New Polymeric Membrane Electrode for Clarithromycin Determination

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Abstract

New polymeric membrane electrodes has been developed for the determination of Clarithromycin. The electrodes were constructed by incorporating the Clarithromycin-tetraphenylborate ion pair complex into a polyvinylchloride matrix plasticized by four plasticizers, Di-octyl phthalate (DOP); Di-butyl phosphate (DBP); Acetophenone (AP); Di-butyl phthalate (DBPH). These electrodes give sub- Nernstian slopes (51.206, 53.930, 58.104 and 58.484 mV/decade) and linear ranges from (1×10^{-5}-1×10^{-3}, 1×10^{-5}-1×10^{-3}, 5×10^{-5}-1×10^{-3} and 1×10^{-5} -1×10^{-3} M) respectively. The best electrode was based on DBPH plasticizer which gave a slope 58.484 mV/decade, correlation coefficient 0.9961, detection limit of 9×10^{-6} M, lifetime 20 day and displayed good stability and reproducibility and used to determine the Clarithromycin in pharmaceutical samples. The interferences measurements were studied using the separated method for selectivity coefficient determination. The pH and life time of the electrodes were also studied.

Keywords: Clarithromycin, Sodium tetraphenylborate, Ion selective electrode (ISE), membrane electrodes.

Introduction

Clarithromycin (CLM), 6-o-methyl-erythromycin or 4-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-a-L-ribohexopyranosyl)oxy]-14-ethyl-12,13-dihydroxy-7methoxy-3,5,7,9,11, 13-hexamethyl-6-[[3,4,6-trideoxy-3-(dimethyl amino)-b-D-xylo hexopyranosyl] oxy] oxacyclotetradecane-2,10-dione,with chemical formula of $C_{38}H_{69}NO_{13}$, as shown in Fig.(1), is white or almost white, crystalline powder with molecular weight 747.953 g/mole, practically insoluble in water, soluble in acetone and methylene chloride, and slightly soluble in methanol.[1] Clarithromycin is a semi-synthetic macrolide antibiotic with good antimicrobial activity against a wide range of gram-positive and gram-negative organisms . It is widely used for the treatment of Mycoplasmas, Haemophilus influenzae, Chlamydia species and Rickettsia [2,3].

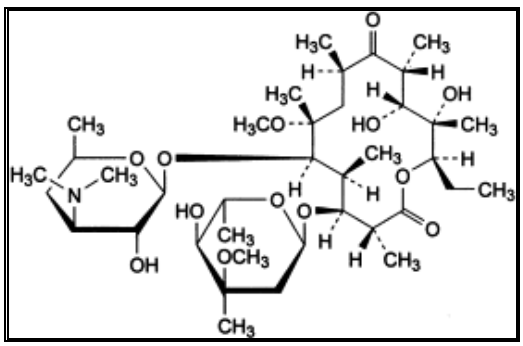


Fig.(1) Structure formula of clarithromycin.

Various analytical methods have been developed to determine clarithromycin in formulations and biological samples, such as spectrophotometric [4, 5], chromatographic [6-8], and HPLC with electrochemical detection[9, 10] methods. The applications of ion selective electrodes continue to be of interest in pharmaceutical analysis because these sensors offer the advantages of simple design and operation, reasonable selectivity, fast response, low cost and applicability to turbid and colored solutions. In this work the sensor is based on Clarithromycin and sodium tetraphenylborate as additive in polyvinyl chloride fabricated with different plasticizers was used for the determination of Clarithromycin in pharmaceutical samples, the properties of prepared electrodes, pH effect and selectivity, was studied.

Experimental

Apparatus

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

Calomel reference electrode were used in this work.

Chemicals and Reagents

Polyvinyl chloride (PVC), (Breon S 110/10 B.P Chemical U. K. Ltd). Clarithromycin was extracted from Claricide tablets according to the literature procedure[11]. Claricide tablets (containing 500 mg clarithromycin) were purchased from Bilim pharmaceuticals (made in Turkey). Sodium tetraphenylborate (NaTPB), molecular weight 342.22, was purchased from Fluka. (DBP), (DBPH), (DOP) and (AP) were purchased from Fluka AG, Switzerland, Tetrahydrofuran (E.Merck), Dichloromethane (density 1.325 g/mL) 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, $CuSO_4$, $MnSO_4$, $Fe_2(SO_4)_3.9H_2O$, $AlCl_3.6H_2O$, sucrose and gelatin were prepared by dissolving 0.2922, 0.3727, 0.7981, 0.7550, 2.81, 1.2071, 1.7115 and 1.50 g in 50 mL of distilled water respectively. A standard solution of 0.01 M Sodium tetraphenylborate (NaTPB) was prepared by dissolving 0.1711 g of pure (NaTPB) in distilled water and completing the solution up to 50 mL.

A stock solution of 1×10^{-3} M Clarithromycin was prepared by dissolving 0.0374 g of pure (CLM) in acetonitrile and water in proportion (1:3) and completing the solution up to 50 mL with the same solvent. The working solutions 10^{-8}-10^{-3} M CLM were prepared by serial appropriate dilution of the stock solution using the same solvent. Stock solutions of 0.1 M of HCl and 0.1 M of NaOH are used for adjusting pH of the solutions.

Procedure

Extraction of Clarithromycin

A quantity of the powdered tablets containing 0.5 g of Clarithromycin was shaken with 10 ml of water and extracted with 20 ml of dichloromethane. The lower dichloro-methane layer was separated and centrifuged, Then the supernatant was filtered and evaporated to dryness[11].

Preparation of membrane

The ion pair was prepared by mixing equal volume of 0.01 M solution of tetraphenylborate with an equimolar solution of CLM dissolved in acetone, The precipitate formed immediately after addition of a few drops of concentrated hydrochloride acid. Then 0.04 g of ionophore was mixed with 0.36 g of plasticizer and 0.17 g of PVC powder; all were dissolved in 5 ml of THF with stirring until a clear viscous solution was obtained[12].

Construction of ion-selective electrodes

The construction of the electrode body and the immobilization were done as described by Craggs et al. [13]. The glass tube was 3/4 filled with 10^{-3} M Clarithromycin 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 general 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^{-3}-10^{-8} M). The calibration curves were prepared by plotting the potential E versus log concentration by using computer program (Microsoft office Excel 2007).

Preparation of Pharmaceutical Samples

All contents of 4 tablets clarithromycin (500 mg) dissolved in 500 mL acetonitrile and then filtered and completing the solution up to 2L with distilled water, the resultant solution is 1.33×10^{-3} M. Other samples prepared by serial dilution.

Calculation of Selectivity coefficient

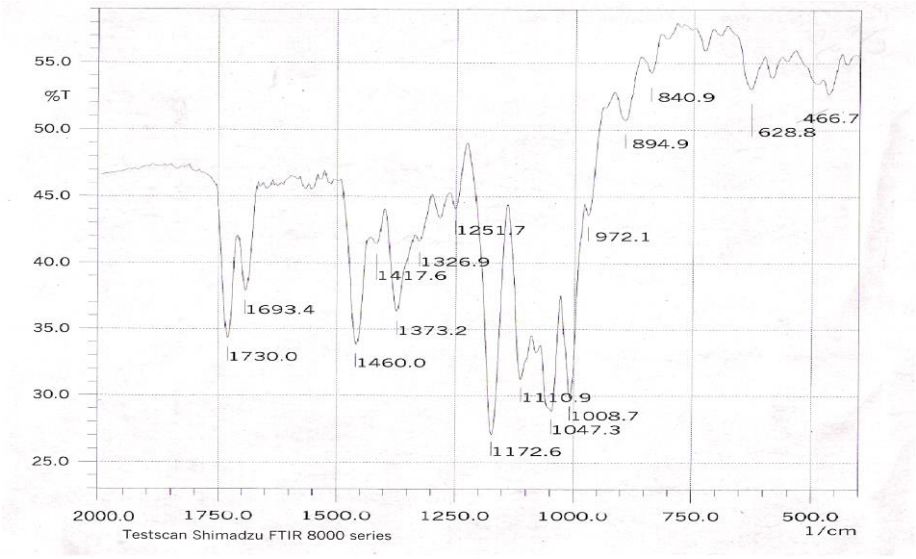
A separate solution method [14] was used for the selectivity coefficient measurement, which calculated according to the equation:

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

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 $a_A = a_B$.

Results and Discussion

Clarithromycin is not available in the local market in the pure form. It is exorbitantly costly and is available in very small amounts only. Therefore, it was extracted by simple laboratory techniques. The FTIR spectrum of the extracted clarithromycin was compared with the reference spectrum of clarithromycin [15], Fig.(2- a and b). The spectrums show a good purity.



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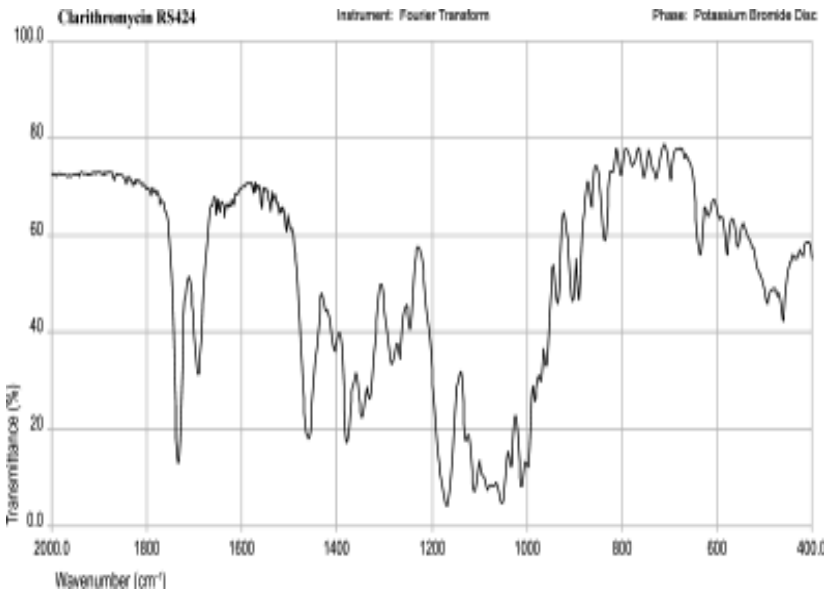

-b-

Fig.(2) a-FTIR spectrum of extracted clarithromycin, b-FTIR reference spectrum of pure clarithromycin.

Four electrodes of clarithromycin (CLM) (E1, E2, E3, E4) based on using clarithromycin (CLM) and tetraphenylborate (TPB) as additive, used four plasticizers: Di-octyl phthalate (DOP); Di-butyl phosphate (DBP); Acetophenone (AP); Di-butyl phthalate (DBPH); with PVC matrix were examined respectively.

Sub-Nernstian slopes were obtained for electrodes based on DOP, DBP and AP (membranes E1, E2 and E3). The slopes are 51.206, 53.930 and 58.104 mV/decade with correlation coefficients of 0.998, 0.9994 and 0.9933 respectively. The linear range for these electrodes 1×10^{-5}-1×10^{-3}, 1×10^{-5}-1×10^{-3} and 5×10^{-5}-1×10^{-3}M with detection limits of 8×10^{-6}, 6×10^{-6} and 2×10^{-5} M, respectively.

The results and other parameters are given in Table (1). The electrodes gave different non-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 [16].

The sensor (E4) displays a linear response from 10^{-5} to 10^{-3} M (CLM) with Nernstian cationic slope of 58.484 mV/decade with lower limit of detection of 9×10^{-6} M, which was calculated at the point of intersection of the extrapolated segments of the two linear parts of the calibration curve of (CLM). Electrode (E4) gave high slope value because the high mixing between the (DBPH) 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 DOP, DBP, AP and DBPH are shown in Fig.(3).

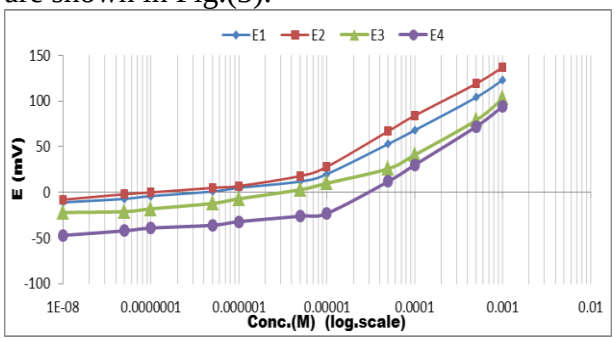

Fig.(3) Calibration curves of Clarithromycin selective electrodes using DOP, DBP, AP and DBPH plasticizer.

Table (1)
The parameters for four (CLM) electrodes.

Electrode	*Slope (mV/Decade)*	*Linear equation*	*Correlation coefficient (r)*	*Linear concentration range (M)*	*Detection limit (M)*	*Response time (sec)*			*Lifetime (day)*
						1×10^{-3} (M)	*5×10^{-4} (M)*	*1×10^{-4} (M)*	
E1 CLM+TPB+ DOP	**51.206**	**y = 22.239ln(x) + 274.35**	**0.998**	1×10^{-5}-1×10^{-3}	8×10^{-6}	30	25	19	24
E2 CLM+TPB+ DBP	**53.930**	**y = 23.422ln(x) + 298.43**	**0.9994**	1×10^{-5}-1×10^{-3}	6×10^{-6}	35	30	21	12
E3 CLM+TPB+ AP	**58.104**	**y = 25.235ln(x) + 274.36**	**0.9933**	5×10^{-5}-1×10^{-3}	2×10^{-5}	41	35	32	45
E4 CLM+TPB+ DBPH	**58.484**	**y = 25.4ln(x) + 266.28**	**0.9961**	1×10^{-5}-1×10^{-3}	9×10^{-6}	36	30	24	20

Effect of pH:-

The effect of pH on the electrode potentials for (CLM) selective membrane electrode (E4) was examined by measuring the potential of the cell in (CLM) solutions at three different concentrations (1×10^{-3}, 5×10^{-4}, 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).

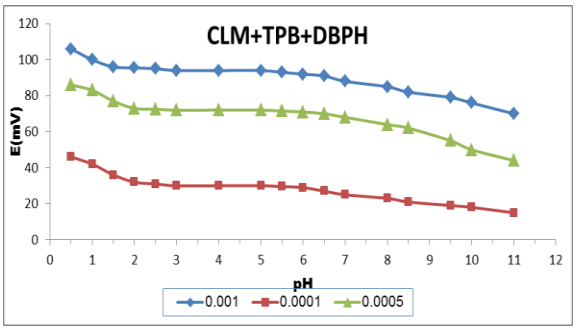

Fig.(4) Effect of pH on the potential of the electrode E4 at concentrations 1×10^{-3}, 5×10^{-4} and 1×10^{-4}M.

At pH values less than 1.5 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 CLM 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 CLM.[17] The working pH were tabulated in Table (2).

Table (2)
Working pH ranges for (CLM) electrode (E4).

Electrode no.	Composition of electrode E4	PH range		
		1×10^{-3} (M)	5×10^{-4} (M)	1×10^{-4} (M)
E4	CLM+TPB+DBPH	1.5-6.5	2.0-6.5	2.0-6.3

Interference studies

In order to investigate the selectivity of the proposed membrane (E4) ion selective electrode toward clarithromycin with respect to various interfering ions by using separate solution method. The values of the selectivity coefficients for separate method are listed in Table (3).

Table (3)
Values of $K^{pot}_{A,B}$ according to separate method by using electrode E4.

Interfering ions	$K^{pot}_{A,B}$
k^{+}	3.66×10^{-2}
Na^{+}	4.28×10^{-2}
Fe^{+3}	3.25×10^{-4}
Al^{+3}	3.01×10^{-4}
Cu^{+2}	1.20×10^{-3}
Mn^{+2}	1.11×10^{-3}
Sucrose	3.12×10^{-2}
Gelatin	3.25×10^{-2}

Sample analyses:-

Two potentiometric techniques were used for the determination of (CLM) 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.

The recovery (Re %), relative error (E_r %) and relative standard deviation (RSD %) for each method are calculated and the results are listed in Table (4). The electrode (E4) was proved to be useful in the potentiometric determination of clarithromycin in pharmaceutical preparations and the data obtained for pharmaceutical samples were listed in Table (5).

Table (4)
Analysis of CLM by potentiometric techniques by using ISE E4.

Parameter	Direct method	SAM
Conc.(M)	1.000×10^{-4}	1.000×10^{-4}
Found(M)	0.988×10^{-4}	0.997×10^{-4}
RSD*%	0.906%	0.663%
Re%	98.84%	99.68%
Er%	-1.16%	-0.32%

Table (5)
Analyses of clarithromycin in pharmaceutical samples.

Parameter	Direct method	SAM
Conc.(M)	1.000×10^{-4}	1.000×10^{-4}
Found(M)	0.988×10^{-4}	0.992×10^{-4}
RSD*%	0.642%	0.666%
Re%	98.76%	99.18%
Er%	-1.24%	-0.82%
S	6.348×10^{-6}	6.610×10^{-6}
$x \pm (ts/\sqrt{N})$	0.988×10^{-4} $\pm0.766\times10^{-6}$	0.992×10^{-4} $\pm0.798\times10^{-6}$

RSD*% for n=5, t=2.7

Conclusions

ISE method included fabrication of membranes for clarithromycin was constructed based on using clarithromycin (CLM) and sodium tetraphenylborate (NaTPB) as additive and many plasticizers. The best electrode for CLM was (E4) electrode, which used to determine CLM 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.

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الخلاصة

تم تطوير أقطاب غشائية بوليمرية لتقدير الكلاريثرومايسين. حضرت الأقطاب من خلال دمج معقد المزدوج الايوني (كلاريثرومايسين–تيترافنيل بوريت) داخل غشاء بوليمر كلوريد الفاينيل ولدنت بأربعة ملدنات، داي اوكتيل فثاليت، داي بيوتيل فوسفيت، أسيتوفينون، داي بيوتيل فثاليت على التوالي. اعطت هذه الأقطاب انحدارا شبه نيرنيستي ٥١,٢٠٦، ٥٣,٩٣٠، ٥٨,١٠٤، ٥٨،٤٨٤ ملي فولت/ حقبة ومدى التركيز الخطى حوالي من ١٠$^{-٥}$ الى ١٠$^{-٣}$. وكان أفضل قطب الذي يعتمد على داي بيوتيل فثاليت كملدن حيث اعطى انحدارا ٥٨,٤٨٤، معامل ارتباط ٩٩٦١، حد كشف 9×10^{-6} وعمره ٢٠ يوم مع استقراريه وتكرارية جيدة، لتقدير الكلاريثرومايسين في المستحضرات الصيدلانية. كذلك تم دراسة التداخلات لحساب معامل الانتقائية بطريقة المحاليل المنفصلة ودراسة حدود الدالة الحامضية وعمر القطب.