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Preparation of liquid electrode based on Amiloride drug complex in PVC matrix membrane

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

By

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We, the Examining Committee, certify that we have read this thesis and examined the student (Maha Abdulateef yahya) in its contents, and that in our opinion it is adequate with (excellent) standing as a thesis for the Degree of Master of Science, in Chemistry.

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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 شمسي وضياء نهاري ..... أخي

إلى من هن بلسم جراحي ..... أخواتي

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

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

مها

بِسْمِ اللهِ الرَّحْمَنِ الرَّحِيمِ

وَهَيِّئْ رَبَّنَآءِاتِنا مِنْ لَّدُنكَ رَحْمَة
لَنَا مِنْ أَمْرِنَا رَشَداً

صَدقَ اللهُ العَظيم
سورة الكهف / جزء من آية 10

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Chapter One: Introduction

Summary:-

Amiloride hydrochloride ion-selective electrodes were constructed in polymeric membrane by using PVC and based on the use of active ion-pair (Amiloride - phosphotungstate) and the other ion pair (Amiloride-tetraphenylborate) with the following plasticizers: Di-butyl phthalate (DBPH), Di-octylphthalate (DOP) and Tri-butyl phosphate (TBP). The properties of these electrodes were studied, including: slope, correlation coefficient, concentration range, detection limit and life time.

These electrodes (A, B and C) based on the ion pair (Amiloride - phosphotungstate) and (D, E and F) based on the ion pair (Amiloride - tetraphenylborate) and the plasticizers used DBPH, DOP and TBP respectively they gave approximately the same linear concentration range from ($1x10^{-5}$ to $1x10^{-2}$) M. The slopes are (54.198, 52.759, 50.91, 49.007, 48.508 and 48.501) mV/decade, and the limit of detection (6×10^{-7}, 1.5×10^{-6}, 7×10^{-6} , 1.75×10^{-5}, 7×10^{-6} and 1.5×10^{-5})M, with the response time (10, 30, 10, 12, 10 and 35) Sec. and the lifetime were about (45, 35, 30, 21, 15 and 10) days respectively. The working pH range found to be (1.9 –7.8) for the concentration of Amiloride solution 1×10^{-3} M by using electrode A (Amilo-PT+DBPH). Also the interferences were studied via selectivity against monovalent, divalent, trivalent ions and interference of the other type of diuretic drugs Hydrochlorothiazide. The separated solution method and fixed interfering mixed method were used to determine the selectivity coefficient $K^{pot}_{A,B}$.

Electrode A (Amilo-PT+DBPH) was used to determine Amiloride in the pharmaceutical samples of Amiloride Maduratic tablets and Saluretic tablets. The analytical methods results showed to be simple, rapid and with a good accuracy by comparing it with UV-spectrophotometric method using F-test.

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Abbreviations

Amilo	Amiloride hydrochloride
Amilo-PT	Amiloride hydrochloride- phosphotungstate complex
Amilo-TPB	Amiloride hydrochloride- Tetraphenylborate complex
CST	Unit of viscosity centistokes=0.01 stock
DMSO	Dimethylsulfoxid
DOP	Dioctylphthalate
DOPP	dioctylphenylphosphonate
FIA	Flow injection analysis
FIM	Fixed interference method
FPM	Fixed primary ion method
FTIR	Fourier transform infrared spectroscopy
F.W.	Formula weight
GC	Gas chromatography
HCT	Hydrochlorothiazide
HPLC	High performance liquid chromatography
ISE	Ion-selective electrode
LC	Liquid chromatography
MPA	Molybdophosphoric acid
MPM	Match potential method
MS	Mass spectrophotometry
NaTPB	Sodium tetraphenylborate
NPOE	nitro phenyl octyl ether
PLS	Partial least squares
PT	Phosphotungastic acid
PVC	Poly vinyl chloride
RSD	Relative standard deviation
E	Relative error
Re	Recovery
SCE	Saturated calomel electrode
SDI	State company for drug industries
SIA	Sequential injection analysis
SPE	Solid phase extraction
Std.	Stndard
TBP	Tributylphosphate
Tg	Glass transition temperature
THF	Tetrahydrofuran
TSM	Two solution method

Chapter One

Introduction

1- Introduction

1-1- Amiloride Hydrochloride:-

1-1-1- Physical and Chemical properties:-

Amiloride Hydrochloride, an antikaliuretic-diuretic agent, is a pyrazine-carbonyl-guanidine[1] that is unrelated chemically to other known antikaliuretic or diuretic agents, it is a yellow solid crystal powder, odourless, it is solubility in water 0.52 g/100 mL; in alcohol 1.96 g/100 mL at 25°C; freely soluble in dimethylsulfoxid (DMSO)[2]; practically insoluble in ether, acetone and chloroform, melting point 240.5 to 241.5°C, pH 3.8 to 5.2, it is the salt of a moderately strong base (pKa 8.7), store in well-closed containers ideally between15 to 30°C, avoid freezing or a temperature greater than 40°C and protect from light[3]. It is designated chemically as 3, 5 - diamino - 6 - chloro- N - (diaminomethylene) pyrazine carboxamide monohydrochloride, dihydrate and has a molecular weight of 302.12. Its empirical formula is $C_6H_8ClN_7O.HCl.2H_2O$ and its structural formula is as show in Fig.1-1.[4]

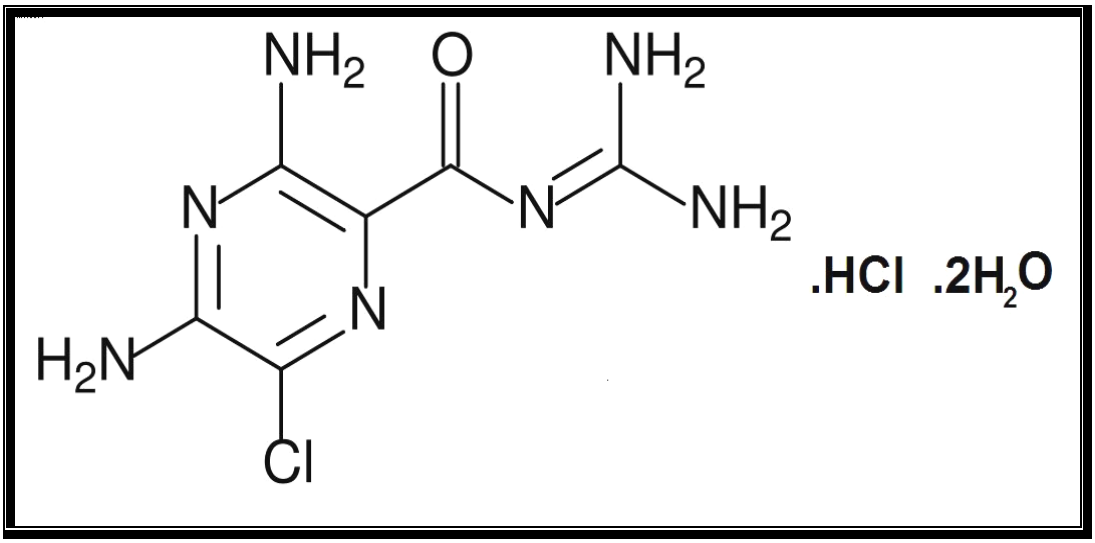


Fig.1-1:- Structure formula of Amiloride hydrochloride.

1-1-2- Classification of diuretics:-

There are several types of diuretics. All diuretics increase the excretion of water from the body, although each class of diuretic does so in a distinct way [5].

- High ceiling diuretics are diuretics that may cause a substantial diuresis up to 20% of the filtered load of NaCl and water.

- Osmotic diuretics, Compounds such as mannitol are filtered in the glomerulus, but cannot be reabsorbed. Their presence leads to an increase in the osmolarity of the filtrate, to maintain osmotic balance; water is retained in the urine.

- Thiazides, drugs such as Hydrochlorothiazide[4] ($C_7H_8ClN_3O_4S_2$; F.W. 297.74) is a white crystalline compound with low solubility in water but readily soluble in alcohol and in dilute aqueous sodium hydroxide. It is act on the distall tubule and inhibits the Sodium-chloride symporter leading to retention of water in the urine.

- Potassium-sparing diuretics, these are diuretics which do not promote the secretion of potassium into the urine; thus, potassium is spared and not lost as much as in other diuretics. Such drugs include spironolactone which is a competitive antagonist of aldosterone. Aldosterone normally adds sodium channels in the principal cells of the collecting duct and late distal tubule of the nephron. Spironolactone prevents aldosterone from entering the principal cells, preventing sodium reabsorption. Other examples of potassium-sparing diuretics are Amiloride and Triamterene. These drugs bind to the sodium channels of the principal cells, inhibiting an aldosterone-induced increase in sodium reabsorption. They are used as adjunctive therapy, together with other drugs, in the treatment of hypertension and management of congestive heart failure.

Amiloride has a weak diuretic effect when used on its own; hence it is usually used in combination with other diuretics. Most other diuretics cause the amount of potassium in the blood to drop. Amiloride doesn't have this effect, as it is a 'potassium-sparing' diuretic type [6]. It is usually added to diuretic treatment to prevent excessive amounts of potassium from being lost.

1-1-3- Indication and usage for Amiloride [7]:-

Amiloride HCl is indicated as adjunctive treatment with Thiazide diuretics or other kaliuretic diuretic agents in congestive heart failure or hypertension to:

1- Help restore normal serum potassium levels in patients who develop hypokalemia on the kaliuretic diuretic.
2- Prevent development of hypokalemia in patients who would be exposed to particular risk if hypokalemia were to develop. Amiloride HCl has little additive diuretic or antihypertensive effect when added to a thiazide diuretic. Amiloride HCl should rarely be used alone. It has weak (compared with Thiazides) diuretic and antihypertensive effects. Used as single agents, potassium sparing diuretics, including Amiloride HCl, result in an increased risk of hyperkalemia[8] (serum potassium levels greater than 5.5 mEq per liter).

1-1-4- Analysis of Amiloride hydrochloride:-

There have been only a few reports on the determination of Amiloride in tablets or in biological fluids[9]. A method for the determination of Amiloride at concentrations between 0.015 and 0.152 mg/L by means of matrix isopotential fluorescence spectrometry and

derivative techniques is proposed, the method was successfully applied to the determination of Amiloride in urine. To obtain maximum sensitivity and adequate selectivity, factors affecting fluorescence intensity were studied in the Amiloride band centered at λex = 362 nm and λem = 415 nm. As a result, the determination was performed in an ethanol–water (1 + 1, v/v) medium at pH 6.3, adjusted by using sodium citrate–citric acid (0.1 M) as buffer solution. A calibration graph was constructed by measuring first derivative values at λex = 357 nm and λem = 392 nm[10]. The determination of Amiloride in human urine by the sequential injection analysis technique (SIA) coupled with solid phase extraction (SPE) microcolumn. SPE microcolumn was used for selective retention of Amiloride, while the urine matrix components were eluted with water carrier flow to the waste, due to the acid-basic and polarity properties of Amiloride molecule and principles of ion-exchange chromatography. The whole procedure comprising raw sample pre- treatment, analyte detection and column reconditioning took 8 min.[11]

A simple and fast method for the simultaneous determination of amiloride and furosemide by digital derivative spectrophotometry. HCl 1×10^{-2} M dissolved in ethanol was used as solvent and to extract drugs from formulations. Subsequently the samples were evaluated directly by first derivative spectrophotometry, using a smoothing factor of 8 and scale factor of 1×10^{-4}. The simultaneous determination of Furosemide and Amiloride can be carried out at 241.4 and 343.6 nm, respectively. In both cases, the zero crossing approach was used. When both compounds are present together in a sample, it is possible to quantify one in the presence of the other, without mutual interference. The determination range was found to be of 6.9×10^{-8} to 16×10^{-5} and 6.8×10^{-8} to 8×10^{-5} M, for Amiloride and Furosemide, respectively. A good level of %RSD of 0.9 and 0.6% was observed for Amiloride and Furosemide, respectively. The ingredients

commonly found in commercial pharmaceutical formulations do not interfere. The proposed method was applied to the determination of these drugs in pharmaceutical formulations.[12]

A detection and excretion study of Amiloride under Conditions of Steroid Screening Procedure. Two chromatographic methods HPLC and GC/MS are used for comparison and determination of the extraction recovery of Amiloride. HPLC analysis of Amiloride was performed in combination with the GC/MS analysis and Amiloride artifact derivatives were detected. The higher percent of extraction (23%) was obtained when ethylacetate used as extragent for L-L extraction. After applying solid phase extraction (SPE) on XAD-2 column extraction recovery yielded to 10% (by GC/MS) and 12% (by HPLC). The drugs containing Amiloride are usually combined with other diuretics as Triamterene or Hydrochlorothiazide. This excretion study was performed after oral administration of a single dose (4 mg) of pure substance. Two excretion maxima were obtained at 3 and 9 hours after administration. In urine samples up to 70 hours after application the Amiloride artifact was detected. The limit of detection of the Amiloride artifact by the screening procedure of steroids is 8 ng/ml. The obtained results show the possibility to include Amiloride in the routine screening procedure of anabolic steroids.[13]

Simultaneous determination of Amiloride and Hydrochlorothiazide in human plasma by liquid chromatography/ mass spectrometry with positive/negative ion-switching electrospray ionization, A new method for simultaneous determination of Amiloride and Hydrochlorothiazide by liquid chromatography/electrospray double mass spectrometry (LC/MS/MS) operated in positive and negative ionization switching mode was developed and validated. Protein precipitation with acetonitrile was selected for sample preparation. The determination was carried out on a Waters Quattro-micro triple-quadrupole mass spectrometer operated in

multiple reactions monitoring mode. The lower limits of quantification were 1×10^{-4} and 1×10^{-3} mg mL^{-1} for Amiloride and Hydrochlorothiazide, respectively, which were lower than other published methods by using ultraviolet (UV), fluorimetric or mass spectrometric detection. The accuracy were studied at three different concentration levels and were always better than 15% (n = 5).[14]

Electrochemical behavior and determination of Amiloride drug in bulk form and pharmaceutical formulation at mercury electrodes, the polarographic behavior of Amiloride hydrochloride has been studied in Britton–Robinson buffers of pH 1.9–11. In acidic medium at pH≤ 2, the dc-polarograms exhibited a single 4-electron cathodic irreversible wave, while at pH values >2, a second two-electron irreversible cathodic wave appeared at a more negative potential. The single or first wave may be attributed to the cleavage of the double bond of the ---CH=NH of the imidino amide group with the release of NH_3. While the second wave may be due to the saturation of the C=O of the carboxamide moiety. A polarographic procedure of sensitivity for the determination of bulk Amiloride drug in Britton–Robinson buffer at pH 2 is described. The calibration graph was obtained over the concentration range 2.5×10^{-5} to 2.5×10^{-4} M Amiloride. The limits of detection and quantitation of the procedure were 1×10^{-5} and 3.3×10^{-4} M bulk Amiloride, respectively. Moreover, a differential-pulse adsorptive cathodic stripping voltammetric procedure has been described to assay of the drug at lower concentration levels. The optimal conditions were: E = −0.9 V, time = 30 sec, pulse-height = 90 mV and Britton–Robinson buffer of pH 8. The calibration graph was obtained over the concentration range 2×10^{-8} to 1×10^{-6} M for bulk Amiloride. Both procedures were successfully applied to the determination of Amiloride in tablets without the necessity for sample pretreatment or any time-consuming extraction or evaporation steps prior

to the drug analysis. [15]

Amiloride Biological Fluid Analysis by reverse-phase HPLC. Because of a lack of sensitivity in reported analytical methods, useful pharmacokinetic data have been sparse. This publication describes a sensitive (1×10^{-3} mg/L), rapid, and reproducible reverse-phase HPLC technique for the quantitative measurement of Amiloride levels in both serum and urine specimens. Sample clean-up is based on the adsorption of Amiloride to commercially available silica cartridges and its selective elution with perchloric acid. The eluted drug is further resolved from endogenous interferences on an analytical column with a mobile phase consisting of methanol:sodium perchlorate (0.1 M, pH 4.0) 40:60 v/v and fluorescence detection (λ_{ex} = 368 nm, λ_{em} = 417 nm). This method has been applied to the analysis of human serum and urine samples. [16]

A high-performance liquid chromatographic method has been developed for Amiloride in rabbit plasma and urine, a mobile phase (flow-rate 2 ml/min) consisting of 32% acetonitrile in 0.15 M perchloric acid, pH 2.2, and spectrofluorometric detection via excitation at 286 nm. A simple extraction step with ethyl acetate eliminates interfering peaks. Short retention times of about 2.3 and 3.8 min are observed for Amiloride and the internal standard, triamterene, respectively. The method can measure at 4×10^{-3} mg/L Amiloride in plasma.[17]

Direct analysis of Amiloride and Triamterene mixtures by fluorescence spectrometry using partial-least squares (PLS) calibration, Triamterene and Amiloride exhibit overlapped spectra and urine produces background fluorescence that precludes the direct determination of these diuretics by conventional fluorimetry. Although, the qualitative composition of the fluorescent metabolites in urine from healthy people is virtually invariable their quantitative composition exhibits some differences. Thus, the shape of the spectrum and the position of the

fluorescence maxima change as the urine is diluted. The determination was performed in a 1:1 (v/v) ethanol /water medium at an apparent pH of 6.3 provided by 0.01 M sodium citrate/citric acid buffer. An excitation wavelength of 365 nm was used for both Amiloride and Triamterene. The corresponding emission maxima were located at 413 and 437 nm for Amiloride and Triamterene, respectively. In order to ensure accurate results, the calibration matrix was implementing a urine sample containing no Triamterene or Amiloride (i.e. urine blank). The concentration of Amiloride was varied from 64×10^{-3} to 32×10^{-2} mg/L and that of Triamterene from 2×10^{-2} to 1×10^{-1} mg/L. Urine dilution was varied from 1:35 to 1:65. Urine was used as the third component of the calibration matrix in order to include the information inherent in changes in the fluorescence spectrum for urine upon dilution. [**18**]

Preparation and Examination of Amine and Amiloride-Ion Selective Electrodes with PVC Matrix Membranes, Ion selective electrodes for Amines and Amiloride were prepared using phosphomolybdic acid ionophore and various plasticizers: di-n-butylphthalate (DBPH), tri-n-butylphosphate (TBP), o-nitrophenyloctyl ether (NPOE), and di-octylphenylphosphonate (DOPP). The response characteristics of these electrodes, including slope of the calibration plot, the corresponding concentration range, detection limit, response time, life time, pH effect, and selectivity were studied. The electrode for Amiloride based on DOPP as the plasticizer exhibited excellent sensitivity; the slope of the calibration plot was 58.4 mV/decade, the detection limit was 1.4×10^{-5} mol L^{-1}, and the suitable pH was 3.8.[**19**]

Simultaneous quantitation of Amiloride Hydrochloride and Hydrochlorothiazide in tablets by UV spectrophotometry coupled with new chemometric regression techniques and artificial neural networks, The chemometrics is an appropriate tool for quick analysis of “Tialorid” tablet

in routine drug control without a complicated process of wavelength selection or derivative calculation. Calibration dataset was constructed from the spectra of 15 solutions containing different concentrations of the ingredients in methanol (0–14 mg/L of their sum), registered in 200– 400 nm range with 0.5 nm intervals. The neural-network models based on absorbances and principal components were also tested. [20]

Different chemometric methods such as classical least squares, principal components regression and partial least squares with one dependent variable applied on UV spectral data and on their first derivatives (1D) were evaluated for the simultaneous quantification of samples containing mixtures of Amiloride hydrochloride, atenolol, Hydrochlorothiazide and Timolol maleate. The four statistically equivalent procedures were successfully applied to the analysis of synthetic samples with two to four analytes and to commercial tablet preparations containing Amiloride hydrochloride and Hydrochlorothiazide alone or in association with Atenolol or Timolol maleate. [21]

Another method was a simple spectrophotometric method for the determination of Amiloride in pharmaceutical preparation and urine. The method is based on the absorption of Amiloride on a cation – exchange resin at pH 4.0 and the direct measurement of its practical UV absorbance in the solid phase at 362 nm (the maximum absorption wavelength) and 412 nm (wavelength at which only the solid support absorbs light), The net absorbance (A) for Amiloride in solid phase was obtained from: $A = A_{362} - A_{412}$. The calibration graphs (for the net absorbance) were linear from 0.8 to 7, 0.08 to 0.7 and 0.02 to 0.15 mg L^{-1} with detection limits of 0.2, 0.02 and 0.006 mg L^{-1} for three sample 10, 100 and 500 mL sample volumes, respectively. The relative standard deviations for ten independent determinations were better than 3.46%. [22]

Potentiometric membrane ion-selective electrodes (IESs) have been used in pharmaceutical and biological analysis. This is mainly due to their simple design, low cost, adequate selectivity, good accuracy and wide concentration range [23]. Electroanalytical methods have a long history of development, Progress in ISE development has occurred rapidly in the past 40 years, with promising innovations still on the horizon. [24]

1-2- Ion-selective electrode (ISE):-

It is an electrochemical sensor, based on a thin selective membrane or film as recognition element and is an electrochemical half-cell equivalent to other half-cell as reference electrode. These devices are distinct for half-cells that involve electrode redox reactions. The potential difference response of the Gibbs energy change associated with perm-selective mass transfer of ions (e.g., by ion-exchange, solvent extraction or some other mechanism) across a phase boundary. The measured potential differences are linearly dependent on the logarithm of the activity of a given ion in solution. [25]

1-3-Ion-selective electrode cell measurements:-

Generally, the cell contains two reference electrodes, “internal” and “external”, and a selective membrane as the recognition element. Conventional notation of the cell is [25]:

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

The measured cell emf, E is described with the Nernst equation [26]:-

$$\mathbf{E = E_o - (RT/nF) \ln a} \qquad \textbf{…1-1}$$

$$\mathbf{E = E_o - (2.303RT/nF) \log a} \qquad \textbf{…1-2}$$

Where E_o = constant for a given cell, E = the total potential (mV) developed between the sensing and reference electrodes, R = gas constant (8.314 joul $mole^{-1}degee^{-1}$), T = temperature in Kelvin 298K (25^0C), n = ionic charge, F = faraday constant (96500 coulombs), a = is the chemical activities for the reduced and oxidized species, at room temperature (25°C) Nernst equation is frequently expressed as:-

$$\mathbf{E = E_o - (59.1/n) Log\ a} \qquad \text{…1-3}$$

The ISE illustrated in Fig.1-2 is a typical electrode in which the transition between the ion and electron takes place at the internal reference electrode (Ag/AgCl) immersed in the inner filling solution (KCl), as follows:

$$AgCl + e^- \leftrightarrow Ag + Cl^-$$

The identical above reaction occurs at the external reference electrode, illustrating the reversible and stable connection between the ionic (Cl^-) and electronic (e) signals. The selectivity is the result of incorporation of specific ionophores in the ion-selective membrane. [27]

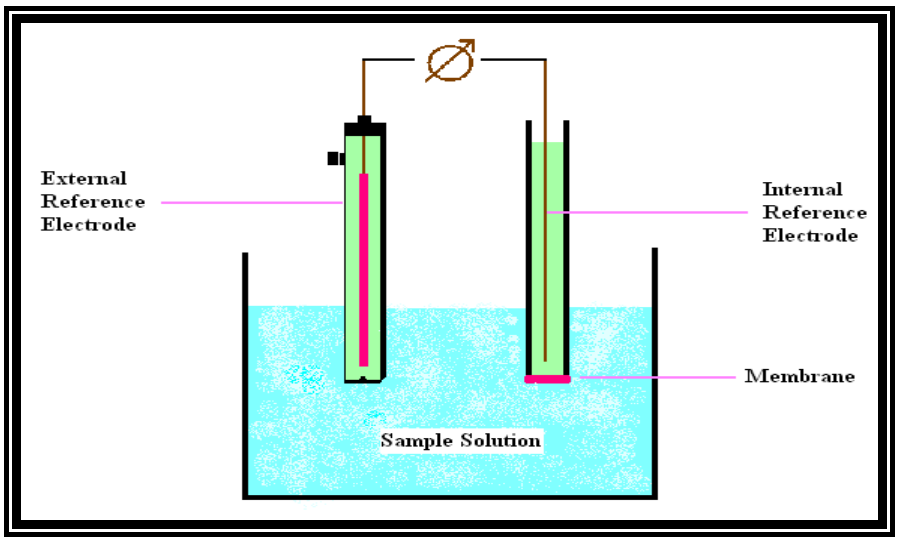


Fig.1-2:- A classical ion-selective electrode in electrochemical cell.

1-4- Classification of Ion Selective Electrode (ISE) membranes:-

Several types of ion-selective electrodes are commercially available, they are classified by the nature of the membrane material used to construct the electrode. The differences in membrane construction make an electrode selective for a particular ion. [28]

1-4-1- Liquid Electrodes (Organic Ion Exchangers and Chelating Agents):-

Initially, to construct the liquid ISE membrane porous materials were soaked in a solution of a water-immiscible, nonvolatile, viscous organic liquid containing the dissolved ionophore or the ion carrier. Recently, polymers have been utilized as homogeneous membrane matrix. The suitability of the polymer to be employed in a sensing membrane (when the required solubility is displayed) is defined by the glass transition temperature (Tg) of the polymer (Tg is the temperature at which an amorphous solid, such as glass or a polymer, becomes brittle on cooling, or soft on heating). The Tg value should be below the room temperature. As a consequence, the designed membranes are fluid enough under the ambient conditions, they allow the diffusion of the membrane components, they present reasonable ionic conductivities and they illustrate the proper mechanical properties to be handled for routine processes.[29] The ionophore (membrane–active recognition) or the ion carrier is the most vital component in a polymeric membrane sensor in terms of selectivity. The ionophore or the membrane–active recognition can be an ion exchanger or a neutral macrocyclic compound. It has molecule–sized dimensions and it contains cavities or semi-cavity to surround the target ions. The binding between the ionophore and the target ion is the molecular-level phenomenon, sensed by the ISE. Therefore, the various

ISE selectivity towards the other ions are regarded to derive from the difference in the binding strengths between the selected ionophore, to be used in the sensor, and the different ions. The ISE function involves the phase transfer of the aqueous ions into an organic medium of the ISE which is typically the plasticized PVC. Actually, the transferred ions interact with the membrane components. In case that the incorporated ionophore is a simple ion exchanging species.[30] The additives, which increase the plasticity or fluidity of the material to which they are added, are called plasticizers. Normally, the composition of the solvent polymeric membranes, used in the ion selective devices, is to exhibit optimal physical properties, ensuring relatively high mobilities for their constituents. The membrane solvent has to be physically compatible with the polymer, so as to give a homogeneous organic phase. Additionally, it may affect the selectivity behavior. In contrast, the selectivity of the carrier-based ISEs are significantly influenced by the membrane solvent. [29]

1-4-2- Solid State Electrodes (Crystalline membrane):-

Solid state electrodes utilize relatively insoluble inorganic salts in a membrane. Solid state electrodes exist in homogeneous or heterogeneous forms. In both types, potentials are developed at the membrane surface due to the ion-exchange process. Examples Chloride-selective electrodes with a poly (pyrrole) solid contact.[31,32]

1-4-3- Glass Membrane Electrodes:-

Glass membrane electrodes are formed by the doping of the silicon dioxide glass matrix with various chemicals. The most common of the glass membrane electrodes is the pH electrode. Glass membrane electrodes are also available for the measurement of sodium ions.[28,31]

1-4-4- Gas Sensing Electrodes (Molecular Selective Electrodes) [28]:-

Gas sensing electrodes are available for the measurement of dissolved gas such as ammonia, carbon dioxide, nitrogen oxide, and sulfur dioxide. These electrodes have a gas permeable membrane and an internal buffer solution. Gas molecules diffuse across the membrane and react with a buffer solution, changing the pH of the buffer. The pH of the buffer solution changes as the gas reacts with it. The change is detected by a combination pH sensor within the housing. Due to the construction, gas sensing electrodes do not require an external reference electrode. Enzyme electrodes definitely are not true ion-selective electrodes but usually are considered within the ion-specific electrode topic, such as urease membrane have been prepared for blood urea analysis.[33] Recently micro fabrication sensors on a flexible substrate minimize the injury in the body, when it is in use.[34,35]

1-5- Reference electrodes:-

A reference electrode has a stable and well defined electrochemical potential (at constant temperature) against which the applied or measured potentials in an electrochemical cell are referred. A good reference electrode is therefore non- polarizable, in other words, the potential of such an electrode will remain stable upon passage of a small current. One could also say that the impedance of an ideal reference electrode is zero. [36, 37]

1-5-1- Types of reference electrodes: - [38]

An absolute standard for the measurement of electrochemical potentials is not available. It is therefore that the equilibrium potential of the so-called Standard Hydrogen Electrode (SHE) is defined as being (0.00) Volt at $a_{H^+}=1$. In practice this means that the following reaction:

$$\mathbf{2\ H^+ + 2\ e^- = H_2\uparrow}$$

Taking place on a Platinum electrode in 1.19 M HCl (H^+ activity = 1) has an equilibrium potential of 0 Volt. The SHE is difficult to use in practice as it involves bubbling H_2 gas through solution, therefore a number of other reference electrodes are available, the most important of which are shortly discussed below:

1-5-1-1- Silver/Silver Chloride (Ag/AgCl in saturated KCl)[37,38]

This is probably the most widely used reference electrode. This electrode consists of an Ag wire in contact with AgCl in a saturated KCl solution. This results in an electrode potential of +0.197 Volt vs SHE at 25° C. Although most electrodes of this type use saturated KCl (3 M) as electrolyte.

$AgCl + e^- = Ag + Cl^-$

The main disadvantage of this reference electrode is the use of chloride, which is unwanted in some cases. Electrodes of this type can be used up to fairly high temperatures (80-100°C).

1-5-1-2- Saturated Calomel Electrode (SCE) (Hg/Hg_2Cl_2 in saturated KCl)[39,40]

Traditionally this was the most widely used electrode until the use of mercury was banned from more and more laboratories. The electrode potential is +0.241 V vs. SHE at 25°C. The following reaction is taking place:

$Hg_2Cl_2 + 2\ e^- = 2\ Hg + 2\ Cl^-$

Compared to the Ag/AgCl electrode, this electrode has the disadvantage that it cannot be used above 50°C due to instability of the Hg_2Cl_2.

1-5-1-3- Mercury/mercury sulphate Electrode (Hg/Hg_2SO_4 in 0.5M H_2SO_4)[36,38]

This reference electrode is used in some cases where the use of chloride ions is not desirable. The electrode potential of this system is +0.680 Volt vs. SHE. The following reaction is taking place:

$$Hg_2SO_4 + 2\ e^- = 2\ Hg + SO_4^{2}$$

1-5-1-4- Mercury/mercury oxide (Hg/HgO in 1 M NaOH)[38]

It used in alkaline solutions only. The electrode potential of this electrode is +0.140 Volt vs. SHE.

1-6- Characterization of ISEs:-

The properties of an ISE are characterized by parameters like:

1-6-1- Calibration curve:-[25, 36, 41]

Fig.1-3 shows the typical ISE calibration curve. It is a plot of the cell emf, E (i.e. the galvanic potential difference measured between the ion-selective electrode and the external reference electrode of a given ion-selective electrode cell assembly vs. the logarithm of the single ionic activity (concentration) of a given species. For uniformity, it is recommended that the cell emf is ascribed to the ordinate (vertical axis) with the more positive potentials at the top of the graph and that paA (-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.

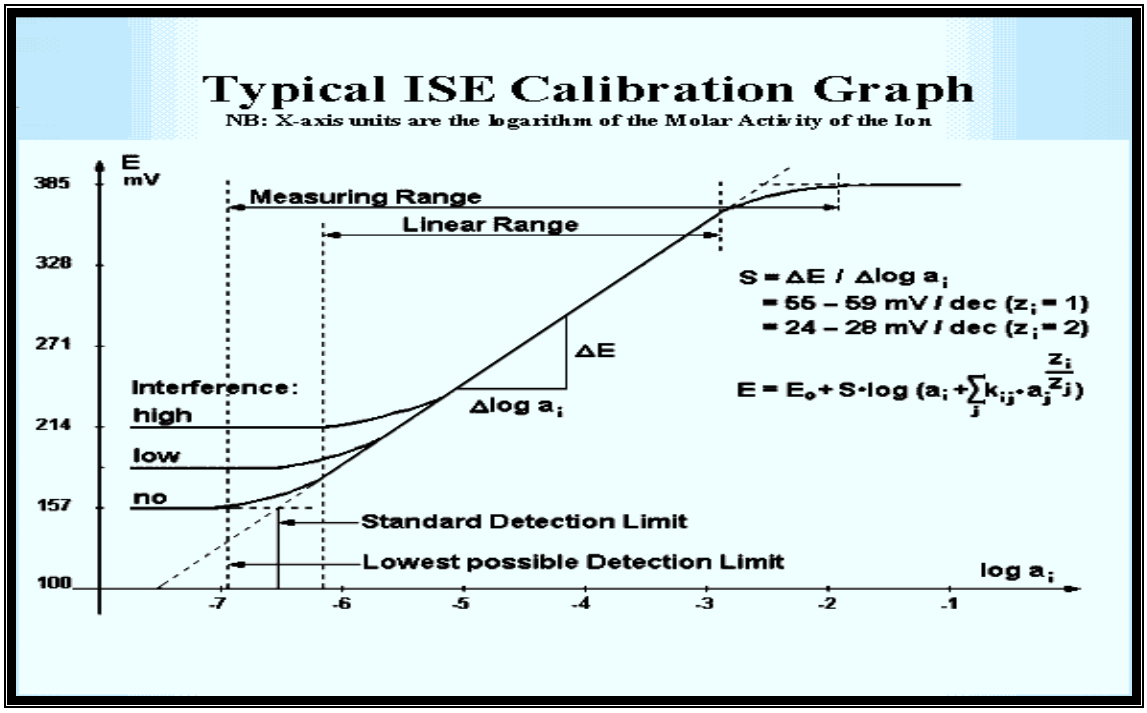


Fig.1-3: Typical ISE calibration graph.

1-6-2-Slope [36, 37] *:-*

The magnitude 2.303RT/nF from equation 1-2 is the slope of the line (from the straight line plot of E versus log (a) which is the basis of ISE calibration graphs) as show in Fig. 1-3.

$$S = (E_1 - E_2) / (\log a_1 - \log a_2) \qquad \text{...1-4}$$

The S value is the slope that equal to $\Delta E/\Delta \log a_i$, and this is an important diagnostic characteristic of the electrode. Generally the slope gets lower as the electrode gets old or contaminated, and the lower the slope the higher the errors on the sample measurements.

1-6-3-Detection limit:- [37, 41, 42]

According to the IUPAC recommendation the detection limit is defined by the cross-section of the two extrapolated linear parts of the ion-selective calibration curve as show in Fig.1-3. In practice, detection limit

on the order of 10^{-5}-10^{-6}M is measured for most of ion-selective electrodes. The observed detection limit is often governed by the presence of other interfering ions or impurities.

1-6-4- Range of linear response: - [37, 41]

At high and very low target ion activities there are deviations from linearity. Typically, the electrode calibration curve exhibits linear response range between 10^{-1}M and 10^{-5}M. As shown in Fig.1-3, through which a linear regression would demonstrate that the data point does not deviate from linearity by more than ± 2 mV.

1-6-5- Response time: - [37, 41, 42]

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 ±1mV for the final equilibrium potential. Generally electrodes with liquid ion-exchanger membrane have longer response time than solid membrane electrode. The factors which influence the response time includes type of membrane are (concentration change, total volume of the test solution, rate of stirring, temperature, interfering species).

1-6-6- Stability and Lifetime:- [43]

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 same factors which influence the response time (solution concentration, the interfering ions, which poison the electrode surface), and 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.

1-6-7-Selectivity:-

An earlier IUPAC data compilation of potentiometric selectivity coefficients $K^{pot}_{A,B}$, for ion-selective electrodes (ISEs) was published in 1979[44]. In 1998 a number of particularly selective ionophores, which are lipophilic complexing agents that are incorporated into ISE membranes to selectively and reversibly bind analyte ions. The potentiometric selectivity coefficients [45] are 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. Several experimental methods for the determination of potentiometric selectivity coefficients are based on Nicolsky–Eisenman equation. Notably, this equation does not correctly describe responses in the activity range in which primary and interfering ions of a different charge significantly contribute to the potential. More complex equations must be applied to describe correctly such mixed ion responses.[46] Potentiometric selectivity coefficients can be measured with different methods that fall into two main groups:

1-6-7-1- Separate solution methods:-[47]

1-6-7-1-1- When ($a_A = a_B$):-[48]

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:

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

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

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

Where (S) is the slope of the electrode.

1-6-7-1-2- When ($E_A = E_B$):- [25,47]

The log$a$ vs E relations 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.

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

1-6-7-2- Mixed solution methods: - [47, 49]

1-6-7-2-1- Fixed interference method (FIM):- [50,51,52]

The potentiometry of a cell comprising an ion - selective electrode and a reference electrode (ISE cell) is measured for solutions of constant activity of the interfering ion, a_B, and varying activity of the primary ion, a_A. The emf values obtained are plotted vs. the logarithm of the activity of the primary ion. The intersection of the extrapolated linear portions of

this plot indicates the value of a_A [as in Fig.1-4] that is to be used to calculate $K^{pot}_{A,B}$ by using equation 1-8.

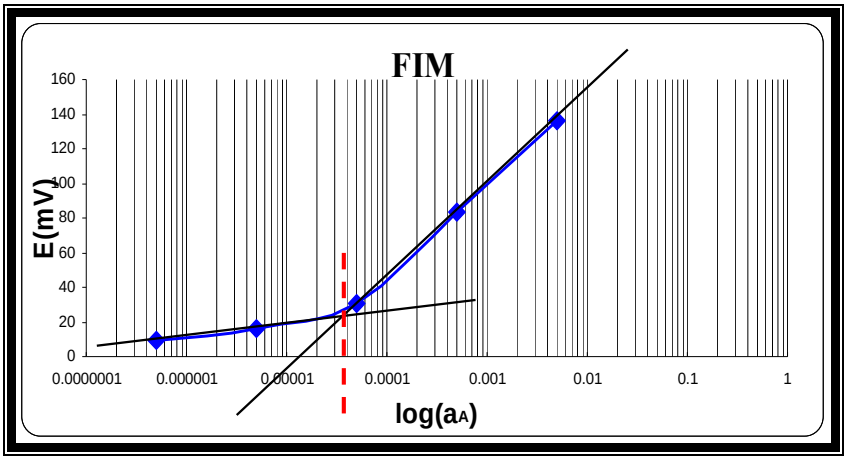


Fig.1-4: Determination of a_A value according to FIM.

1-6-7-2-2- Fixed primary ion method (FPM):- [47,53]

The potentiometry of a cell comprising an ion-selective electrode and a reference electrode (ISE cell) is measured for solutions of constant activity of the primary ion, a_A, and varying activity of the interfering ion, a_B. The emf 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 $K^{pot}_{A,B}$ using equation 1-8.

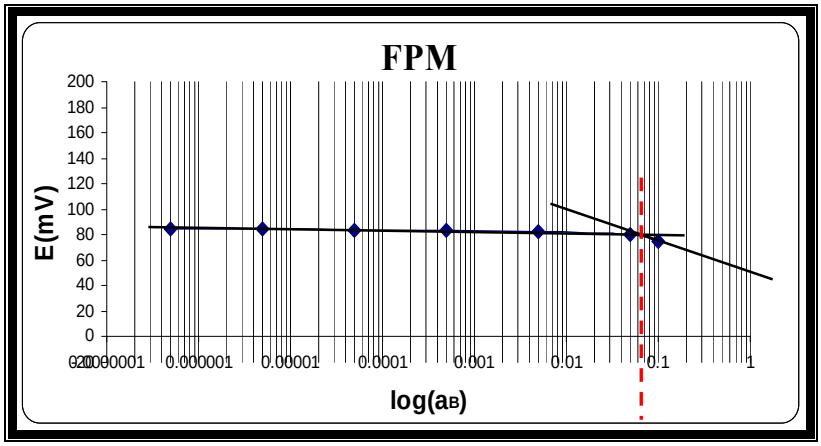


Fig.1-5:- Determination of a_B value according to FIM.

1-6-7-2-3- Two solution method (TSM):-[47,54]

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:

$$\mathbf{K^{pot}_{A,B} = a_A (e^{\Delta E\, z_A\, F / (R\, T)} - 1) / (a_B)^{\, z_A/z_B}} \qquad \mathbf{... 1\text{-}9}$$

1-6-7-2-4- Matched potential method (MPM):-[47,55,56]

Among the mixed solution methods, this method was recommended in 1995 by IUPAC as a method that gives analytically relevant practical $K^{pot}_{A,B}$ values, This theory 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 e.m.f. (Δe.m.f.) 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

$$\mathbf{K^{pot}_{A,B} = \Delta a_A / a_B} \qquad \mathbf{\dots 1\text{-}10}$$

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

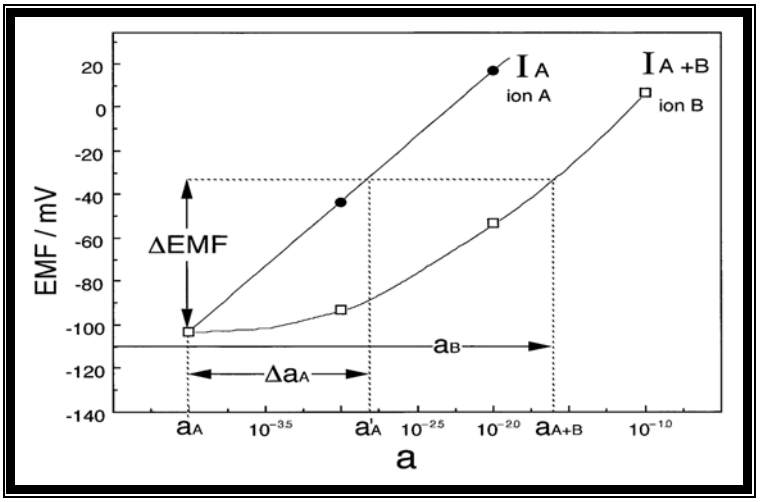


Fig.1-6:- Determination of selectivity coefficients by the MPM.

1-7- Measurement Techniques:- *[36,42]*

There are multiple techniques analytical in the use of ion selective electrodes measurement and depends on sample composition, precision & accuracy and time requirement in the process of measurement. The most important and widely used techniques for such studies are; direct, standard and titration methods.

1-7-1- Direct Potentiometry Method:- [36,40,57]

Direct potentiometry is the simplest and most widely used method of using ISEs. Simply measure the electrode response in an unknown solution and read the concentration directly from the calibration graph as in Fig.1-3 by using equation 1-4 for the slope, 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.

1-7-2- Incremental Methods:-

There are three main types of incremental methods in general use:-[36]

81-7-2-1- Standard (or Known) Addition method (SAM):-

This method is generally more accurate than direct method for concentration measuring in the sample, but it is more time-consuming because of the calibration involved [42]. A small volume of a know solution 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:

$$C_U = C_S / 10^{\Delta E/S} [1+ (V_U / V_S)] - (V_U / V_S) \quad \ldots 1\text{-}11$$

Where C_U, C_S, V_U and V_S are the concentration and volume of unknown and standard solution respectively.

1-7-2-2- Multiple standard addition method (MSA):- [36, 58]

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

$$E= E^o + S \text{ Log } a_A \times V_S/ V_U \quad \ldots 1\text{-}12$$

Where S is the slope of the electrode, V_S, V_U, are the volumes of added standard (titrante) 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:

$$\text{Antilog } E/S = \text{constant} \times a_A V_S /V_U \quad \ldots 1\text{-}13$$

Where antilog (E^o/S) is constant thus the antilog (E/S) is proportional to V_S. A plot of antilog (E/S) against V_S, a straight line is obtained, the intercept of which with the volume axis denote the end point of the unknown concentration in an addition method. In this method several addition of standard solution to the same sample to be measured in orders to increase the accuracy and decreases the errors.

1-7- 3- Potentiometric titration method:-[36]

Potentiometric titration method have been also used for the evaluation of the performance of ISE 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%. The principle of ion selective electrode titrations is based on the fact that in a stoichiometric reaction between two species in solution, the end point of the reaction is characterized by the total disappearance of one of the species or first appearance of a product of the reaction. The sample is titrated with a suitable titrant and the increase or decrease in titrant activity is followed with an ion-selective electrode response, to locate the equivalence point as in Fig.1-7. [59]

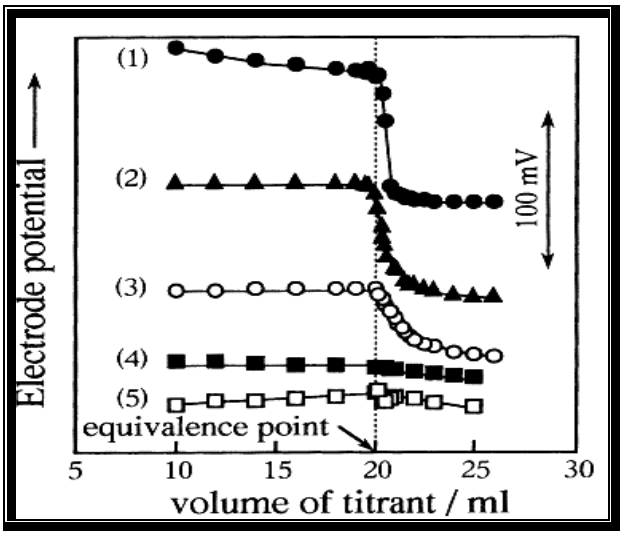


Fig.1-7:- Potentiometric titration curves by using ion-selective electrodes.

1-8- Sources of Error: - [60,61]

***1-8-1- Diffusion*: -** 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. This difference creates an additional potential resulting in error. To compensate for this type of error it is important that a positive flow of filling solution move through the junction and that the junction not become clogged or fouled [62].

***1-8-2- Sample Ionic Strength*: -** Covington [63] points out that the total ionic strength of a sample affects the activity coefficient and that it is important that this factor stay constant. In order accomplish this; the addition of an ionic strength adjuster is used. 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.

1-8-3- Temperature* [60]*: - 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%.

1-8-4- pH* [60]*: - Some samples may require conversion of the analyte to one form by adjusting the pH of the solution (e.g. ammonia). Failure to adjust the pH in these instances can lead to significant measurement errors.

1-8-5- Interferences* [60]*: - The background matrix can effect the accuracy of measurements taken using ISEs. Covington[63] points out that some interference may be eliminated by reacting the interfering ions prior to analysis.

1-9- General Application of ISEs: - [36]

Ion-selective electrodes (ISEs) have become important and reliable devices for chemical, pharmaceutical and biomedical analysis; they are inexpensive and easy to use, and have a wide range of application. The following list is a brief survey of some of the major applications in which electrodes have been used:

1-9-1- Agriculture [64]***:-*** Soil and fertilizer analysis for Nitrate, Ammonium and Potassium to optimize the use of fertilizer.
-Dissolved Oxygen and pH in ponds for fish breeding.

1-9-2- Medical diagnosis and hygiene control:- Potassium in urine.[65,66]

-Contamination control for various ions. [67]

1-9-3- Pollution Monitoring [68, 69]***:-*** pH of acid rain, soil, surface water.

- Contamination of surface water and ground water with ammonium and nitrate.

- Contamination of waste water with cyanide, cadmium, mercury and copper.

1-9-4- Explosives [70]***:-*** Fluoride, chloride and nitrate have been measured in explosives and their combustion products.

1-9-5- Food processing (food quality control) [71]***:-*** Nitrate in meat and vegetables. [72]

-Chloride, Sodium, Nitrate in baby food.
-Fluoride and Calcium in milk and other milk products.
-Cadmium in fish.

1-9-6- Cosmetics industry: - Fluoride and pH of tooth paste. [73]
-pH of hair shampoo.[74]
-Many other ions determined in cosmetic research and development.

1-9-7- Industrial production:- Salinity and pH of boiler feed water.[71]

-Cyanide in plating baths.[75]

-Process specific ions like, Sodium[76], Lithium[77], Potassium[78], Silver[79], Nickel[80], Calcium[81], Zinc[82], Copper[83,84], Mercury[85], Manganese[86], Magnesium[87], Cobalt[88], Ferric[89], Aluminum[90.91], Cesium[92], Chloride[93], Fluoride[94], Bromide.[95]

1-10- Applications of ISEs in pharmaceutical samples:-

Potentiometric membrane sensors have been more extensively used in pharmaceutical analysis [96]. Most of the ISEs, which are sensitive to medically important ionic compounds, belong to the class of ion-pair based liquid membrane electrodes were; Ampicillin[97], Atenolol[98], phenylpiperazine antidepressant (Nefazodone)[99], Promethazine hydrochloride[100], Scopolamine[101] and Methylene Blue[102] are PVC membrane electrodes that were based on ion-pair with phosotungstic acid, when other drugs like Acebutolol[103], Methacycline hydrochloride[104], Tiapride[105], Chloropromazine Amitriptyline[106], Hydralazine[107], Chlorprothixene[108], Naphazoline[109] were PVC membrane electrodes based on ion-pair with tetraphenylborate. Another drugs such as Ibuprofen[110] with methyltrioctylammonium chloride as ion-pair constructed a PVC membrane electrode, Nimesulide[52]is based on the molybdophosphoric acid (MPA) as ion pair, Cetylpyridinium chloride[111] with Ferric Thiocynate as active ion-pair PVC matrix membrane electrode, Cyproheptadine hydrochloride[112] with Tetrakis(4-chloro phenyl borate) as active ion-pair, Naproxen[113] and Tetraheptylammonium as ion-pair with drug, Vitamin (B_1)[114] with anion reineckate as ion-pair, Ephedrine[115] form Ephedrine-5-nitro- barbiturate as active ion-pair in PVC membrane electrode, Another Atenolol[116] ion-selective electrode is

prepared incorporates PVC membrane with atenolol-tetrakis(*p*-chlorophenyl) borate ion-pair complex, Histamine[117] with tetraphenyl-porphyrins as Ionophore, Cefuroxime[118] selective electrodes for batch and FIA (Flow Injection Analysis) were tetraoctylammonium or bis(triphenylphosphoranylidene) ammonium used as ion exchanger ionophore), An ion-selective electrode for the determination of some multidrug resistance reversers chlorpromazine, clomipramine, imipramine and verapamil in pharmaceuticals[119]. Another new conventional coated-wire ISEs used for flow-injection potentiometric determination of triamterene[120] based on triamterene–tetraphenylborate ion-pair.

1-11- Spectrophotometric Method:-

Spectrophotometric techniques are used to measure the concentration of solutes in solution by measuring the amount of light that is absorbed by the solution in a cuvette placed in the spectrophotometer. A spectrophotometer measures the intensity of a light beam after it is directed through and emerges from a solution.[121] Photometric methods whether UV, visible, or FTIR, are characterized by their sensitivity and selectivity. The visible and UV regions are usually of greater practical application of drugs because the molar absorptivity exhibited are usually of high order of magnitude than those in the FTIR. Thus greater sensitivity can be obtained at these spectra region. [122] A spectrophotometer is employed to measure the amount of light that a sample absorbs. The instrument operates by passing a beam of light through a sample and measuring the intensity of light reaching a detector. The beam of light consists of a stream of photons, represented by the purple balls in the simulation shown below. When a photon encounters an analyte molecule (the analyte is the molecule being studied), there is a chance the analyte will absorb the photon. This

absorption reduces the number of photons in the beam of light, thereby reducing the intensity of the light beam. [123]

First, the intensity of light (I_o) passing through a blank is measured. The blank is a solution that is identical to the sample solution except that the blank does not contain the solute that absorbs light. This measurement is necessary, because the cell itself scatters some of the light. Second, the intensity of light (I) passing through the sample solution is measured. Third, the experimental data is used to calculate two quantities: the transmittance (T) and the absorbance (A).

$$T = I/I_o$$

$$A = - \log 10T$$

The wavelength range of Ultraviolet (UV) radiation starts from 200 nm and ends to 400 nm. The radiation of UV has sufficient energy to excite valance electrons in many atoms or molecules from their ground state to higher energy levels .The excited electrons transfer from a bonding to an anti bonding orbital. The UV radiation may be absorbed by saturated compounds that containing atoms with unshared electron pair on bonding such as Nitrogen, Oxygen, Sulfur, Halogen ...etc, are capable of $n\text{-}\delta^*$ transition (150-250) nm. Most unsaturated organic compounds are based upon transitions of (n or π) electrons to π^* excited state. The visible region 400-900 sometime to near IR 1100 nm was used for all colored compounds. [124]

Wide applications of UV/Visible spectroscopy include numbers of inorganic metals, organic compounds, and biochemical species absorbed ultraviolet or visible radiation and are thus amenable to direct quantitative determination. The typical applications of UV absorption spectroscopy include the determination of poly nuclear aromatic compounds such as steroids, painting materials, Vitamins, drugs and there are various applications of visible spectrophotometric methods have been developed

for analysis of different colored metal complexes and colored compounds.[125]

1-12- Aim of the work:-

This project was aimed to construct and characterize several ion-selective electrodes for the potentiometric determination of Amiloride hydrochloride. The construction of a poly(vinyl chloride) (PVC) membrane comprised an ion-pair formed between Amiloride and phosotungstic acid (PT) and the other ion-pair used sodium tetraphenylborate (NaTPB), utilize the solvent mediators or plasticizers, Di-butylphthalate (DBPH), Di-octylphthalate (DOP) and Tri-butyl phosphate (TBP). The constructed electrodes characteristic parameters that include slope, linear concentration range, detection limit, response time and lifetime will be investigated; The best electrode characteristic parameters used to find working pH range, selectivity and potentiometric measurements including direct method, standard addition method and titration method will be studied and compared with UV-spectrophotometric determination of Amiloride (Normal calibration curve) by using F-test.

Chapter Two

Experimental Part

2- Experimental part

2-1- Instruments and equipment:-

1- Expandable ion analyzer, ORION, model EA 940, (U. S. A.).

2- Reference electrode single junction, ORION, model 90-01

3- Combined glass electrode Orion No.91-02, (Swiss made).

4- Ultra sonic devise (ultrasonicator) for dissolving samples, (W. Germany).

5- Silver- silver chloride wire.

6- Micropipettes (200-1000µl) and 25µl (Swiss made).

7- Clear PVC tubing (6 mm o.d.).

8- Magnetic stirrer.

9- Ultra pure water manufacturing devise, TORAYPURE, Model LV-08 (Mihama, Japan).

10- FTIR-8300 Fourier transforms infrared spectrophotometer Shimadzu. (Japan).

11- Double-beam UV-Visible spectrophotometer model (UV-1650 PC) SHIMADZ (Japan), interfaced with computer via a SHIMADZU UV probe data system program (Version 1.10).

2-2- Chemicals:-

1- Pure Standard Amiloride hydrochloride ($C_6H_8CIN_7O.HCl.2H_2O$ F.W.302.12) was a gift from the State Company of Drug Industries and Medical Appliances (Samara- IRAQ-SDI).

2- Commercial* drugs: Saluretic tablets (Amiloride hydrochloride 5mg + Hydrochlorothiazide 50mg) made in Cairo-Egypt and the same content in Maduratic Indian tablets.

Note: * mean the trade name of drug.

3- Dodeca –Tungstophosphoric acid (PT) ($H_3PO_4.12WO_3.XH_2O$; F.W. 2880.2), (BDH).

4- Sodium tetraphenylborate (NaTPB) ($C_{24}H_{20}BNa$; F.W. 342.22), (BDH).

5- Tetrahydrofuran (C_4H_8O; F.W. 72.11), (E.Merck).

6- Polyvinyl chloride (PVC) of relatively high molecular weight (Breon S 110/10 B.P Chemical U. K. Ltd).

7- The plasticizers were obtained from Fluka AG, (Switzerland), their composition and viscosity were tabulating in Table 2- 1.

Table 2-1:- Shows the plasticizer which were used and their chemical composition and their viscosity.

Plasticizer's name	Chemical composition	viscosity	company
Di-butylphthalate (DBPH)	**$C_6H_4[CO_2CH_3(CH_2)_3]_2$**	**14.44 CST**	**Fluka**
Di-octylphthalate (DOP)	**$C_6H_4[CO_2C_8H_{17}]_2$**	**82.98 CST**	**Fluka**
Tri-butylphosphate (TBP)	**$(C_4H_7O)_3PO$**	**3.114 CST**	**Fluka**

8- Other chemicals such as hydrochloric acid (HCl; F.W. 36.45; sp.gr. 1.184; 37% HCl; ≈12M), sodium hydroxide (NaOH; F.W. 40.00 pellets), sodium chloride (NaCl; F.W. 58.45), potassium chloride (KCl; F.W. 74.58), magnesium chloride ($MgCl_2.6H_2O$; F.W. 203.218), manganese(11) sulfate, anhydrous ($MnSO_4$; F.W. 151), copper(11)sulfate, anhydrous ($CuSO_4$; F.W. 159.60) and Ferric(III) Sulfate ($Fe_2(SO_4)_3.9H_2O$;F.W. 506.027). All chemicals and solvents were of an analytical reagent grade obtained from BDH, Fluka and Aldrich companies. Other needed to prepare was Hydrochlorothiazide (HCT) $C_7H_8ClN_3O_4S_2$; F.W. 297.74 that used in selectivity methods to find if it interfered with Amiloride drug, this drug provided from the commercial drug (Esidrex 25mg) manufactured by Novartis Farmaceutica S.A., Spain for Novartis Pharma AG, Basle, Switzerland. Deionized distilled water was used throughout the study.

2-3- Preparation of standard solutions:-

2-3-1- standard solution for ISE:-

All solutions were prepared in doubly distilled deionized water.

1- A standard solution of 0.01M Amiloride hydrochloride was prepared by dissolving 0.151g of standard Amiloride hydrochloride in distill water and completing the solution up to 50ml, (ultrasonicator) equipment was used to assist the dissolving of the drug. The other Amiloride standard solutions were prepared by subsequent dilution of the stock solution, ranged (10^{-7}-10^{-2} M).

2- The stock standard solution of 0.01M PT was prepared by dissolving 1.44g in distilled water and diluted up to 50 mL.

3- A stock standard solution of 0.01M NaTPB was prepared by dissolving 0.171g in distilled water and diluted up to 50 mL.

4- Stock solutions of 0.1 M of NaCl, KCl, $MgCl_2.6H_2O$, $MnSO_4$, $CuSO_4$, and $Fe_2(SO_4)_3.9H_2O$ were prepared by weighted (0.584, 0.745, 2.0311, 1.51, 1.596 and 5.056 g) respectively and dissolved by distill water in 100mL volumetric flask. More diluted solutions were prepared by dilution from the stock solutions.

5- A stock standard solution of 0.01M Hydrochlorothiazide(HCT) prepared by dissolving 0.297g in methanol about 10 mL and completes the volume to 100 mL with distill water another diluted solution prepared from the stock.

6- A standard solution of 0.1M HCl was prepared by diluting 0.833mL 0f 12M HCl concentrated stoke solution to 100mL, this 0.1M HCl used to acidified the Amiloride solutions to lower their pH and the other standard solution of 0.1M NaOH was prepared by weighting 0.4g of NaOH and dissolving it to 100mL by water then it used to increasing the pH of the Amiloride solutions by those additions of HCl in a time and NaOH in other

time, the pH controlled by using pH electrode, the pH effect can be studied.

2-3-2- Standard Solution for UV Spectrophotometric Studies:-

- Stock standard solutions of 200 mg/L drug (Amloride hydrochloride) were prepared by dissolving 0.002 g with deionized water to 100 mL, several 10 mL standard solutions ranged from 2-60 mg/L were freshly prepared by dilution.

2-4- Preparation of Ion-pair Compounds:-

The Amiloride hydrochloride ion-selective electrode is prepared based on the use of ion-pair compound (Amilo-PT) as the electro-active substance. The preparation of ion-pair was performed by mixing 50 ml of 0.01 M solution of Amiloride hydrochloride with 50 ml of 0.01 M PT with stirring. The resulting precipitate was filtered off, washed with water, dried at room temperature for two days. The composition of the ion-pair compound (Amilo-PT) was confirmed using FTIR and UV spectrum.

The other ion-pair compound (Amilo-NaTPB) was prepared by the same way, mixing 50 ml of 0.01 M solution of Amiloride hydrochloride with 50 ml of 0.01 M NaTPB with stirring. The resulting precipitate was also filtered off, washed with water, dried at room temperature for two days. The composition of the ion-pair compound, (Amilo-NaTPB) was confirmed also by using FTIR and UV spectrum.

2-5- Fabrication of the Electrodes:-

The electrodes for Amiloride hydrochloride were prepared using electro active complexes (Amilo-PT), (Amilo-NaTPB) with different plasticizers showed in Table 2-1.

The ISE nature and characteristics are considerably influenced by the nature and the amount of each component. As far as the polymeric membrane is concerned, it separates the test solution from the inner compartment, containing the target ion solution. [126,127]

The method of immobilization the ion-pair compounds into the PVC matrix membrane as described by Craggs et al [128]. A (0.040g) of Amilo-PT (ionophore or ion-pair) matrix was mixed with (0.360g) of plasticizer and (0.17g) of PVC powder; all were dissolved in (6-7 mL) of THF with stirring until a clear viscous solution was obtained. The same process was performed for the other ion-pair (Amilo-NaTPB). The resultant solution poured into a glass casting ring about (30 mm) in length and (35 mm) in diameter. It consists of two pieces; one of them was the glass cylinder and the other was glass plate. The two pieces was pasted together using PVC-THF viscous mixture to make sure no loss in the membrane mixture. The top side of the cylinder was covered with a pad of filter paper. Then all of the contents were left for two days to allow slow evaporation of the solvent and formation sensing membranes. A disc of the membrane was cut equal to the external diameter of a PVC tube (≈3 cm length, 6 mm i.d) as shown in Fig. 2-1 (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 (5). Next step is connection into a glass tube, step (6). 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 10^{-3}M Amiloride

hydrochloride as internal solution before fixing the cover, step (7). The electrode was then conditioned by placing it in 10^{-3}M Amiloride solution for 1 h before use. When not in use, they were stored in air. The master membrane was used to prepare several others [129].

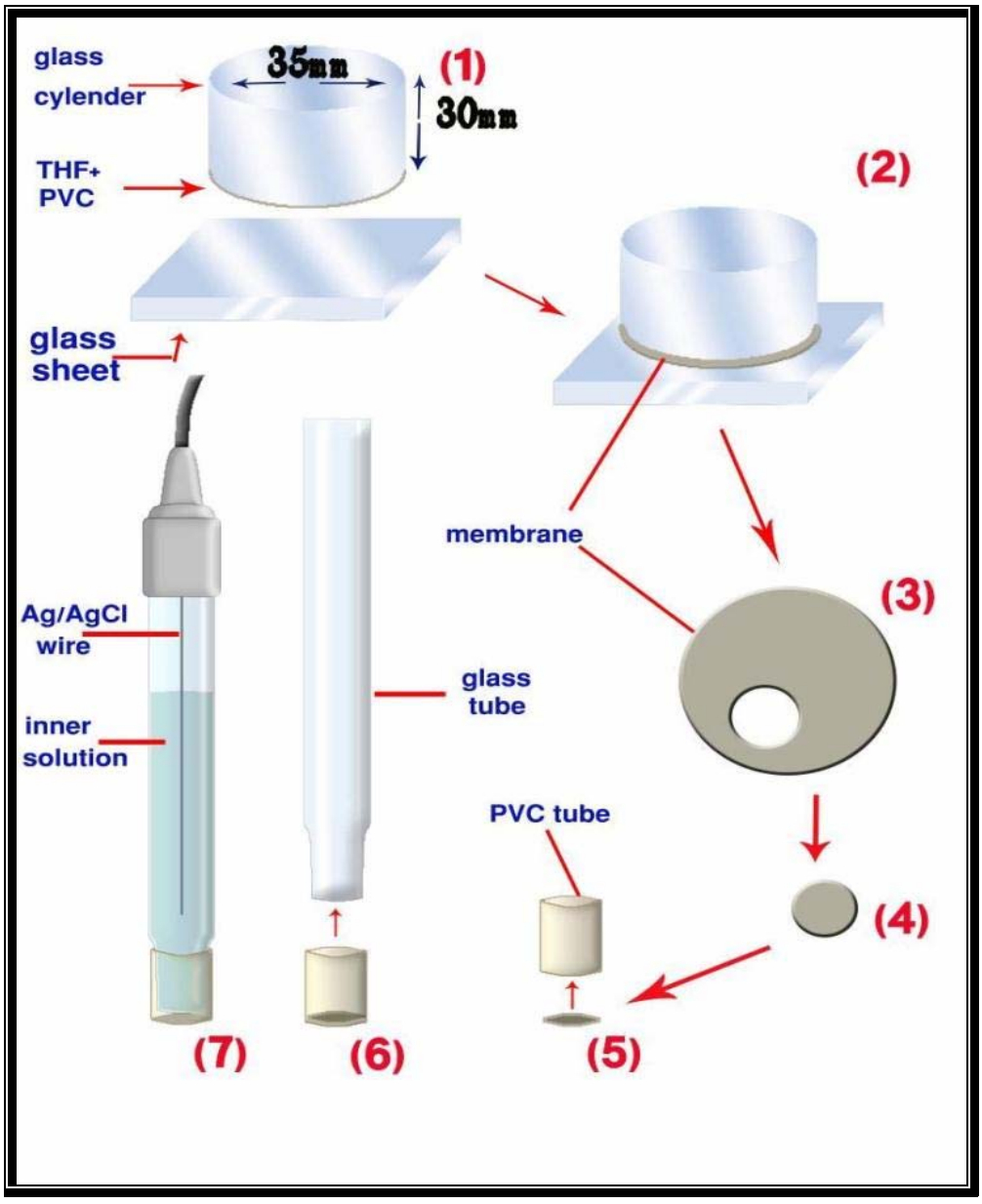


Fig. 2-1:- Assembling the Ion-selective Electrode.

2-6-Potential measurement:-

The potentiometric cell was arranged by immersing the electrode and reference electrode in a beaker (50ml) containing certain amount of drug 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 drug solutions ranged from 10^{-7}-10^{-2} M. The calibration curves were prepared by plotting the potential (E) versus log concentration by using computer program (Microsoft office Excel 2003).

From the calibration curve, the characterization parameters [25,36,41] of an ISE were obtained, including; slope, correlation coefficient, concentration range and detection limit.

The effect of pH on the response of membrane was examined by measuring the potential of the standard solutions of concentrations (10^{-4}, 10^{-3}, 10^{-2}) M at different pH ranged from 1 to 12; obtained by addition of small volumes of 0.1M hydrochloric acid and/or 0.1M sodium hydroxide solutions. The life time of each membrane was calculated, that is the decrease in response with the time. [43]

2-7-Selectivity measurements:-

The influence of some inorganic ions (Na^+, K^+, Mg^{2+}, Mn^{2+}, Cu^{2+}, Fe^{3+}) and additive drug (HCT) may be present in the commercial drugs, on the response of the Amiloride hydrochloride sensors was investigated. The selectivity coefficients were determined by:

2-7-1- Separate solution method [47,48]:-

According to this method a 25 mL of 10^{-3} M solution of the prepared Amiloride (A), and 25 mL of 10^{-3} M from each other interfering ion (B)

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

2-7-2- Mixed solution method [Fixed interference method (FIM)]:- [49,50,51]

In this method a 10 mL of Amiloride (A) solution from each 10^{-6} to 10^{-2} M are mixed with 10mL from 0.1M interfering ion (B) in 50 mL beaker. The potential were measured for each solution. The activities of Amiloride are found after mixing as show in Fig. 1- 4. The selectivity coefficient ($K^{pot}_{A,B}$) are calculated according to equation 1- 8. The activities of interfering ion (a_B) are calculated after dilution:-

$a_B = (0.1M \times 10mL)/ 20mL = 5\times10^{-2}$ M

All of the operation of this method was repeated by using 0.01M fixed interference ion concentration instead of 0.1M, but after dilution the activity calculated of (a_B) is 5×10^{-3} M.

2-8- Sample analysis:-

2-8-1- Direct method [36,57]*:-*

The potentiometry of sample is measured directly using Amiloride hydrochloride indicator electrode. The concentration was then calculated using calibration curve of standard Amiloride hydrochloride.

2-8-2- Incremental Methods [36,42]*:-*

a- Standard (or known) single point addition the sample of 25 mL with concentration of $1x10^{-3}$ M is introduced followed by addition of 0.5 mL of 0.01 M increment of Amiloride. The potential were measured before and after addition. The concentration of the sample is calculated using equation 1-11 for a single point increment.

b- Multiple standard addition method [47] is an extension of standard addition method the sample of 25 mL of $1x10^{-3}$ M is introduced followed

by addition of 0.5 mL of 0.01 M increments. The potential is recorded before and after each addition. The multi addition method was plotted between antilog (E/S) and the added volume of standard solution.

2-8-3- Potentiometric titration method [36, 59]:-

A precipitation titration was performed for the Amiloride sample under study. In this method a 15 ml sample solution containing Amiloride hydrochloride 0.01M was titrated against 0.01 M PT solution. Potential was measured after each addition using the prepared electrode. A direct plot of potential as a function of reagent 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, the maximum is the end point.

2-9- Preparation of pharmaceutical formulation:-

Teen tablets were crushed, mixed in a mortar and weighted accurately it found that the weight of average was equal to 0.2334 and 0.2364g for Maduratic and Saluretic respectively. and the weight of three tablets (0.7002 and 0.7038 g) which contain approximately 0.015 mg from Amloride hydrochloride (each one tablet contained 0.005g) it dissolved by deionized water and using ultrasonicator for ~ 5min then filtrate and washing the precipitate, the filtrate was collected in 50 mL volumetric flask the resulting solution contain ~ 0.001 M Amloride hydrochloride.

Chapter Three

Results and Discussion

3- Results and Discussion

3-1- Influence of membrane composition:-

The polymer could provide the required physical properties (e.g. elasticity and mechanical stability). It should be stressed that these ISEs exhibited a Nernstian response, owing to the possible ionic impurity presence in the used PVC as well as in the presence of the other membrane components. Moreover, membranes with no ionic sites did not respond to the target ion concentration, as they incorporate almost completely pure membrane ingredients. [27]

Membrane solvents (plasticizers) are used to increase the plasticity of membrane, which is required to give a homogeneous organic phase. This ingredient also affects the selectivity behavior of the ISE membrane. In the case of ligand-free ISE membranes or those based on an ion exchanger, incapable of specific interactions with a target ion, what governs the selectivities is the difference between the standard free energies of the ions in the aqueous and the organic phases. [29, 130]

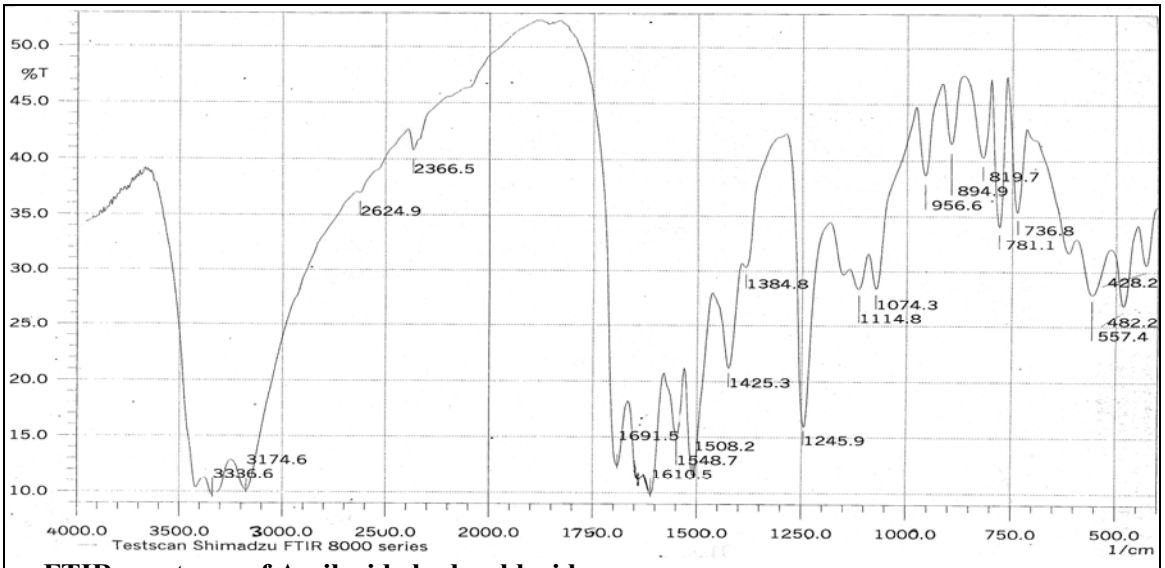
The ionophore or the membrane–active recognition element can be an ion exchanger or a neutral macrocyclic compound, having molecule–sized dimensions and containing cavities or semi-cavity to surround the target ions [131,132]. The characteristics of the ligand as well as the substrates are crucial in molecular recognition. Complex formation is very important; the interaction between two species can be either attractive or repulsive. The former one results in complex formation. Stability, formation mechanism and formation or dissociation rates are the main parameters in complexation. The complex stoichiometry is another effective factor used to describe a complex. [133]

3-1-a- The FTIR spectra for Amiloride, Amilo-PT and Amilo-TPB:-

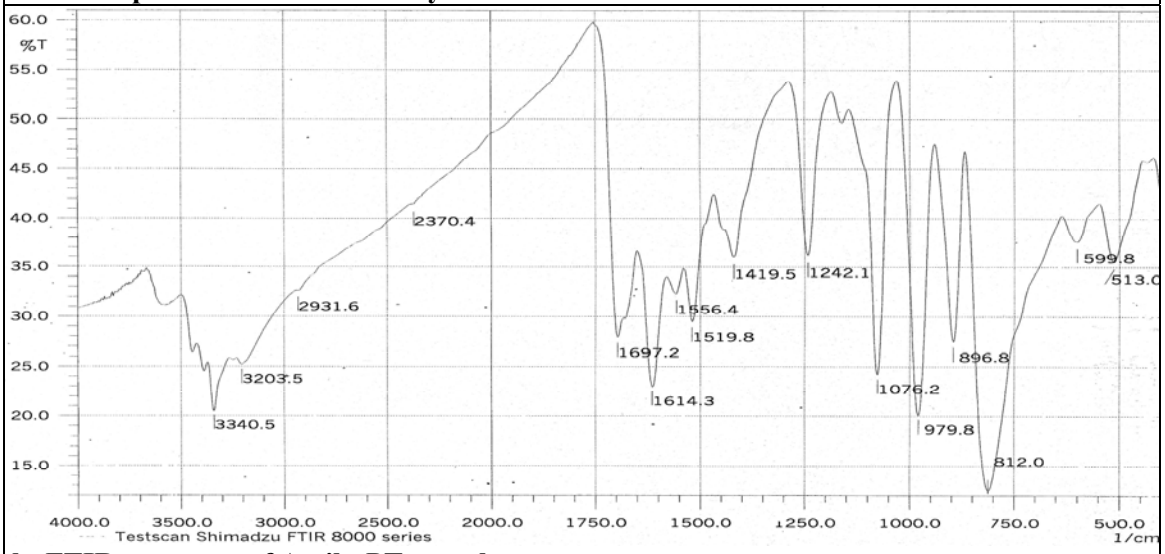
The complexes are obtained by conversion Amiloride hydrochloride to Amilo-PT and Amilo-TPB characterized by their FTIR spectra as shown in (Fig.3-1-a by using KBr and Fig.3-2-a by using CsI) are the FTIR spectra of Amiloride hydrochloride alone, (Fig.3-1-b using KBr and Fig.3-2b using CsI) characterized Amiloride phosphotungastate (Amilo-PT) light brown precipitate and the second complex is obtained by conversion Amiloride hydrochloride into Amiloride tetraphenylborate (Amilo-TPB) white precipitate which was characterized by it's FTIR spectra as shown in (Fig.3-1-c for KBr and Fig.3-2-c for CsI). The IR spectra indicate and improved the formation of the complex Amilo-PT, and Amilo-TPB. The functional groups for Amiloride hydrochloride and the complexes are listed in Table 3-1.

Table 3-1:- The functional groups obtained from the spectrum for Amiloride hydrochloride, Amiloride- phosphotungstic acid and Amiloride-tetraphenylborate complexes from FTIR charts that used KBr disc.

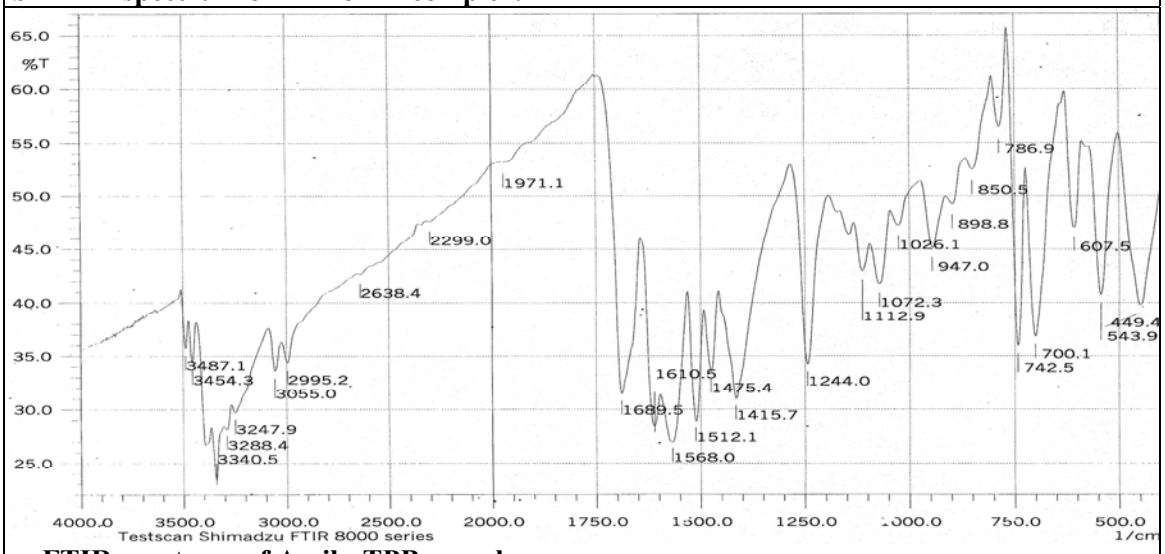
Functional group	Amiloride hydrochloride	Complex (Amilo-PT)	Complex (Amilo-TPB)
Primary Amine N-H Symmetric, Asymetric	3336.6	3340	3340.5-3454.3
Aromatic C-H (sp^2)	3174.6	3203.5	3055
Secondary amine N-H	____	____	3247.9
Amide C=O	1691.5	1697.2	1689.5
Shiff C=N	~1645	(1614.3)	_____
Pyrimidine[134] C=N	1548.7	1556.4	1568
Aryl amine C-N	1245.9	1242.1	____
*(w)*Alkyl amine C-N	1114.8-1074.3	1076.2	____



a- FTIR spectrum of Amiloride hydrochloride.

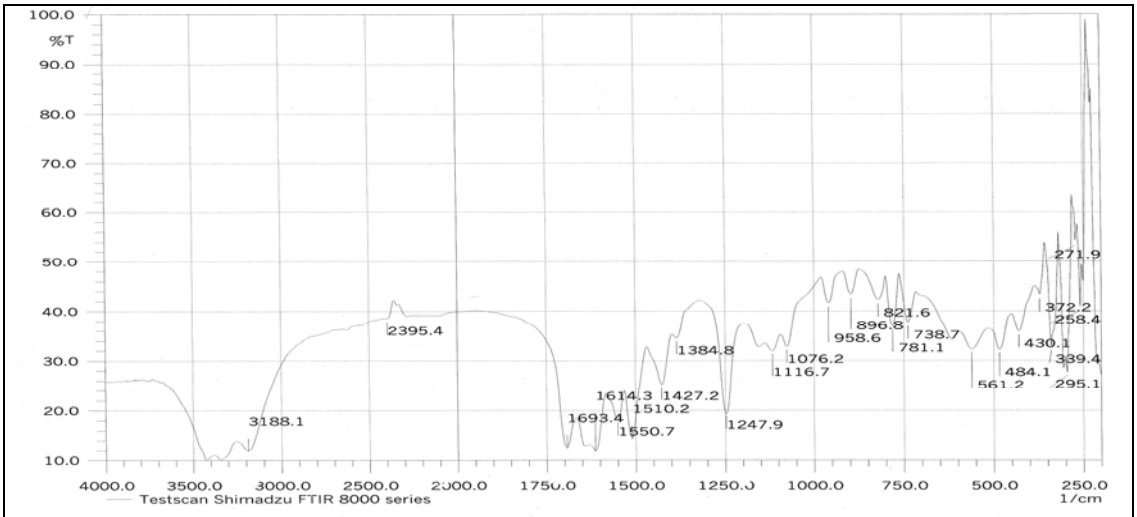


b- FTIR spectrum of Amilo-PT complex.

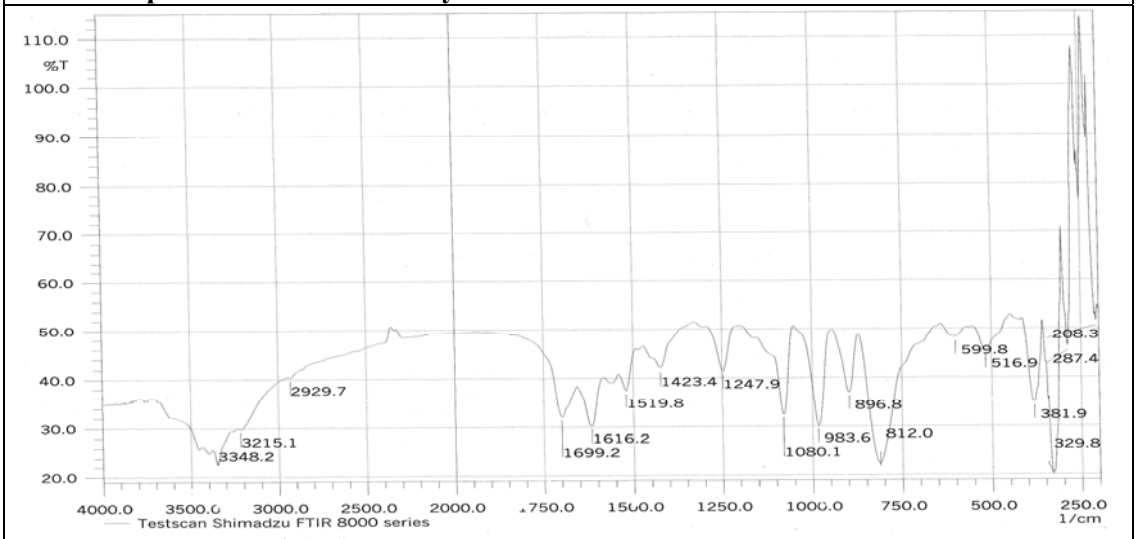


c- FTIR spectrum of Amilo-TPB complex.

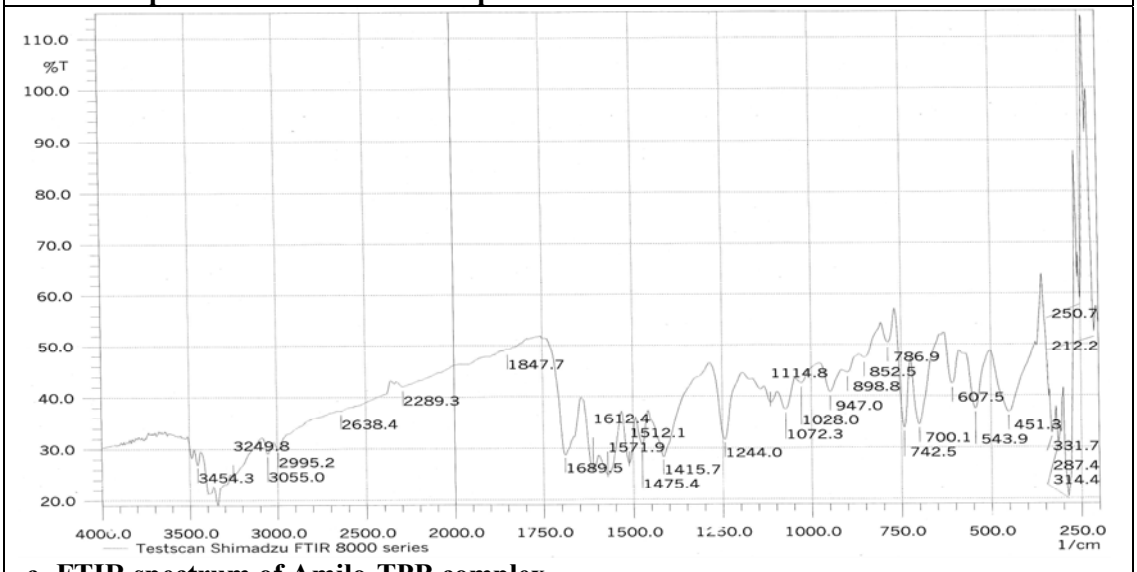
Fig.3-1: a- FTIR spectrum of (Amilo), b- (Amilo-PT), c- (Amilo-TPB) by using KBr.



a- FTIR spectrum of Amiloride hydrochloride.



b- FTIR spectrum of Amilo-PT complex.



c- FTIR spectrum of Amilo-TPB complex.

Fig.3-2: a- FTIR spectrum of (Amilo), b- (Amilo-PT), c- (Amilo-TPB) by using CsI.

3-1-b- The UV- spectra for Amiloride, Amilo-PT and Amilo-TPB:-

In spectrophotometry UV- region Amiloride hydrochloride detected by three wavelengths (361,286 and 213) as show in Fig.3-3 and in the complexes spectra show that there are differences in peaks a shift in the wavelengths 361nm and 286 nm and disappearance of the wavelength 213nm as show in Fig.3-4 and Fig.3-5, Table 3-2 show the three compounds and their wavelengths.

From the IR charts, UV spectra and the differences in the physical properties like solubility and colors of the Amiloride and the two complexes the formation of complexes were improved.

Table 3-2:- The Wavelengths of the UV- spectra of Amiloride, Amilo-PT and Amilo-TPB complexes.

The compound	Amiloride	Amilo-PT	Amilo-TPB
Wavelengths (λ) (max)/(nm)	361	365	367
	286	271	289
	213	-----	-----

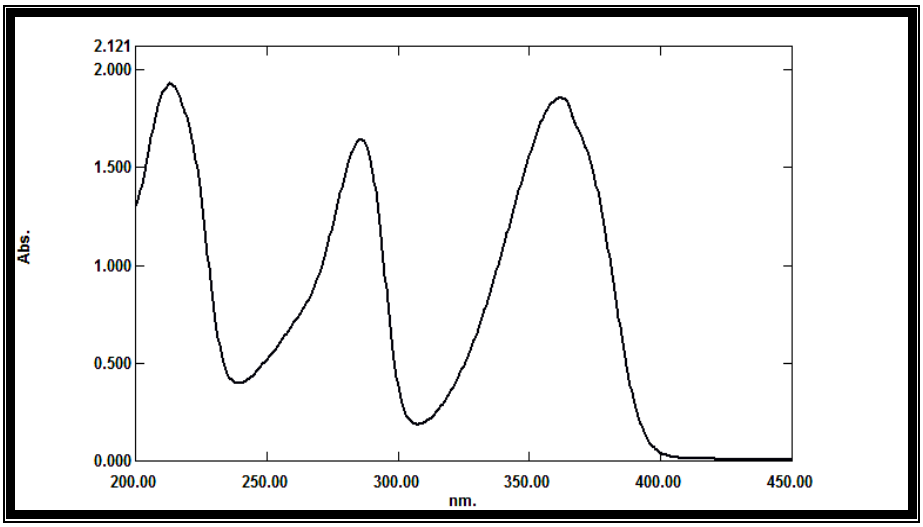


Fig.3-3:- UV- Spectrum for Amiloride sample solution.

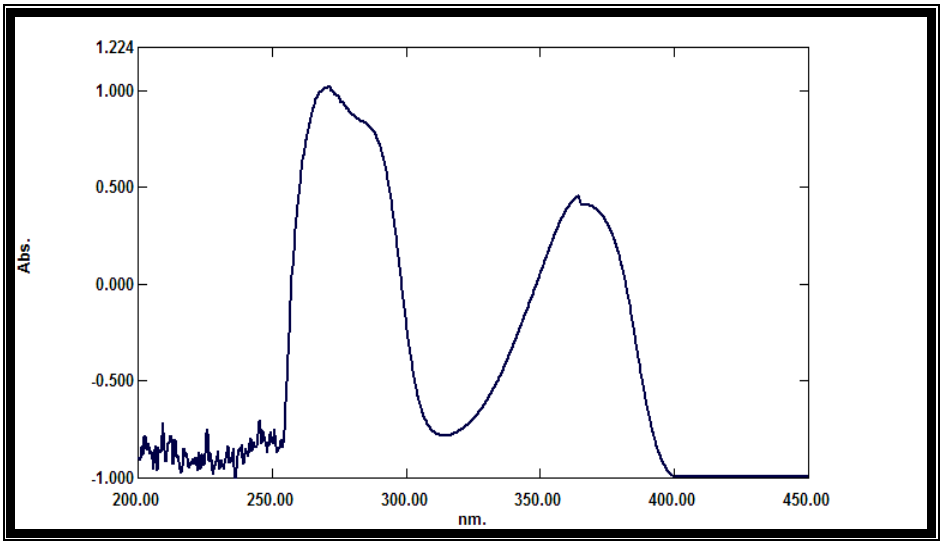


Fig.3-4:- UV- Spectrum for Amilo - PT complex sample solution.

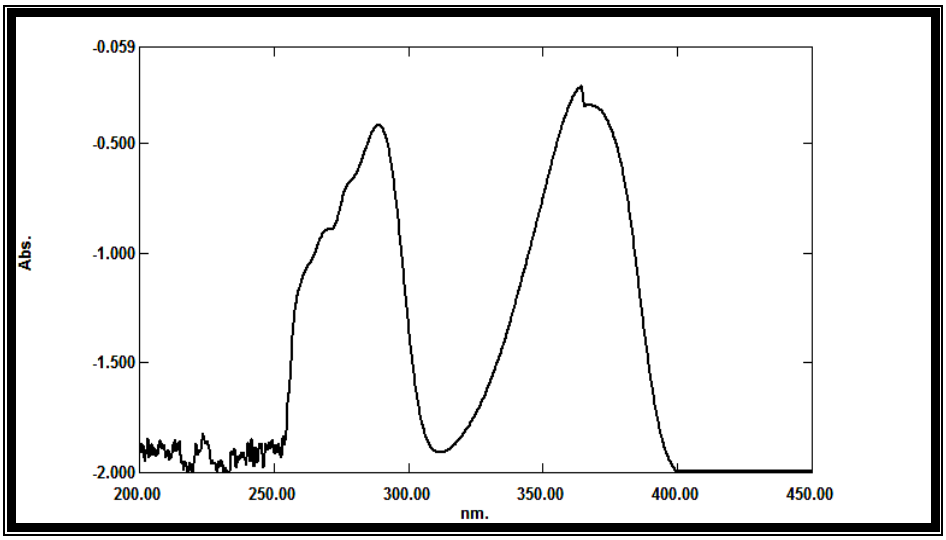


Fig.3-5:- UV- Spectrum for Amilo - TPB complex sample solution.

3-2- Sensor Characteristics:-

The potentiometric response characteristics of sensors are based on Amiloride- phosphotungstate (Amilo-PT) ion-pair complexes and Amiloride- tetraphenyle borate as (Amilo-TPB) as the electro-active material[27] and three plasticizers; di-butylphthalate (DBPH), di-octylphthalate (DOP) and tributylphosphate (TBP) in PVC matrix were examined. The effects of different plasticizers and ion-pair complexes were studied with respect to the slope[36], response time, linear concentration

range, life time[32] and detection limit[37,41]. The electrode with good characteristics was used for further studies.

The working characteristics for the investigated A, B, C, D, E, F electrodes were assessed on the basis of the calibration curves which were obtained by measuring of the e.m.f. values of the set of Amiloride hydrochloride solutions ranged (10^{-2} –10^{-7})M. These electrodes show sub-Nernstain response to the Amiloride hydrochloride activity in different concentration ranges depending on the properties of the plasticizers and ion-pair complexes.

The first ion-pair complex (Amilo-PT) used to construct three electrodes (A, B and C) by using three different plasticizers (DBPH, DOP and TBP respectively) which their calibration curves shown in Fig.3-6.

The second ion-pair complex (Amilo-TPB) used to construct other three electrodes (D, E and F) by using the same three different plasticizers (DBPH, DOP and TBP respectively) which their calibration curves shown in Fig. 3-7. The equations of the linear range and their slope, correlation coefficient and relative standard deviation of there were listed in Table 3-3.

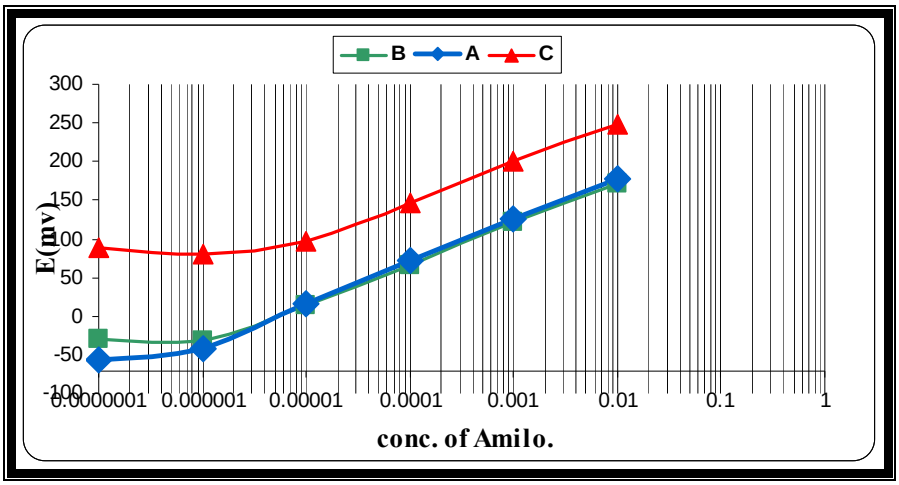


Fig.3-6:- Calibration curves of Amiloride hydrochloride selective electrodes (A, B and C) using (Amilo-PT) ion-pair complex.

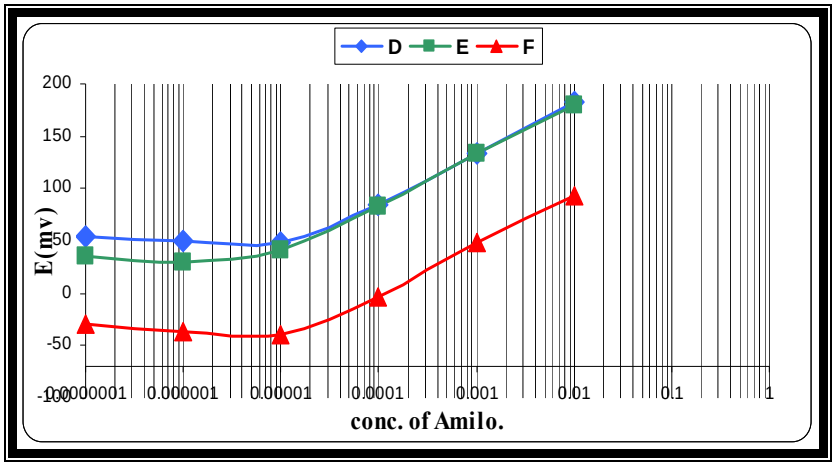


Fig.3-7:- Calibration curves of Amiloride hydrochloride selective electrodes (D, E and F) using (Amilo-TPB) ion-pair complex.

Table3-3:- The equation of calibration curves and their slope, Correlation coefficient and relative standard deviation of their slope.

Electrode Membrane	Linear equation	Slope (mV/Decade)	RSD*%	Correlation coefficient (R)
A-Amilo-PT +DBPH	y= 23.534 Lnx + 287.49	54.198	0.275	0.9999
B-Amilo-PT +DOP	y= 22.909 Lnx + 277.75	52.759	0.674	0.9998
C-Amilo-PT +TBP	y= 22.106 Lnx + 351.4	50.910	0.836	0.9998
D-Amilo-TPB +DBPH	y = 21.28 Lnx + 280	49.007	0.358	1.000
E-Amilo-TPB +DOP	y= 21.063 Lnx + 277.83	48.508	0.708	0.9995
F-Amilo-TPB +TBP	y= 21.063 Lnx + 191.17	48.501	0.852	0.9991

*** The result of three times repeated.**

The magnitude of these slopes of Amiloride selective electrodes were non- Nernstian slope but they can be considered worked and good for detection of Amiloride and show a linearity response of a series from 10^{-5}-10^{-2}M Amiloride solutions and there are several pharmaceutical application of ion selective electrode that have non- Nernstian slope proved that they can be used those electrodes in detection of drugs like the determination of Scopolamine[101] in Some Pharmaceutical Formulations by using ISE has a slope of 54.5 mV /decade, other ion selective membrane electrode for

Methylene Blue[102] and its pharmaceutical applications has a slope of 51.5 mV/decade. PVC membrane sensors for potentiometric determination of Acebutolol[103] has slope 51.5 and 53 mV /decade, also the determination of Methacycline Hydrochloride[104] by using ISE has the slope equal 52.9 mV/decade. Cefuroxime[118] ion selective electrode used in pharmaceutical preparations has a slope of −50.4 mV /decade.

The parameters of Amiloride hydrochloride electrodes which include the slope, linear concentration range, detection limit, response time and life time of the six electrodes (A, B, C, D, E and F) are listed in Table 3-4, from the table electrode A (Amilo-PT with DBPH) is the best electrode which used to determined Amiloride and studying other parameters like pH effect and selectivity.

Table 3-4:- The parameters of Amiloride hydrochloride electrodes.

Membrane Composition	Slope (mV/Decade)	Linear Concentration Range (M)	Detection Limit (M)	Response time (sec)			Lifetime (day)
				10^{-2} (M)	10^{-3} (M)	10^{-4} (M)	
A- Amilo-PT +DBPH	**54.198**	**10^{-5} – 10^{-2}**	**6.000×10^{-7}**	**8**	**10**	**20**	**45**
B- Amilo-PT +DOP	**52.759**	**3×10^{-6}– 10^{-2}**	**1.500×10^{-6}**	**20**	**30**	**35**	**35**
C- Amilo-PT +TBP	**50.910**	**10^{-5} – 10^{-2}**	**7.000×10^{-6}**	**5**	**10**	**15**	**30**
D- Amilo-TPB +DBPH	**49.007**	**6×10^{-5}– 10^{-2}**	**1.750×10^{-5}**	**20**	**35**	**40**	**21**
E- Amilo-TPB +DOP	**48.508**	**3×10^{-5}– 10^{-2}**	**7.000×10^{-6}**	**7**	**10**	**20**	**15**
F- Amilo-TPB +TBP	**48.501**	**5×10^{-5} –10^{-2}**	**1.500×10^{-5}**	**6**	**12**	**20**	**10**

3-3- Response Time of Amiloride ISEs:-

The average response time is defined as the time required for the electrode to reach a stable potential within ±1 mV of the final equilibrium value, after successive immersion of the electrode in different Amiloride solutions, each having a 10-fold difference in concentration. This time was found to be shorter for a concentration of 10^{-2} M, higher for 10^{-3} M and 10^{-4} M, this may explained that dilute solution 10^{-4} M reach the equilibrium on the surface of the membrane slower than concentrated one 10^{-2} M and 10^{-3} M. The response time of Amiloride membranes are listed in Table 3-4.

3-4- Effect of pH:-

The effect of pH on the response of the Amiloride electrode (A) was examined by measuring the variation in the potential against pH range from 0.7 to 11.0 by using 0.1M HCl to lower pH to 0.7 and by monitoring the potential stability with addition of 0.1M NaOH until the pH reach to 11, the pH effect studied for the three different Amiloride hydrochloride concentrations 10^{-4}, 10^{-3} and 10^{-2} M as shown in Fig.3-8.

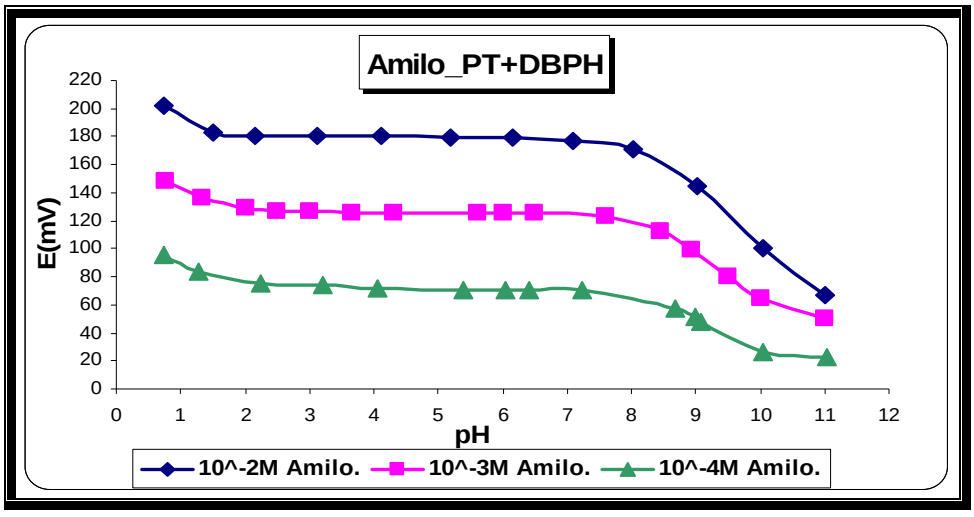


Fig.3-8:- Effect of pH on the potential of the electrode A (Amilo-PT+DBPH) at concentrations 10^{-2}, 10^{-3} and 10^{-4} M.

At pH values lower than 1.0 or in very high acidity, the electrodes responses has been increased rather irregularly. This may be due to that the electrodes responses to H^+ activities as well as analyte ions. A drift in potential was noticed at pH > 8. This attributed to the poisoning of the membrane by formation a white precipitated tungsten oxides or sodium phosphotungastate [100]. The working pH ranges of Amiloride electrode A are listed in Table 3-5.

Table 3-5:- Working pH ranges for Amiloride electrode A.

Membrane Composition	***pH range***		
Amilo-PT +DBPH	**10^{-2}M**	**10^{-3}M**	**10^{-4}M**
	1.8 – 7.8	1.9 – 7.8	2.3 – 7.2

3-5- Selectivity methods:-

3-5-1- Separate solution method [47, 48]:-

When ($a_A = a_B$) the potential of a cell comprising an ion-selective electrode and a reference electrode is measured with two separate solutions, one containing Amiloride solution at the activity a_A (but no B), the other one containing the interfering ion B at the same activity $a_A=a_B$ (but no A). When measured values of E_A and E_B, respectively, the value of $K^{pot}_{A,B}$ is calculated by the equation 1-7. The influence of some inorganic cations on the response characteristic of the six electrodes was investigated. The results of selectivity coefficients are summarized in Tables 3-6 to 3-11, which show the calculated $K^{pot}_{A,B}$, and $K^{pot}_{A,B}$ of the electrodes based on DBPH, DOP and TBP plasticizers with corresponding potential of 10^{-3} M Amiloride (E_A= 126, 121, 200, 133, 134 and 48 mV respectively) and their slopes (54.198, 52.759, 50.910, 49.007, 48.508 and 48.501 mV/decade respectively).

Table3-6: Selectivity coefficient values for Amilo-PT+DBPH, when E_A=126 mV and the slope of 54.198 mV/decade.

Interfering-Ion	E_B *(mV) of* $10^{-3}M$	*Log* $K_{A,B}$	$K_{A,B}$
K^+	-30	-2.878	0.00132
Na^+	-60	-3.431	3.699×10^{-4}
Mg^{2+}	-34	-4.452	3.53×10^{-5}
Mn^{2+}	-45	-4.655	2.212×10^{-5}
Cu^{2+}	-52	-4.784	1.643×10^{-5}
Fe^{3+}	50	-3.402	3.96×10^{-4}
Hydrochlorothiazide	65	-1.125	0.075

Table3-7: Selectivity coefficient values for Amilo-PT+DOP, when E_A=121mV and the slope of 52.759 mV/decade.

Interfering-Ion	E_B *(mV) of* $10^{-3}M$	*Log* $K_{A,B}$	$K_{A,B}$
Na^+	-35	-2.958	0.0011
K^+	-35	-2.957	0.001104
Mg^{2+}	-30	-4.632	4.344×10^{-5}
Mn^{2+}	-23	-4.229	5.896×10^{-5}
Cu^{2+}	-25	-4.267	5.403×10^{-5}
Fe^{3+}	10	-4.103	7.872×10^{-5}
Hydrochlorothiazide	63	-1.099	0.079

Table3-8: Selectivity coefficient values for Amilo-PT+TBP, when E_A=200mV and the slope of 50.910 mV/decade.

Interfering-Ion	E_B *(mV) of* $10^{-3}M$	*Log* $K_{A,B}$	$K_{A,B}$
Na^+	60	-2.750	0.00177
K^+	45	-3.044	9.024×10^{-4}
Mg^{2+}	103	-3.405	3.932×10^{-4}
Mn^{2+}	100	-3.464	3.433×10^{-4}
Cu^{2+}	95	-3.562	2.738×10^{-4}
Fe^{3+}	150	-2.981	0.00104
Hydrochlorothiazide	148	-1.021	0.0951

Table3-9: Selectivity coefficient values for Amilo-TPB+DBPH, when E_A=133mV and the slope of 49.007 mV/decade.

Interfering-Ion	E_B *(mV) of* 10^{-3}*M*	*Log* $K_{A,B}$	$K_{A,B}$
Na^+	30	-2.101	0.00791
K^+	36	-1.982	0.0104
Mg^{2+}	38	-3.438	3.643×10^{-4}
Mn^{2+}	40	-3.397	4.002×10^{-4}
Cu^{2+}	26	-3.683	2.0731×10^{-4}
Fe^{3+}	80	-3.081	8.289×10^{-4}
Hydrochlorothiazide	78	-1.122	0.0754

Table3-10: Selectivity coefficient values for Amilo-TPB+DOP, when E_A=134mV and the slope of 48.508 mV/decade.

Interfering-Ion	E_B *(mV) of* 10^{-3}*M*	*Log* $K_{A,B}$	$K_{A,B}$
Na^+	23	-2.288	0.00514
K^+	25	-2.247	0.00566
Mg^{2+}	30	-3.643	2.269×10^{-4}
Mn^{2+}	33	-3.582	2.617×10^{-4}
Cu^{2+}	29	-3.664	2.164×10^{-4}
Fe^{3+}	78	-3.154	7.007×10^{-4}
Hydrochlorothiazide	82	-1.072	0.0847

Table3-11: Selectivity coefficient values for Amilo-TPB+TBP, when E_A=48mV and the slope of 48.501 mV/decade.

Interfering-Ion	E_B *(mV) of* 10^{-3}*M*	*Log* $K_{A,B}$	$K_{A,B}$
Na^+	-46	-1.938	0.0115
K^+	-53	-2.082	0.00827
Mg^{2+}	-37	-3.252	5.59×10^{-4}
Mn^{2+}	-35	-3.211	6.147×10^{-4}
Cu^{2+}	-41	-3.335	4.623×10^{-4}
Fe^{3+}	-5	-3.092	8.076×10^{-4}
Hydrochlorothiazide	-8	-1.154	0.07

None of the investigated cations interfere, due to very small values of $K^{pot}_{A,B}$, all values of $K^{pot}_{A,B}$ are less than (0.1). This reflects a very high selectivity of all six electrodes towards Amiloride hydrochloride.

3-5-2- Mixed solution method:-

By using the fixed interference method (FIM),[49,50,51] The potentiometry of a cell comprising an ion-selective electrode and a reference electrode (ISE cell) is measured for solutions of constant activity of the interfering ion (a_B) 5×10^{-2}M with varying activity of the primary ion that is for the Amiloride (a_A). The potentials E (mV) values obtained are plotted vs. the logarithm of the activity of the primary ion. The intersection of the extrapolated linear portions of this plot indicates the value of (a_A) from Fig.3-9 to Fig.3-14 can be used to calculate $K^{pot}_{A,B}$ according to equation (1-8) [$K^{pot}_{A,B}= a_A/ (a_B)^{Z_A/Z_B}$] where Z_A and Z_B are the charges of primary ion A and interfering ion B respectively, all results of $K^{pot}_{A,B}$ were in Table 3-12.

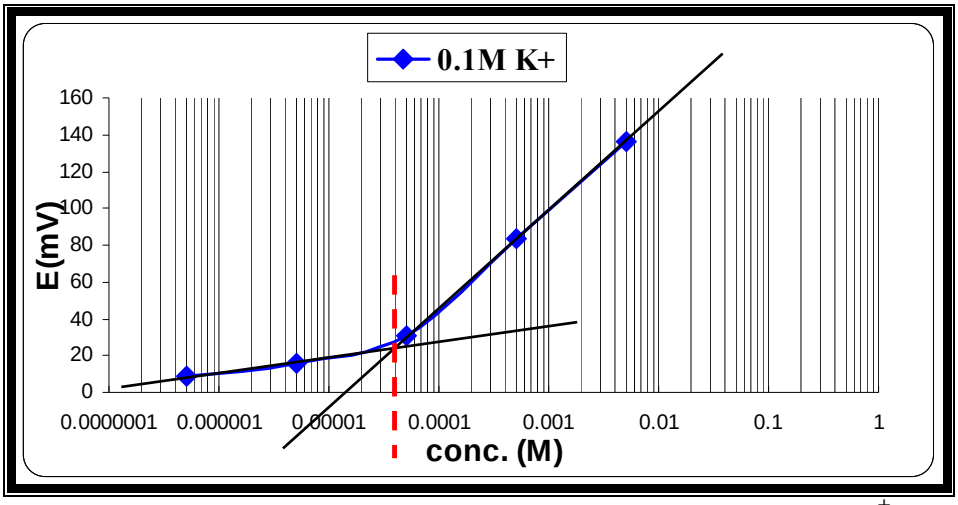


Fig.3-9:- FIM calibration curve for electrode A (Amilo-PT+DBPH), K^+ (5×10^{-2}M) as interfering ion $a_A=4\times10^{-5}$M.

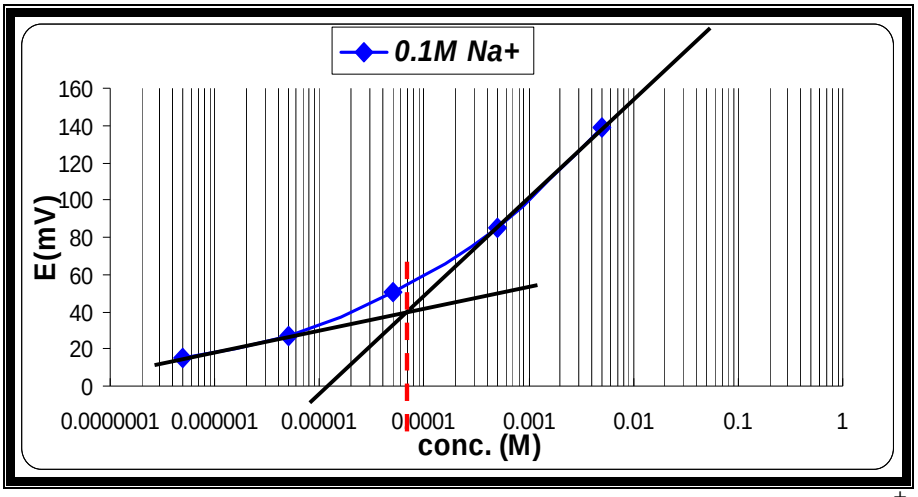


Fig.3-10:- FIM calibration curve for electrode A (Amilo-PT+DBPH), Na^{+} (5×10^{-2} M) as interfering ion $a_A=7\times10^{-5}$M.

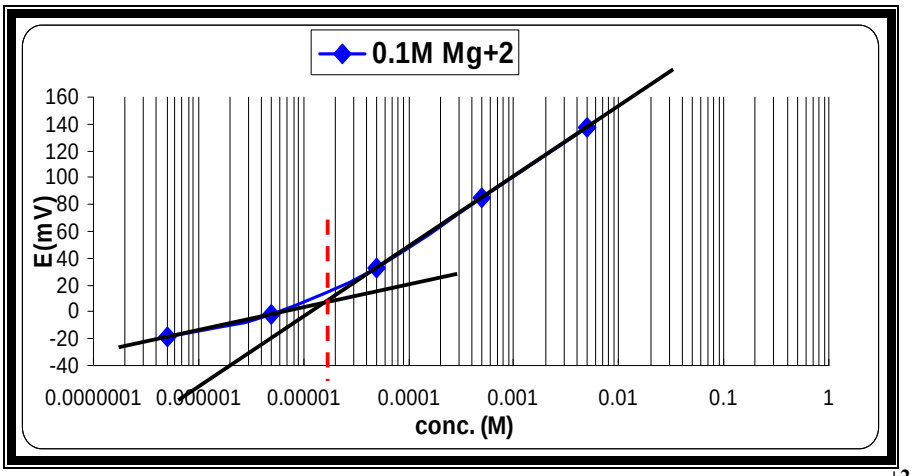


Fig.3-11:- FIM calibration curve for electrode A (Amilo-PT+DBPH), Mg^{+2}(5×10^{-2} M) as interfering ion $a_A=1.9\times10^{-5}$M.

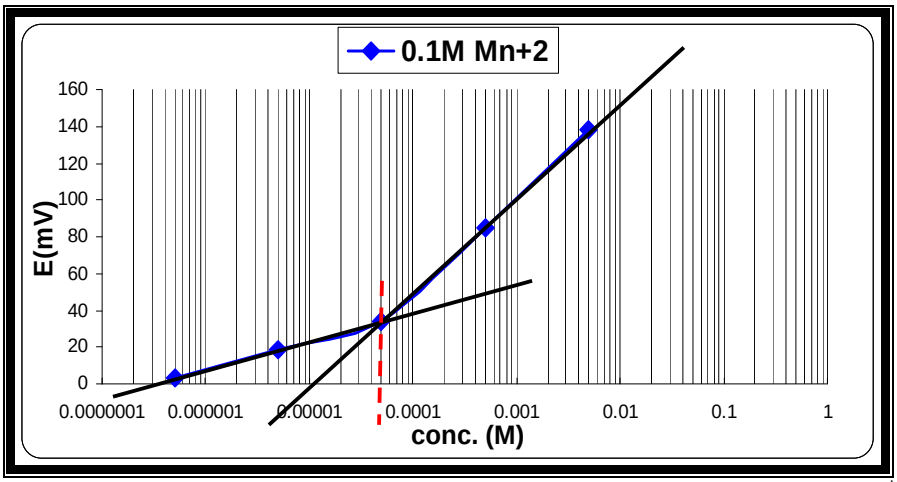


Fig.3-12:- FIM calibration curve for electrode A (Amilo-PT+DBPH), Mn^{+2}(5×10^{-2} M) as interfering ion $a_A=5\times10^{-5}$M.

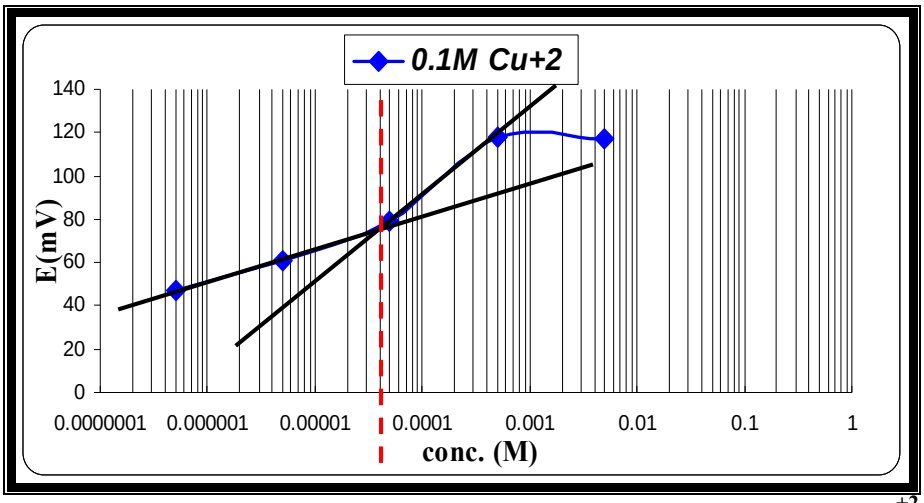


Fig.3-13:- FIM calibration curve for electrode A (Amilo-PT+DBPH), Cu^{+2}(5×10^{-2} M) as interfering ion $a_A=4\times10^{-5}$M

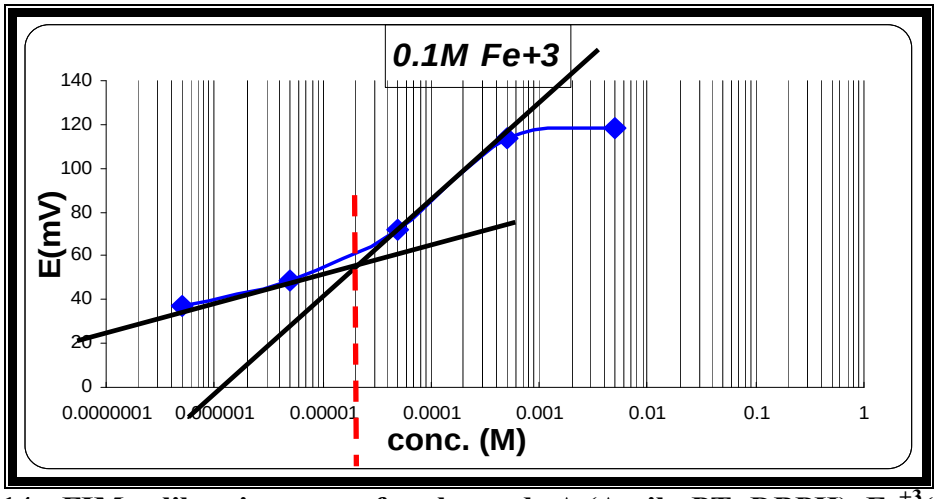


Fig.3-14:- FIM calibration curve for electrode A (Amilo-PT+DBPH), Fe^{+3}(5×10^{-2} M) as interfering ion, $a_A=2\times10^{-5}$ M.

Another fixed interfering ions (5×10^{-3}M) used in this method, as shown from Fig.3-15 to Fig.3-21, with varying concentration samples of Amiloride for the same electrode A (Amilo-PT+DBPH), $K^{pot}_{A,B}$ are also calculated in the same way and listed in Table 3-12.

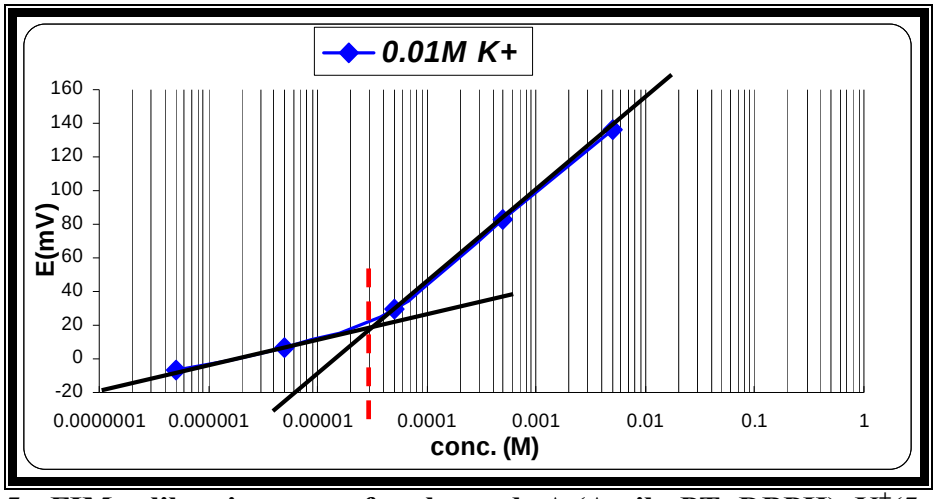


Fig.3-15:- FIM calibration curve for electrode A (Amilo-PT+DBPH), $K^{+}(5\times10^{-3}M)$ as interfering ion $a_A=3\times10^{-5}M$.

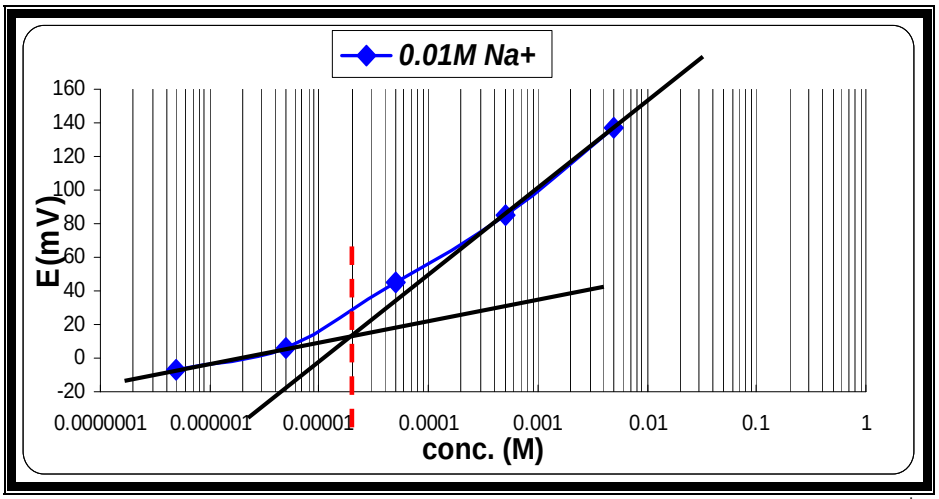


Fig.3-16:- FIM calibration curve for electrode A (Amilo-PT+DBPH), Na^{+} $(5\times10^{-3}$ M) as interfering ion $a_A=2\times10^{-5}M$.

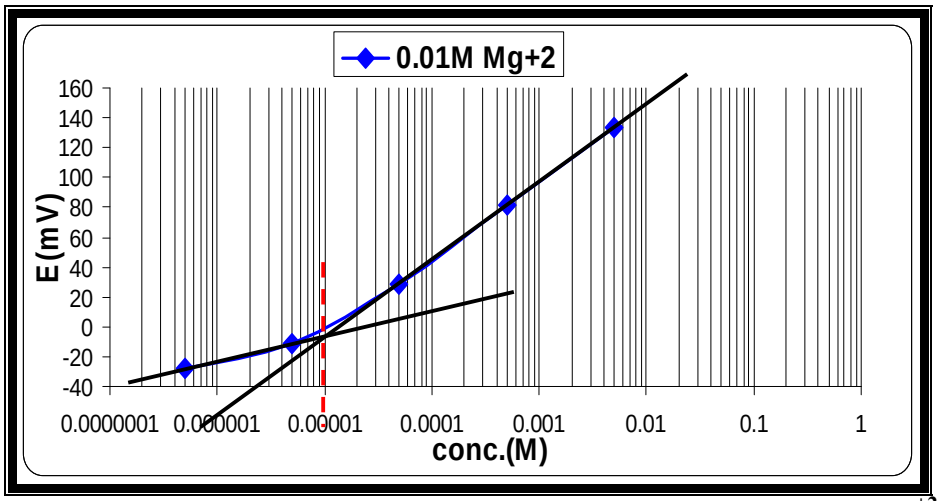


Fig.3-17:- FIM calibration curve for electrode A (Amilo-PT+DBPH), $Mg^{+2}(5\times10^{-3}$ M) as interfering ion $a_A=1\times10^{-5}M$.

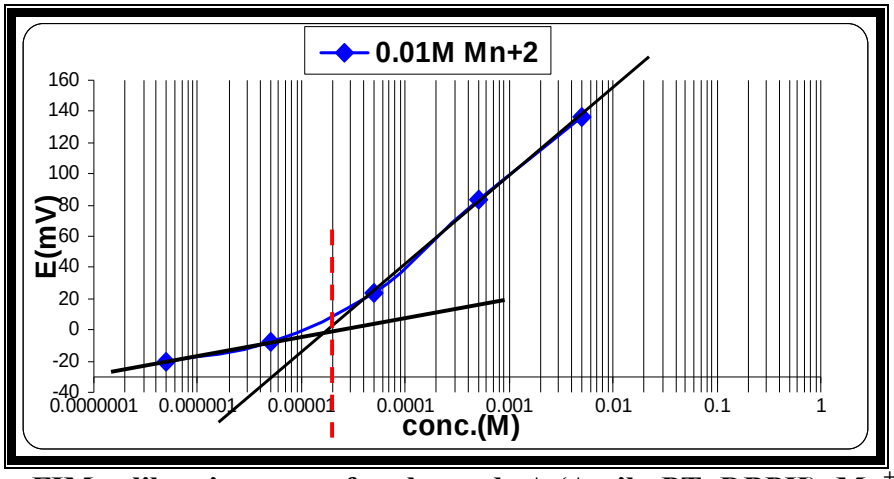


Fig.3-18:- FIM calibration curve for electrode A (Amilo-PT+DBPH), Mn^{+2}(5×10^{-3} M) as interfering ion $a_A=2\times10^{-5}$M.

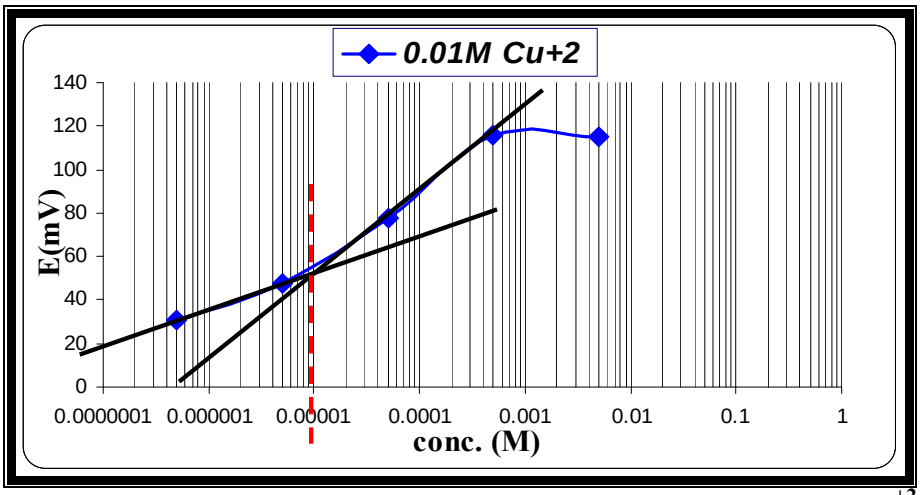


Fig.3-19:- FIM calibration curve for electrode A (Amilo-PT+DBPH), Cu^{+2}(5×10^{-3} M) as interfering ion $a_A=1\times10^{-5}$M.

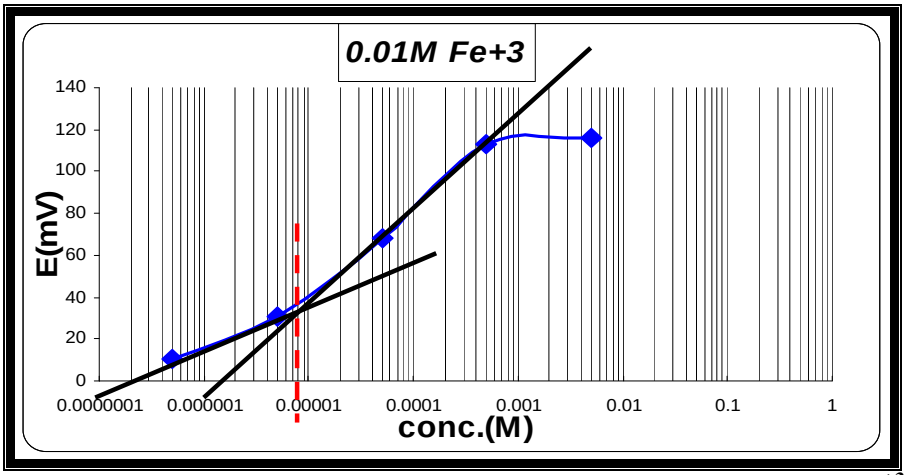


Fig.3-20:- FIM calibration curve for electrode A (Amilo-PT+DBPH), Fe^{+3}(5×10^{-3} M) as interfering ion $a_A=8\times10^{-6}$M.

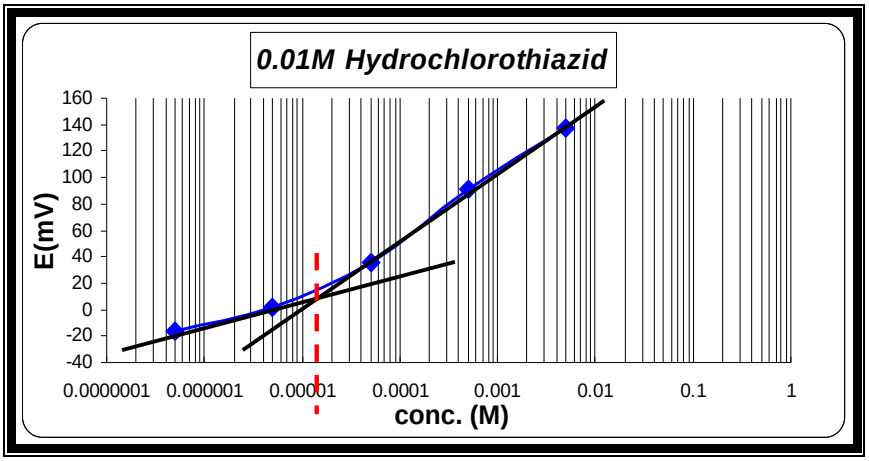


Fig.3-21:- FIM calibration curve for electrode A (Amilo-PT+DBPH), Hydrochlorothiazid (5×10^{-3}M) as interfering ion $a_A=1.5\times10^{-5}$M.

Table 3-12: Values of $K^{pot}_{A,B}$ calculated from the equation $K^{pot}_{A,B} = a_A/(a_B)^{ZA/ZB}$ according to FIM.

Interfering ions	a_B= (5×10^{-2} M)		a_B= (5×10^{-3} M)	
	a_A	$K^{pot}_{A,B}$	a_A	$K^{pot}_{A,B}$
K^+	4×10^{-5}	8.000×10^{-4}	3×10^{-5}	6.000×10^{-3}
Na^+	7×10^{-5}	1.400×10^{-3}	2×10^{-5}	4.000×10^{-3}
Mg^{+2}	1.9×10^{-5}	8.497×10^{-5}	1×10^{-5}	1.414×10^{-4}
Mn^{+2}	5×10^{-5}	2.236×10^{-4}	2×10^{-5}	2.828×10^{-4}
Cu^{+2}	4×10^{-5}	1.788×10^{-4}	1×10^{-5}	1.414×10^{-4}
Fe^{+3}	2×10^{-5}	5.428×10^{-5}	8×10^{-6}	4.678×10^{-5}
Hydrochlorothiazide	——	——	1.5×10^{-5}	3.000×10^{-3}

There are another benefit from the fixed interfering mixed method beside the selectivity we can see the drift in the calibration curve when interfering ion reacts with analyte (Amilo) such as ($Cu^{+2}$and Fe^{+3}) as shown in Fig. 3-13, 3-14, 3-19 and 3-20.

3-6- Sample analyses:-

Four potentiometric techniques were used for the determination of Amiloride, direct measurement, standard addition (SAM), multi-standard addition (MSA) and titration by using electrode A. The recovery (Re %), relative error (E_r %) and relative standard deviation (RSD %) for each method are calculated.

3-6-1- Direct method:-

The calibration curve was constructed (for electrode A) and the concentration of the unknown was calculated from the linear equation y=23.534Lnx+287.49 of the calibration curve which has the slope (S) ± S.D. = 54.19 ± 0.0177 and the intercept ± S.D. = 287.49 ± 0.133 for n=5, and the results are listed in Table 3-13.

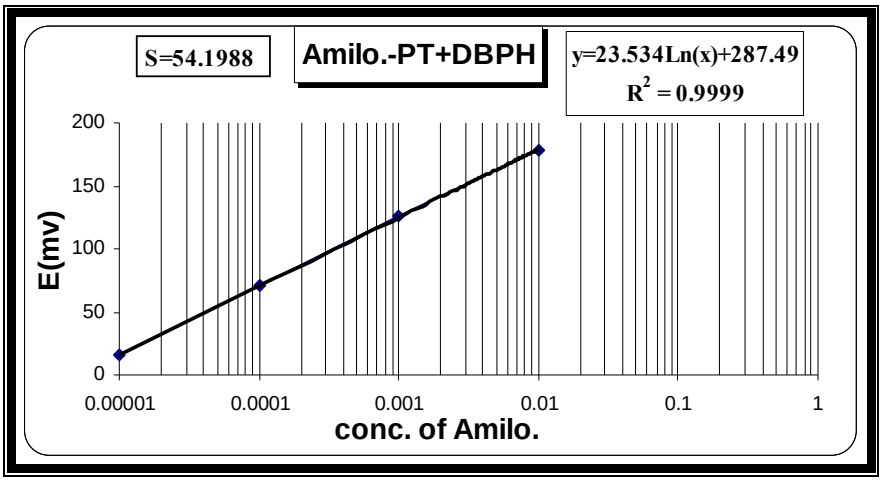


Fig.3-22:- Calibration curve of electrode A (Amilo-PT+DBPH).

Table 3-13:- Calculation for five samples of Amiloride hydrochloride standard solution 10^{-4} M using direct method for electrode (A) where slope=54.198.

Potential reading for the sample, E(mV)	The conc. of Amilo. sample calculated from linear equation/(M)	Average	Re%	E_r%	RSD%
70.71	0.999×10^{-4}	0.994×10^{-4}	99.9%	-0.1%	0.366%
70.62	0.995×10^{-4}		99.5%	-0.5%	
70.66	0.997×10^{-4}		99.7%	-0.3%	
70.54	0.992×10^{-4}		99.2%	-0.8%	
70.5	0.990×10^{-4}		99.0 %	-1.0%	

3-6-2- Incremental Methods:-

3-6-2-1- Standard addition method (SAM):-

It carried out by a procedure that 0.5 ml increment of 10^{-2}M Amiloride as standard was added to 20 ml of sample as unknown. The results of calculation SAM for the Amiloride hydrochloride using electrode (A) and equation 1-11, Recovery, Relative error and Relative standard deviation for five addition of Amiloride hydrochloride are listed in Table 3-14.

Table 3-14:- Calculation for five additions Amilo. standard solution using (SAM) for electrode (A) where slope=54.198, at concentration of sample 10^{-3} M.

V_S (mL) added	E/(mV)	ΔE	(V_U/V_S)	C_U/(M)	Re%	E_r%	RSD%
0.0	126	--------	0.0	----------	----------	----------	0.372%
0.5	130.7	4.7	40.0	0.994×10^{-3}	99.4%	-0.6%	
1.0	134.4	8.4	20.0	0.999×10^{-3}	99.9%	-0.1%	
1.5	137.6	11.6	13.3	0.990×10^{-3}	99.0%	-1.0%	
2.0	140.2	14.2	10.0	0.990×10^{-3}	99.0%	-1.0%	
2.5	142.4	16.4	8.0	0.993×10^{-3}	99.3%	-0.7%	

3-6-2-2- Multi standard addition method (MSA):-

The calibration curve for MSA for electrode (A) was shown in Fig. 3-23 by plotting antilog(E/S) versus the volume of the five addition of standard Amiloride. From the equation of the calibration curve the volume (mL) at intercept with X axis for the curve was calculate. 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 3-15.

Vol.(mL) of std. add	E/mV	Antilog (E/S)
0	126	211.25
0.5	130.7	257.94
1.0	134.4	301.85
1.5	137.6	345.811
2.0	140.2	386.199
2.5	142.4	424.036

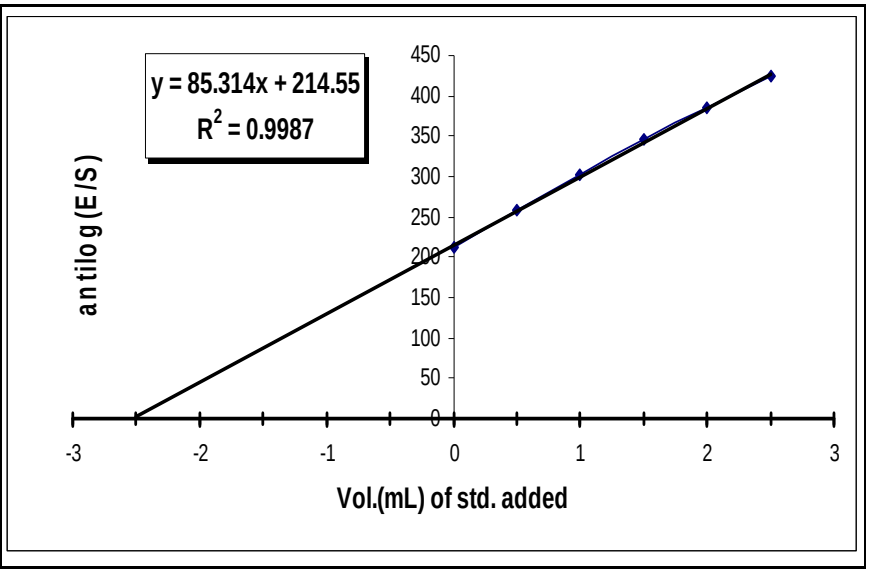


Fig.3-23:- Calibration curve of antilog (E/S) versus the volume added of standard (0.01 M) for determination of 25mL Amiloride hydrochloride solution 10^{-3} M by (MSA).

Table 3-15:- The linear equation of the calibration curve using MSA, and correlation coefficient, volume at intercept, the concentration of sample (C_U), Re% and E_r% of the unknown sample.

Linear equation	R	Volume at intercept (mL)	C_U(M)	Re%	E_r%
Y=85.314x+214.55	0.9993	2.514	1.006×10^{-3}	100.6%	0.6%

3-6-2-3-Titration method:-

The potentiometric titration for 15 mL of 0.01M Amiloride hydrochloride sample solution with 0.01M phosphotungistic acid as titrant solution as shown in (Fig.3-24 and 3-25), the results of titration (Re%, E_r% and RSD%) are listed in Table 3-16.

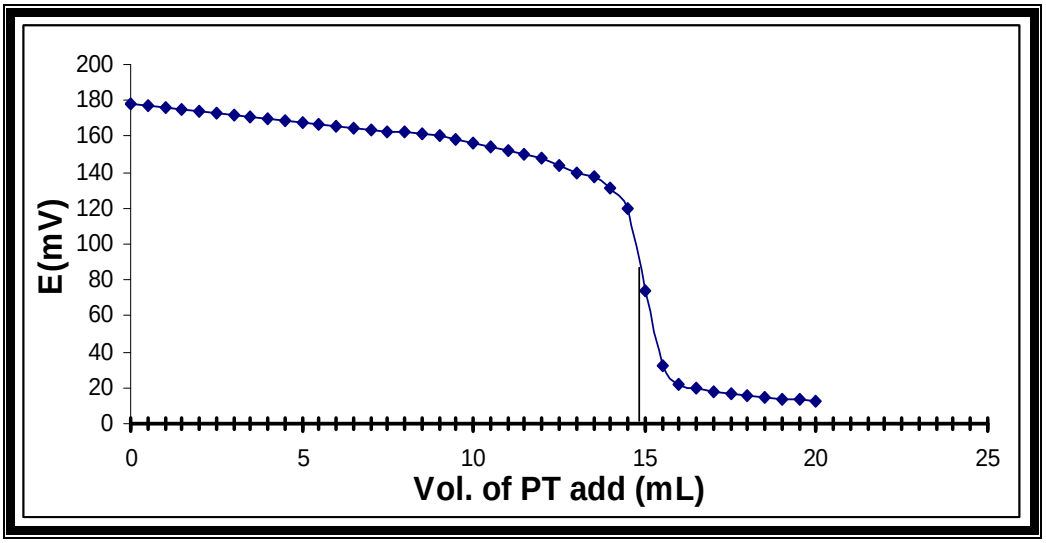


Fig.3-24:- Titration curve of electrode A (Amilo-PT+DBPH) for 15 mL sample solution 0.01 M Amiloride hydrochloride with 0.01 M of PT as a titrant solution.

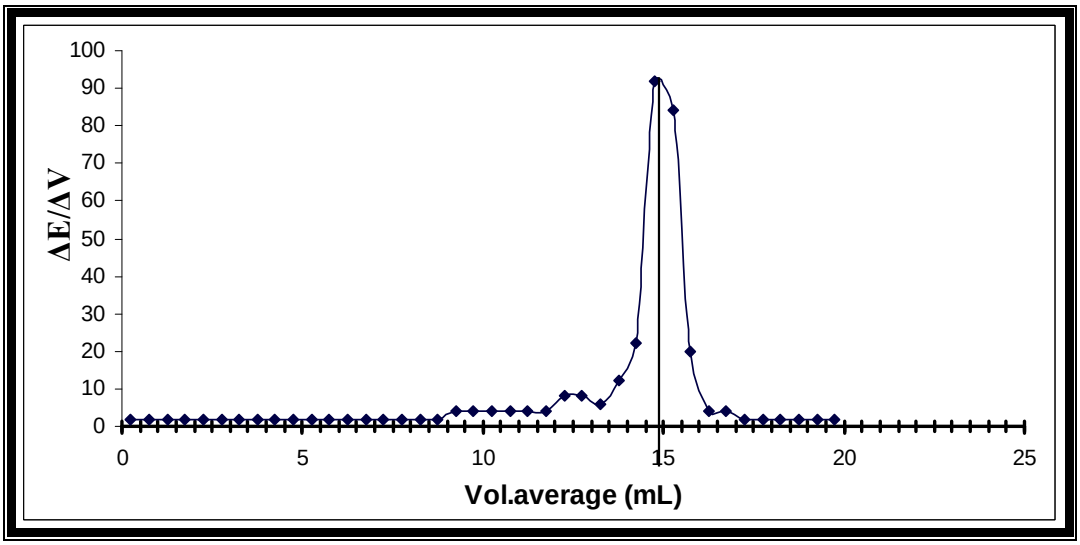


Fig.3-25:- Titration curve of electrode A (Amilo-PT+DBPH) by using first derivative, a 15 mL sample solution 0.01 M Amiloride hydrochloride with 0.01 M of PT as a titrant solution.

Table 3-16:- Standard Amiloride hydrochloride sample analysis results by using titration method for electrode A (Amilo-PT+DBPH).

Titration Fig.	Vol. mL at the end point	C_U(M)	Re%	E_r%	RSD*%
Fig.3-24	14.8	0.986×10^{-2}	98.66 %	-1.33 %	0.500%
Fig.3-25	14.9	0.993×10^{-2}	99.30%	-0.70%	

RSD% for the unknown concentration from the two figures.

3-7-Analytical Application of the Selected Electrode(A):-

Accuracy of the proposed electrode was assisted by determining Amiloride's solutions using the above methods and the data obtained for pharmaceutical samples were listed in Table 3-17 for Maduratic tablets and Table 3-18 for Saluretic tablets which were in the level of the certificate of British pharmacopeia's 2000, that Amiloride hydrochloride contains not less than 98.0 per cent and not more than the equivalent of 101.0 percent.[4]

Table 3-17:- Sample analyses of Maduratic tablets pharmaceutical Amiloride using electrode A (Amilo-PT +DBPH).

Parameter	Direct method	SAM	MSA	Titration method
Conc. (M)	1.000×10^{-3}	1.000×10^{-3}	1.000×10^{-3}	1.000×10^{-3}
Found(M)	0.995×10^{-3}	0.991×10^{-3}	1.008×10^{-3}	0.990×10^{-3}
%RSD*	0.474%	0.408%	-------	0.631%
Re%	99.5%	99.1%	100.8%	99.0%
E_r%	-0.5%	-0.9%	0.8%	-1.0%

Each concentration represents an average of at least three measurements.

Table 3-18:- Sample analyses of Saluretic tablets pharmaceutical Amiloride using electrode A (Amilo-PT +DBPH).

Parameter	Direct method	SAM	MSA	Titration method
Conc. (M)	1.000×10^{-3}	1.000×10^{-3}	1.000×10^{-3}	1.000×10^{-3}
Found*(M)	0.986×10^{-3}	0.988×10^{-3}	1.01×10^{-3}	0.983×10^{-3}
%RSD*	0.576%	0.510%	-------	0.768%
Re%	98.6%	98.8%	101.0%	98.3%
E_r%	-1.4%	-1.2%	1.0%	-1.7%

Each concentration represents an average of at least three measurements.

3-8- Sample analyses by using UV-Spectrophotometry:-

In spectrophotometry UV- region Amiloride hydrochloride detected by three wavelengths (361,286 and 213) by using normal calibration curves as show in Fig.3-26:-

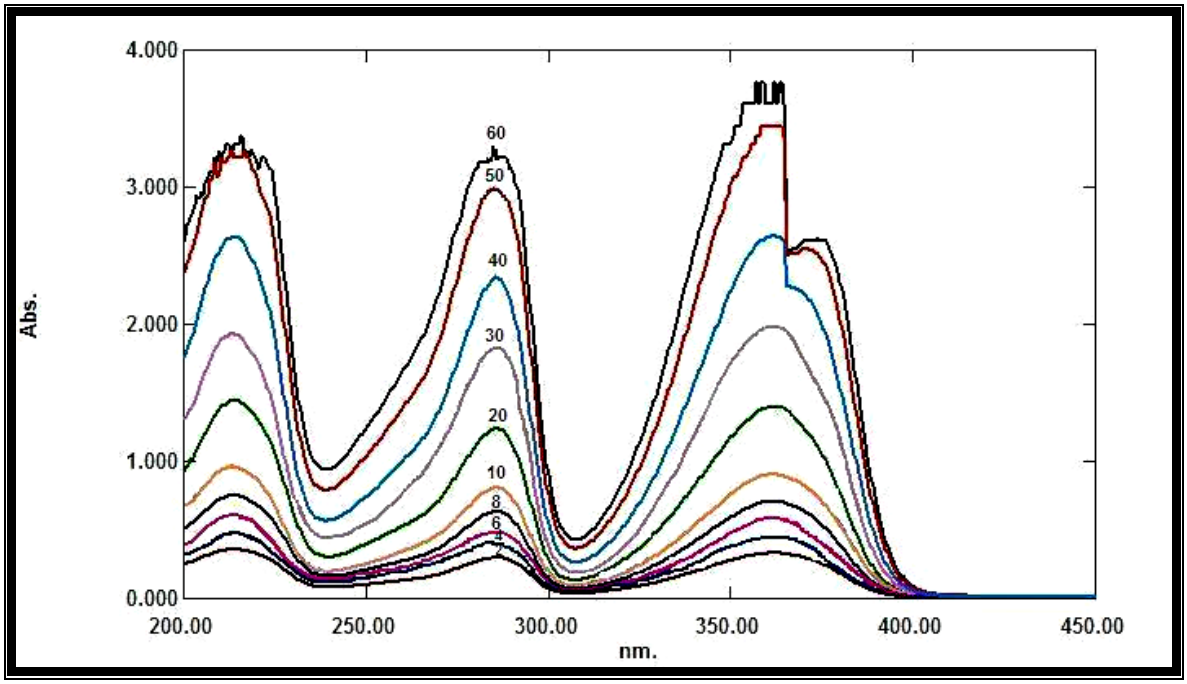


Fig.3-26:- UV- Spectra for Amilo. solutions at different concentration ranged from 2 to 60 mg/L.

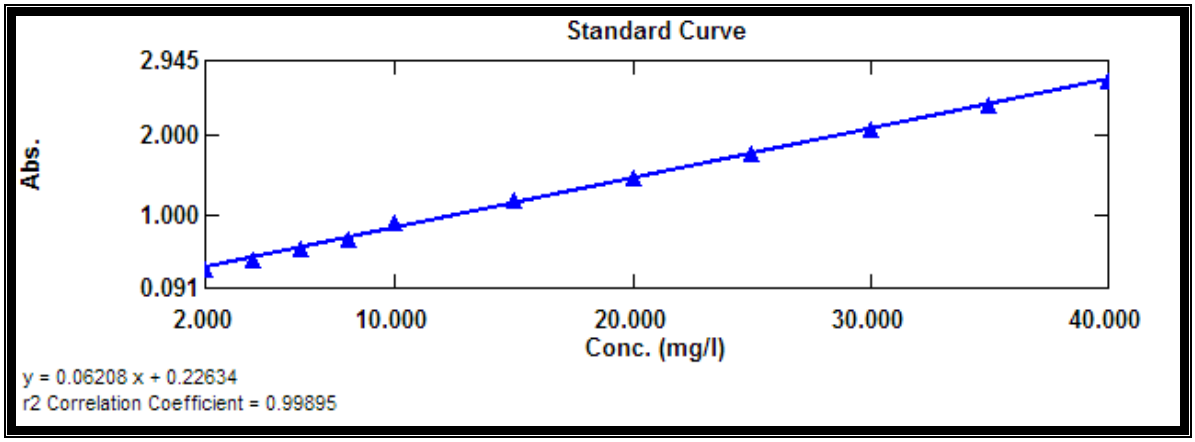


Fig.3-27:- calibration curve for Amiloride at λ 361 at different concentration ranged from 2 to 40 mg/L.

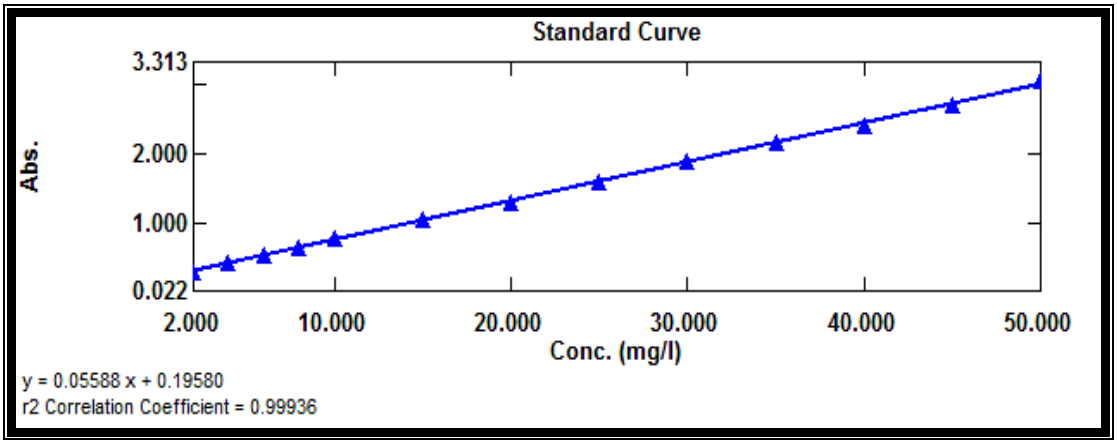


Fig.3-28:-calibration curve for Amiloride at λ 286 at different concentration ranged from 2 to 50 mg/L.

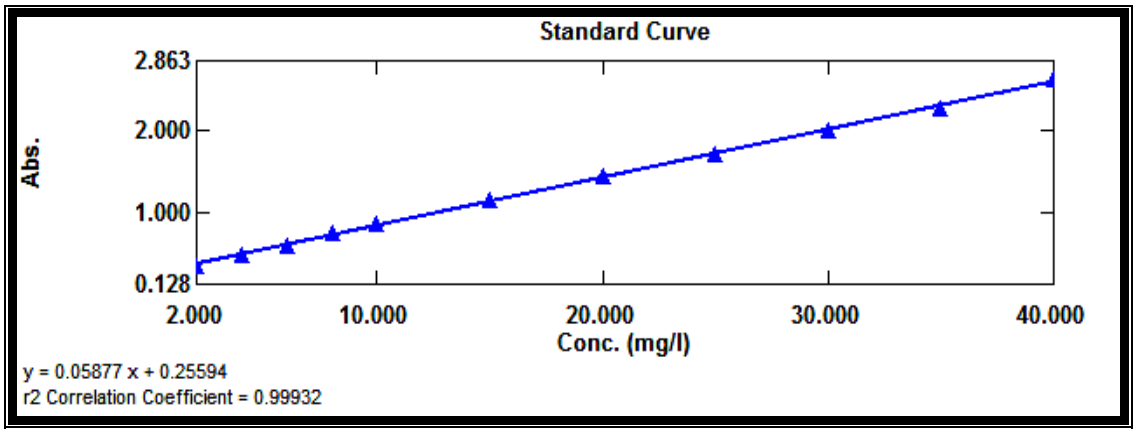


Fig.3-29:- calibration curve for Amiloride at λ 213 at different concentration ranged from 2 to 40 mg/L.

Table 3-19:- The concentration range of the three wavelengths and their linear equations, correlation coefficient, standard deviation of slope and intercept respectively.

Wavelength (λmax)/nm	Linear conc. Range mg/L	Linear equation	r^2	S.D.(σ)* of the slope	S.D.(σ)* of the intercept
361	2-40	Y= 0.06208X+ 0.22634	0.99895	2.511×10^{-4}	7.990×10^{-3}
286	2- 50	Y= 0.05588X+ 0.19580	0.99936	3.407×10^{-4}	4.0926×10^{-3}
213	2-40	Y= 0.05877X+ 0.25594	0.99932	3.214×10^{-4}	3.7004×10^{-3}

The result of three times repeated.

The best wavelength 286 nm with wider concentration range and best (r^2 = 0.99936) as shown in the normal calibration curve. Fig.3-28 used to determine prepared Amiloride hydrochloride sample solutions (1×10^{-4}M that equal to 30.2 ppm) by direct method, by reading the absorbance of the

unknown samples and calculated there concentration from the linear equation of the calibration curve the results are listed in Table 3-20.

Table 3-20:- Calculation for five samples of Amiloride hydrochloride standard solution 10^{-4} M (30.2mg/L) by using direct method for normal calibration curve of UV-spectrophotometry.

Absorbance	C_U (mg/L)	C_U (M)	Re%	E_r%	RSD*%
1.884	30.200	0.999×10^{-4}	99.9%	-0.1%	0.497 %
1.897	30.441	1.0076×10^{-4}	100.76%	0.76%	
1.882	30.173	0.9987×10^{-4}	99.87%	-0.13%	
1.887	30.262	1.0017×10^{-4}	100.17%	0.17%	
1.875	30.048	0.994×10^{-4}	99.4%	-0.6%	

%RSD for five unknown concentrations in (M).

3-9- Comparison between ISE and UV-Spectrophotometric Methods:-

The comparison between ISE and UV-Spectrophotometric Methods by using direct method as listed in Table 3-21 shows that the ISE method was better than UV- Spectrophotometric Method.

Table 3-21:- The comparison between ISE and UV-Spectrophotometric Methods.

Parameters	ISE method	UV- spectrophotometric method
Linear range(M)	$1.000\times10^{-5} - 1.000\times10^{-2}$	$6.620\times10^{-6} - 1.655\times10^{-4}$
Detection limit(M)	6.000×10^{-7}	3.056×10^{-8}
RSD%*	0.366%	0.497 %

***for five unknown concentrations of direct method of ISE and UV-spectro.**

And the results of UV- spectrophotometric method compared with direct method of ISE by F-test [135] as listed in Table 3-22, from the Table found that the value of F equal to 1.863 that was smaller than the value in the table of F- test at 95% confidence level for (n-1) that was equal to 6.39 when n= 5 means that the newer ISE method was better than UV-spectrophotometry.

Table 3-22:- Calculation of F-test between the two methods ISE and UV-spectrophotometry.

C_U(M) from direct method of ISE	S.D.(σ)*	C_U(M) from direct method of UV-spectrophotometry	S.D.(σ)*	The (F) magnitude
0.999×10^{-4}	3.6469×10^{-7}	0.999×10^{-4}	4.978×10^{-7}	1.863
0.995×10^{-4}		1.0076×10^{-4}		
0.997×10^{-4}		0.9987×10^{-4}		
0.992×10^{-4}		1.0017×10^{-4}		
0.990×10^{-4}		0.994×10^{-4}		

*** The standard deviation S.D. are for five readings (n= 5).**

3-10- Conclusions:-

Amiloride-selective PVC membrane electrodes based on ion pair complexes of Amilo-PT and the other ionophore Amilo-TPB and (DBPH, DOP and TBP) as plasticizers were constructed, and used as a method for potentiometric determination. The best electrode for Amiloride was based on Amilo-PT active complex and DBPH as plasticizer which gives excellent electrode parameters as well as good result in determination of Amiloride Also there is no interference for some cations (Na^{+1}, K^{+1}, Mg^{+2}, Mn^{+2}, Cu^{+2}, Fe^{+3} and (HCT drug), it also has the working pH in the range (2-7). The practical utility of the electrode has been demonstrated by use it as indicator electrode in potentiometric precipitation titration of Amiloride solution with phosphotungistic acid solution. Direct method, Standard addition method and multi standard addition method have been also successfully applied and showing a very good results. The proposed electrode was successfully applied to the determination of Amiloride hydrochloride in pharmaceutical preparation (Maduratic and Saluretic). The analytical method proposed proved to be simple, rapid and of good accuracy compared to the UV-spectrophotometric method by normal calibration curve.

3-11-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 methods for preparation ion exchanger (ionophore) by using silicotungstic acid ($SiO_2.12WO_3.xH_2O$) or potassium tetrakis(p-chlorophenyl) borate (KTPClPB) instead of phosphotungstic acid ($H_3PO_4.12WO_3.xH_2O$) and sodium tetraphenylborate $NaB(C_6H_5)_4$.

2- Other plasticizers to get better idea on their influence on the electrode performance.

3- Other types of matrixes as alternative to PVC matrix.

4- 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.

Appendices:-

There are some statical relations needed in the Thesis[135]:-

1- Standard Deviation (SD):-

$$\mathbf{SD} = \left[\sum(x_i - \bar{x})^2 / N - 1\right]^{1/2}$$

Where:

X_i = concentrations of individual deviations.

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

N = no. of degrees of freedom.

2- Relative Standard Deviation (RSD%):-

$$\mathbf{RSD\%} = \left[SD / \bar{x} \right] \times 100$$

3- Relative Error (E_r%)

$$\mathbf{E_r\%} = (d/u) \times 100$$

Where:

d = Absolute Error, the difference between the measurment quantity (X_i) and the true or accepted value of the quantity (u).

4- Recovery (Re %):-

$$\mathbf{Recovery\ (Re)\ \%} = (x_i/u) \times 100$$

5- **F-test:-**

$$\mathbf{F} = S_a^2 / S_b^2$$

Where: S_a, S_b are the standard diviations for first and second methods respectivily, ($S_a > S_b$).

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الخلاصة

تم تصنيع عدة أقطاب پوليمريه من مادة البولي ڤاينايل كلورايد حساسة للاميلورايد هيدروكلورايد بالاعتماد على المعقد المحضر (Amiloride-phosphotungistate) والمعقد الثاني (Amiloride-tetraphenylborate) كمادة فعالة. هذه المادة الفعالة تكون مذابة في عدة مواد ملدنه منها :

Di-butylphthalate (DBPH), Di-octylphthalate (DOP), Tri-butyl phosphate (TBP),

وقد تم دراسة خواص هذه الأقطاب و التي شملت(ميل منحني المعايرة و معامل الارتباط و مدى التراكيز و حد التحسس و عمر القطب) ومن خلال الدراسة أظهرت النتائج أن أقطاب الاميلورايد: **Amilo-PT+DOP, Amilo-PT+TBP, Amilo-TPB+ Amilo-PT+DBPH, Amilo-TPB +TBP, DBPH, Amilo-TPB + DOP,** لها تقريبا نفس مدى التركيز الخطي $1x10^{-5}$ – $1x10^{-2}$ مولاري وإنحدار ,54.198 50.91,52.759, 48.501,48.508,49.007 ملفولت/حقبة على التوالي. و حد التحسس (1.5×10^{-5} ,7×10^{-6}, 6×10^{-7}, 1.5×10^{-6}, 7×10^{-6} ,1.75×10^{-5}) مولاري, زمن الإستجابة (35, 10, 12 10, 30, 10) ثانية للتركيز 10^{-3} مولاري و عمر القطب (45, 35, 30, 21 , 15, 10) يوم على التوالي. و مدى الدالة الحامضية وجد بحدود(7.8 – 1.9) لتركيز محلول الاميلورايد 10^{-3} مولاري باستخدم القطب Amilo-PT+DBPH (A). أيضا درست التداخلات لبعض الأيونات الاحادية والثنائية والثلاثية و تداخل نوع آخر من الادوية المدررة مثل الهايدوكلوروثايازايد بوساطة طريقة المحاليل المنفصلة وطريقة المحاليل الممزوجة لتعين معامل الانتقائية ($K^{pot}_{A,B}$) و كذلك استخدم القطب (A) في التقدبرات الإجهادية لتعين الاميلورايد في الأدوية التجارية و اظهرت النتائج بأن الطريقة المستخدمة بسيطة, سريعة و دقيقة مقارنةً بطريقة الاطياف .

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

تحضير قطب سائل مستند على أساس معقد دواء الاميلورايد في صفيحة غشاء البولي فاينايل كلورايد

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

من قبل
مها عبد اللطيف يحيى
بكالوريوس كيمياء 2006 (جامعة النهرين)

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