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Construction and study of new polymeric membrane electrodes for clarithromycin determination

A Thesis submitted to the College of Science of Al-Nahrain University in partial fulfillment of the requirements for the Degree of Master of Science in Chemistry

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بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

يَرۡفَعِ ٱللَّهُ ٱلَّذِينَ ءَامَنُواْ مِنكُمۡ
وَٱلَّذِينَ أُوتُواْ ٱلۡعِلۡمَ دَرَجَٰتٖۚ وَٱللَّهُ
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الإهداء

إلى الذي لم يأل جهدا في تربيتي منذ الصغر ..... أبي الغالي

إلى التي نذرت شبابها من اجلي ..... أمي الغالية

إلى من غرست في نفسي الامل الى القلب الكبير ..... زوجتي الغالية

إلى شمسي وضياء نهاري ..... ولدي

إلى من هم بلسم جراحي ..... أخوتي وأخواتي

إلى كل من فرح بي وشجعني على هذه الدراسة ..... الاصدقاء والأقارب

إليهم جميعا اهدي ثمرة جهدي عرفانا بفضلهم .....

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Summary

This piece of research includes constructing and characterizing two kinds of ion-selective electrodes (ISEs) based on PVC matrix membrane. **First**, four ion-selective electrodes for clarithromycin (CLM) which based on clarithromycin-tetraphenylborate (CLM-TPB) ion-pair complex as the electro-active materials were prepared. **Second**, four ion-selective electrodes for clarithromycin which based on using clarithromycin-tetraiodomercurate (CLM-TIM) ion-pair complex as the electro-active materials were also prepared. In both kinds of ISEs, some of the selected plasticizers were employed such as; Di-octyl phthalate (DOP), Di-butyl phosphate (DBP), Acetophenone (AP) and Di-butyl phthalate (DBPH) in PVC matrix.

This thesis has mainly been structured in three different chapters, each one containing the following information:

Chapter one provides a short historical review with the analytical performance characteristics of ISEs are described. The applications of ISEs in pharmaceutical and clarithromycin analyses are well-arranged in tables and the general and specific objectives of thesis are reported.

Chapter two corresponds to the experimental part. Reagents, instruments, procedures and detail protocols for the preparation of two kinds of ISEs used in this study are reported.

Chapter three contains the experimental results and discussion that lead to the possibility of successful applications the constructed ISEs in pharmaceuticals preparation and clarithromycin measurements. It is reporting the construction of two kinds of clarithromycin ISEs;

The first kind ISEs were: CLM-TPB+DOP (E1), CLM-TPB+DBP (E2), CLM-TPB+AP (E3) and CLM-TPB+DBPH (E4), give the linear range 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), the slopes of (51.206, 53.930, 58.104 and 58.484 mV/decade) respectively, with detection limits of (8×10^{-6}, 6×10^{-6}, 2×10^{-5} and 9×10^{-6} M), response time of 10^{-3} M (30, 35, 41 and 46 second) and the lifetime were about (24, 30, 12 and 20 days) respectively.

The second, were: CLM-TIM+DOP (E5), CLM-TIM+DBP (E6), CLM-TIM+AP (E7) and CLM-TIM+DBPH (E8), give the linear range from (1×10^{-5}-1×10^{-3}, 5×10^{-5}-1×10^{-3}, 5×10^{-5}-1×10^{-3} and 1×10^{-5}-1×10^{-3} M), the slopes of (48.445, 42.970, 52.692 and 49.442 mV/decade) respectively, with detection limits of (5×10^{-6}, 5.5×10^{-5}, 5×10^{-5} and 1.5×10^{-5} M), response time of 10^{-3} M (26, 32, 30 and 48 second) and the lifetime were about (28, 22, 16 and 25 days) respectively.

The best electrode is (E4) used to determine the clarithromycin in pure and pharmaceuticals samples. The working pH for (E4) electrode was ranged from (1.5-6.5), the selectivity coefficients ($K^{pot}_{A,B}$) of ISEs for the CLM have been studied in the presence of interference ions (Na^{+}, K^{+}, Mn^{+2}, Cu^{+2}, Fe^{+3}, Al^{+3}, sucrose and gelatin) by using separated solution and fixed interfering methods and the results was ranged from (4.28×10^{-2}-8.82×10^{-5}), which revealed that there was no effect of the interferences on the determination of CLM in tablets by using the constructed ISE. Therefore the ISE (E4) gave a good electrochemical characterization among the others and it has been used successfully for the determination of clarithromycin in the Claricide tablets using different potentiometric methods.

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List of abbreviation

AP	Acetophenone
CLM	Clarithromycin
CST	stock unit of viscosity gm/sec.cm.density
DBP	Dibutylphosphate
DBPH	Dibutylphthalate
DOP	Dioctylphthalate
e.m.f	Electromotive force
$E_{rel.}$	Relative error
F.W.	Formula weight
FIM	Fixed interference method
FPM	Fixed primary ion method
FTIR	Fourier transform infrared spectroscopy
HPLC	High performance liquid chromatography
ISE	Ion-selective electrode
LC	liquid chromatography
MPM	Match potential method
MSA	Multi Standard addition method
mV	Millivolt
PVC	Poly vinyl chloride
Rec.	Recovery
RSD	Relative standard deviation
δ	Standard deviation
SAM	Standard addition method
SCE	Saturated calomel electrode
SHE	Standard hydrogen electrode
Std.	Standard
THF	Tetrahydrofuran
TIM	Potassium tetraiodomercurate
TPB	Sodium tetraphenylborate
TSM	Two solution method

Chapter One

Introduction

Introduction

1-1-Ion selective electrodes (ISEs):-

Ion-Selective Electrodes (ISEs) are part of a group of relatively simple and inexpensive analytical tools which are commonly referred to as Sensors, based on thin films or selective membranes as recognition elements.

ISE is an electrochemical half-cell equivalent to other half-cells of the different kinds. These devices are distinct from systems that involve redox reactions, although they often contain a second kind electrode as the "inner" or "internal" reference electrode. The potential difference response has, as its principal component, the Gibbs energy change associated with perm selective mass transfer (by ion-exchange, solvent extraction or some other mechanism) across a phase boundary.

The ion selective electrodes must be used in conjunction with a reference electrode (i.e. "outer" or "external" reference electrode) to form a complete electrochemical cell. The measured potential differences (ISE versus outer reference electrode potentials) are linearly dependent on the logarithm of the activity of a given ion in solution [1]. The pH electrode is the most well-known and simplest member of this group and can be used to illustrate the basic principles of ISEs [2].

1-2- The advantages of (ISEs) [3]:-

1- Ion-Selective Electrodes are relatively inexpensive and simple to use and have an extremely wide range of applications and wide concentration range.

2- The most recent plastic-bodied all-solid-state or gel-filled models are very strong and permanent and ideal for use in either field or laboratory environments.

3- Under the most favorable conditions, when measuring ions in relatively dilute aqueous solutions and where interfering ions are not a problem, they can be used very rapidly and easily(e.g. simply dipping in lakes or rivers, dangling from a bridge or dragging behind a boat).

4- They are particularly useful in applications where only an order of magnitude concentration is required, or it is only necessary to know that a particular ion is below a certain concentration level.

5- They are invaluable for the continuous monitoring of changes in concentration: e.g. in potentiometric titrations or monitoring the uptake of nutrients, or the consumption of reagents.

6- They are particularly useful in biological/medical applications because they measure the activity of the ion directly, rather than the concentration.

7- In applications where interfering ions, pH levels, or high concentrations are a problem, then many manufacturers can supply a library of specialized experimental methods and special reagents to overcome many of these difficulties.

8- With careful use, frequent calibration, and an awareness of the limitations, they can achieve accuracy and precision levels of $\pm$ 2 or 3% for some ions and thus compare favorably with analytical techniques which require far more complex and expensive instrumentation.

9- ISEs are one of the few techniques which can measure both positive and negative ions.

10- They are unaffected by sample color or turbidity.

11- ISEs can be used in aqueous solutions over a wide temperature range. Crystal membranes can operate in the range 0° C to 80° C and plastic membranes from 0° C to 50° C.

1-3-Limitation in ISE measurements:-

1-3-1-Diffusion [3]:-

Orion Research points out that difference in the rates of diffusion of ions based on size can lead to some error. In the example of sodium iodide, sodium diffuses across the junction at a given rate. Iodide moves much slower due to its larger size. To compensate for this type it is important that a positive flow of filling solution move through the junction.

1-3-2-Sample ionic strength [1,4]:-

The total ionic strength of a sample affects the activity coefficient and that it is important that this factor stay constant. This adjustment is large, compared to the ionic strength of the sample, such that variation between samples becomes small and the potential for error is reduced.

1-3-3- Temperature [1,5]:-

It is important that temperature be controlled as variation in this parameter can lead to significant measurement errors. A single degree (C) change in sample temperature can lead to measurement errors greater than 4%.

1-3-4- pH [3,6]:-

Some samples may require conversion of the analyte to one form by adjusting the pH of the solution. Failure to adjust the pH in these instances can lead to significant measurement errors.

1-3-5-Interferences [3,6]:-

The background matrix can affect the accuracy of measurements taken using ISE's. Covington was pointed out that some interference may be eliminated by reacting the interfering ions prior to analysis.

1-4- The applications of ISEs [3]:-

Ion-selective electrodes are used in a wide variety of applications for determining the concentrations of various ions in aqueous solutions. The following is a list of some of the main areas in which ISEs have been used.

1-4-1- Agriculture [7,8]:

a) Determination of nitrate, potassium, calcium and chloride in soils.
b) Analysis of additives in animal foodstuffs.
c) Analysis of plant materials for nitrate, potassium, chloride, fluoride, iodide, cyanide and calcium.
d) Measurement of nitrate content of fertilizers.

1-4-2- Biomedical and clinical laboratories [9,10]:

a) Determination of various species including calcium, potassium, chloride in serum, blood, plasma and other body fluids. Electrodes are particularly suitable as they monitor ion activity which is considered to be more biologically significant than concentration.
b) Analysis of fluoride in skeletal structures.
c) Investigation of fluoride in dental studies.
d) Sweat chloride measurement as a screening test for cystic fibrosis.

1-4-3- Paper and pulp [3]:

a) Sulphide is measured at every stage of the pulping and recovery cycle in liquors, as well as in mill effluents.
b) Analysis of chloride in pulping liquors.

1-4-4-Pollution monitoring [11,12]:

Levels of cyanide, fluoride, sulphide and chloride can be measured in effluents, natural waters and waste-matters. The use of electrodes for continuous, unattended and trouble-free monitoring makes them increasingly suitable for pollution monitoring.

1-4-5-Detergent manufacture [13]:

The measurements of calcium, water hardness and barium can be used to study the effects of detergents on water quality.

1-4-6-Education and research [13]:

a) Electrodes of all types are being used as sensors in many experiments to study reaction mechanisms, kinetics, equilibria, activity coefficients and solubility.
b) Electrodes are simple and inexpensive enough to be used by undergraduates as part of analytical chemistry training.
c) Electrodes are particularly suitable for nuclear applications since they are unaffected by radiation and can be remotely operated. Fluoride finds wide application in fuel reprocessing solutions.

1-4-7-Explosives [3]:

Fluoride, chloride and nitrate have been measured in explosives and their combustion products.

1-4-8-Food processing [3,14]:

a) Determination of nitrate and nitrate in meat preservatives.
b) Determination of salt content of meat, fish, milk, dairy products, fruit juices, beer and brewing water.
c) Analysis of fluoride in drinking water, mineral drinks, fish protein, tea, beer and brewing water.
d) Measurement of calcium in milk and dairy products and beer.
e) Determination of potassium in fruit juices and wine-making.
f) Monitoring the potential corrosive effect of nitrate in canned foods.
g) Determination of water hardness in brewing water.

1-4-9-Metallurgy and electroplating [3,13]:

a) Analysis of etching baths for fluoride and chloride.
b) Measurement of sulphate and aluminium in anodizing baths.
c) Monitoring of urinary fluoride concentrations in people involved in the extraction of aluminium.

1-5- Applications of ISEs in pharmaceutical drugs:-

The ion selective membranes are widely used for pharmaceutical analysis with advantages of determining sample directly, rapidly and simplicity. Table (1-1) shows some applications of ion selective electrodes in pharmaceuticals.

Table 1-1:-Some applications of ion selective electrodes in pharmaceuticals.

Pharmaceuticals	Membrane components	Linear rang (M)	Detection Limit(M)	Slope (mV/decade)	Ref.
Azithromycin	Azithromycin-tetraiodomercurate ion pair complex into a polyvinylchloride matrix plasticized by nitrobenzene.	1×10^{-2}-7×10^{-6}	5×10^{-6}	29.8	15
Ketoconazole	Ketoconazole-tetraphenylborate ion pair complex into NPOE as plasticizer.	6.3×10^{-3}-7.1×10^{-6}	5×10^{-6}	72	16
Fluconazole	Fluconazole ion pair complex by use dibutyl phthalate as plasticizer.	5×10^{-5}–5×10^{-2}	4×10^{-5}	57.0	17
Methacycline Hydrochloride	Methacycline-tetraphenylborate as the electro active substance and di-octyl phthalate as the plasticizing.	3×10^{-2}-6×10^{-6}	3.4×10^{-6}	52.9	18
Clozapine	Clozapine-phosphotungstate ion pair complex by use di-octyl sebacate as plasticizer.	1×10^{-5}–1×10^{-2}	3.7×10^{-6}	57.46	19
Cimetidine	The electrode incorporates PVC-membrane with cimetidine-phospohotungstate ion pair complex.	1×10^{-5}–1×10^{-2}	5×10^{-6}	58	20
Tramadol	Tramadol-tetraphenyl borate complexes by use dibutyl phosphate as plasticizer.	1×10^{-5}-1×10^{-1}	3×10^{-5}	58.06	21
Ketamine	Ketamine hydrochloride has been constructed using a modified PVC membrane plasticized with (NPOE).	1×10^{-5}-1×10^{-2}	5×10^{-6}	59	22
Diclofenac	The diclofenac complex with hexa-decylpyridinium bromide was plasticized with di-butyl phthalate.	1×10^{-5}-1×10^{-2}	4×10^{-6}	59	23

Pharmaceuticals	Membrane components	Linear rang (M)	Detection Limit(M)	Slope (mV/decade)	Ref.
Nimesulide	Nimesulide - molybdophosphoric acid as the electro-active material in PVC matrix in presence of bis(2-ethyl hexyl) phthalate (BEP) as a plasticizer.	1×10^{-6}–1×10^{-2}	3.2×10^{-7}	55.6	24
Promethazine Hydrochloride	Based on promethazine-Molybdophosphoric acid ion pair in PVC matrix.	1×10^{-1}–1×10^{-5}	6×10^{-6}	57.27	25
Chloramphenicol Sodium Succinate	Based on chloramphenicol palmitate and sodium tetraphenyl borate in PVC matrix.	1×10^{-1}–1×10^{-4}	5×10^{-5}	53.98	26
Ibuprofen	Based on β-cyclodexterin as an potential ionophore incorporated in to poly(vinylchloride) (PVC).	2×10^{-3}–3×10^{-8}	5.01×10^{-9}	58.8	27
Cyproheptadine HCL	Prepare ion pair with tetrakis(4-chloro phenyl borate by use this plasticizer o-NPOE.	1×10^{-2}-3.5×10^{-4}	4.8×10^{-5}	58.2	28
cefditoren pivoxil	Coated wire electrode based on ion exchangers; phosphor-molybdic acid, phosphotungstic acid and a mixture of both.	1×10^{-2}–1×10^{-7}	1.48×10^{-9} 5.01×10^{-8} 5.01×10^{-8}	56.29 54.60 58.17	29
Chlorprothixen	Prepare ion pair with sodium tetraphenylborate by use o-NPOE as plasticizer.	1×10^{-2}-6.3×10^{-4}	1.5×10^{-5}	53	30
Cetylpyridinium chloride	Prepare ion pair with Ferric thiocynate and use di-octylphthalate as plasticizer.	1×10^{-3}–1×10^{-6}	8×10^{-7}	57.5	31
Scopolamine	$(Scopolamin)_3$-tungstophosphoric acid by use o-NPOE as plasticizer.	1×10^{-2}–1×10^{-6}	8×10^{-7}	54.5	32

Pharmaceuticals	Membrane components	Linear rang (M)	Detection Limit(M)	Slope (mV/decade)	Ref.
Dextrometho-rphan	Dextromethorphan-molybdophosphoric acid as the electro-active substance and dioctylphtalate as the plasticizing.	2×10^{-6}–1×10^{-1}	1×10^{-6}	59.5	33
Amiloride	Amiloride-molybdophosphoric acid as an active material based on dibutyl phthalate as plasticizer.	1×10^{-4}-1×10^{-1}	2.5×10^{-6}	58.5	34
Tiapride	Tiapride-tetraphenylborate	1×10^{-5}-1×10^{-2}	4.3×10^{-6}	57.5	35
Atenolol	Atenolol-phosphotungstate as an active material by used dioctyl phthalate as plasticizer.	3×10^{-4}-5×10^{-2}	5×10^{-5}	55.91	36
Acebutolol	Acebutolol-tetraphenylborate	1×10^{-3}-1×10^{-6}	6×10^{-6}	51.5	37

1-6- Ion selective electrode cell measurements:-

The cell consists of both an indicator and reference electrode. Since the potential of the reference electrode is constant, the potential developed at the indicator electrode that contains information about the amount (activity) of analyte in a sample. An electrochemical sensor based on a thin selective membrane or film as recognition element is an electrochemical half-cell equivalent to other half-cells of the zeroth (inert metal in a redox electrolyte).

The components of electrochemical cell are shown in Fig. 1-1. The potential developed at the membrane is the result of either an ion exchange process or an ion transport process occurring at each interface between the membrane and solution.

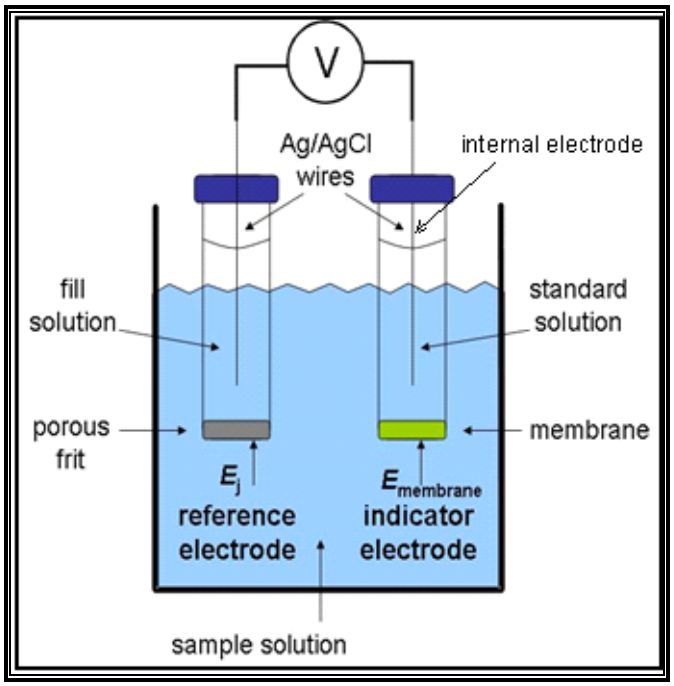


Figure 1-1:- A classical ion selective electrode in electrochemical cell [38].

Generally, the cell contains two reference electrodes, "internal" and "external", and a selective membrane as the recognition element.

However, besides this conventional type of the cell with solution contact on both sides of the membrane there are ISE cell arrangements with wire contact to one side of the membrane. Conventional notation of the cell is [38]:-

External ref. │ test solution │ membrane │ internal solution │ internal ref.

The measured cell e.m.f, E is described with the Nernst equation [39]:-

$$\mathbf{E = E^0 - (RT/nF) \ln a} \qquad \mathbf{\ldots 1\text{-}1}$$

$$\mathbf{E = E^0 - (2.303RT/nF) \log a} \qquad \mathbf{\ldots 1\text{-}2}$$

Where E^0 = constant for a given cell, E = the total potential developed between the sensing and reference electrode (mV), R = gas constant (8.314 joule $mole^{-1}deg^{-1}$), T= temperature in Kelvin (298ºK or 25^0C), n = ionic charge, F = faraday constant (96485 coulombs), a = is the ion activity. At room temperature (25^0C) Nernst equation is frequently expressed as:-

$$\mathbf{E = E^0 - (59.2 / n) \log a} \qquad \mathbf{\ldots 1\text{-}3}$$

Cell design according to the basic rule of designing of electrolytical cells, with a condition that the current passed through the electrolytical cell equals zero, as showed in Fig. 1-1. The exchange that occurs between the internal and external solution across the membrane depends on ionic exchange and the active ionophore which used in the membrane [40].

$$\mathbf{E_{total} = E^0 + E_{junction} - E_{membren}} \qquad \mathbf{\ldots 1\text{-}4}$$

1-7- Classification of ion-selective electrodes [1]:-

1-7-1- Primary ion selective electrodes:

1-7-1-1-Crystalline electrodes:

May be homogeneous or heterogeneous:

(a) Homogeneous membrane electrodes are ion-selective electrodes in which the membrane is a crystalline material prepared from either a single compound or a homogeneous mixture of compounds (i.e., Ag_2S, $AgI + Ag_2S$).

(b) Heterogeneous membrane electrodes are formed when an active substance or mixture of active substances, is mixed with an inert matrix, such as silicone rubber or polyvinyl chloride (PVC), to form the sensing membrane.

1-7-1-2-Non-crystalline electrodes:

In these electrodes, the ion-selective membrane consists of a matrix containing an ion-exchanger [see (a) and (b) below) and is usually interposed between two aqueous solutions. The matrix may be porous (e.g., cellulose ester) or nonporous (e.g., glass or inert polymeric material such as PVC).

(a) Glass electrodes [41,42]:

Are ion-selective electrodes in which the sensing membrane is a thin piece of special glass. The chemical composition of the glass determines the selectivity of the membrane. In this group are:

(i) *hydrogen ion-selective electrodes,*

(ii) *monovalent cation-selective electrodes.*

A common form of the glass membrane electrode is depicted in Fig. 1-2. In use for measurement of pH, the glass membrane separates two liquid phases. One is inside the electrode as shown and it is typically 0.1 F hydrochloric acid.

The other is the test solution into which the electrode is dipped. Two reference electrodes are employed, one in each of the two solutions.

The difference in potential between the two reference electrodes is measured by means of an electronic voltmeter, the read-out device of which is commonly calibrated directly in pH units.

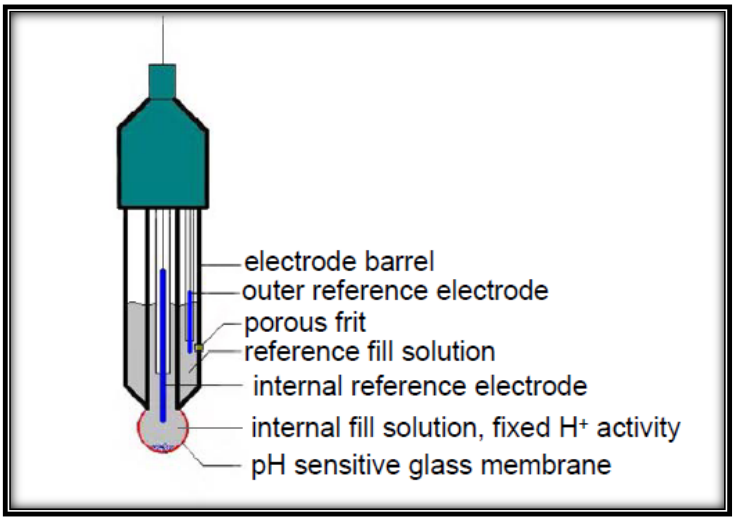


Figure 1-2: a glass pH electrode.

(b) Electrodes with mobile charged sites [43]:

(i) ***Positively charged hydrophobic cations*** (e.g., quaternary ammonium salts or salts of transition metal complexes such as derivatives of 1,10-phenanthroline), which, dissolved in a suitable organic solvent and held on an inert support (e.g., cellulose ester or PVC), provide membranes that are sensitive to changes in the activities of anions.

(ii) ***Negatively charged hydrophobic anions*** (e.g., of type $(RO)_2PO_2^-$) or bulky anions (e.g., tetrakis (p-chlorophenyl) borate), which, when dissolved in a suitable organic solvent and held in an inert support (e.g., cellulose ester or PVC), provide membranes that are sensitive to changes in the activities of cations.

(iii) ***Uncharged 'carrier' electrodes*** **based on solutions of molecular complexing agents** (e.g., antibiotics, macrocyclic compounds or other sequestering agents), which can be used in membrane preparations to give selectivity to certain cations.

(iv) ***Hydrophobic ion-pair electrodes*** **consisting of a dissolved hydrophobic ion-pair in plasticized PVC** (e.g., a cation drug tetra-phenyl borate, or a tetra-alkyl ammonium surfactant anion) responding to component activities in the solution (e.g., containing the cation drug chloride or sodium salt of the surfactant).

1-7-2- Compound or multiple membrane (multilayer) ion selective electrodes:-

1-7-2-1- Gas sensing electrodes [44,45]:

Gas sensing electrodes as shown in Fig. 1-3; respond to dissolved gases in solution. They have a plastic body that is usually made of poly tetra-fluorethylene. The dissolved gas diffuses across the membrane into a small volume of buffer. The reaction of the gas with the buffer causes a pH change which is sensed by an internal glass pH electrode. Carbon dioxide and ammonia are among the species measured by gas sensing electrodes.

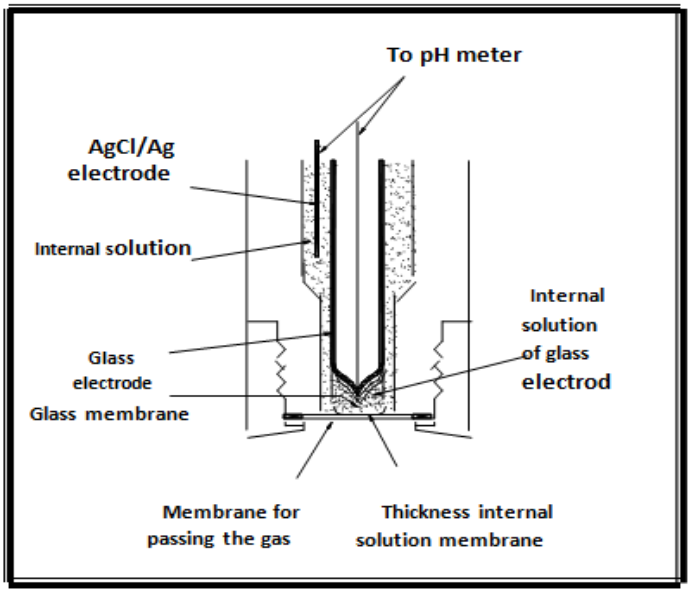


Figure 1-3:- A gas sensing electrodes type [45].

1-7-2-2- Enzyme electrodes [46]:-

Enzyme electrodes definitely are not true ion selective electrodes but usually are considered within the ion specific electrode topic. Such an electrode has a double reaction mechanism, an enzyme reacts with a specific substance, and the product of this reaction (usually ammonia or carbon dioxide) is detected by a true ion selective electrode, such as a pH selective electrode. All these reactions occur inside a special membrane which covers the true ion selective electrode. An example is glucose selective electrodes.

1-8- Reference electrodes [47]:-

Reference electrodes are applicable in instances where the electrical potential is to be imposed or measured in a solution. Also, it has a stable and well defined electrochemical potential against which the applied or measured potentials in an electrochemical cell are referred, Reference electrode is necessary to complete electrochemical cell. It is preferable to use a double-junction reference electrode for ISE applications. Standard reference half cells have KCl based electrolyte filling solutions, as shown in Fig. 1-4. This is a distinct disadvantage when, for example, potassium or chloride is being measured. To overcome this, a double junction reference is used in which the escaping KCI is retained in a second chamber containing a non-interfering electrolyte, which in turn escapes into the test solution at the second junction.

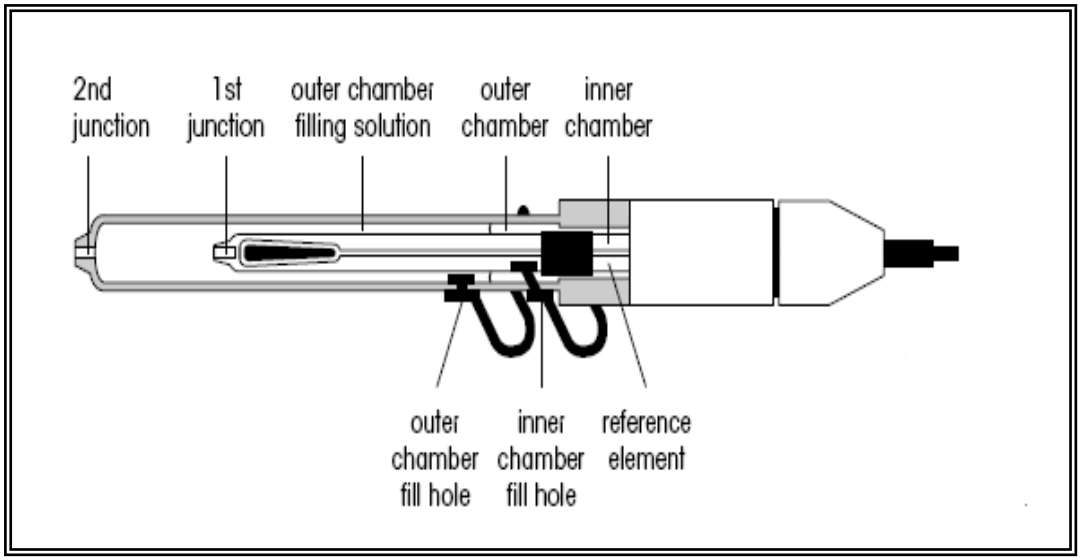


Figure 1-4: Double junction reference electrode.

Mostly electrodes of the second kind are used as reference electrodes. On account of its environmental compatibility, the $Ag/AgCl/Cl^{-1}$ electrode is preferably used. The $Hg/Hg_2Cl_2/Cl^{-1}$ (calomel) electrode, introduced by Ostwald as early as1894, has advantages with regard to potential stability and the influence of light, but it is only applicable up to temperatures of about 80 °C. This electrode is commercially available, while, in contrast, the $Tl(Hg)/TlCl/Cl^{1-}$ system, the Thalamid electrode, has been banned because of its toxicity [48].

1-9- Characterization of ISEs

1-9-1- Selectivity [49-51]:-

Selectivity of ion selective electrodes is quantitatively related to equilibria at the interface between the sample and the electrode membrane. Moreover, they are also required for the optimization of ionophore structures and membrane compositions. For example, errors arise when the response to a weakly interfering ion is also influenced by the primary ion leaching from the membrane.

Wrong selectivity coefficients may be also obtained when the interfering agent is highly preferred and the electrode shows counter ion interference. A detailed recipe to determine correct potentiometric selectivity coefficients unaffected by such biases is presented. The potentiometric selectivity coefficient is expressed according to the Nicolsky-Eisenman equation as:

$$\mathbf{E = E^0 + R\,T / Z_A F \ln [a_A + \Sigma K_{A,B} (a_B)^{Za/Zb}]} \qquad \mathbf{\ldots 1\text{-}5}$$

Where E is the measured potential; E^0 is a constant that includes the standard potential of the electrode, the reference electrode potential, and the junction potential; (z_A, z_B, a_A and a_B are the charge numbers and activities of the primary ion, A, and the interfering ion, B respectively); and $K_{A,B}$ is the potentiometric selectivity coefficient for the primary ion A against the interfering ion B. This selectivity coefficient can be determined using either separate solutions or match solutions method, containing both the analyte A, and the interfering B ions. Potentiometric selectivity coefficients can be measured with different methods that fall into two main groups:

1-9-1-1- Separate solution methods:-

1-9-1-1-1- When ($a_A = a_B$) [52]:-

The potential of a cell comprising an ion selective electrode and a reference electrode is measured with two separate solutions, one containing the ion A at the activity a_A (but no B), the other one containing the ion B at the same activity $a_A = a_B$ (but no A). If the measured values are E_A and E_B, respectively, the value of $K^{pot}_{A,B}$ is calculated from the equation:

$$\mathbf{\log K^{pot}_{A,B} = (E_B - E_A)\, Z_A\, F / R\,T \ln 10 + (1\text{-}z_A/z_B) \log a_A} \qquad \mathbf{\ldots 1\text{-}6}$$

or for any electrode in general, where (Z_A F/ R T ln10) =1/S

$$\log K^{pot}_{A,B} = (E_B - E_A)/S + (1 - z_A/z_B)\log a_A \quad \ldots 1\text{-}7$$

Where (S) is the slope of the electrode.This method is recommended only if the electrode exhibits a Nernstian response. It is less desirable because it does not represent as well the actual conditions under which the electrodes are used.

1-9-1-1-2- When ($E_A = E_B$)[53]:-

The potential of an ISE for the primary and interfering ions are obtained independently. Then, the activities that correspond to the same electrode potential value are used to determine the $K^{pot}_{A,B}$ value and it equal:

$$K^{pot}_{A,B} = a_A / (a_B)^{Za/Zb} \quad \ldots 1\text{-}8$$

1-9-1-2- Mixed solution methods:-

1-9-1-2-1- Fixed interference methods (FIM) [54,55]:-

The potential of a cell comprising an ion selective electrode and a reference electrode is measured with solution of constant level of interference, a_B, and varying activity of the primary ion, a_A.

The potential values obtained are plotted vs the activity of the primary ion as shown in Fig. 1-5. The intersection of the extrapolation of the leaner portions of this curve will indicate the values of a_A which are to be used to calculate $K^{pot}_{A,B}$ from the equation:

$$K^{pot}_{A,B} = a_A / (a_B)^{Za/Zb} \quad \ldots 1\text{-}9$$

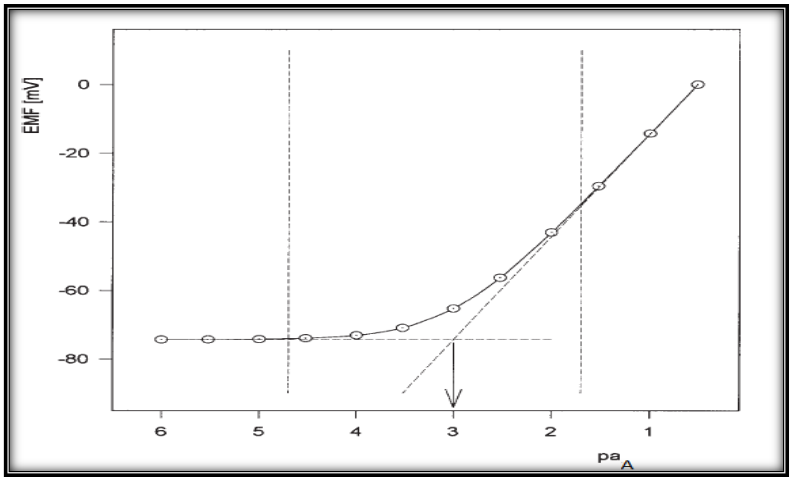


Figure 1-5:- Determination of a_A value according to FIM [56].

1-9-1-2-2- Fixed primary ion method (FPM) [57,58]:-

The e.m.f of the cell comprising and a ion selective electrode and reference electrode (ISE cell) is measured for solutions of constant activity of the primary ion, a_A and a varying activity of the interfering ion, a_B. The e.m.f. values obtained are plotted vs. the logarithm of the activity of the interfering ion. The intersection of the extrapolated linear portions of this plot indicates the value of a_B that is to be used to calculate $K^{pot}_{A,B}$ from the equation 1-8.

1-9-1-2-3- Two solutions method (TSM) [57,53]:-

This method involves measuring potentials of a pure solution of the primary ion, E_A, and a mixed solution containing the primary and interfering ions E_{A+B}. The $K^{pot}_{A,B}$ is calculated by inserting the value of the potential difference, $\Delta E=E_{A+B} -E_A$, into the following equation:

$$\mathbf{K^{pot}_{A,B} = a_A (e^{\Delta E\, zA\, F / (R\, T)} - 1) / (a_B)^{Za/Zb}} \qquad \textbf{... 1-10}$$

1-9-1-2-4- Matched potential method (MPM) [53,59]:-

A theory is presented that describes the matched potential method (MPM) for the determination of the potentiometric selectivity coefficients $K^{pot}_{A,B}$ of ion-selective electrodes when the charge of the primary ion not equal to charge of interfering ions and used in case no possible do achieve Nernstain responses for a given interfering ion. This method is based on electrical diffuse layers on both the membrane and the aqueous side of the interface, a solution of the primary ion A with a fixed activity is used as the reference solution. The activity a_A is calculated from the ionic strength of the solution. While the primary ion is added step by step, the potential change is measured and plotted against a_A (curve I_A) in Fig. 1-6, another curve, I_{A+B}, is obtained from the potential change by stepwise adding the interfering ion B to the reference solution with the same composition as on curve I_A.When the change in potential (ΔE) on curve I_A at a'_A matches that on curve I_{A+B} at a_{A+B}, the ratio between the activities of the primary

ion A relative to the interfering ion B denotes the selectivity coefficient $K^{pot}_{A,B}$. The selectivity coefficient $K^{pot}_{A,B}$ is thus obtained as

$$\mathbf{K^{pot}_{A,B} = \Delta a_A / a_B} \qquad \mathbf{\ldots 1\text{-}11}$$

Which $\Delta a_A = (a_A' - a_A)$

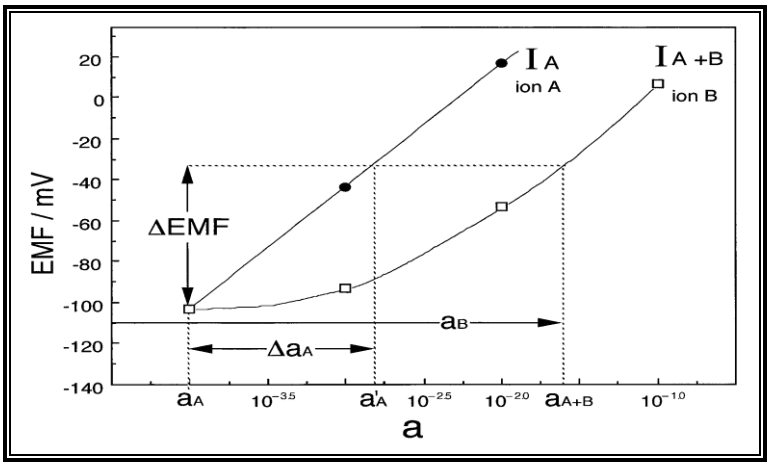


Figure 1-6: Determination of selectivity coefficients by the matched potential method [53].

1-9-2- Calibration curve [3,39,60]:-

The operation of ion selective electrodes is based on the fact that there is a linear relationship between the electrical potential developed between an ISE and a reference electrode (RE) immersed in the same solution, and the logarithm of the activity (or "effective concentration") of the ions in the solution**.** This relationship is described by the Nernst equation:

$$\mathbf{E = constant \pm (2.303\ RT/\ nF)\ log\ a} \qquad \mathbf{\ldots 1\text{-}12}$$

Fig. 1-7 shows, a typical plot of the cell e.m.f (i.e. the galvanic potential difference measured between the ISE and the external RE of a given ion-selective electrode cell assembly) versus the logarithm of the single ionic activity (concentration) of a given species.

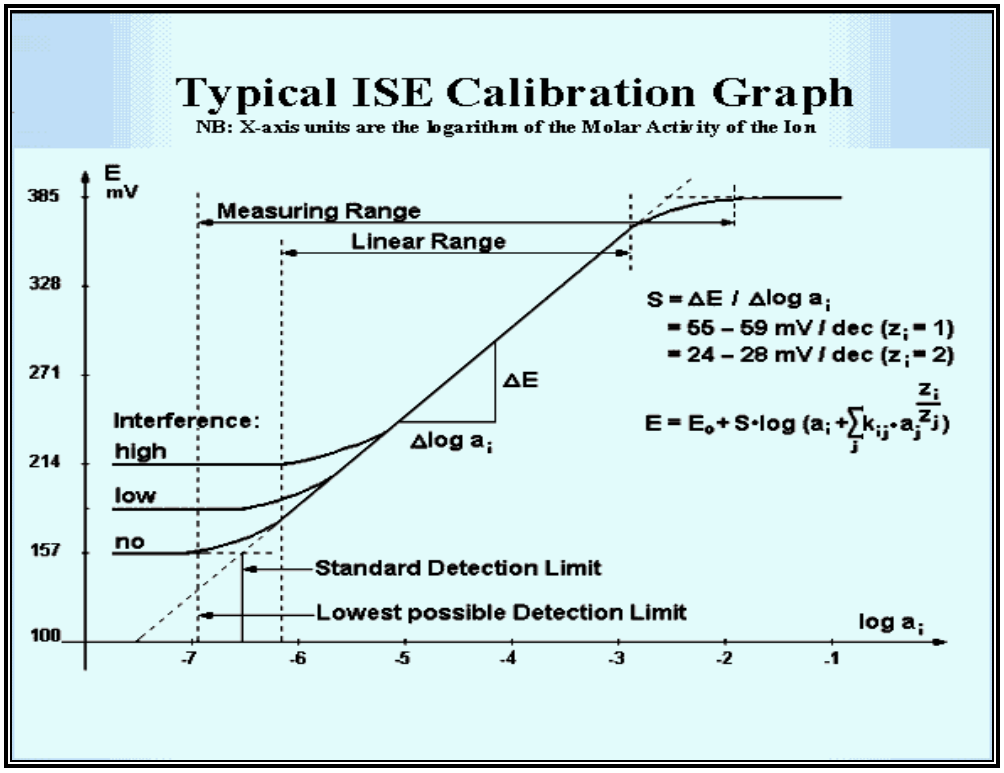


Figure 1-7: Typical ISE calibration graph [3].

For uniformity, it is recommended that the cell e.m.f is ascribed to the ordinate (vertical axis) with the more positive potentials at the top of the graph and that pa_A (-log activity of the measured species A) or pc_A (-log concentration of the measured species A) is ascribed to the abscissa (horizontal axis) with increasing activity or concentration to the right[1]. The linear rang is a part of the calibration curve through which a linear regression would demonstrate that the data points do not deviate from linearity by more than 2 mV. For many electrodes this range can extend from 1 Molar down to 10^{-6} or even 10^{-7} Molar.

1-9-3- Slope [7,1]:-

The gradient of the line is formed by plotting the electrode response in millivolts against the logarithm of the activity (or concentration) of the measured ion. The theoretical Nernstian slope at 25°C is 59.16 mV/decade changes in activity for monovalent ions and 29.58mV/decade for divalent ions.

In practice the slope is generally lower than (59.16 and 29.58mV/decade.) this is due to the inefficiency of the ion selective membranes and the failure to meet ideal conditions. Measured slopes generally lie in the range 54±5 and 26±3 mV/decade, respectively and will have a negative for anion. Measured slopes lower than this, or a gradual reduction of slope during use, are indicative of contamination of the ISE membrane. The slope of an electrode can be determined by measuring the mV response in two standard solutions with concentrations (activities) of a_1 and a_2, (effectively creating a calibration graph of mV (E) against the log of the concentration). The slope of the line is calculated from:

$$\mathbf{S = (E_1 - E_2) / (Log\ a_1 – Log\ a_2)} \qquad \text{….. 1-13}$$

And the intercept from:

$$\mathbf{c = E_1 - S\ Log\ a_1} \qquad \text{…..1-14}$$

Thus, if an unknown sample is measured and found to have a potential of E_3 mV the concentration can be calculated from: Log a = $(E_3-c)/S$. This is essentially the calculation used in the direct potentiometry method of sample measurement.

1-9-4- Detection limit [1]:-

According to the IUPAC recommendation, the detection limit is defined by the intersection of the two extrapolated linear parts of the ion selective calibration curve as shown in Figure 1-6. In practice, detection limit on the order of 10^{-6}-10^{-5}M is measured for most of ion selective electrodes. The observed detection limit is often governed by the presence of other interfering ions and the purity of standard solutions used for calibration curve.

1-9-5- Range of linear response [3]:-

The linear range of the electrode is defined as that part of the calibration curve through which a linear regression would demonstrate that the data points do not deviate from linearity by more than 2 mV. For many electrodes this range can extend from about 0.1 Molar down to 10^{-6} or even 10^{-7} Molar.

1-9-6- Response time [61,62]:-

In earlier IUPAC recommendations, it was defined as the time between the instant at which the ion selective electrode and a reference electrode are dipped in the sample solution and the first instant at which the potential of the cell becomes equal to its steady-state value within ±1mV for the final equilibrium potential. Generally electrodes with liquid ion-exchanger membrane have longer response time than solid membrane electrode. This may be due to the slow rate of reaction between the determined ion and the ion-exchanger which lead to slower transport of the ions across the membrane-solution interface. However, the main factors that influenced on the response time include; the type of membrane, the rate of change of solution activity and the presence of interference which all slow the response time of these electrodes.

1-9-7- Stability and Lifetime [49]:-

The stability and lifetime are features associated with the response behavior of ISEs. A number of problems affect the stability and lifetime of PVC based electrodes. They include the solution concentration, the interfering ions, which poison the electrode surface, the limited solubility of the active material, and its solvent, which affect the content of the membrane to leak away. All these lead to a positive or negative drift in the response and slope values, indicating that the electrode is approaching the end of its life.

1-10- Methods of measurement [3]:-

Many measurement techniques are based on ion selective electrodes have been described. The most important and widely used techniques for such studies are; direct method, incremental methods and potentiometric titration method.

1-10-1- Direct potentiometry method [56]:-

Direct potentiometric method is the simplest and most widely used for the quantitative measurements using ISEs. Simply measure the electrode response in an unknown solution and calculate the concentration directly from the regression line of the calibration curve or manually by using a special type of graph paper called the semi-log (or log/mm) paper is used. Semi-log paper comes in one cycle, two cycles, three cycles…etc.

Each cycle is an exact repetition of single cycle. Each single cycle corresponds to an order of magnitude or decade, or by using special computer graphics and calculations (eg. Microsoft Office Excel). A big advantage of this method is that it can be used to measure large batches of samples covering a wide range of concentrations very rapidly without having to change range, recalibrate or make any complicated calculations.

1-10-2- Standard additions method (SAM) [49,63]:-

This method is generally more accurate than the direct method for concentration measuring in the sample, but it is more time-consuming because of the calibration involved. In this method, the ISE cell assembly is immersed in the sample and the equilibrium cell potential is recorded, then a known volume of a standard solution of the determinant is added to the first volume and the electrode potential is re-measured, from which the potential difference (ΔE) is found. By solving the following equation the unknown concentration can be obtained:

$$C_U = C_S / 10^{\Delta E/S} [1+ (V_U / V_S)] - (V_U / V_S) \quad \ldots 1\text{-}15$$

Where C_U: the concentration of unknown solution, C_S: the concentration of standard solution, V_U: the volume of unknown solution, V_S: the volume of standard solution and S: the slope of electrode. Standard addition can be applied to most analytical techniques and is used instead of a calibration curve to solve the matrix effect problem. The standard solution is added to the unknown solution so any impurities in the unknown are accounted for in the calibration.

1-10-3- Multiple standard additions method (MSA) [64]:-

It is an extension of standard additions method. The response of ISE to certain analyte A, in solution free from interfering ions can be represented by Nernst equation:

$$\mathbf{E= E^0 + S\ Log\ a_A \times V_S / V_U} \qquad \textbf{...1-16}$$

Where S is the slope of the electrode, V_S, V_U, are the volumes of added standard and unknown (sample) respectively; V_U is usually set to be hundred times more than V_S. Rearranging of equation and taking the antilog gives:

$$\mathbf{Antilog\ E/S = constant \times a_A V_S / V_U} \qquad \textbf{...1-17}$$

Where antilog E/S is constant thus the antilog E/S is proportional to V_S. A plot of antilog E/S as a measure of a_A along the ordinate against V_S along abscissa yields a straight line which can be extrapolated back to an intercept on the standard volume. The concentration of sample (unknown) can be calculated:

$$\mathbf{C_U = V_s \times C_S / V_U} \qquad \textbf{…1-18}$$

Where C_U and C_S are the concentration of unknown and standard, respectively, V_s is the volume of standard.

1-10-4- Advantages and Disadvantages of the Incremental Methods [3]:-

1- Incremental methods are particularly useful for the 'one-off' analysis since they generally require only one standard solution and two potential measurements.

2- Since the standard solution is always in at least 10:1 excess, the effects of the matrix in the sample are practically eliminated. Ionic strength adjustors are not necessary.

3- Temperature differences between the sample and the standard become unimportant, since again the dilution effect will quickly reduce the sample temperature to that of the standard.

4- An increased range of species can be measured using the sample subtraction technique, since measurements can be made on samples for which there is no ion selective electrode.

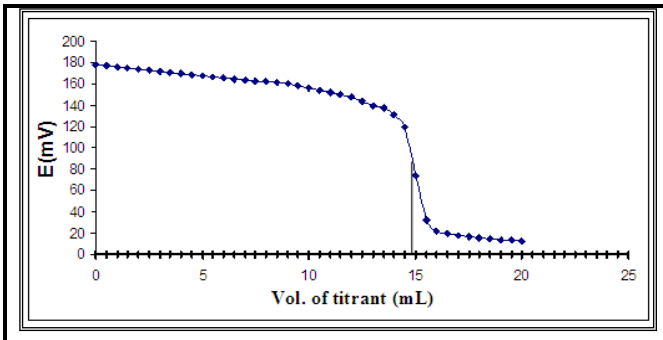
5- A disadvantage of all incremental addition methods is the fact that the approximate value of the 'unknown' concentration must be known so that the correct standard solution can be used.

6- The use of incremental techniques also introduces the need for the accurate measurement of volumes of both sample and standard.

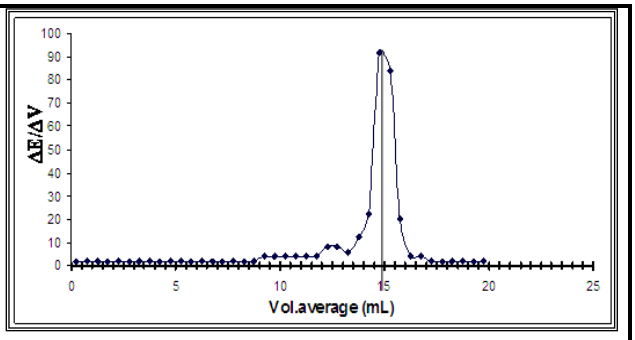
1-10-5- Potentiometric titration method [65]:-

Potentiometric titration method has also been used for the evaluation of the performance of ion selective electrode in which the ion selective electrode is only used as an indicator and the accuracy is derived from the classical titration process can yield answers to within 0.1-0.5%. Potentiometry is generally valuable as a technique for detecting the end-point of titrations where there is often a drastic change in the concentration of the reactants and thus a big shift in the electrode potential.

These end point determinations can often be made more precisely than other ISE methods because they depend on the accuracy of the volumetric measurements rather than the measurement of the electrode potential. The sample is titrated with a suitable titrant and the increase or decrease in titrant activity is followed with an ISE response, to locate the equivalence point as in Fig. 1-8.



(a)-Titration curve.



(b)-Titration curve by using first derivative.

Figure 1-8: Potentiometric titration curves by using ion-selective electrodes: (a)-titration curve, (b)-by using first derivative [66].

1-11- Clarithromycin:

Clarithromycin (CLM), 6-o-methyl-erythromycin or 4-[(2,6-Dideoxy-3-C-methyl- 3-O-methyl- L-ribohexopyranosyl) oxy] -14-ethyl -12,13-dihydroxy -7-methoxy- 3,5,7,9,11,13-hexamethyl -6-[[3,4,6-trideoxy -3-(di-methyl amino)-D-xylohexopyranosyl] oxy] oxa-cyclo-tetra-decane-2,10-dione, which has the empirical formula ($C_{38}H_{69}NO_{13}$), as shown in Fig. 1-9, is white or almost white, crystalline powder with molecular weight 748 g/mole, practically insoluble in water, soluble in acetone and in methylene chloride, slightly soluble in methanol [67].

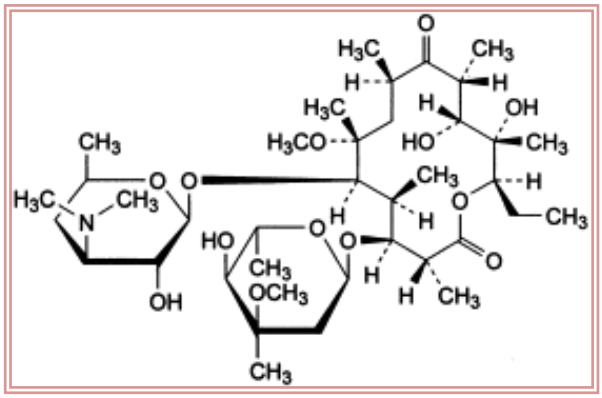


Figure 1-9: Structure formula of clarithromycin [67].

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 [68,69].

1-12- Analysis of Clarithromycin:-

Various analytical methods have been developed to determine clarithromycin in formulations and biological samples, such as spectro-photometric and high performance liquid chromatography with electrochemical and spectrophotometric detection methods. Table 1-2, shows some of these methods.

Table 1-2: Some methods for the analyses of Clarithromycin.

Method	Reagents used	Linearity	Detection limit	Ref.
Spectrophotometry	The methods involved formation of yellow colored chloroform extractable ion-association complexes of clarithromycin with bromothymol blue (BTB) and cresol red (CR) in buffered aqueous solution at pH 4. λ= 410 nm for (BTB), λ= 415 nm for (CR)	0.1-20 µg/ mL For (BTB) 2-20 µg/ mL For (CR)	0.06 µg/ mL For (BTB) 0.6 µg/ mL For (CR)	70
	Based on the formation of a binary complex between clarithromycin and eosin in aqueous buffered medium. λ= 542 nm	330 µg/mL	40 µg/mL	71
	The drug forms a green chromogen with p-dimethyl amino benzaldehyde with sulphuric acid. λ= 600 nm	10-70 µg/mL	8 µg/mL	72
HPLC with spectrophotometric detection	Chromatography was carried out using a CN column (250 mm × 4.6 mm, 5 µm) under isocratic elution with acetonitrile–50 mM aqueous sodium dihydrogen phosphate (32:68, v/v), pH 4.5. λ= 205 nm	0.031–2 µg/mL	0.01 µg/mL	73
	The analyses were run on a C_{18} column using a mobile phase comprised of potassium di-hydrogen phosphate (50 mM, pH 6.8, contained 0.7% tri-ethylamine), acetonitrile, and methanol (30:45:25, v/v/v). λ= 275 nm	0.1–10 µg/mL	0.03 µg/mL	74

Method	Reagents used	Linearity	Detection limit	Ref.
HPLC with electrochemical detection	Column was used with a mobile phase consisting of acetonitrile/aqueous phosphate buffer (pH 7, 0.086 M) (45:55 v/v). The coulometric detection was used at 850 mV using a screen voltage of 600 mV.	0.16-20.3 µg/mL	0.15 µg/mL	75
	The drug was extracted from plasma sample spiked with internal standard under an alkaline condition with tert.-butyl methyl ether. The detector cell potential for the oxidation of the drugs was set at +950 mV.	0.03-3.0 µg/mL	0.01 µg/mL	76
	Clarithromycin and the internal standard were separated with acetonitrile–methanol–0.05 M potassium phosphate buffer (pH 7.0) (41:6:53, v:v).Electrochemical oxidation of clarithromycin occurred at 0.87 V vs. Ag/AgCl reference electrode with glassy carbon electrode.	0.1 -4.0 µg/mL	0.03 µg/mL	77
	The mobile phase consisted of acetonitrile with 0.045M H_3PO_4 (37:63, v/v) adjusted to pH 6.7. Detections were monitored on an electrochemical detector operated at a potential of 0.85 V with glassy carbon electrode against Ag/AgCl reference electrode.	0.05-5.0 µg/mL	0.02 µg/mL	78

1-13-Aim of the work:-

This project was aimed to construct and characterize several types of ion-selective electrodes for the potentiometric determination of Clarithromycin (CLM) in pure and pharmaceuticals. These electrodes utilize plasticizers as the solvent mediators such as; di-butylphthalate (DBPH), di-butylphosphate (DBP), di-octyl phthalate (DOP) and Acetophenone (AP). The constructed electrodes characteristic parameters that include slope, linear range, detection limit, lifetime, selectivity, and working pH range will be investigated. Also, the statistical treatments were applied for the analytical results.

The best combination of clarithromycin (ionophore), solvent mediator, and PVC matrix will be chosen. Potentiometric measurements including direct method, standard additions method and titration method will be studied.

Chapter Two

Experimental Part

Experimental part

2-1- Instruments and equipment:-

1- A digital pH/ion meter (inoLab 740 with terminal 740 – WTW, Germany).
2- Fourier transforms infrared spectrophotometer (FTIR-8300 SHIMADZU, Japan).
3- Hotplate Magnetic stirrer (LMS-1003, Daihan Labtech).
4- Sartorius Handy 4digits Analytical Balance (GMBH, H110, Germany).
5- pH combination glass electrode (SenTix® 82 WTW, Germany).
6- Calomel reference electrode.
7- Silver-silver chloride wire.
8- Clear PVC tubing (6 mm o.d.).

2-2- Chemicals:-

The chemical compounds were used throughout the study as shown in Table2-1.

Table 2-1: Shows types of used chemicals compounds.

Chemicals	Molecular formula	Molecular Weight (M)	Density g/mL at 25°C	Viscosity (CST)	Purity	Company
Sodium tetra-phenyl borate (NaTPB)	$C_{24}H_{20}BNa$	342.22	---	---	98%	Fluka
Poly vinyl chloride	$((CH_2\text{-}CHCl)_2)_n$	High molecular weight	---	---	99.5%	Fluka
Hydrochloric acid	HCl	36.45	1.19	---	36%	Fluka
Sodium hydroxide	NaOH	40.00	---	---	98%	BDH
Acetonitrile	CH_3CN	41.05	0.786	---	99%	Sigma
Di-chloromethan	CH_2Cl_2	84.93	1.325	---	98.5%	Sigma
Tetra-hydrofuran (THF)	C_4H_8O	72.11	0.889	---	99%	Fluka
Acetone	$(CH_3)_2CO$	58.08	0.791	---	99%	Fluka
Di-butylphosphate(DBP)	$(C_4H_7O)_2PO(OH)$	210.21	1.06	1I2.88	97%	Fluka
Di-butylphthalate(DBPH)	$C_6H_4[CO_2CH_3(CH_2)_3]_2$	278.34	1.043	14.44	99%	Fluka
Di-octylphthalate(DOP)	$C_6H_4[CO_2C_8H_{17}]_2$	390.56	0.985	82.98	99.5%	Fluka
Acetophenone (AP)	$CH_3CO(C_6H_5)$	120.15	1.03	1.62	98%	Fluka
Potassium iodide	KI	166	---	---	99.5%	BDH
Mercury (II) chloride	$HgCl_2$	271.52	---	---	99%	BDH
Potassium hydroxide	KOH	56.1	---	---	98%	BDH
Potassium chloride	KCl	74.55	---	---	98.5%	BDH
Sodium chloride	NaCl	58.45	---	---	99%	BDH
Copper (II) sulfate anhydrous	$CuSO_4$	159.62	---	---	98%	Fluka
Manganese (II) sulfate anhydrous	$MnSO_4$	151	---	---	99%	Fluka
Ferric (III) sulfate	$Fe_2(SO_4)_3.9H_2O$	562	---	---	99%	BDH
Aluminum (III) chloride	$AlCl_3.6H_2O$	241.43	---	---	98.5%	Fluka
Sucrose	$C_{12}H_{22}O_{11}$	342.30	---	---	99%	BDH

2-3- Extraction of Clarithromycin:-

Clarithromycin was extracted from Claricide tablets according to the literature procedure [79]. Claricide tablets (containing 500 mg clarithromycin) were purchased from Bilim pharmaceuticals (made in Turkey).

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 dichloromethane layer was separated and centrifuged, and then the supernatant was filtered and evaporated to dryness at room temperature, the resulting precipitate was investigated using FTIR.

2-4- Preparation of potassium tetra-iodomercurate [80]:-

Potassium tetra-iodomercurate (KTIM), Nessler's reagent, is generally prepared from potassium iodide and mercury chloride. A hot saturated solution (60 g/L) of mercury chloride is added to solution of potassium iodide (10 g dissolved in 10 mL water), until the precipitate formed, then adding 80 mL of 9M potassium hydroxide solution and diluted to 200mL. The resulting solution is then cooled and then 5.55mL of this solution was diluted to 50mL to prepare 0.01M.

2-5- Preparation of standard solutions:-

1- A stock solution of 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. The working solutions 10^{-8}-10^{-3} M of CLM were prepared by serial appropriate dilution of the stock solution using the same solvent.

2- A standard solution of 0.01 M Sodium tetra-phenyl borate (NaTPB) was prepared by dissolving 0.1711 g of pure (NaTPB) in acetone and completing the solution up to 50 mL.

3- A 0.1 M hydrochloric acid was prepared by diluting 0.8 mL of 12 M HCl concentrated stoke solution to 100 mL by distilled water, and 0.1 M of NaOH was prepared by weighting 0.4 g of NaOH and dissolving it in 100 mL by distilled water.

4- All solutions were prepared in distilled water, stock solutions of 0.1 M of NaCl, KCl, $CuSO_4$, $MnSO_4$, $Fe_2(SO_4)_{3.}9H_2O$, $AlCl_3.6H_2O$, sucrose and gelatein were prepared by weighted (0.2922, 0.3727, 0.7981, 0.7550, 2.81, 1.2071, 1.7115 and 1.50 g) and dissolved by distilled water in 50 mL volumetric flask. More diluted solutions were prepared by dilution from the stock solutions as required.

2-6- Preparation of ion-pair compounds:-

Two type of electro-active substance were prepared, the preparation of ion-pair of (CLM-TPB) was performed by mixing equal volume of 0.01 M solution of tetra-phenyl borate with an equimolar solution of clarithromycin dissolved in acetone, a white precipitate formed immediately after addition of a few drops of concentrated hydrochloride acid. The ion-pair of (CLM-TIM) was prepared using the same method and a semi white precipitate formed.

The resulting precipitates were filtered off, washed with water, dried at room temperature for two days using the vacuum desiccator.

2-7- Preparation of clarithromycin electrodes:-

Eight clarithromycin ion-selective electrodes were prepared based on the use of ion-pair compounds clarithromycin-tetraphenylborate (CLM-TPB) and clarithromycin-tetraiodomercurate (CLM-TIM) as the electro-active substance with four plasticizers. The method of immobilization the ion-pair compounds into the PVC matrix membrane as described by Craggs et al [81].

A 0.040 g of electro-active precipitates was mixed with 0.360 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.

2-8- Assembling the ion-selective electrode:-

The above 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 (to make sure no loss in the membrane mixture) Figure 2-1. The top side of the cylinder was covered with a pad of filter paper on which a heavy weight was placed. The assembly was left for 2-3 days to allow graduate evaporation of the solvent.

The glass ring with adhering membrane was carefully removed from the glass plates as shown in Figure 2-1 (3^{rd} step). The membrane was then detached away from the edge of the ring. A disc of the membrane was cut equal to the external diameter of a PVC tube; step (4).One of sides of PVC tubing was flatted and smoothed by placing it on glass plate moisture with THF with aid of vertical rotation.

The disc then mounted with a forceps on the polished end, the outer edge of the disc membrane was carefully sealed to the end of the PVC tube, step (7). Next step is connection into a glass tube, step (8).

The other side of the glass tube was assembled with plastic cover in which Ag/AgCl wire was inserted through it, the tube was filled 3/4 with 1×10^{-3} M clarithromycin solution before fixing the cover, step (9). The electrode was then conditioned by placing it in 1×10^{-3} M solution containing the CLM to be measured (at least 2 hour's) before using.

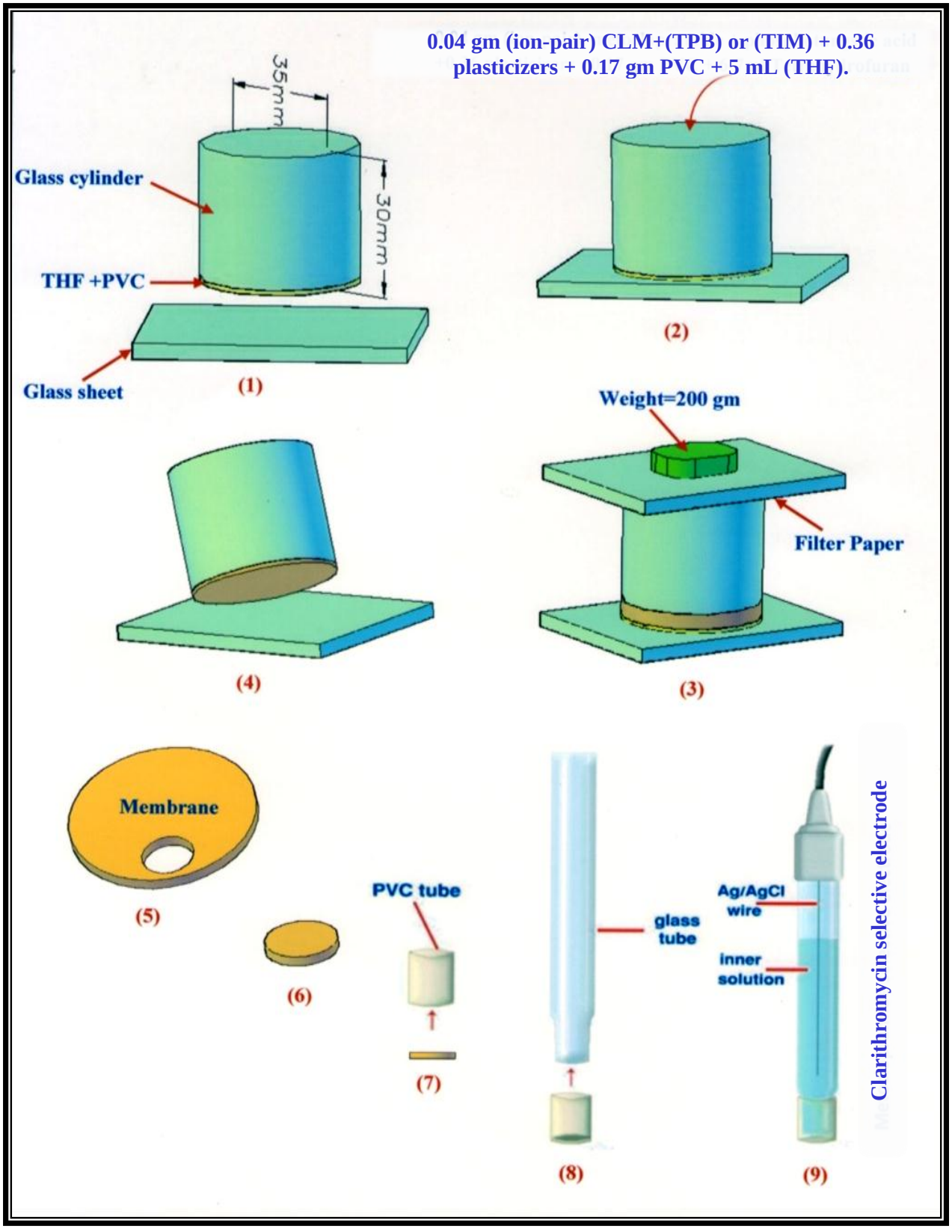


Figure 2-1:- Assembling the ion selective electrode.

2-9- Potential measurement:-

The potentiometric cell was arranged by immersing the electrode and reference electrode in 50 mL containing certain amount of analyte solutions 25 mL. The cell was equipped with a magnetic stirrer. The potential measurements were carried out at room temperature. 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 2010). From the calibration curve, the characterization parameters of an ISE were obtained, including; concentration range; slope and detection limit.

The lifetime of each membrane was calculated, when the positive or negative drift in the slope values, indicating that the electrode is approaching the lifetime.

2-10- pH effect:-

The effect of pH on the response of membrane was examined by measuring the potential of the standard solutions of CLM at concentrations (1×10^{-3} , 5×10^{-4}, 1×10^{-4} M) at different pH ranged from 1 to 12 were obtained by addition of small volumes of hydrochloric acid and/or sodium hydroxide solutions.

2-11- Selectivity measurements [49-51]:-

The selectivity coefficients of the ion-selective electrodes for: some inorganic ions (Na^{+}, K^{+}, Mn^{2+}, Cu^{2+}, Fe^{3+}, Al^{3+}), sucrose and gelatins, were determined by:-

2-11-1- The separate solution methods [52]:-

In this method, a 25 mL of $1x10^{-3}$ M solution of the prepared analyte (A) (clarithromycin) and 25 mL of $1x10^{-3}$ M from each other interfering ion (B) (Na^{+}, K^{+}, Mn^{2+}, Cu^{2+}, Fe^{3+}, Al^{3+}, sucrose and gelatins).

The potential of each solution is measured separately. The selectivity coefficient was calculated from the equation 1-7.

2-11-2- Mixed solution methods [fixed interference method (FIM)] [54,55]:-

In this method, a 10 mL of analyte (A) solution (clarithromycin) from each (1×10^{-8} to 1×10^{-3} M) are mixed with 10 mL from (1×10^{-3} M) interfering ion (B) in 50 mL beaker. The potential were measured for each solution. The activities of analyte (A) are found after mixing as shown in Figure 1-5. The selectivity coefficient ($K^{pot}_{A,B}$) are calculated according to equation 1-9. The activities of interfering ion (a_B) are calculated after dilution:-

$a_B = (1\times10^{-3}\ M\times 10\ mL)/ 20\ mL = 5\times10^{-4}\ M$.

2-12- Sample analyses [3]:-

2-12-1- Direct method [63]:-

The potentiometry of sample is measured directly using clarithromycin indicator electrodes. The concentration was then calculated using calibration curve of standard clarithromycin.

2-12-2- Standard additions method (SAM) [63,49]:-

In this method, the sample of 20 mL with concentration of 1×10^{-4} M is introduced followed by addition of 0.5 mL of 1×10^{-3} M increment of clarithromycin solution. The potential were measured before and after addition. The concentration of the sample is calculated using equation 1-15 for a single point increment.

2-12-3- Multiple standard additions method [49,64]:-

This method is an extension of standard additions method, the sample of 20 mL of 1×10^{-4} M is introduced followed by addition of 0.5 mL of 1×10^{-3} M of clarithromycin solution. The potential is recorded before and after each addition. The multi additions method was plotted between antilog (E/S) and the added volume of standard solution.

2-12-4- Potentiometric titration method [65]:-

A precipitation titration was performed for the clarithromycin sample under study. In this method, a 15 mL sample solution containing clarithromycin 1×10^{-4} M was titrated against 1×10^{-4} M Sodium tetraphenylborate (NaTPB) solution. Potential was measured after each addition using the prepared electrode.

A direct plot of potential as a function of titrant volume, the midpoint in the steeply rising portion of the curve is estimated visually and taken as end point. A second approach to end point detection is to calculate the change in potential per unit volume of titrant $\Delta E/\Delta V$ plotted versus the average volume of titrant, the maximum is the end point.

2-13- Preparation of pharmaceutical formulation:-

All contents of 10 tablets of clarithromycin (500 mg) powdered and weighted accurately to be found 5.8692 g, then 0.0878 g of this powder dissolved in 250 mL acetonitrile and then filtered and completing the solution up to 1 L with distilled water, the resultant solution is 1×10^{-4} M.

Chapter Three

Results and Discussion

Results and Discussion

3-1- Extraction of clarithromycin:-

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 [82], as shown in Fig. 3-1(a) for the extracted clarithromycin and Fig. 3-1 (b) demonstrates the reference spectrum. The spectrums show a good purity.

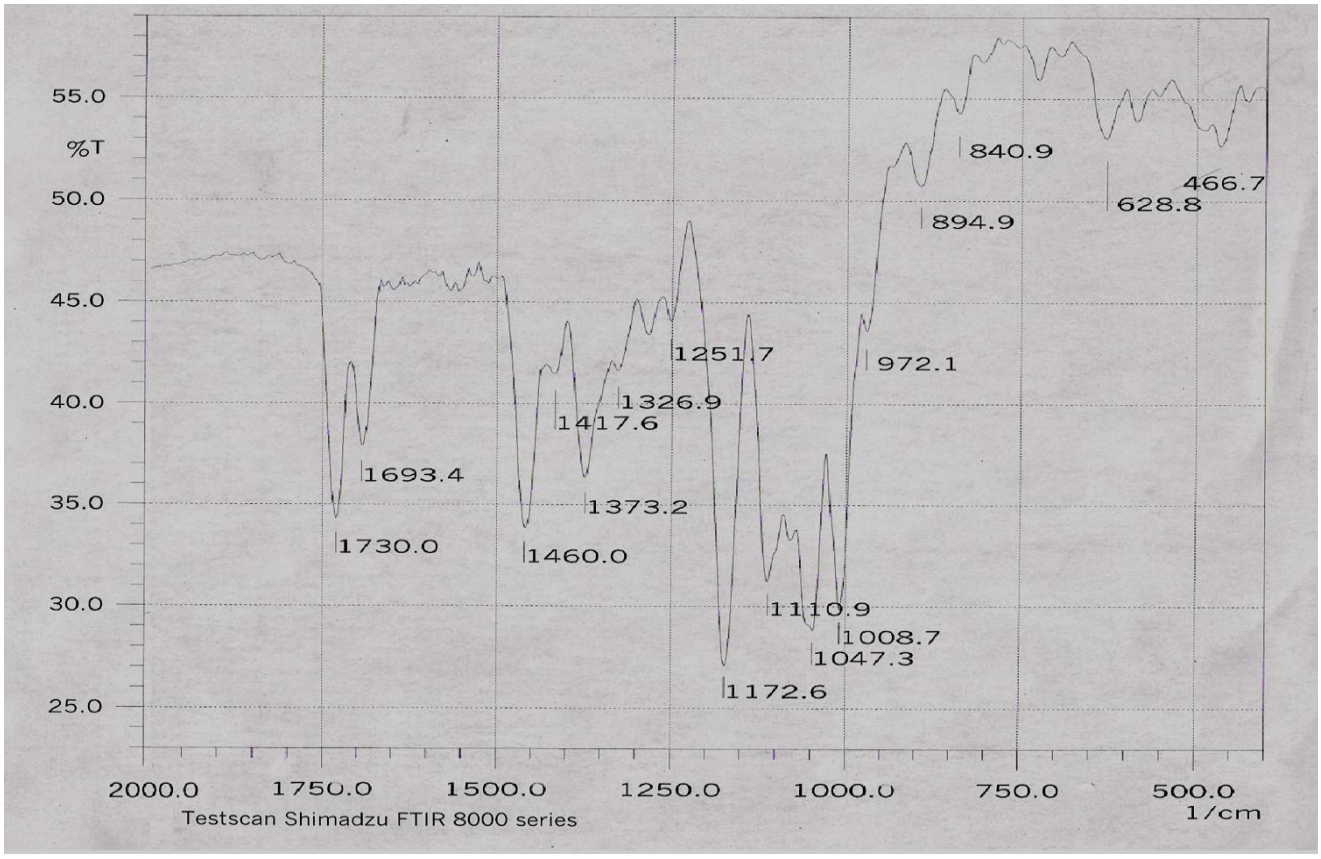


Fig.(3-1) a- FTIR spectrum of extracted clarithromycin.

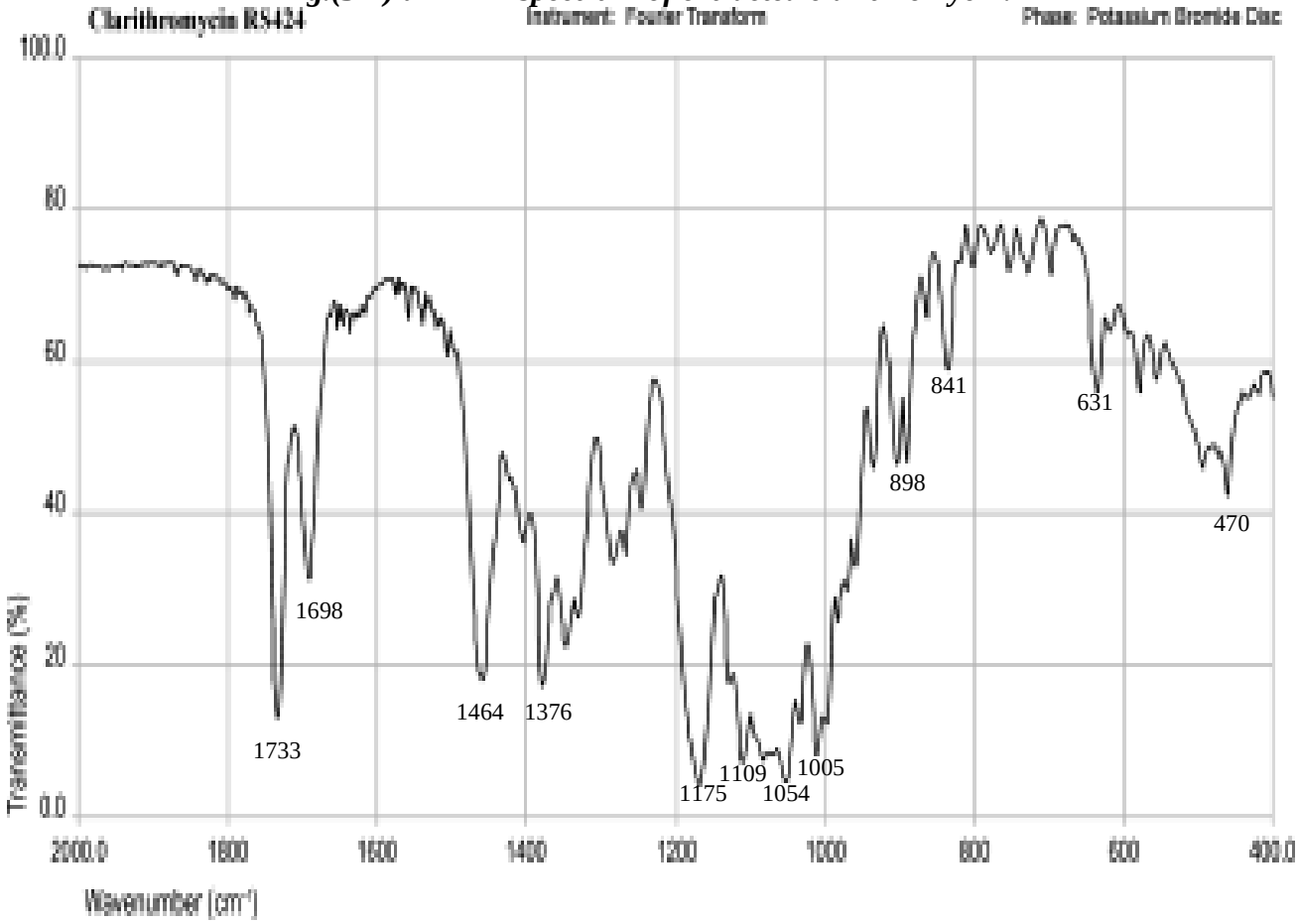


Fig.(3-1) b- FTIR reference spectrum of pure clarithromycin.

3-2- Sensor Characteristics:-

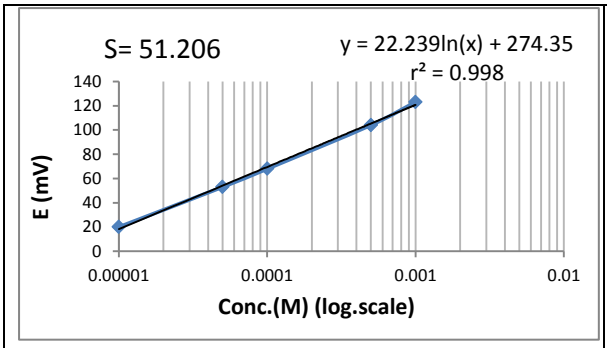
Eight electrodes of clarithromycin were prepared four of them based on clarithromycin-tetraphenylborate (CLM-TPB) as the electro-active material and the other four electrodes based on clarithromycin-tetraiodomercurate (CLM-TIM) as the electro-active material. These ion-pair complexes were examined with four plasticizers such as: Di-octyl phthalate (DOP); Di-butyl phosphate (DBP); Acetophenone (AP); Di-butyl phthalate (DBPH) in PVC matrix.

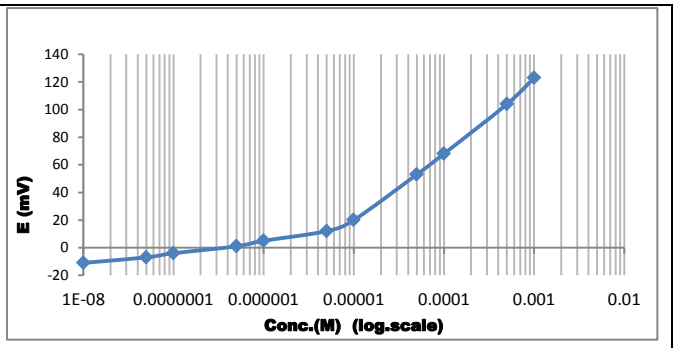
The effects of different plasticizers were studied with respect to the linear concentration range, slope, detection limit, response time and lifetime.

The potential values of these electrodes were plotted versus the logarithm of concentration of drug. All membranes were soaked in 1×10^{-3} M of clarithromycin solution for one hour for condition by the membrane.

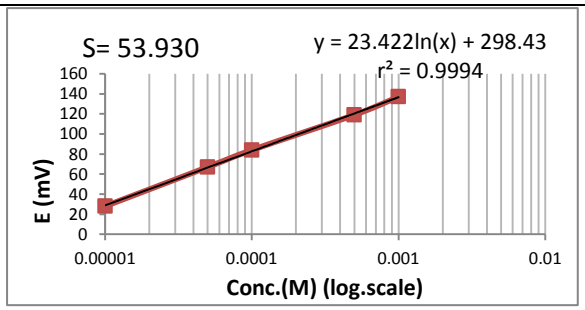
Clarithromycin-tetraphenylborate electrodes (E1, E2, E3 and E4) using these plasticizers (DOP, DBP, AP and DBPH) respectively, which their calibration curves shown in Fig. 3-2(a, b, c, d) respectively. These electrodes gave the slopes of (51.206, 53.930, 58.104 and 58.484) mV/decade respectively. The linear range for these electrodes (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) with detection limits of (8×10^{-6}, 6×10^{-6}, 2×10^{-5} and 9×10^{-6} M) respectively.

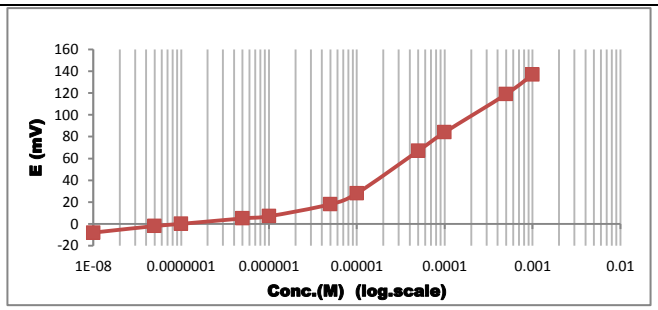
The other electrodes are for Clarithromycin-tetraiodomercurate ion-pair. These electrodes are (E5, E6, E7 and E8) using the plasticizers (DOP, DBP, AP and DBPH) respectively, which their calibration curves shown in Fig. 3-3(a, b, c, d) respectively. These electrodes gave the slopes of (48.445, 42,970, 52.692 and 49.442) mV/decade respectively. The linear range for these electrodes (1×10^{-5}-1×10^{-3}, 5×10^{-5}-1×10^{-3}, 5×10^{-5}-1×10^{-3} and 1×10^{-5}-1×10^{-3} M), with detection limits of (5×10^{-6}, 5.5×10^{-5}, 5×10^{-5} and 1.5×10^{-5} M) respectively. The results of all electrodes were summarized in Table 3-1.



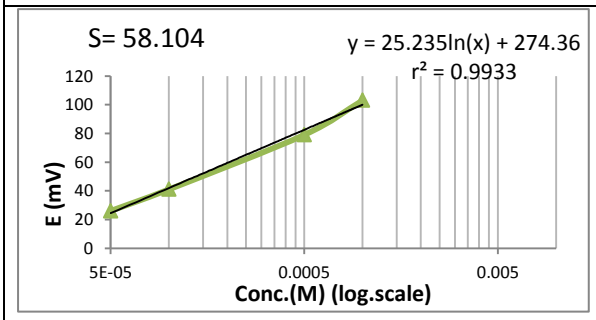


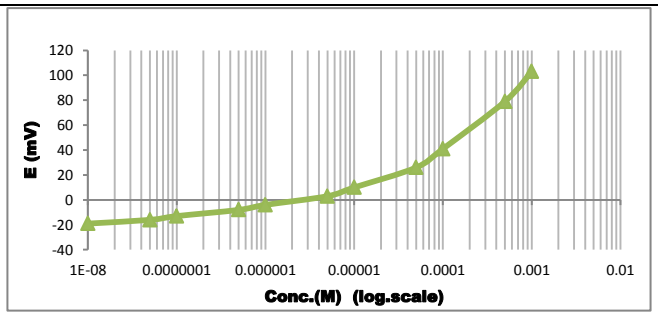
a: E1 electrode (CLM-TPB+ DOP)



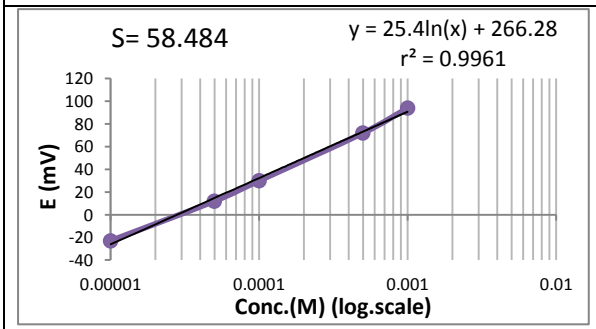


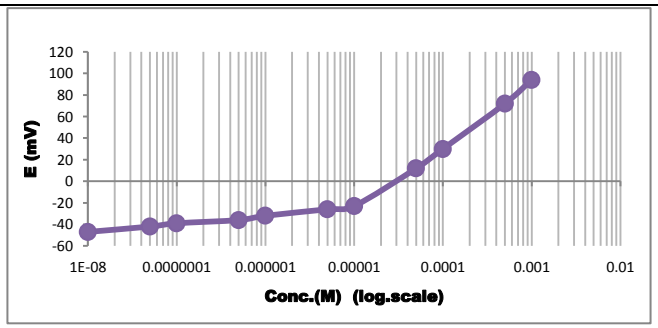
b: E2 electrode (CLM-TPB+ DBP)





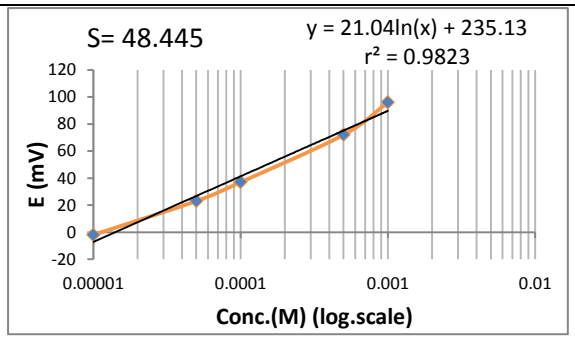
c: E3 electrode (CLM-TPB+ AP)

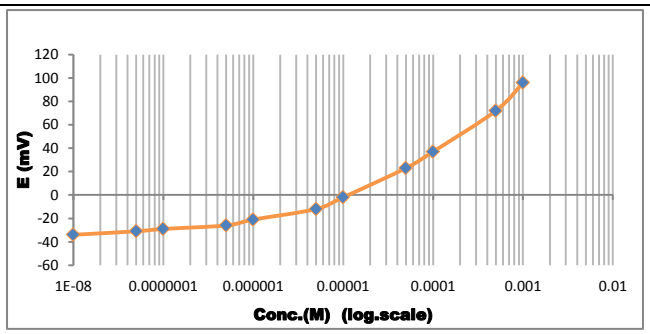




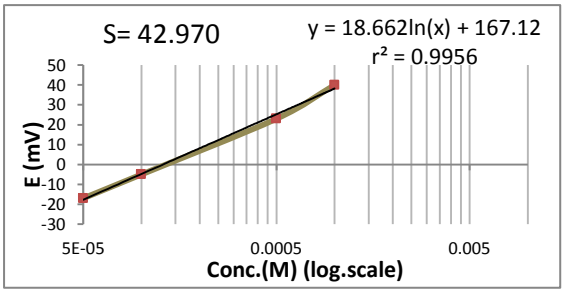
d: E4 electrode (CLM-TPB+ DBPH)

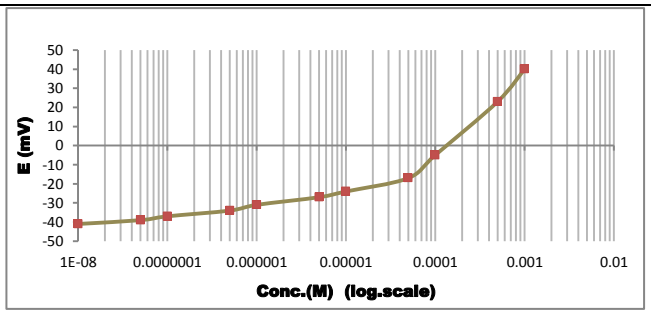
Figure 3-2:-The calibration curves of clarithromycin-tetraphenylborate electrodes with different plasticizers: a-E1 electrode, b-E2 electrode, c-E3 electrode and d-E4 electrode.



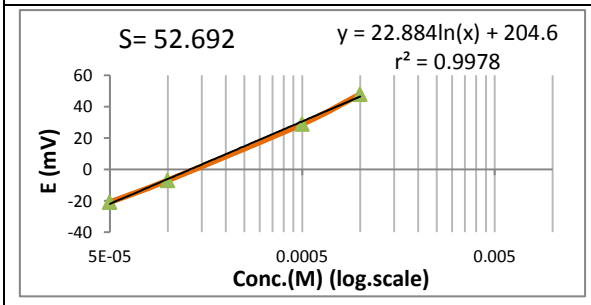


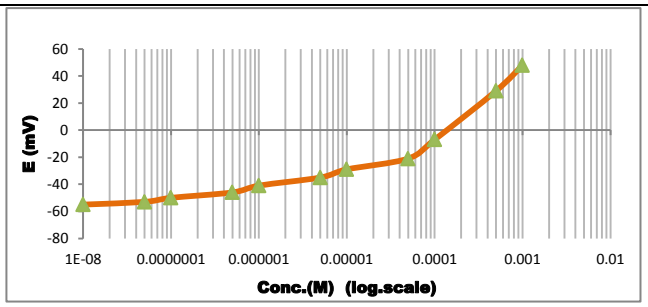
a: E5 electrode (CLM-TIM+ DOP)



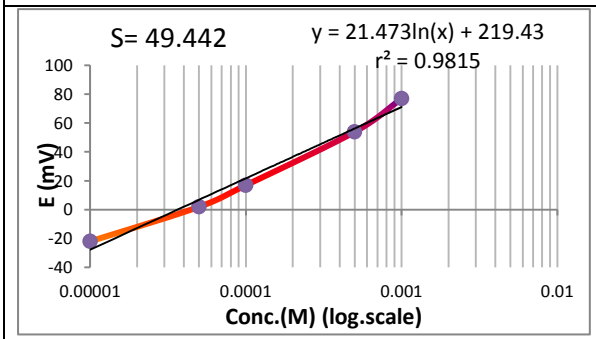


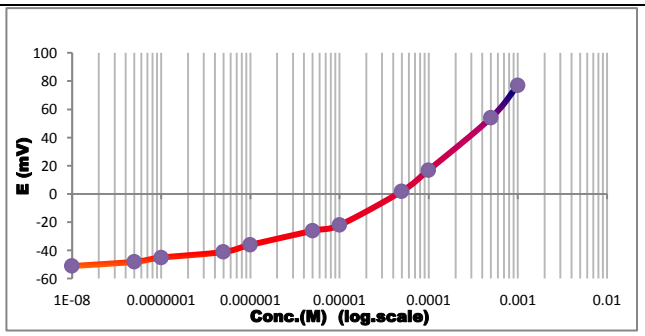
b: E6 electrode (CLM-TIM+ DBP)





c: E7 electrode (CLM-TIM+ AP)





d: E8 electrode (CLM-TIM+ DBPH)

Figure 3-3:-The calibration curves of clarithromycin-tetraiodomercurate electrodes with different plasticizers: a-E5 electrode, b-E6 electrode, c-E7 electrode and d-E8 electrode.

The sensor (E4), which has the composition (CLM-TPB+DBPH), is the best electrode, it displays a linear response from 10^{-5} to 10^{-3} M (CLM) with Nernst slope 58.484 mV/decade, lower limit of detection of 9×10^{-6} M and correlation coefficient 0.9980. Electrode (E4) gave the best Nernst slope value because the high mixing between the (DBPH) and (PVC) due to the compatibility of the plasticizer used and also the composition of the electro-active compound.

Table 3-1: The parameters for eight (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**	**0.9989**	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**	**0.9996**	1×10^{-5}-1×10^{-3}	6×10^{-6}	35	30	21	30
E3 CLM-TPB + AP	**58.104**	**y = 25.235ln(x) + 274.36**	**0.9966**	5×10^{-5}-1×10^{-3}	2×10^{-5}	41	35	32	12
E4 CLM-TPB+ DBPH	**58.484**	**y = 25.4ln(x) + 266.28**	**0.9980**	1×10^{-5}-1×10^{-3}	9×10^{-6}	36	30	24	20
E5 CLM-TIM+ DOP	**48.445**	**y = 21.04ln(x) + 235.13**	**0.9911**	1×10^{-5}-1×10^{-3}	5×10^{-6}	26	25	20	28
E6 CLM-TIM+ DBP	**42.970**	**y = 18.662ln(x) + 167.12**	**0.9977**	5×10^{-5}-1×10^{-3}	5.5×10^{-5}	32	37	33	22
E7 CLM-TIM + AP	**52.692**	**y = 22.884ln(x) + 204.6**	**0.9988**	5×10^{-5}-1×10^{-3}	5×10^{-5}	30	28	22	16
E8 CLM-TIM+ DBPH	**49.442**	**y = 21.473ln(x) + 219.43**	**0.9907**	1×10^{-5}-1×10^{-3}	1.5×10^{-5}	48	39	30	25

Table 3-2: The results for linear regression of the (CLM) selective electrode E4.

Linear range (M)	***Slope**(mV/Decade) at 95% for (n-2)* $b\mp S_b t$**	***Intercept**(mV/Decade) at 95% for (n-2)* $a\mp S_a t$**	$t_{cal.}$ *at 95% for (n-2)*	$t_{cal.}$ *at 95% for (n-2)*	*Correlation coefficient (r)*	*Linearity r^2%*
1×10^{-5}-1×10^{-3}	**58.484∓5.453**	**266.28∓21.73**	**27.68**	**2.57**	**0.9980**	**99.61%**
			$t_{cal.} \gg t_{tab.}$			

The analytical method was accepted according to the calculated value of **t** is greater than the tabulated value.

3-3- 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.(3-4). 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 . The working pH were tabulated in Table (3-3).

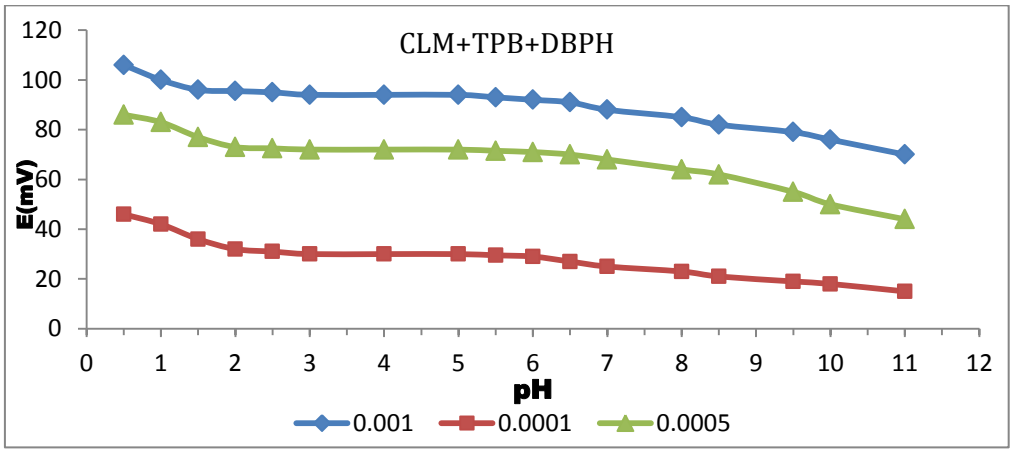


Figure 3-4: Effect of pH on the potential of the electrode E4 at concentrations 1×10^{-3}, 5×10^{-4} and 1×10^{-4}M.

Table (3-3)
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**

3-4- Effect of temperature:-

The effect of temperature on the electrode potentials for (CLM) selective membrane electrode (E4) was studied by adjusting different temperatures. The best temperature for the working of electrode (E4) was at room temperature.

3-5- Interference studies:-

In order to investigate the selectivity of the proposed membrane (E4) ion selective electrode toward clarithromycin with respect to various interfering ions two methods of selectivity are used:-

3-5-1- Separate solution methods:-

In this case ($a_A = a_B = 10^{-3}$ M) The potential is measured with two separate solutions, one containing the clarithromycin at the concentration 1×10^{-3} M and the other one containing the interfering ion at the same concentration. The value of $K^{pot}_{A,B}$ is calculated by using the equation 1-7, by measurement the values of E_A and E_B. When E_A= 94 mV and the slope is 58.484 mV/decade. The results of selectivity coefficients are summarized in Table 3-4.

Table 3-4:
Values of $K^{pot}_{A,B}$ according to separate method by using electrode E4.

Interfering ions	*E_B (mV) When $a_B = 10^{-3}$*	*log $K^{pot}_{A,B}$*	*$K^{pot}_{A,B}$*
k^{+}	10	-1.43629	3.66×10^{-2}
Na^{+}	14	-1.3679	4.28×10^{-2}
Fe^{+3}	7	-3.48759	3.25×10^{-4}
Al^{+3}	5	-3.52178	3.01×10^{-4}
Cu^{+2}	11	-2.91919	1.20×10^{-3}
Mn^{+2}	9	-2.95339	1.11×10^{-3}
Sucrose	6	-1.50469	3.12×10^{-2}
Gelatin	7	-1.48759	3.25×10^{-2}

From the Table 3-3, all values of $K^{pot}_{A,B}$ are less than (0.1), This reflects a very high selectivity of this electrodes towards clarithromycin.

3-5-2- Mixed solution methods (FIM):-

The potential of cell is measured for the solutions at constant concentration of the interfering ion (a_B) at first used 1×10^{-3} M that calculated in 20 mL total volume after mixed it with varying concentration of the clarithromycin (a_A). The potential values obtained are plotted vs. the logarithm of the concentration of the clarithromycin.

From Fig. 3-5 to Fig. 3-12 the intersection of the extrapolated linear portions of this plot determine the value of (a_A), which can be used to calculate $K^{pot}_{A,B}$ using equation 1-9, all results of $K^{pot}_{A,B}$ were listed in Table 3-5.

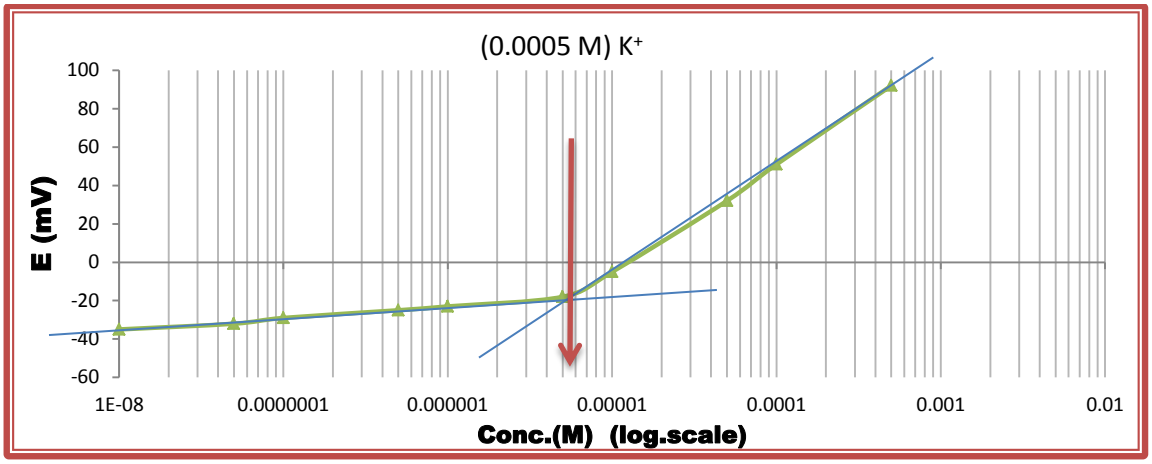


Figure 3-5: FIM calibration curve for E4 electrode, K^{+1} ($5\times10^{-4}M$) as interfering ion $a_A = 5.5\times10^{-6}M$.

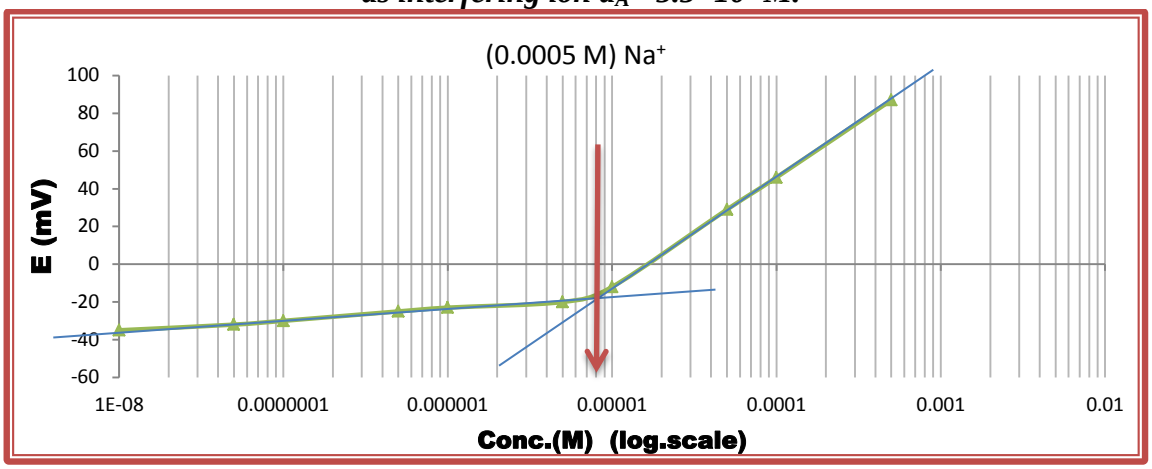


Figure 3-6: FIM calibration curve for E4 electrode, Na^{+1} ($5\times10^{-4}M$) as interfering ion $a_A = 8\times10^{-6}M$.

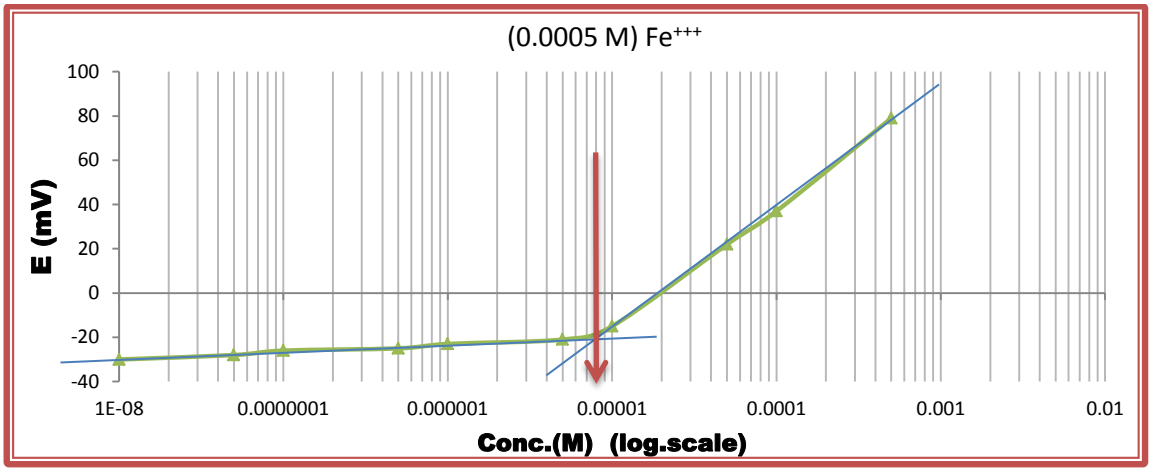


Figure 3-7: FIM calibration curve for E4 electrode, Fe^{+3} ($5\times10^{-4}M$) as interfering ion $a_A = 8\times10^{-6}M$.

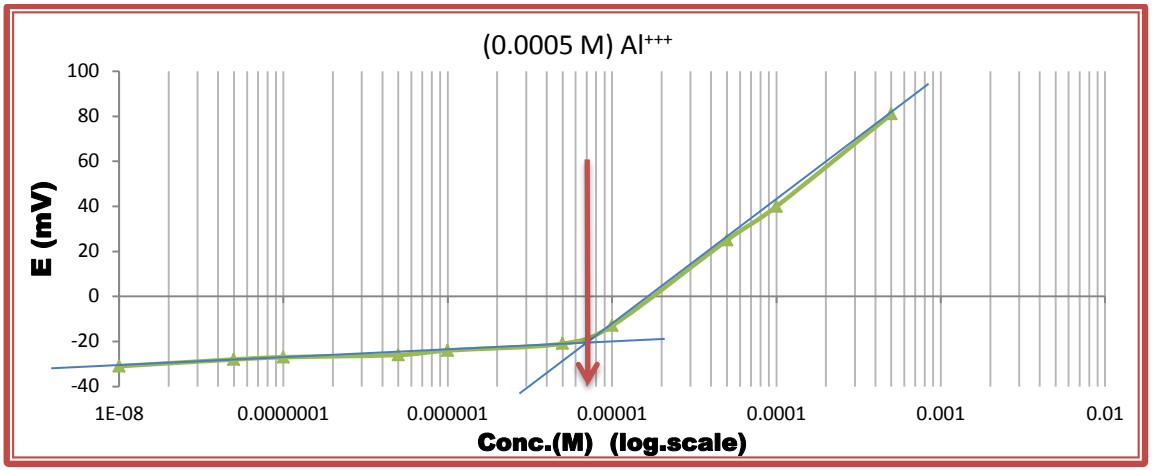


Figure 3-8: FIM calibration curve for E4 electrode, Al^{+3} ($5\times10^{-4}M$) as interfering ion $a_A = 7\times10^{-6}M$.

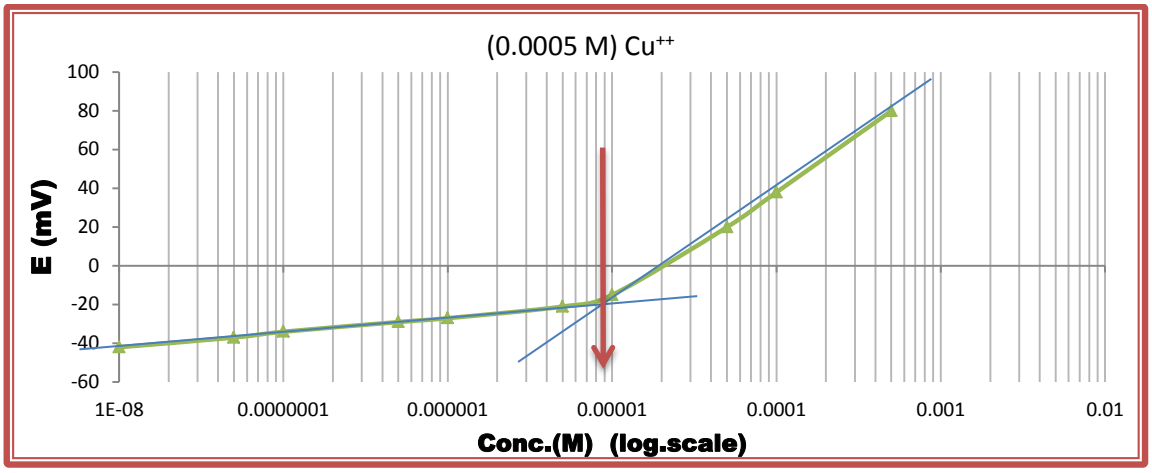


Figure 3-9: FIM calibration curve for E4 electrode, Cu^{+2} ($5\times10^{-4}M$) as interfering ion $a_A = 9\times10^{-6}M$.

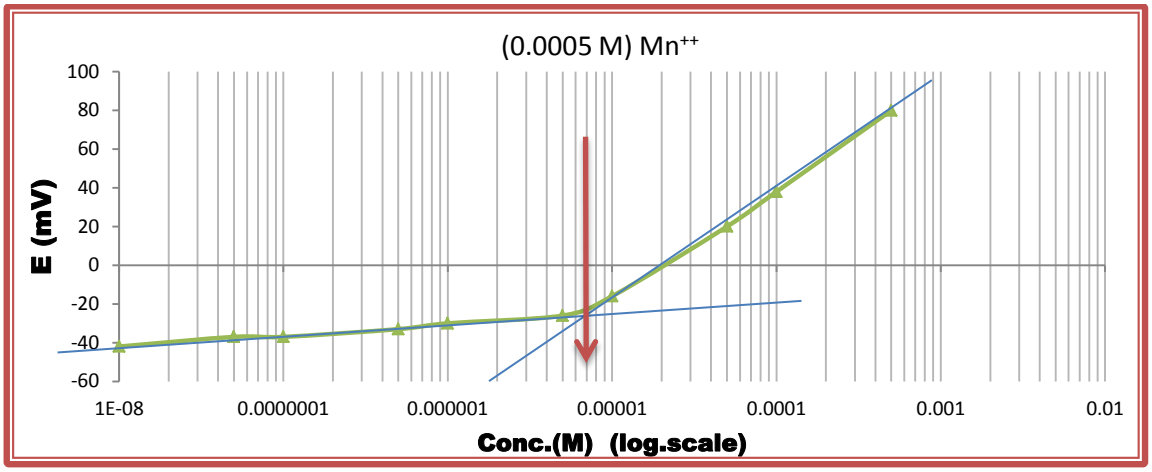


Figure 3-10: FIM calibration curve for E4 electrode, Mn^{+2} ($5\times10^{-4}M$) as interfering ion $a_A = 7\times10^{-6}M$.

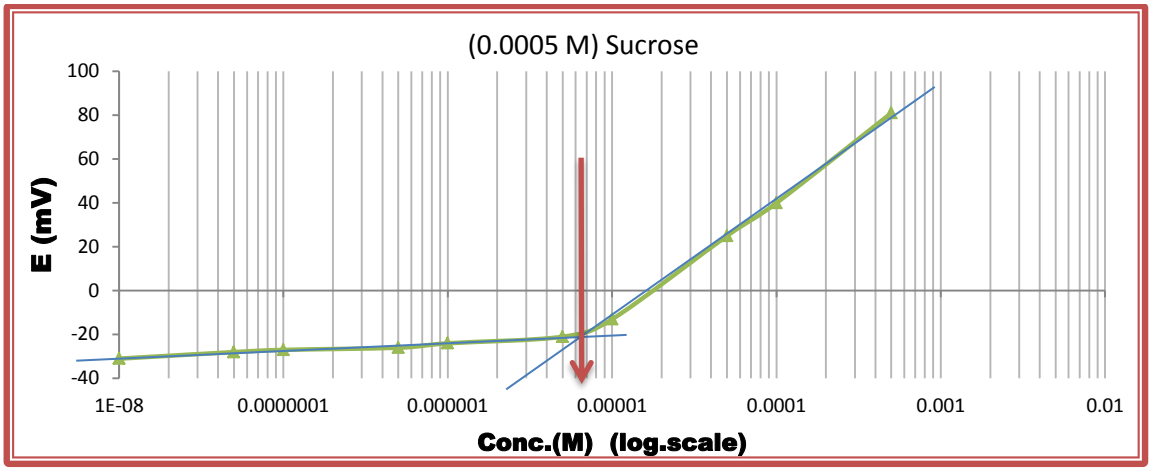


Figure 3-11: FIM calibration curve for E4 electrode, Sucrose (5×10^{-4}M) as interfering ion $a_A = 6.5\times10^{-6}$M.

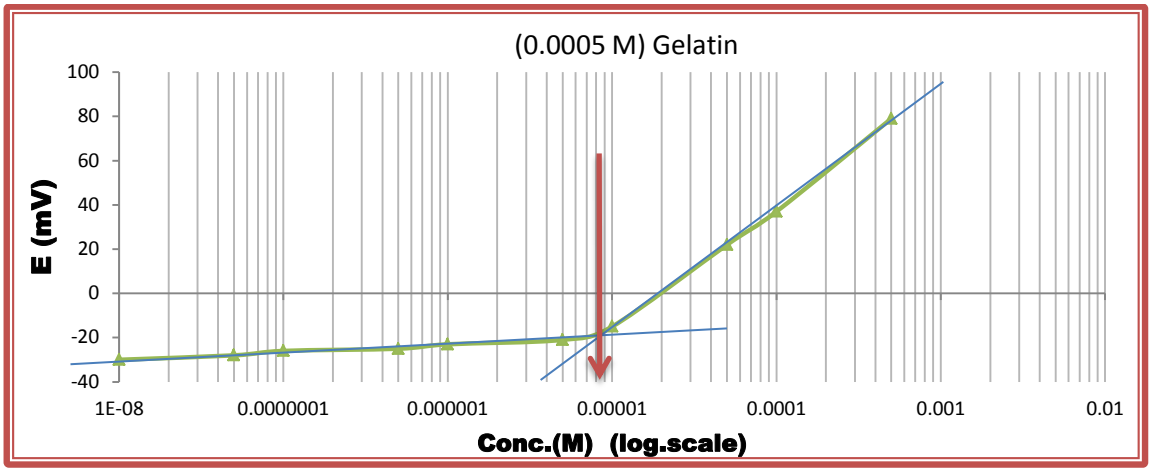


Figure 3-12: FIM calibration curve for E4 electrode, Gelatin (5×10^{-4}M) as interfering ion $a_A = 8.5\times10^{-6}$M.

Table 3-5:- Values of $K^{pot}_{A,B}$ according to FIM.

Interfering ions	$a_B = 5\times10^{-4}$ *M*	
	a_A	$K^{pot}_{A,B}$
k^+	5.5×10^{-6}	1.1×10^{-2}
Na^+	8×10^{-6}	1.6×10^{-2}
Fe^{+3}	8×10^{-6}	1.008×10^{-4}
Al^{+3}	7×10^{-6}	8.819×10^{-5}
Cu^{+2}	9×10^{-6}	4.025×10^{-4}
Mn^{+2}	7×10^{-6}	3.13×10^{-4}
Sucrose	6.5×10^{-6}	1.3×10^{-2}
Gelatin	8.5×10^{-6}	1.7×10^{-2}

3-6- Sample analyses:-

3-6-1- Direct method: -

The calibration curve was constructed (for E4 electrode), as shown in Fig. 3-13, and the concentration of the unknown was calculated from the linear equation y=25.4Ln(x) + 266.28 of the calibration curve which has the slope S = 58.484 mV/decade and the intercept = 266.28, for n=5, the results are listed in Table 3-6.

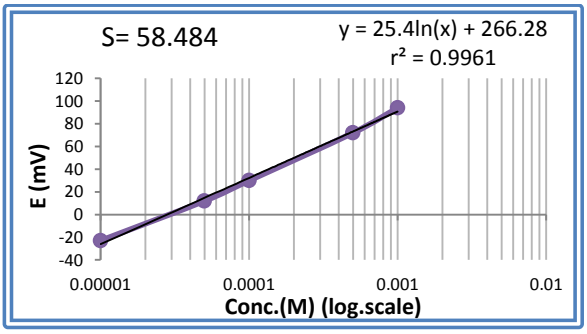


Figure 3-13:- Calibration curve of E4 electrode.

Table 3-6: The results of five measurements of Clarithromycin standard solution 10^{-3} M using direct method for E4 electrode, where slope=58.484 mV/decade.

Potential reading E(mV)	***Conc. Of (CLM) sample calculated from linear equation (M)***	***Rec. %***	***$E_{rel.}$%***	***δ_{n-1}****	***S.E.M*** $\bar{x}\pm(t_{0.05}\frac{\delta n-1}{\sqrt{n}})$ *at 95% CL for (n-1)*	***RSD %***
90.49	0.987×10^{-3}	98.69	-1.30	6.416×10^{-6}	$0.987\times10^{-3}\pm0.797\times10^{-4}$	0.649%
90.38	0.983×10^{-3}	98.27	-1.72			
90.30	0.980×10^{-3}	97.96	-2.03			
90.67	0.994×10^{-3}	99.39	-0.60			
90.66	0.994×10^{-3}	99.36	-0.63			

δ_{n-1}**:standard deviation, t=2.78 for N=5,* $\bar{x}$ *= 0.987*$\times10^{-3}$.

3-6-2- Standard additions method (SAM):-

It carried out by a procedure that 0.5 mL increment of 10^{-3}M clarithromycin as standard was added to 20 mL of sample as unknown. The results of calculation (SAM) for the clarithromycin using (E4) electrode and equation 1-15, recovery, relative error and relative standard deviation for five additions of clarithromycin are listed in Table 3-7.

Table 3-7: The results for five additions of clarithromycin standard solution using (SAM) for E4 electrode, where slope=58.484 mV/decade, at concentration of sample 10^{-4} M.

V_s (mL) added	E (mV)	ΔE	(V_u/V_s)	Antilog (ΔE/S)	C_u (M)	Rec. %	Erel%	δ_{n-1}*	S.E.M $\bar{x}\pm(t_{0.05}\frac{\delta n-1}{\sqrt{n}})$ at 95% CL for (n-1)	RSD %
0	30	-----	0	1	----	-----	-----	7.208×10^{-7}	$0.991\times10^{-4} \pm 0.896\times10^{-6}$	0.727
0.5	35.11	5.11	40	1.2228	0.986×10^{-4}	98.6	-1.3			
1.0	39.14	9.14	20	1.4331	0.990×10^{-4}	99.1	-0.9			
1.5	42.45	12.45	13.3	1.6328	0.993×10^{-4}	99.3	-0.7			
2.0	45.15	15.15	10	1.8157	1.003×10^{-4}	100.3	0.3			
2.5	47.83	17.83	8	2.0177	0.984×10^{-4}	98.4	-1.6			

δ_{n-1}*:standard deviation, t=2.78 for N=5.*

3-6-3- Multiple standard addition method (MSA):-

The calibration curve for MSA for (E4) electrode was shown in Figure 3-14 by plotting antilog (E/S) versus the volume of the five additions of standard clarithromycin. From the equation of the calibration curve the volume (mL) at intercept with X axis for the curve was calculate, the concentration of the unknown sample (C_U) was calculated using the equation 1-18.The volume at intercept with X axis, concentration of the unknown sample (C_U), the analysis results %Re and $\%E_r$ are listed in Table 3-8.

V_s (mL) added	E (mV)	Antilog (E/S)
0	30	3.26
0.5	35.11	3.98
1.0	39.14	4.67
1.5	42.45	5.32
2.0	45.15	5.92
2.5	47.83	6.57

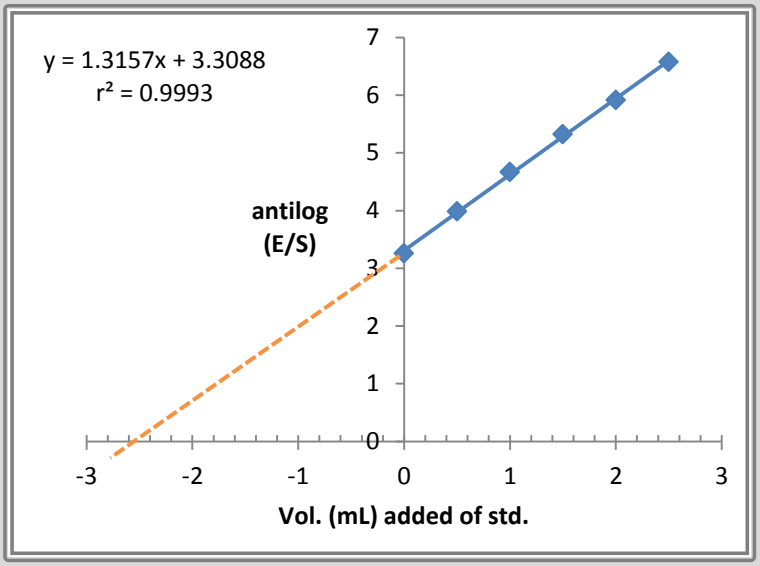


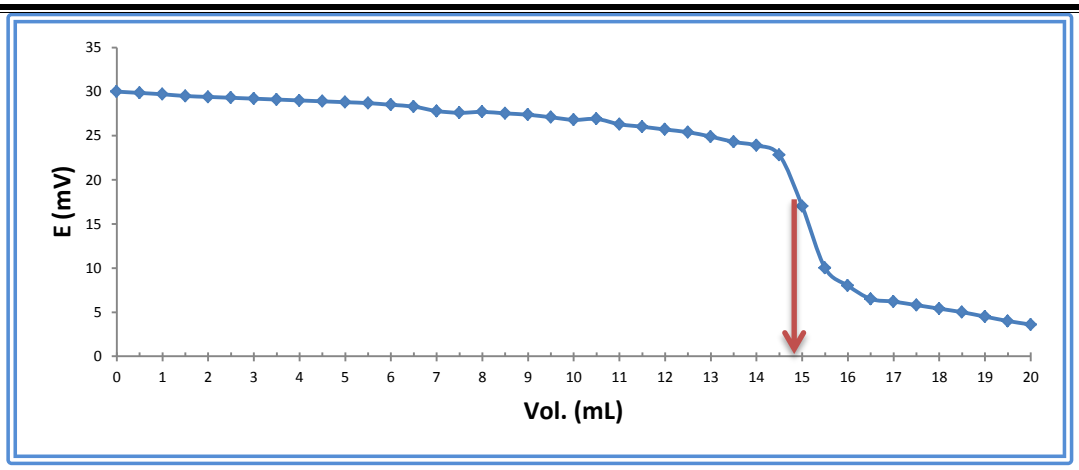
Figure 3-14: Calibration curve of antilog (E/S) versus the volume added of standard 10^{-3}M for determination of clarithromycin solution 10^{-4}M by (MSA).

Table 3-8: The linear equation of calibration curve uses MSA, correlation coefficient, volume at intercept, the concentration of sample (C_U), Re% and E_r% for the unknown sample.

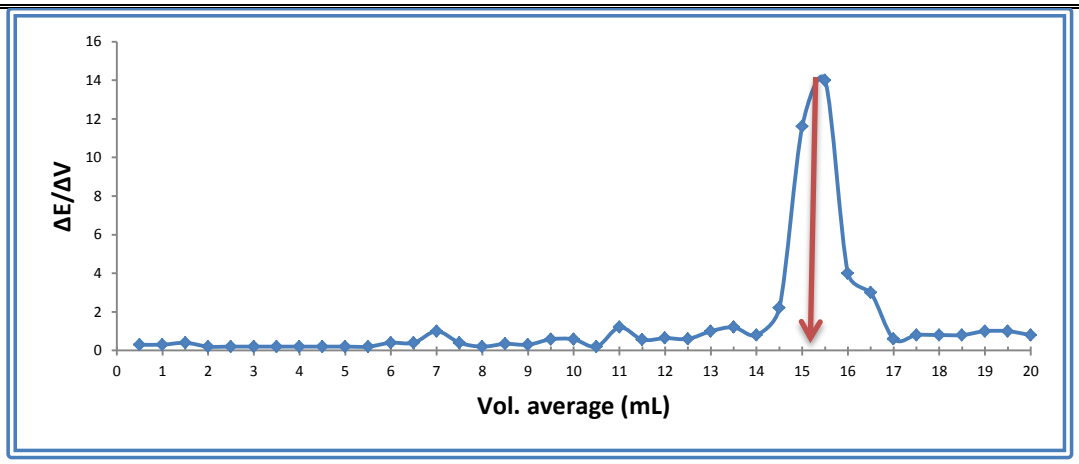
Linear equation	r	Volume at intercept (mL)	C_u (M)	Rec. %	Erel. %
y = 1.3157(x)+ 3.3088	0.9996	2.514	1.005×10^{-4}	100.56	0.56

3-6-4- Titration method:-

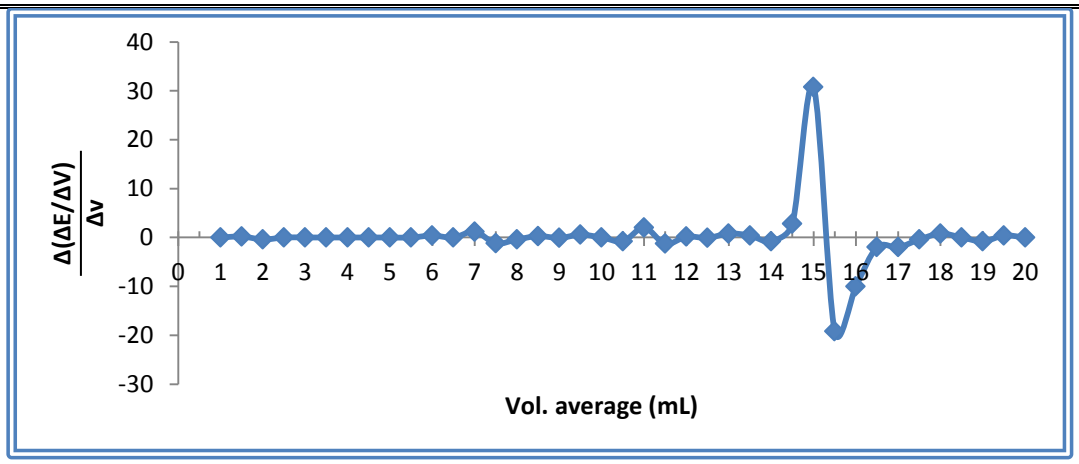
The potentiometric titration for 15 mL of 1×10^{-4} M clarithromycin sample solution with 1×10^{-4} M Sodium tetraphenylborate as titrant solution as shown in Figure 3-15 (a and b), the results of titration (Re%, E_r% and RSD%) are listed in Table 3-9.



a- Titration curve



b- Titration curve by using first derivative



c- Titration curve by using second derivative

Figure 3-15: Titration curve of E4, 15 mL sample solution 1×10^{-4} M clarithromycin with 1×10^{-4}M of (NaTPB) as a titrant solution.

Table 3-9: The results of using titration method for standard clarithromycin sample for E4 electrode.

Titration Figure	Vol. (mL) at the end point	C_u (M)	Rec. %	Erel. %	δ_{n-1}	RSD*%
Figure 3-15(a)	14.8	0.986×10^{-4}	98.66	-1.33	1.677×10^{-6}	1.113
Figure 3-15(b)	15.1	1.006×10^{-4}	100.66	0.66		
Figure 3-15(c)	15.3	1.020×10^{-4}	102.00	2.00		

RSD% for the unknown concentration from the three figures.*

3-7- Analytical application of the selected electrode:

3-7-1- Direct method: -

From the calibration curve of the electrode E4 the concentration of the unknown was calculated from the linear equation y=25.4Ln(x) + 266.28 of the calibration curve, the results are listed in Table 3-10.

Table 3-10: The results of five measurements of the same pharmaceutical sample of Clarithromycin solution using direct method for E4 electrode, where slope=58.484 mV/decade.

Potential reading E(mV)	Conc. Of (CLM) sample calculated from linear equation (M)	Rec. %	Erel.%	δ_{n-1}*	S.E.M $\bar{x}\pm(t_{0.05}\frac{\delta n-1}{\sqrt{n}})$ at 95% CL for (n-1)	RSD %
32.21	0.995×10^{-4}	99.49	-0.50	1.233×10^{-6}	$1.007\times10^{-4}\pm1.533\times10^{-6}$	1.224%
32.82	1.019×10^{-4}	101.91	1.91			
32.16	0.993×10^{-4}	99.30	-0.69			
32.62	1.011×10^{-4}	101.12	1.11			
32.77	1.017×10^{-4}	101.71	1.71			

δ_{n-1}*:standard deviation, t=2.78 for N=5, $\bar{x} = 1.007\times10^{-4}$.

3-7-2- Standard additions method (SAM):-

It carried out by a procedure that 0.5 mL increment of 10^{-3}M clarithromycin as standard was added to 20 mL of sample as unknown. The results of calculation (SAM) for the clarithromycin using (E4) electrode and equation 1-15, recovery, relative error and relative standard deviation for five additions of clarithromycin are listed in Table 3-11.

Table 3-11: The results for five additions of clarithromycin standard solution using (SAM) for E4 electrode, where slope=58.484 mV/decade, at concentration of sample 10^{-4} M.

V_s (mL) added	E (mV)	ΔE	(V_u/V_s)	Antilog (ΔE/S)	C_u (M)	Rec. %	Erel.%	δ_{n-1}*	S.E.M $\bar{x}\pm(t_{0.05}\frac{\delta n-1}{\sqrt{n}})$ at 95% CL for (n-1)	RSD %
0	30	-----	0	1	----	-----	-----	9.282×10^{-7}	$1.014\times10^{-4}\pm 1.150\times10^{-6}$	0.915
0.5	34.97	4.97	40	1.2161	1.014×10^{-4}	101.41	1.4			
1.0	38.86	8.86	20	1.4174	1.024×10^{-4}	102.40	2.4			
1.5	42.38	12.38	13.3	1.6281	0.999×10^{-4}	99.97	-0.03			
2.0	44.93	14.93	10	1.8000	1.020×10^{-4}	102.03	2.03			
2.5	47.42	17.42	8	1.9854	1.013×10^{-4}	101.32	1.32			

δ_{n-1}: at t=2.78 for N=5*

3-7-3- Multi standard addition method (MSA):-

The calibration curve for MSA for (E4) electrode was shown in Figure 3-16 by plotting antilog (E/S) versus the volume of the five additions of standard clarithromycin. From the equation of the calibration curve the volume (mL) at intercept with X axis for the curve was calculate. The concentration of the unknown sample (C_U) was calculated using the equation 1-18, The volume at intercept with X axis, concentration of the unknown sample (C_U), the analysis results %Rec. and $\%E_{rel.}$ are listed in Table 3-12.

V_s (mL) added	E (mV)	Antilog (E/S)
0	30	3.25
0.5	34.97	3.96
1.0	38.86	4.62
1.5	42.38	5.30
2.0	44.93	5.86
2.5	47.42	6.46

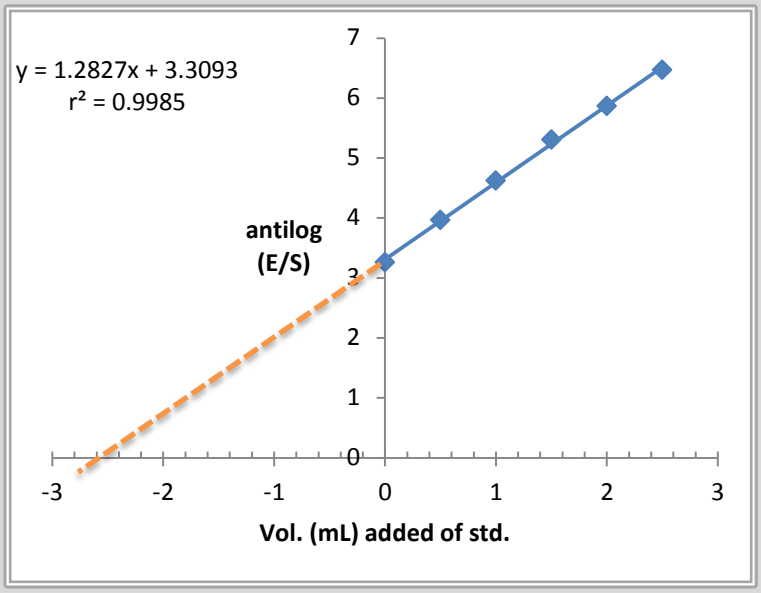


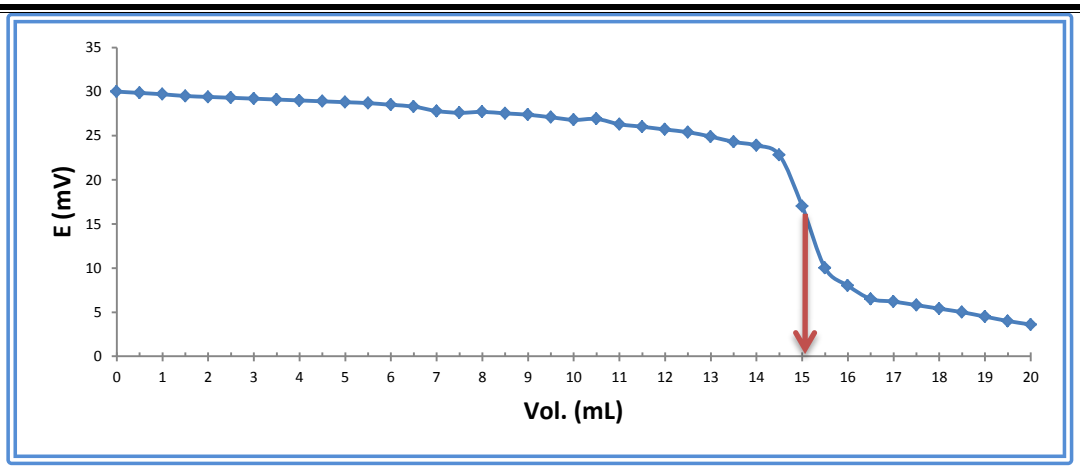
Figure 3-16: Calibration curve of antilog (E/S) versus the volume added of standard 10^{-3}M for determination of clarithromycin solution 10^{-4}M by (MSA).

Table 3-12: The linear equation of calibration curve uses MSA, correlation coefficient, volume at intercept, the concentration of sample (C_U), Re% and E_r% for the unknown sample.

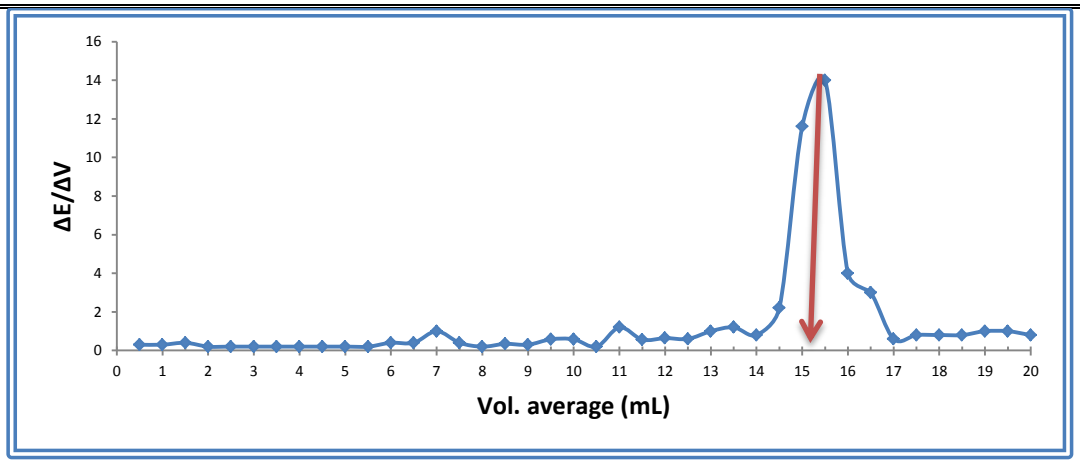
Linear equation	r	Volume at intercept (mL)	C_u (M)	Rec. %	Erel. %
y = 1.2827(x)+ 3.3093	0.9992	2.579	1.032×10^{-4}	103.19	3.19

3-7-4- Titration method:-

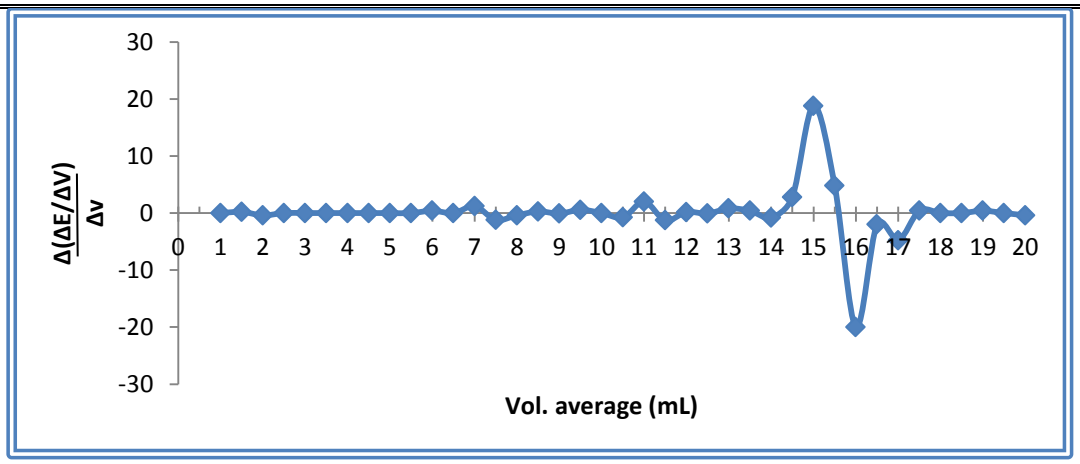
The potentiometric titration for 15 mL of 1×10^{-4} M clarithromycin sample solution with 1×10^{-4} M Sodium tetraphenylborate as titrant solution as shown in Figure 3-16 (a and b), the results of titration (Rec.%, $E_{rel.}$% and RSD%) are listed in Table 3-13.



a- Titration curve



b- Titration curve by using first derivative



c- Titration curve by using second derivative

Figure 3-17: Titration curve of E4, 15 mL sample solution 1×10^{-4} M clarithromycin with 1×10^{-4}M of (NaTPB) as a titrant solution.

Table 3-13: The results of using titration method for clarithromycin sample for E4 electrode.

Titration Figure	Vol. (mL) at the end point	C_u (M)	Rec. %	Erel. %	δ_{n-1}	RSD*%
Figure 3-15(a)	15.1	1.006×10^{-4}	100.66	0.66	1.387×10^{-6}	0.909
Figure 3-15(b)	15.2	1.013×10^{-4}	101.33	1.33		
Figure 3-15(c)	15.5	1.033×10^{-4}	103.33	3.33		

RSD% for the unknown concentration from the three figures.*

Table 3-14: Summary of sample analyses of claricide tablets pharmaceutical using E4 electrode.

Parameter	Direct method	SAM	MSA	Titration Method
Conc.(M)	1.000×10^{-4}	1.000×10^{-4}	1.000×10^{-4}	1.000×10^{-4}
Found(M)	1.007×10^{-4}	1.014×10^{-4}	1.032×10^{-4}	1.017×10^{-4}
RSD %	1.224%	0.915%	----------	0.909 %
Rec.%	100.7%	101.4%	103.19%	101.77 %
$E_{rel.}$%	0.7%	1.4%	3.19%	1.77 %

3-8- Conclusions:-

The two kinds of electrodes were prepared in this study based on PVC matrix for clarithromycin determination; the first kind included fabrication of membranes for clarithromycin (CLM) was constructed by tetraphenylborate (TPB) with drug as ion-exchanger and many plasticizers: Di-butyl phthalate (DBPH), Di-octyl phthalate (DOP), Di-butyl phosphate (DBP) and Acetophenone (AP) in PVC matrix membrane, and the second kind included fabrication of membranes for clarithromycin (CLM) was constructed by tetraiodomercurate (TIM) with drug as ion-exchanger with the same plasticizers. The best electrode was (E4) electrode which used to determine clarithromycin in the pharmaceutical sample.

Also there is no interference for some interfering ion (Na^{+}, K^{+}, Mn^{2+}, Cu^{2+}, Fe^{3+}, Al^{3+}, sucrose and gelatin), it also has the working pH in the range (1.5–6.5). The practical utility of the electrode has been demonstrated by use it as indicator electrode in potentiometric precipitation titration of clarithromycin solution with sodium tetraphenylborate solution. Direct method, standard additions method and multi standard additions method have been also successfully applied and showing a very good results. The results of these analytical methods were showed to be simple, rapid and with good accuracy.

3-9- Future Work:-

Based on the above ion selective electrode studies, a future work can be applied on other ISE's which can be fabricated using:

1- Other types of drugs and antibiotic, with different properties and chemical structure, to obtain wide selectivity over drugs and multiple drugs.
2- Other methods for preparation ion-exchanger (ionophore).
3- Other plasticizers to get better idea on their influence on the electrode performance.
4- Other types of matrixes as alternative to PVC matrix.
5- Other physical properties of membrane: percentage of components proportions in membrane, through fixing one of the components and changing the other, and thickness by increasing the weight of the components or changing the diameter of a glass casting ring.
6- Application of these membranes in analyses of other drug samples with similar active groups.
7- Study the selectivity behavior using other methods and also by using more interfering ions.
8- Using new analytical techniques such as flow injection technique.

1- Standard Deviation (δ_{n-1})

$$\delta_{n-1} = \sqrt{\frac{\sum(X_i - \bar{X})^2}{N-1}}$$

Where:

X_i = concentration of individual deviations.

$\bar{X}$ = Mean of concentration.

N = no. of measurements.

2- Relative Standard Deviation (RSD%)

$$\text{RSD\%} = \frac{\delta_{n-1}}{\bar{X}} \times 100$$

3- Relative Error ($E_{rel.}$%)

$$\text{Error} = \bar{X} - \mu$$

$$E_{rel.}\% = \frac{\text{Error}}{\mu} \times 100$$

Where:

$E_{rel.}$% ; Error relative between the results average (X) and the true value (μ).

4- Recovery (Rec.%)

$$\text{Recovery (Rec.) \%} = \frac{\mu - E}{\mu} \times 100$$

5- Standard Error mean (S.E.M.)

$$Cl\ of\ \mu = \bar{x} \pm t_{0.05} \left(\frac{\delta_{n-1}}{\sqrt{n}}\right)$$

6- Limit of detection (LOD)

$$LOD = \frac{3\delta_{n-1}X}{\bar{X}}$$

7- Linear regression:

$$S_b = \frac{S_{y/x}}{\sqrt{\sum_i (x_i - x)^2}} \quad , \quad S_a = S_{y/x} \sqrt{\frac{\sum_i x_i^2}{n \sum_i (x_i - x)^2}}$$

$$S_{y/x} = \sqrt{\frac{\sum_i (y_i - \hat{y}_i)^2}{(n-2)}} \quad , \quad \hat{y}_i = bx_i + a$$

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الخلاصة

هذا الجزء من البحث يتضمن تصنيع نوعين من الاقطاب الانتقائية السائلة بالاعتماد على مادة البولي فاينيل كلورايد. **اولاً** اربع اقطاب انتقائية للكلاريثرومايسين والتي تعتمد على المعقد (clarithromycin-tetraphenylborate) كمادة فعالة تم تحضيرها. **ثانياً** اربع اقطاب انتقائية للكلرثرومايسين ايظا والتي تعتمد على المعقد (clarithromycin-tetraiodomercurate) كمادة فعالة وفي كلا النوعين تم استخدام المواد الملدنة مثل:

Di-octyl phthalate (DOP), Di-butyl phosphate (DBP), Acetophenone (AP) and Di-butyl phthalate (DBPH).

تتضمن هدة الاطروحة ثلاثة فصول وكل فصل يحتوي على المعلومات التالية:

الفصل الاول يشمل نبذة مختصرة عن خواص الاقطاب الانتقائية وكذلك تطبيقات الاقطاب الانتقائية في المستحضرات الصيدلانية وايضاً يتضمن تحليلات الكلاريثرومايسين واستخداماته.

الفصل الثاني يشمل الجزء العملي وايضا يوضح المواد الكيميائية المستخدمة والاجهزة وطريقة تحضير النوعين من الاقطاب الانتقائية المستعملة في البحث.

الفصل الثالث يشمل النتائج العملية ومناقشتها للاقطاب المصنعة التي تسفر عن امكانية التطبيقات الناجحة للاقطاب المصنعة في التحضيرات الصيدلانية وقياسات الكلاريثرومايسين الذي يشمل نوعين من الاقطاب المصنعة للكلاريثرومايسين. اقطاب النوع الاول وهى:

CLM-TPB+DOP (E1), CLM-TPB+DBP (E2), CLM-TPB+AP (E3), CLM-TPB+DBPH (E4).

حيث أعطت مدى التراكيز الخطية (5×10^{-5}-1×10^{-3}, 1×10^{-5}-1×10^{-3}, 1×10^{-5}-1×10^{-3}, 1×10^{-5}-1×10^{-3}) مولاري وانحدار (58.484, 58.104, 53.930, 51.206) ملي فولت/ حقبة وحد تحسس (9×10^{-6}, 2×10^{-5}, 6×10^{-6}, 8×10^{-6}) مولاري وزمن استجابة (46, 41, 35, 30) ثانية لتركيز محلول الكلاريثرومايسين 10^{-3} مولاري وعمرالقطب (29, 12, 30, 24) يوم بالتوالي.

اقطاب النوع الثاني وهى:

CLM-TIM+DOP (E5), CLM-TIM+DBP (E6), CLM-TIM+AP (E7), CLM-TIM+DBPH (E8).

حيث اعطت مدى التراكيز الخطية (5×10^{-5}-1×10^{-3}, 5×10^{-5}-1×10^{-3}, 1×10^{-5}-1×10^{-3}, 1×10^{-5}-1×10^{-3}) مولاري وانحدار (49.442, 52.692, 42.970, 48.445) ملي فولت/ حقبة وحد تحسس (1.5×10^{-5}, 5×10^{-5}, 5.5×10^{-5}, 5×10^{-6}) مولاري وزمن استجابة (48, 30, 32, 26) ثانية لتركيز محلول الكلاريثرومايسين 10^{-3} مولاري وعمرالقطب (25, 16, 22, 28) يوم بالتوالي.

لقد وجد ان افضل قطب هو E4 حيث استخدم لتقدير الكلاريثرومايسين في المستحضرات الصيدلانية. ان مدى الدالة الحامضية للقطب E4 بحدود (1.5-6.5) ولقد تم دراسة معامل الانتقائية $K^{pot}_{A,B}$ للاقطاب الانتقائية للمتدخلات التالية (Na^{+}, K^{+}, Mn^{+2}, Cu^{+2}, Fe^{+3}, Al^{+3}, sucrose and gelatin) بواسطة طريقة المحاليل المنفصلة وطريقة المحاليل الممزوجة حيث تراوحت بين (4.28×10^{-2}-8.82×10^{-5}) وكذلك تم استخدام القطب E4 في التقديرات الاجهادية لتعيين الكلرثرومايسين في الادوية التجارية .

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين/كلية العلوم
قسم الكيمياء

تصنيع ودراسة اقطاب بوليمرية جديدة لتقدير الكلاريثروميسين

رسالة
مقدمة إلى كلية العلوم- جامعة النهرين
وهي جزء من متطلبات نيل درجة الماجستير في الكيمياء

من قبل
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١٤٣٤ هـ 2013 م