzenesulfonamide, $C_{10}H_{11}N_3O_3S$, as shown in Fig.(1), is white or almost white, crystalline powder with molecular weight 253.279 g/mole, practically insoluble in water, freely soluble in acetone, sparingly soluble in ethanol. It dissolves in dilute solutions of sodium hydroxide and in dilute acids. Sulfamethoxazole is a sulfonamide bacteriostatic antibiotic. It is most often used as part of a synergistic combination with trimethoprim in a 5:1 ratio in co-trimoxazole.[1] Various analytical methods have been developed to determine sulfamethoxazole in formulations and biological samples, such as spectrophotometric [2,3], chromatographic[4,5], and HPLC (High Performance Liquid Chromatography)[6,7,8] methods. The applications of ion selective electrodes continue to be of interest in pharmaceutical analysis [9] and ions [10,11] because these sensors offer the advantages of simple design and operation, reasonable selectivity, fast response, low cost and applicability to turbid and colored solutions [12,13]. In this work the sensor is based on Sulfamethoxazole -tengestophosphoric acid as ion pair in polyvinyl chloride plasticized with different plasticizers were used for the determination of Sulfamethoxazole in pharmaceutical samples, The properties of prepared electrodes, pH effect and selectivity, were studied.

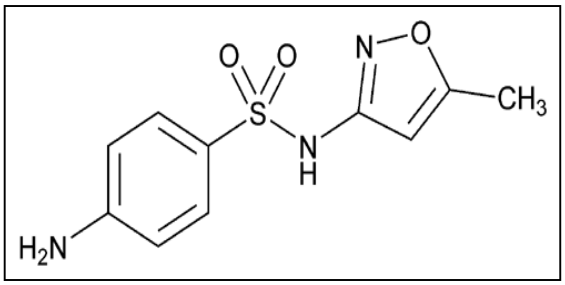


Fig. (1) Structure formula of sulfamethoxazole.

Experimental Part

Equipment

A digital pH/ion meter (inoLab 740 with terminal 740 – WTW, Germany) was used for all potentiometric and pH measurements. Hotplate Stirrer (LMS-1003, Daihan Labtech), Sartorius Handy 4digits Analytical Balance, Fourier transforms infrared spectrophotometer (FTIR-8300 SHIMADZ, Japan), pH combination electrodes (SenTix® 82 WTW, Germany), Silver-silver chloride wire and Saturated Calomel reference electrode were used in this work.

Reagents and Solutions

-Sulfamethoxazole standard was a gift from the State Company of Drug Industries and Medical Appliances (Samara IRAQ-SDI). Polyvinyl chloride (PVC) of relatively high molecular weight. Trimole tablets each tablet (containing 400 mg Sulfamethoxazole) were purchased from Bilim pharmaceuticals (made in Emirates). Tengestophosphoric acid (TPA), Molecular Weight 2880.2g/mole, was purchased from Fluka. DOPH, TBP, AP and NB were purchased from Fluka AG, Switzerland, Tetrahydrofuran (E.Merck). Acetone from Sigma-Aldrich. Other chemicals and solvents were of an analytical reagent grade obtained from BDH.

-Stock solutions of 0.1 M for each of NaCl, KCl, $Cu(NO_3)_2.3H_2O$, $MnSO_4$, $Fe_2(SO_4)_3.9H_2O$, $AlCl_3.6H_2O$, trimethoprim, starch, sucrose and gelatin were prepared by dissolving 0.2922, 0.3722, 1.2077, 0.7550, 2.8100, 1.2066, 1.4516, 0.8108, 1.7115 and 1.5000 g in 50 mL of distilled water respectively.

-A standard solution of 0.01 M Tengestophosphoric acid (TPA) was prepared by dissolving 1.4401 g of pure (TPA) in distilled water and completing the solution up to 50 mL.

-A stock solution of 10^{-2} M Sulfamethoxazole was prepared by dissolving 0.1266 g of pure (SMZ) in acetone and distilled water in proportion (1:4) and completing the solution up to 50 mL .The working solutions 10^{-8}-10^{-2} M SMZ were prepared by serial appropriate dilution of the stock solution using the same solvent.

-Stock solution of 0.1 M of HCl and 0.1 M of NaOH which are used for adjusting pH of the solutions.

Procedure

Preparation of ion pair:-

The ion pair was prepared by mixing equal volume of 0.01 M solution of tengestophosphoric acid (TPA) dissolved in distilled water with an equimolar solution of sulfamethoxazole(SMZ) dissolved in acetone, The precipitate formed after 24 hr.

Preparation of membrane:-

0.0400 g of ion pair was mixed with 0.3600 g of plasticizer and 0.1700 g of PVC powder; all were dissolved in 5 ml of THF with stirring until a clear viscous solution was obtained **[14].**

Construction of ion-selective electrodes:-

The construction of the electrode body and the immobilization were done as described by Craggs et al. **[14].** This method include that the viscous solution poured into a glass casting ring about 30mm length and 35mm in diameter. It consists of two pieces; one of them is the glass cylinder and the other is glass plate. The two pieces was pasted together by using (PVC-THF) viscous mixture. The glass tube was 3/4 filled with 10^{-3} M Sulfamethoxazole solution as an internal filling solution, the membrane was conditioned by immersing in a standard solution of the same concentration for at least 4 hour before measurements.

Potential Measurements

The potential was carried out at room temperature. The electrochemical cell may be represented as follows:

Ag/AgCl |internal filling solution|| membrane ||test solution| SCE

A calibration curve was constructed for each electrode using standard analyte solutions ranged from (10^{-8}-10^{-2} M). The calibration curves were prepared by plotting the potential E versus concentration (M) log scale by using computer program (Microsoft office Excel 2010).

Preparation of Pharmaceutical Samples

Ten tablets of trimole each tablet containing 400 mg of sulfamethoxazole weighted accurately and grinded it found that the weight of average of one tablet was 0.6656 g to prepare 10^{-2} M from sulfamethoxazole 0.2107g was dissolved in 10 mL acetone and then filtered and completing the solution up to 50 mL with distilled water. Other samples prepared by dilution.

Calculation of Selectivity coefficient

A separate solution method**[15]** was used for the selectivity coefficient measurement, which calculated according to the equation:

$$\log K^{pot}_{A,B}=(E_B-E_A)/S+(1-z_A/z_B)\log a_A \quad \ldots\ldots (1)$$

E_A, E_B; z_A, z_B; and a_A, are the potentials, charge numbers, and activities for the primary A ion, respectively, at $a_A = a_B$.

The selectivity coefficients were also measured by the mixed method (Fixed interference method) (FIM) [16,17] according to the equation:

$K^{pot}_{A,B}=a_A/(a_B)^{zA/zB}$ ..... (2)

Results and Discussion

Four electrodes of sulfamethoxazole (SMZ) (E1, E2, E3, E4) based on using SMZ, TPA and used four plasticizers: DOPH, TBP, AP and NB with PVC matrix were examined respectively.

The sensor (E2) displays a linear response from 10^{-7} to 10^{-2} M (SMZ) with Nernstian cationic slope of 58.381mV/decade with lower limit of detection of 9×10^{-8}M, which was calculated at the point of intersection of the extrapolated segments of the two linear parts of the calibration curve of SMZ. Electrode (E2) gave high slope value because the high mixing between the TBP and PVC due to the compatibility of the plasticizer used to the electro-active compound in both structure and composition. A typical plot for calibration curves of electrodes based on four plasticizers DOPH, TBP, AP and NB are shown in Fig.(2).

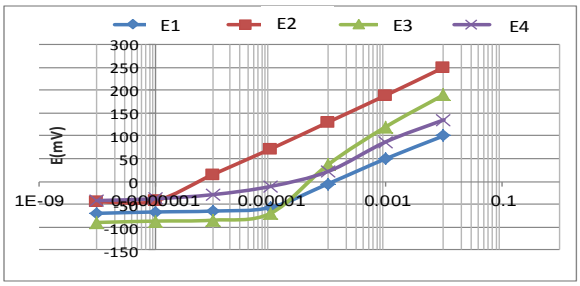


Fig.(2) Calibration curves of Sulfamethoxazole selective electrodes using DOPH, TBP, AP and NB plasticizer.

The FTIR spectrum of the complex was compared with the reference spectrum of sulfamethoxazole [18], Fig.(3- a and b). The spectrums show a good purity and the functional groups obtained from the spectrum were shown in Table (1).

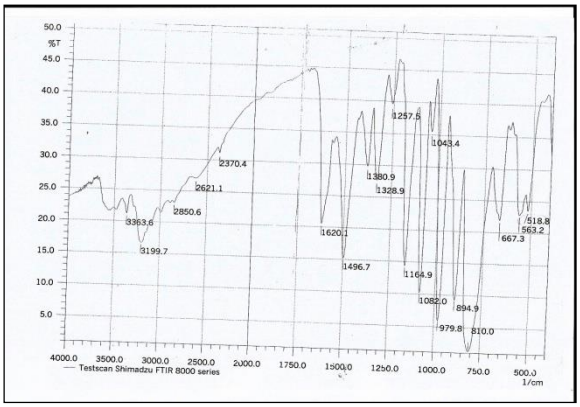
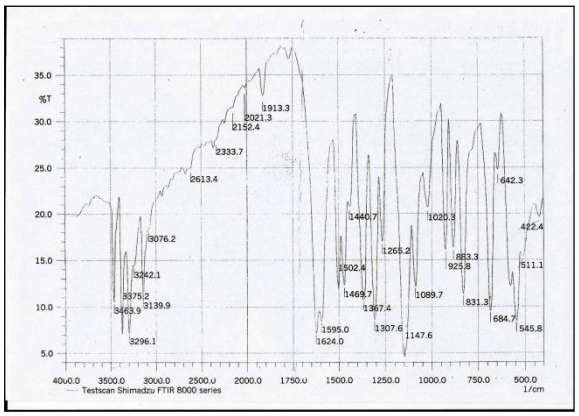


Fig.(3) a- FTIR spectrum of complex (SMZ-TPA).



b- FTIR reference spectrum of pure SMZ.

Table (1)
The functional groups obtained from the spectrum for each SMZ and complex [SMZ-TPA].

Functional groups	*Sulfamethoxazole (SMZ) cm^{-1}*	*Complex [SMZ-TPA] cm^{-1}*
ν (N-H)	3463	3199
ν (S=O)	1307	1328
Bending (N-H)	1595	1620
ν (N=C)	1624	1620

The slopes were obtained for electrodes based on DOPH, AP and NB (membranes E1, E3 and E4), and the values were 52.008, 56.909 and 50.309mV/decade with correlation coefficients of 0.9991, 0.9994 and 0.9981 respectively. The linear range for these electrodes 1×10^{-5}-1×10^{-2}, 1×10^{-5}-1×10^{-2} and 1×10^{-5}-1×10^{-2} M with detection limits of 9×10^{-6}, 6×10^{-6} and 7×10^{-6} M, respectively. The results and other parameters are given in Table (2). The electrodes gave different Nernst slopes, this could be due to the different

viscosities of plasticizers; for example, the high viscosity decrease the ion-exchange process and the low viscosity causes rapid leaching of the membrane components to the external solution [19].

Table (2)
The parameters for four (SMZ) electrodes.

Electrode	Slope (mV/Decade)	Linear equation	Correlation coefficient (r)	Linear concentration range (M)	Detection limit (M)	Response time (sec)			Lifetime (day)
						1×10^{-2} (M)	1×10^{-3} (M)	1×10^{-4} (M)	
E1 SMZ+TPA+ DOPH	52.008	y = 22.583ln(x) + 210	0.9991	1×10^{-5}-1×10^{-2}	9×10-6	26	30	37	29
E2 SMZ +TPA+ TBP	58.381	y = 25.35ln(x) + 364.17	0.9997	1×10^{-7}-1×10^{-2}	9×10-8	24	28	36	27
E3 SMZ +TPA+ AP	56.909	y = 24.711ln(x) + 263.4	0.9994	1×10^{-5}-1×10^{-2}	6×10-6	29	36	42	20
E4 SMZ +TPA+ NB	50.309	y = 21.8451ln(x) + 234.3	0.9981	1×10^{-5}-1×10^{-2}	7×10-6	22	25	33	9

Effect of pH:-

The effect of pH on the electrode potentials for SMZ selective membrane electrode E2 was examined by measuring the potential of the cell in SMZ solutions at three different concentrations (1×10^{-2},1×10^{-3},1×10^{-4}) M in which the pH ranged from (0.5-11.0). The pH adjusted by adding appropriate amounts of hydrochloric acid and/or sodium hydroxide solution. The results shown in Fig.(4).

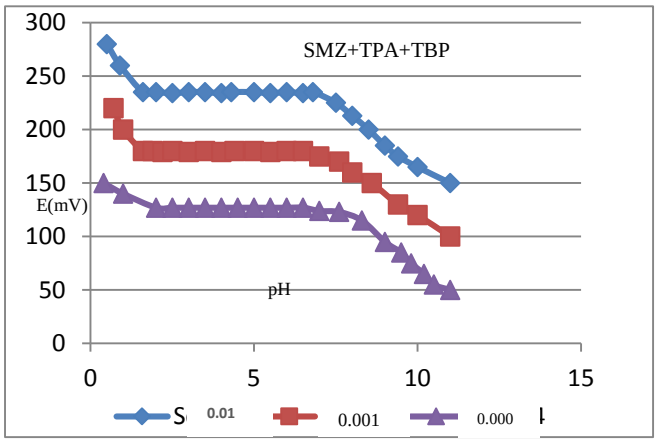


Fig.(4) Effect of pH on the potential of the electrode E2 at concentrations 1×10^{-2}, 1×10^{-3} and 1×10^{-4}M.

At pH values less than 1.6 or in very high acidity, the electrode response has been increased rather irregularly. This may be due to that the electrode response to H^+ activities as well as SMZ ions and in an alkaline solution (pH greater than 7) the electrode response has been decreased, may attribute to the decreasing in the solubility of SMZ.[20] The working pH were tabulated in Table (3).

Table (3)
Working pH ranges for (SMZ) electrode (E2). for (1×10^{-2} , $1\times10^{-3}$and 1×10^{-4}) M of SMZ.

Electrode no.	Composition of electrode E2	pH range		
		1×10^{-2} (M)	1×10^{-3} (M)	1×10^{-4} (M)
E2	SMZ+TPA+TBP	1.6-6.8	1.6-6.5	2.0-6.5

Interference studies:-

In order to investigate the selectivity of the proposed membrane (E2) ion selective electrode toward sulfamethoxazole with respect to various interfering ions by using: separate solution method according to equation (1) and mixed solution method according to equation (2). The values of the selectivity coefficients for separate method and mixed method are listed in Table (4) and Fig.(5) show the calibration curve of fixed interfering method sulfamethoxazole selective electrode (E2) for sodium ion (Na^+).

Table (4)
Values of $K^{pot}_{A,B}$ according to separate method and FIM by using electrode E2.

Interfering ions	Separate method	Mixed method
	$logK^{pot}_{A,B}$	$K^{pot}_{A,B}$	$K^{pot}_{A,B}$
Na^{+}	-2.119	7.59×10^{-3}	3.40×10^{-6}
K^{+}	-2.205	6.23×10^{-3}	3.00×10^{-6}
Cu^{+2}	-4.102	7.91×10^{-5}	3.35×10^{-7}
Mn^{+2}	-4.239	5.76×10^{-5}	3.57×10^{-7}
Fe^{+3}	-4.825	1.49×10^{-5}	5.42×10^{-7}
Al^{+2}	-4.894	1.27×10^{-5}	1.62×10^{-7}
Trimethoprim	-2.239	5.75×10^{-3}	6.00×10^{-6}
Starch	-2.464	3.43×10^{-3}	1.80×10^{-6}
Sucrose	-2.670	2.13×10^{-3}	1.40×10^{-6}
Gelatin	-2.705	1.97×10^{-3}	1.30×10^{-6}

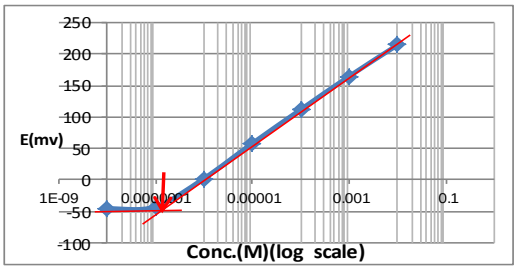


Fig.(5) Calibration curve of fixed interfering method sulfamethoxazole selective electrode (E2).

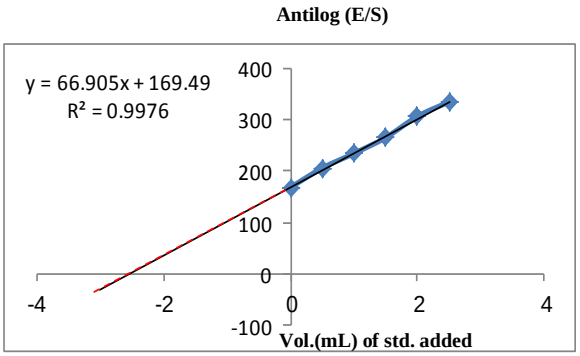


Fig.(6) Calibration curve of antilog (E/S) versus the volume added of standard 10^{-3}M for determination of 25 mL sulfamethoxazole solution 10^{-4}M by (MSA).

Sample analyses:-

Four potentiometric techniques were used for the determination of sulfamethoxazole including. Direct method and standard addition method (SAM) follows the equation:

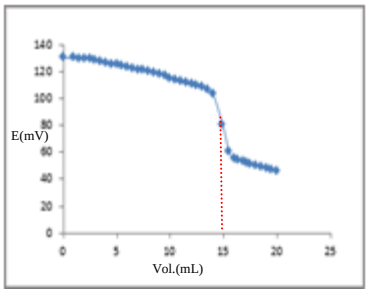
$$C_U = C_S / 10^{\Delta E/S} [1+ (V_U / V_S)] - (V_U / V_S)$$

Where C_U, C_S, V_U and V_S are the concentration and volume of unknown and standard solution respectively and Multiple standard additions (MSA) follows the equation:

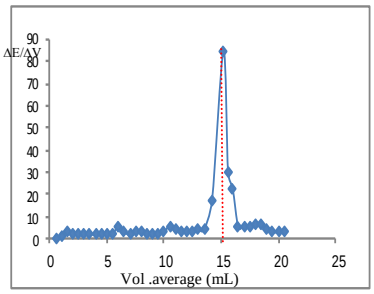
$$C_U = V_S \times C_S / V_U$$

Where C_U and C_S are the concentration of unknown and standard, respectively, V_S is the volume of standard solution. The method was carried out as in Fig.(6).

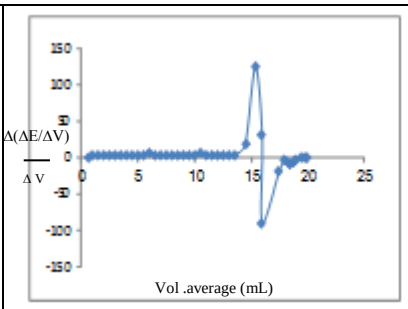
by plotting antilog (E/S) versus the volume of the five addition of standard sulfamethoxazole, used to of concentration can be covered as compared with working range calibration curve for MSA used to determine the concentration of sulfamethoxazole. For potentiometric titration a 10^{-4} M of tengestophosphoric acid were used as a titrant. A typical titration plot were shown in Fig.(7) (a ,b and c)



a-Titration curve using electrode (E2)



b-First derivative Ttitration curve using electrode (E2)



c-Second derivative Titration curve using electrode (E2)

Fig.(7)(a, b and c) Titration curves of sulfamethoxazoleselective electrode using TBP plasticizer.

The recovery (Re %), relative error (E_r %) and relative standard deviation (RSD %) for each method are calculated and the results are listed in Table (5). The electrode (E2) was proved to be useful in the potentiometric determination of sulfamethoxazole in pharmaceutical preparations and the data obtained for pharmaceutical samples were listed in Table (6).

Table (5)
Analysis of SMZ by potentiometric techniques by using ISE E2.

Parameter	*Direct method**	*SAM**	*Multi SAM**	*Titration method***
Conc.(M)	1.000×10^{-4}	1.000×10^{-4}	1.000×10^{-4}	1.000×10^{-4}
Found(M)	0.996×10^{-4}	0.988×10^{-4}	1.013×10^{-4}	1.011×10^{-4}
RSD%*	0.411%	0.532%	---------	1.659%
Re%	99.660%	98.860%	101.320%	101.110%
Er%	-0.340%	-1.14%	1.320%	1.110%
S	4.098×10^{-7}	5.263×10^{-7}	---------	1.677×10^{-6}
x ± (ts/ √ N)	$0.996\times10^{-4} \pm 0.494\times10^{-6}$	$0.988\times10^{-4} \pm 0.635\times10^{-6}$	---------	$1.011\times10^{-4} \pm 0.416\times10^{-5}$

Table (6)
Analyses of SMZ inpharmaceutical samples.

Parameter	*Direct method**	*SAM**	*Multi SAM**	*Titration method****
Conc.(M)	1.000×10^{-4}	1.000×10^{-4}	1.000×10^{-4}	1.000×10^{-4}
Found(M)	0.998×10^{-4}	0.998×10^{-4}	1.002×10^{-4}	0.999×10^{-4}
RSD%*	0.647%	0.753%	---------	0.919%
Re%	99.820%	99.8%	100.200%	99.95%
Er%	-0.180%	-0.200%	0.200%	-0.05%
S	6.457×10^{-7}	7.516×10^{-7}	---------	9.192×10^{-7}
x ± (ts/√N)	$0.998\times10^{-4} \pm 0.779\times10^{-6}$	$0.998\times10^{-4} \pm 0.907\times10^{-6}$	---------	$0.999\times10^{-4} \pm 0.825\times10^{-5}$

RSD% for n=2, t=12.7***
RSD**% for n=3, t=4.3
RSD*% for n=5, t=2.7

Conclusions

ISE method included formation of membranes for sulfamethoxazole was constructed based on using sulfamethoxazole (SMZ) and tengestophosphoric acid (TPA) as ion pair dissolving in many plasticizers. The best electrode for SMZ was (E2) electrode which used to determine SMZ in the pharmaceutical samples. Also there is no interference for some interfering ions. The proposed analytical method is proved to be simple and rapid, with good accuracy.

References

[1] British pharmacopoeia; "*Medicinal and Pharmaceutical Substances"*; Published by The Stationery Office on behalf of the Medicines and Healthcare products Regulatory Agency (MHRA); Vol. 1 & 2; pp. 1406-1407; 2014.

[2] Jasmin S.; Rasul M.J.; Suraya M.; "*Extractive Spectrophotometric Methods for Determination of Sulfamethoxazole in Pharmaceutical Formulations Using Bromothymol Blue and Cresol Red"*; J. Chin. Chem. Soc. 55, 1107-1112, 2008.

[3] Mohamed E.; Safaa El-Din H.E.; *"Spectrophotometric methods for estimation of sulfamethoxazole based on charge transfer complexes"*; Canadian J. Anal. Sci. Spec. 52, 316-324, 2007.

[4] Jiang Y.; Wang J.; Li H.; Wang Y.; Gu J.; *"Determination of sulfamethoxazole in human plasma by liquid chromatography-electrospray ionization tandem mass spectrometry"*; J Pharm Biomed Anal. 43, 1460-1464, 2007.

[5] Punnamchand L.; Poornima D.H.; *"A simple, rapid, and sensitive HPTLC method for the estimation of sulfamethoxazole: Application to single dose clinical study"*; J Planar Chrom. 24, 534-538, 2012.

[6] Wei L.; Huijuan J.; Kang Z.; *"Determination of sulfamethoxazole in rat plasma by HPLC–UV method with pre-column derivatization"*; Talanta 71, 385-390, 2007.

[7] Wibawa J.I.D.; Shaw P.N; Barrett D.A; *"Quantification of sulfamethoxazole and decladinose metabolites in rat plasma, gastric juice and gastric tissue using high-performance liquid chromatography with electrochemical detection"*; J. Chromatogr. B 783, 359-366, 2006.

[8] Pappa-Louisi A.; Papageorgiou A.; Zitrou A.; Sotiropoulos S.; Georgarakis E.; Zougrou F.; *"Study on the electrochemical detection of the macrolide antibiotics sulfamethoxazole and trimethoprim in reversed-phase high-performance liquid chromatography"*; J. Chromatogr. Biomed. Appl. 755, 57-64, 2001.

[9] Khaleda H. Al-Saidi and Haider Q. Munshid.; "*New Polymeric Membrane Electrode for Clarithromycin Determination*"; journal of Al-Nahrain University-Sciencem JNUS.Vol.16,No.2,pp.30-36, 2013.

[10] Sulekh Chandra, Kusum Sharma, and Adarsh Kumar; *"Mg(II) Selective PVC Membrane Electrode Based on Methyl Phenyl Semicarbazone as an Ionophore"*; Journal of Chemistry, Vol. 2013, Article ID 189464, 7 pages, 2013.

[11] Khaleda H. Al-Saidi and Zainab Watheq Ahmed.; "*Construction of Indium (III) Selective electrodes based on promethazine Hydrochloride in a PVC Matrix Membrane*".; AL-Mustansiriya J.Sci. Vol.22, No.4, pp.147-158, 2011.

[12] C. Rundle, *"A Beginners Guide to Ion-Selective Electrode Measurements"*, Nico 2000 Ltd, London, UK, 2008.

[13] J. Koryta, "*Theory and applications of ion-selective electrodes part II*", *Anal. Chem. Acta*, Vol. 91, No. 1, pp. 1-85, 1977.

[14] Craggs A.; Moody G.J.; Thomas, J.D.R.; *"PVC matrix membrane ion-selective electrodes. Construction and laboratory experiments"*; Chem. Educ. 8, pp. 541-544, 1974.

[15] Umezawa, Y.; Umezawa, K. and Sato, H., "*Selectivity Coefficients for Ion Selective Electrodes", Pure Appl. Chem.*, Vol.67, pp. 508–518, 1995.

[16] Umezawa, Y.; Umezawa, K.; Bhlmann, P.; Tohda, K. and Amemiya, S., *"Potentiometry Selectivity Coefficients of Ion-Selective Electrodes*", *Pure and Appl. Chem*., Vol. 72, No. 10, pp. 1851–2082, 2000.

[17] Susan, S.; Mohammad, T. and Hossein, N., *Wiley Interscience Journal*, Vol. 96, Issue 1-2, pp. 65-74, 2006.

[18] Najwa I.A.; Abdul-Muhsin A.; Moen I.A.; Nabil S.N.; "*Construction and Characterization of Indium Liquid Ion Selective Electrodes Based on Crown Ethers in a PVC Matrix Membrane*"; Turk. J. Chem. 29, 687-696, 2005.

[19] British pharmacopoeia; "*Infrared Reference Spectra*"; Published by The Stationery Office on behalf of the Medicines and Healthcare products Regulatory Agency (MHRA); Vol. 4; p 10638; 2014.

[20] Skoog D.A.; West D.M.; Holler F.J.; *"Fundamentals of Analytical Chemistry"*; Eighth edition, p.621, 2009.

الخلاصة

تم تحضير أقطاب بوليمرية حساسة لتقدير السلفاميثواوكسازول (E1, E2, E3, E4) معتمدة على معقد المزدوج الايوني (سلفاميثواوكسازول –حامض التنكستوفوسفوريك) كمادة فعالة مع المعقد بوليمر كلوريد الفاينيل لتكوين الغشاء .وتكون مذابة في اربعة مواد ملدنه هي : داي اوكتيل فثاليت، تراي بيوتيل فوسفيت، أسيتوفينون ونايتروبنزين على التوالي. وقد اعطت هذه الأقطاب انحدارا ٥٢,٠٠٨، ٥٨,٣٨١، ٥٦,٩٠٩، ٥٠,٣٠٩ ملي فولت/ حقبة ومدى التركيز الخطى $١٠^{-٢}-١٠^{-٥}$, $١٠^{-٢}-١٠^{-٧}$, $١٠^{-٢}-١٠^{-٥}$, $١٠^{-٢}-١٠^{-٥}$. وكان أفضل قطب E2 الذي يعتمد على تراي بيوتيل فوسفيت كملدن حيث اعطى انحدارا ٥٨,٣٨١، معامل ارتباط ٠.٩٩٩٦ حد كشف ٩×١٠^{-٨} وعمره ٢٧ يوم مع استقراريه وتكرارية جيدة، لتقدير السلفاميثواوكسازول في المستحضرات الصيدلانية. كذلك تم دراسة التدخلات لحساب معامل الانتقائية بطريقة المحاليل المنفصلة وطريقة المحاليل الممزوجة بوجود الايونات و المواد التالية sucrose, starch, trimethoprim, Fe^{+3}, Al^{+3}, Cu^{+2}, Mn^{+2}, K^{+}, Na^{+} gelatin ودراسة حدود الدالة الحامضية وعمر القطب.