Preparation and Potentiometric Study of Amiloride Hydrochloride Selective Electrodes and Their Application in Determining Some Drugs

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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+ DBPH, Amilo-TPB + Amilo-PT+DBPH, Amilo-TPB +TBP, DOP, لها تقريبا نفس مدى التركيز الخطي $1x10^{-5}$ – $1x10^{-2}$ مولاري وإنحدار 54.198, 52.759,50.91, 49.007,48.508,48.501 ملفولت/حقبة على التوالي. و حد التحسس (6×10^{-7}, 7×10^{-6}, 1.5×10^{-5}, 1.75×10^{-5}, 7×10^{-6}, 1.5×10^{-6}) مولاري، زمن الإستجابة (10, 30, 10, 12, 10, 35) ثانية للتركيز 10^{-3} مولاري و عمر القطب (10, 15 , 21, 30, 35, 45) يوم على التوالي. و مدى الدالة الحامضية وجد بحدود(7.8 – 1.9) لتركيز محلول الاميلورايد 10^{-3} مولاري باستخدم القطب (A) Amilo-PT+DBPH. أيضاً 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 التقديرات الإجهادية لتعين الاميلورايد في الأدوية التجارية و اظهرت النتائج بأن الطريقة المستخدمة بسيطة، سريعة و دقيقة مقارنةً بطريقة الاطياف .

ABSTRACT

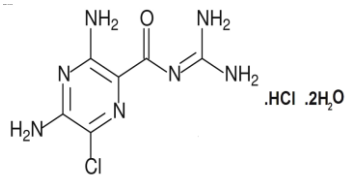
Amiloride hydrochloride ion-selective electrodes were constructed in polymeric membrane by using PVC and based on the use of active ion-pair (Amiloride-phosphotungstate) and the other ion pair (Amiloride- tetraphenyl -borate). The plasticizers used were Di-butyl phthalate (DBPH), Di-octylphthalate (DOP) and Tri-butyl phosphate (TBP). The electrodes (A, B and C) based on the ion pair (Amiloride -phosphotungstic acid) 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, 50.910, 49.007, 48.508 and 48.501) mV/decade, and the detection limit were (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. And 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). The measurement interferences in the presence of $Na^{+,}$ K^{+}, $Mg^{2+,}$ Mn^{2+}, $Cu^{2+,}$, Fe^{3+} and Hydroclorothiazide were studied using separate and mixed methods for selectivity coefficient determination. The pH and life time of the electrodes were also studied. The analytical methods results showed to be simple, rapid and with a good accuracy by comparing it with UV-spectrophotometric method by using F-test.

Key Words Amiloride hydrochloride ion-selective, phosphotungstic acid, tetraphenylborate, Amiloride determination.

INTRODUCTION

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 is 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(4) .



There have been only a few reports on the determination of Amiloride in tablets or in biological fluids, such as fluorescence spectrometry (5,6). The sequential injection analysis technique (SIA).(7) . A simple and fast method for the derivative spectrophotometry (8, 9). The chromatographic methods HPLC and GC/MS.(10-13).The electrochemical methods voltammetry(14) and Potentio - metry(15). UV spectrophotometry coupled with new chemometric regression techniques (16, 17). 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 (18).

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. (19). Analytical techniques are commonly employed for this purpose by using drug-selective electrodes (15, 20-25).

MATERIALS AND METHODS

Instruments and equipment:

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

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

3-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).

Reagents and solutions

1- Pure Standard Amiloride hydrochloride ($C_6H_8CIN_7O.HCl.2H_2O$ F.W.302.12) and Hydrochlorothiazide (HCT) $C_7H_8ClN_3O_4S_2$; F.W. 297.74) were 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.

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)
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 1.

Table-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, All solutions were prepared in doubly distilled water.

1-A stock standard solution of 0.01 M Amiloride hydrochloride was prepared by dissolving 0.151 g of standard Amiloride hydrochloride and completing the solution up to 50 ml, (ultrasonicator) equipment was used to assist the dissolving of the drug. The other

Amiloride standard solutions were prepared by serial 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 distilled water in 100mL volumetric flask. More diluted solutions were prepared by dilution from the stock solutions.

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

6-A solution of ≈ 0.1M HCl was prepared by diluting 0.833mL 0f 12M HCl concentrated stoke solution to 100mL. 0.1M NaOH was prepared by weighting 0.4g of NaOH and dissolving to 100mL distilled water.

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

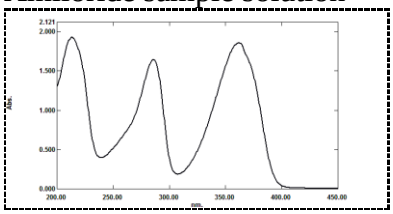
Procedure

Preparation of Ion-pair Compounds:

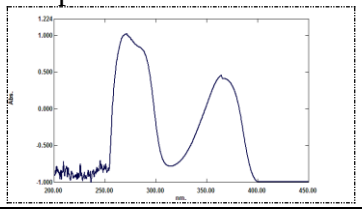
The Amiloride hydrochloride ion-selective electrode is prepared based on the use of ion-pair compound (Amilo-PT) as the electro-active substance. The preparation of ion-pair was performed by mixing 50 ml of 0.01 M solution of Amiloride hydrochloride with 50 ml of 0.01 M PT with stirring. The resulting 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 UV and FTIR spectra as shown in Fig. 1 and Fig 2 respectively.

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 UV and FTIR spectra as shown in Fig. 1 and Fig 2 respectively..

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



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



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

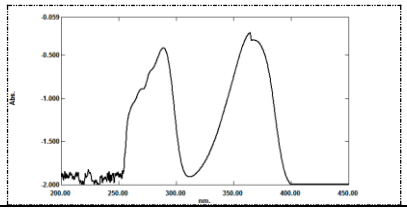
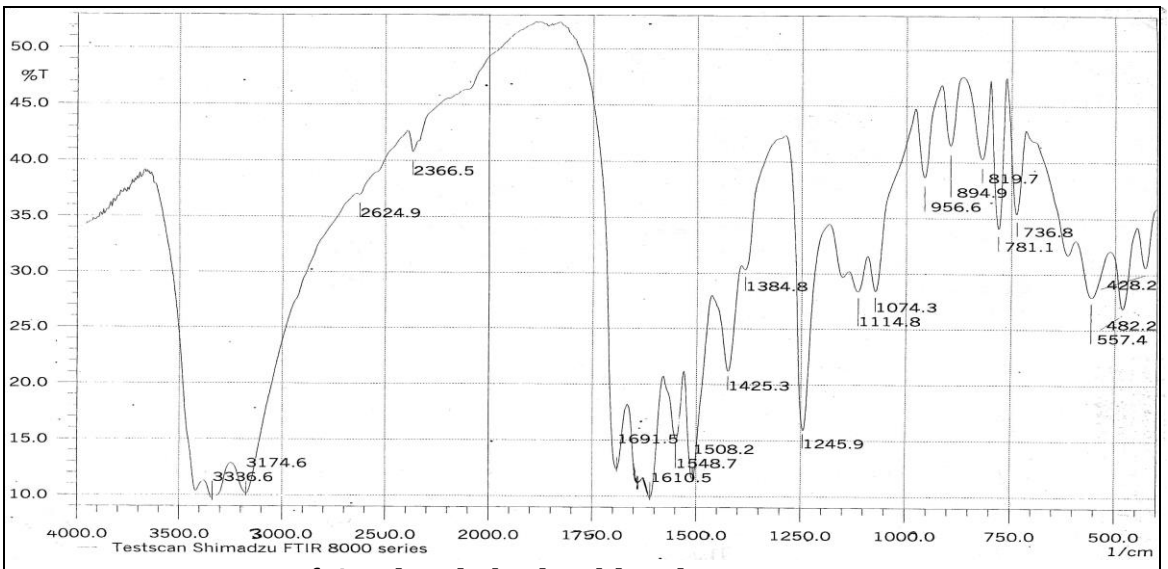
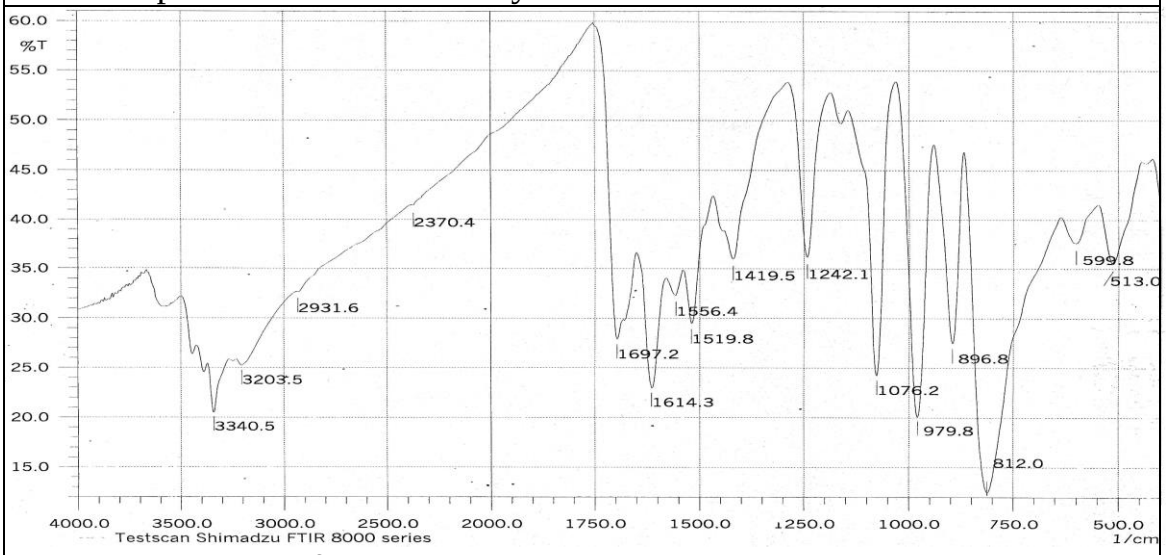


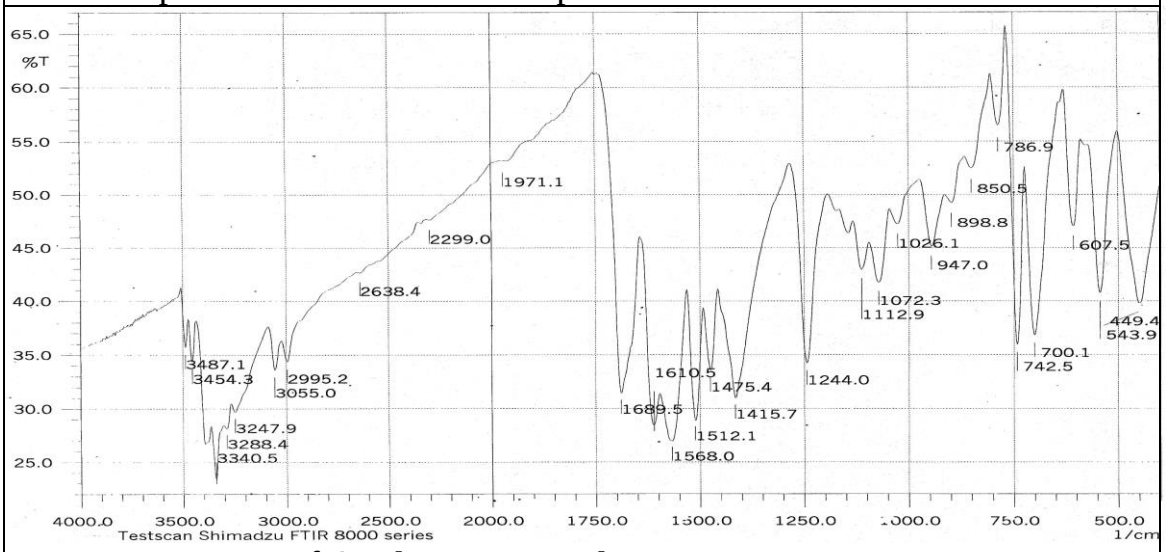
Fig.-1: UV spectra, a- Amiloride, b- Amilo-PT complex, c- Amilo-NaTPB



a- FTIR spectrum of Amiloride hydrochloride.



b- FTIR spectrum of Amilo-PT complex.



c- FTIR spectrum of Amilo-TPB complex.

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

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 (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. The method of immobilization ion-pair compounds into the PVC matrix membrane as described by Craggs et al (26). 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) The other side of the glass tube was assembled with plastic cover in which Ag/AgCl wire was inserted through it, tube was filled with 10^{-3}M Amiloride hydrochloride as internal solution before fixing the cover. The electrode was immersed in 10^{-3}M Amiloride solution for at lest one hour before use. When not in use, they were stored in air. The master membrane was used to prepare several others.

Selectivity measurements

A separate solution method was used for the selectivity coefficient measurement, and was calculated according to the equation(27, 28)

$$\log K_{pot} = [(E_B - E_A)/(2.303RT/zF)] + (1 - z_A/z_B) \log a_A \qquad (1)$$

E_A, E_B; z_A, z_B; and a_A, a_B are the potentials, charge numbers, and activities for the primary A and interfering B ions, respectively, at $a_A = a_B$. The selectivity coefficients were also measured by the mixed solution method and was calculated according to the equation] (29-31)

$$K^{pot}_{A,B} = a_A / (a_B)^{ZA/ZB} \qquad (2)$$

RESULTS AND DISCUSSION

The complexes were obtained by conversion Amiloride hydrochloride to Amilo-PT and Amlo-TBP characterized by their UV and FTIR spectra, , Amiloride phosphotungastate (Amilo-PT) light brown precipitate and the second complex is obtained by conversion Amiloride hydrochloride into Amiloride tetraphenylborate (Amilo-TPB) white precipitate the complex Amilo-PT, and

Amilo-TPB. were stable and water insoluble ion-pair complex though readily soluble in organic solvents such as tetrahydrofuran. The complex was incorporated into a PVC membrane and three plasticizers; di-n-butylphthalate (DBPH), di-octylphthalate (DOP) and tri-n-butylphosphate (TBP). 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.

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). 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, which their calibration curves shown in Fig. 4. The equations of the linear range and their slope, correlation coefficient and relative standard deviation of there were listed in Table 2.

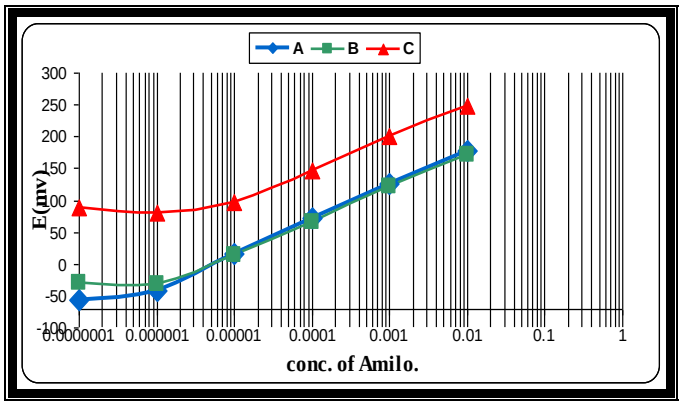


Fig.-3:Calibration curves of Amiloride hydrochloride selective electrodes (A, B and C) using (Amilo-PT) ion-pair complex.

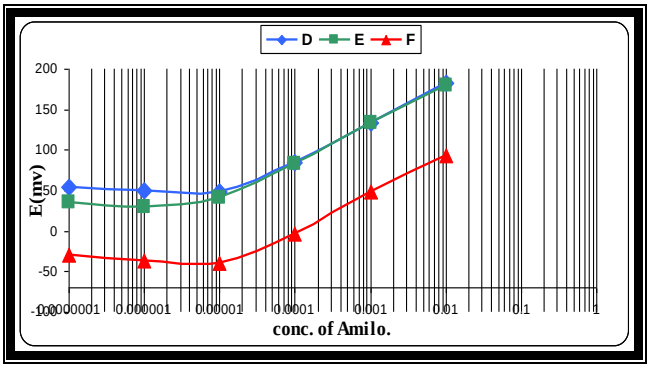


Fig.-4: Calibration curves of Amiloride hydrochloride selective electrodes (D, E and F) using (Amilo-TPB) ion-pair complex.

Table-2: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	1.0000
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 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, F) are listed in Table 3, from the table electrode A (Amilo-PT with DBPH) is the best electrode which used to determined Amiloride.. Non-Nernstian slopes were obtained for electrodes non-Nernstian slope behaviors could be attributed to the high viscosity of plasticizers. slow down the ion exchange process between ion pair complex in membrane with the external solution of amiloride hydrochloride. Moreover, the steric effect of the alkyl group on the DBPH may decrease the bond strength of the ion pair complex. The TBP, which has a low viscosity (3.11 cSt), leads to leaching of the complex from the membrane or may have a high steric effect on methyl groups. Near Nernstian slopes . 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(32) in Some Pharmaceutical Formulations by using ISE has a slope of 54.5 mV /decade, other ion selective membrane electrode forr PVC Membrane Sensors for potentiometric determination of Acebutolol(33) has slope 51.5 and 53 mV /decade, also the determination of Methacycline Hydrochloride(34) by using ISE has the slope equal 52.9 mV/decade and there was also Promethazine Hydrochloride (25) ion selective electrodes used in pharmaceutical preparations have a slopes of 40-56 mV /decade.

Table-3: 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	$10^{-5} - 10^{-2}$	6×10^{-7}	8	10	20	45
B- Amilo-PT +DOP	52.759	$3\times10^{-6} - 10^{-2}$	1.5×10^{-6}	20	30	35	35
C- Amilo-PT +TBP	50.910	$10^{-5} - 10^{-2}$	7×10^{-6}	5	10	15	30
D- Amilo-TPB +DBPH	49.007	$6\times10^{-5} - 10^{-2}$	1.75×10^{-5}	20	35	40	21
E- Amilo-TPB +DOP	48.508	$3\times10^{-5} - 10^{-2}$	7×10^{-6}	7	10	20	15
F- Amilo-TPB +TBP	48.501	$5\times10^{-5} - 10^{-2}$	1.5×10^{-5}	6	12	20	10

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. The curves are shown in Fig.5.

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. The working pH ranges of Amiloride electrode A are listed in Table 4.

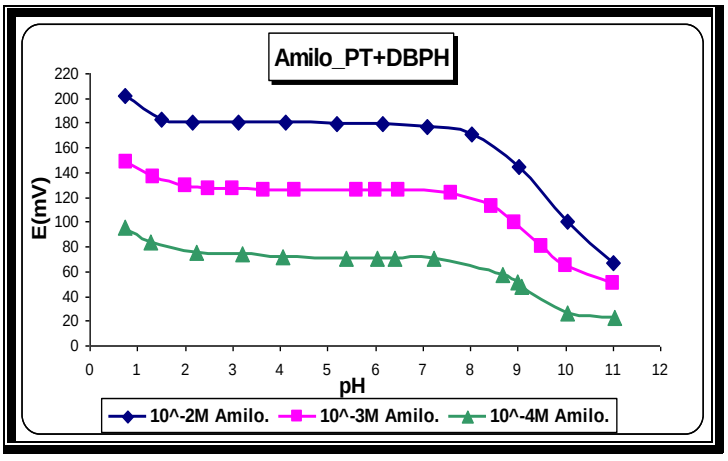


Fig.-5: Effect of pH on the potential of the electrode A (Amilo-PT+DBPH) at concentrations 10^{-2}, 10^{-3} and 10^{-4} M.

Table-4: 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

Selectivity methods:

Separate solution methods (27,28):

The influence of some possible interfering inorganic cations such as Na^+, K^+, Mg^{2+}, Mn^{2+} ,Cu^{2+} and Fe^{3+} and Hydrochlorothiazide druge which presented with amiloride in the pharmaceutical formulation for amiloride sensors (A, B, C, D, E and E) on the electrode response was studied. The selectivity of the electrodes based on DBPH, DOP and TBP were measured by the separate solution methods.. The potentiometric selectivity coefficients were calculated using equation 1 at Amiloride and interfering concentrations were10^{-3} .The values of the selectivity coefficients electrodes are listed in Table 5. The selectivity coefficients were very small. This means that there is no interference of these cations with the response of amiloride electrodes.

Table 5: Selectivity coefficient values for Amiloride electrode using - Separated solution methods

Sensors / Interfering-Ion	A Amilo-PT+DBPH	B Amilo-PT+DOP	C Amilo-PT+TBP	D Amilo-TPB+DBPH	E Amilo-TPB+DOP	F Amilo TPB+TBP
Na^+	1.320×10^{-3}	1.100×10^{-3}	1.770×10^{-3}	7.910×10^{-3}	5.140×10^{-3}	1.150×10^{-2}
K^+	3.699×10^{-4}	1.104×10^{-3}	9.024×10^{-4}	1.040×10^{-2}	5.660×10^{-3}	8.270×10^{-3}
Mg^{2+}	3.530×10^{-5}	4.344×10^{-5}	3.932×10^{-4}	3.643×10^{-4}	2.269×10^{-4}	5.59×10^{-4}
Mn^{2+}	2.212×10^{-5}	5.896×10^{-5}	3.433×10^{-4}	4.002×10^{-4}	2.617×10^{-4}	6.147×10^{-4}
Cu^{2+}	1.643×10^{-5}	5.403×10^{-5}	2.738×10^{-4}	2.0731×10^{-4}	2.164×10^{-4}	4.623×10^{-4}
Fe^{3+}	3.96×10^{-4}	7.872×10^{-5}	1.040×10^{-3}	8.289×10^{-4}	7.007×10^{-4}	8.076×10^{-4}
Hydrochlorothiazide	7.500×10^{-2}	7.900×10^{-2}	9.510×10^{-2}	7.540×10^{-2}	8.470×10^{-2}	7.000×10^{-2}

Mixed solution methods

By using the fixed interference method (FIM),(29-31) 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) at first used 5×10^{-2}M then mixed it with varying activity of the primary ion that is for the Amiloride (a_A). The potentiometry 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.6 as an example for all interfering ions, that is used to calculate $K^{pot}_{A,B}$ from equation (2) all results of $K^{pot}_{A,B}$ were in Table 6.

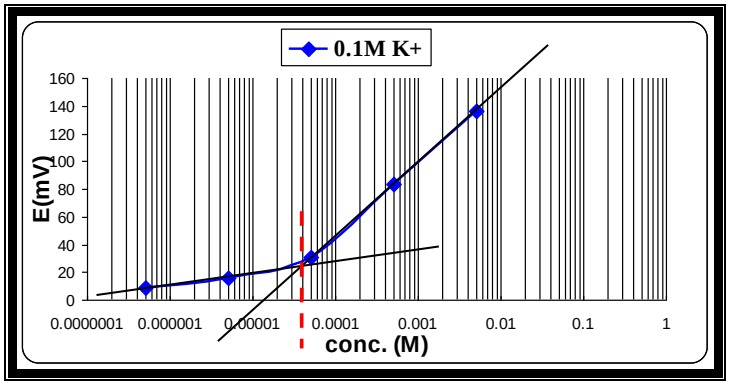


Fig.-6: FIM calibration curve for electrode A (Amilo-PT+DBPH), K^+ (5×10^{-2}M) as interfering ion $a_A=4\times10^{-5}$M.

Table-6: 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	$a_B= (5\times10^{-2}$ M)		$a_B= (5\times10^{-3}$ M)	
	a_A	$K^{pot}_{A,B}$	a_A	$K^{pot}_{A,B}$
K^+	$4.000\ x10^{-5}$	8.000×10^{-4}	3.000×10^{-5}	6.000×10^{-3}
Na^+	7.000×10^{-5}	1.400×10^{-3}	2.000×10^{-5}	4.000×10^{-3}
Mg^{+2}	1.900×10^{-5}	8.497×10^{-5}	1.000×10^{-5}	1.414×10^{-4}
Mn^{+2}	5.000×10^{-5}	2.236×10^{-4}	2.000×10^{-5}	2.828×10^{-4}
Cu^{+2}	4.000×10^{-5}	1.788×10^{-4}	1.000×10^{-5}	1.414×10^{-4}
Fe^{+3}	2.000×10^{-5}	5.428×10^{-5}	8.000×10^{-6}	4.678×10^{-5}
Hydrochlorothiazide	——	——	1.500×10^{-5}	3.000×10^{-3}

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 (RC %), relative error (RE %) and relative standard deviation (RSD %) for each method are calculated and listed in table 7.

Direct method

The calibration curve was constructed (for electrode A) in Fig.7, 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. = 54.1988 ± 0.0177 and the intercept □□S.D. = 287.49□□□□□□□□, for n=5, and the results are listed in Table 7.

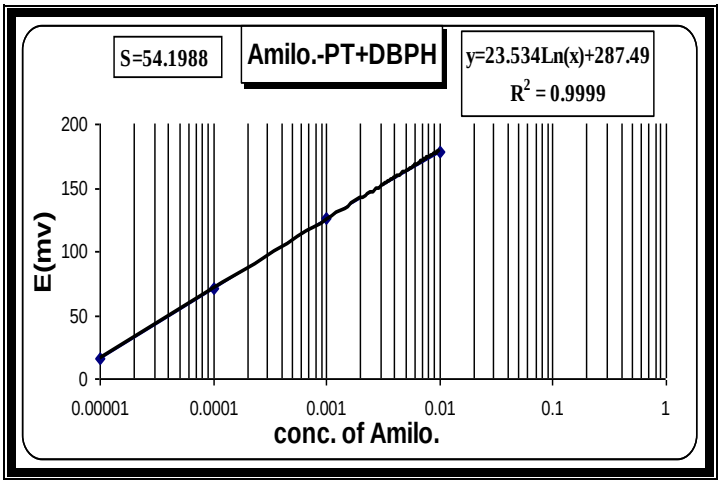


Fig.-7: Calibration curve of electrode A (Amilo-PT+DBPH).

Standard addition method (SAM)

That 0.5 ml increment of standard solution of 10^{-2}M Amiloride was added to 20 ml of unknown sample. By solving the following equation the unknown concentration can be obtained.

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

Where: C_U: concentration of unknown solution, C_S: concentration of standard solution, V_U: volume of unknown solution, V_S: volume of standard solution, S: slope of electrode. The Recovery, Relative error and Relative standard deviation for five addition of Amiloride hydrochloride are listed in Table 7.

Multi standard addition method (MSA)

The calibration curve for MSA for electrode (A) was shown in Fig. 8 by plotting of antilog(E/S) versus the volume of the five addition of Amiloride hydrochloride of 0.05 mL of $1x10^{-2}$ M. the analysis results RC% and RE% were listed in Table 7

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

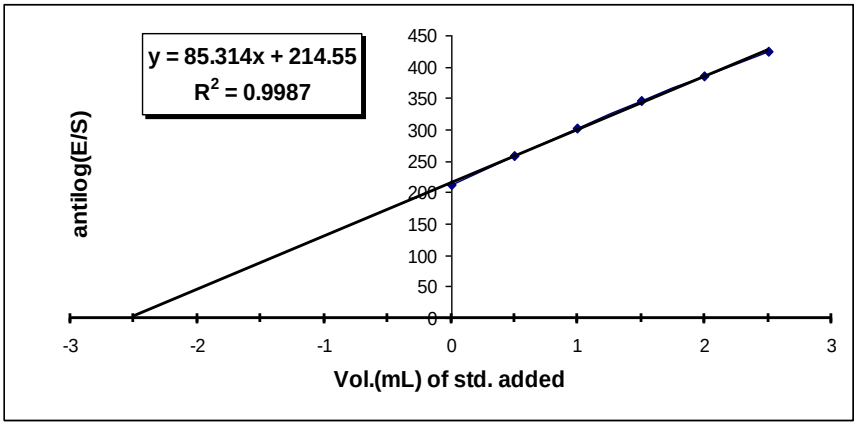


Fig.-8:Calibration curve of antilog (E/S) versus the volume added of standard (0.01 M) for determination of 25mL Amiloride hydrochloride solution 10^{-3} M by (MSA).

Titration method:

The potentiometric titration for 15 mL of 0.01M Amiloride hydrochloride sample solution with 0.01M phosphotungistic acid as titrant solution, using two methods for indication end point from potentiometric titration curves as shown in (Fig.8), the results of titration (RC%, RE% and RSD%) were listed in Table 7.

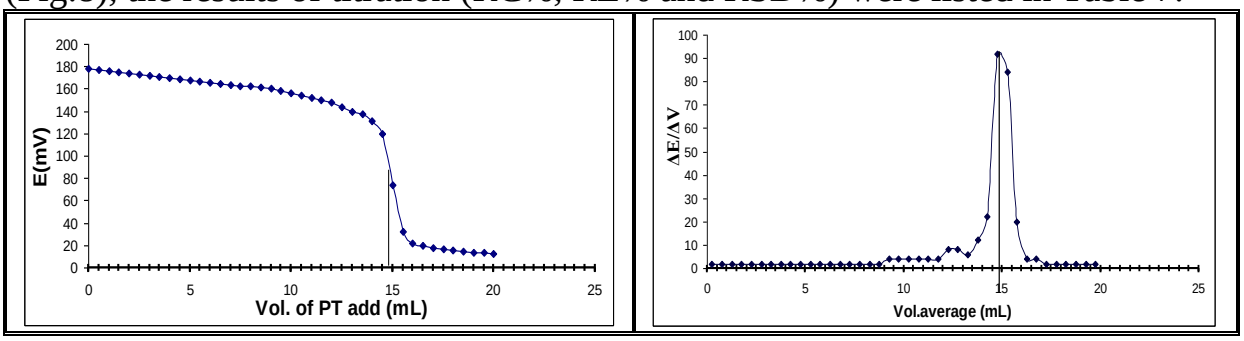


Fig.-9:Titration curve of electrode A (Amilo-PT+DBPH) for 15 mL sample solution 0.01 M Amiloride hydrochloride with 0.01 M of PT as a titrant solution.

Table -7: Determination of Amiloride in the standard sample ($1.00x10^{-4}$M) by potentiometric methods using Amiloride selective electrode A

Membrane.	Samples	potentiometric methods			
		Titration	Direct	SAM*	MSA*
A (Amilo-PT+DBPH	$1.00x10^{-4}$	0.986×10^{-3}	0.999×10^{-3}	0.994×10^{-3}	1.006×10^{-3}
	RSD%	0.500%	0.366%	0.372%	4.280
	RE%	-1.33 %	-0.10%	-0.60%	0.60%
	%RC	98.66 %	99.900%	99.40%	100.60%

Each concentration represents an average of 3 measurements* The results of five additions

Analytical Application of the Selected Electrode:-

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 8 for Maduratic and Saluretic tablets.

Table-8:Sample analyses of Maduratic and Saluretic tablets pharmaceutical Amiloride using electrode A (Amilo-PT +DBPH).

Parameter	Maduratic tablets				Saluretic tablets			
	Direct method	SAM	MSA	Titration method	Direct method	SAM	MSA	Titration method
Conc. (M)	1.000×10^{-3}	1.000×10^{-3}	1.000×10^{-3}	1.000×10^{-3}	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}	0.986×10^{-3}	0.988×10^{-3}	1.01×10^{-3}	0.983×10^{-3}
RSD%*	0.474%	0.408 %	-------	0.631%	0.576%	0.51%	-------	0.768%
RC%	99.5%	99.1%	100.8 %	99%	98.6%	98.8%	101%	98.3%
RE %	-0.5%	-0.9%	0.8%	-1%	-1.4%	-1.2%	1%	-1.7%

*Each concentration represents an average of at least three measurements.

Sample analyses by using UV-Spectrophotometry:-

The standard methods detected in British pharmacopeia's 2000[4] are spectrophotometric methods. UV spectrum of Amiloride hydrochloride as shown in Fig 9. and the equations and other parameters of the calibration curves are listed in table 9

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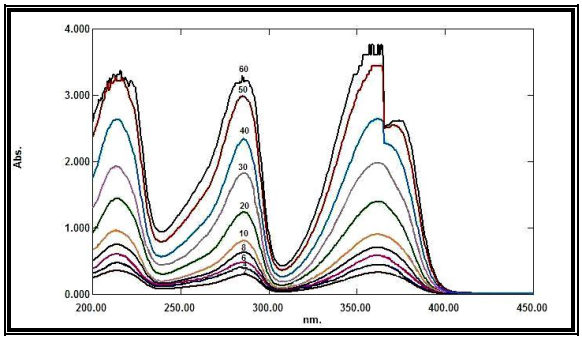

Fig.-10: UV- Spectra for Amilo. solutions at different concentration ranged from 2 to 60 mg/L.

Table -9 : The concentration range of the three wavelengths and their linear equations, correlation coefficient, standard deviation of slope and intercept respectively.

Wavelength (λmax)/nm	Range of conc. mg/L	Linear equation	r^2	r	S.D of the slope	S.D of the intercept
361	2-40	Y= 0.06208X+ 0.22634	0.99895	0.99947	2.511×10^{-4}	7.990×10^{-3}
286	2- 50	Y= 0.05588X+ 0.19580	0.99936	0.99968	3.407×10^{-4}	4.0926×10^{-3}
213	2-40	Y= 0.05877X+ 0.25594	0.99932	0.999664	3.214×10^{-4}	3.7004×10^{-3}

*The result of three times repeated.

The best wavelength 286 nm with highr concentration range and best (R^2 = 0.99936) as shown in the calibration curve in Table 10 used to determine Amiloride hydrochloride sample solutions (1×10^{-4}M that equal to 30.2 ppm) by direct method. The analysis results of three samples are average recovery, relative error and relative standard deviation are 99.87%, -0.13% and 0.497 % respectively.

Comparison between ISE and UV-Spectrophotometric Methods:-

The comparison between ISE and UV-Spectrophotometric methods by using direct method are listed in Table 10 shows that the ISE method was better than UV- Spectrophotometric Method. The F value found 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 10 :- The comparison between ISE and UV-Spectrophotometric Methods.

Parameters	ISE method	UV- spectrophotometric method
Linear range	$1\times10^{-5} - 1\times10^{-2}$	$6.62\times10^{-6} - 1.655\times10^{-4}$
Detection limit	6×10^{-7}	6.62×10^{-6}
RSD%*	0.366%	0.497 %
S^2	3.6469×10^{-7}	4.978×10^{-7}

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

Conclusions:-

Amiloride-selective PVC membrane electrode (Amilo-PT +DBPH) was used for potentiometric determination. which gives excellent electrode parameters as well as good results in determination of Amiloride and no interference with several cations and (HCT drug),. The analytical method proposed proved to be simple, rapid and of good accuracy compared to the UV-spectrophotometric method by normal calibration curve.

REFERENCES

1. György,S. & Budvári-Bárány,Z.,"Pharmaceutical Chemistry of Antihyper - tensive Agents", CRC Press, :92-98(1990).
2. 2-Dhasmana, D.," Effect of amilorid on ouabain induced arrhythmias in vivo in guinea-pigs. Indian Journal of Pharmacology, 32:102-107 (2000).
3. 3-Ellenhorn. M. & Barceloux, D., "Potassium-Sparing Diuretics", Medical Toxicology, New York, (1988).
4. 4-"British pharmacopoeia on CD-ROM", version 4, Copyright by Crown Ltd., London,(2000).
5. Jose,A.;Murillo,P.;Aurelia,A.&Pablo,F.,Direct Determination of Amiloride in Urine Using Isopotential Fluorimetry" Analyst,122:247–252(1997).
6. Murillo,P.;Alanon,M.&Fernandez,L." Direct analysis of amiloride and triamterene mixtures by fluorescence spectrometry using partial-least squares calibration", Analytica chimica acta, 449:179 -187(2010).
7. Huclová, J.; Satínský, D.; Pavlícek, O.; Vedralová, L. and Karlícek, R.," Using on-line solid phase extraction for determination of amiloride in human urine by sequential injection technique", Analytica Chimica Acta., 573, : 376-82(2006).
8. Toral, M.;Pope, S.;Quintanilla, S.&Richter P.,"Simultaneous determination of amiloride and furosemid in pharmaceutical formulation by first digital derivative spectrophotometry" International journal of pharmaceutics,249, (2) : 117-126(2002).
9. Ortega-Barrales,P.;Pellerano,G.;Vazquez,F.&Molina-Díaz,A.,"Rapid and sensitive determination of Amiloride by cation exchange pre-concentration and direct solid-phase UV detection." Analytical Letter, 35, :1491-1504(2002).
10. Schänzer,W.;Geyer, H.;Gotzmann, A. and Mareck-Engelke, U.," Recent advances in doping analysis"Sport und Buch Strauß,8:197-202 (2000).
11. Min,S.;Taijun,H.;Hua,Z.;Li,W.;Ping,G. & Pengcheng, M.," Simultaneous determination of amiloride and hydrochlorothiazide in human plasma by liquid chromatography/tandem mass spectrometry with positive/negative ion-switching electrospray ionization", John Wiley & Sons,21 Issue 21: 3427– 3434(2007).

12. Vincek,W.; Hessey,G.;Constanzer,M.& ayne ."Amiloride:Biological Fluid Analysis by Reverse-Phase HPLC" Pharmaceutical Research,2:143-145 (1985).
13. 13- Yip, M.; Coates, P. and Thiessen, J.," High-performance liquid chromatographic analysis of amiloride in plasma and urine," J Chromatogr., 307, (2):43-50(984)
14. El-Hefnawy,G.;El-Hallag,I.;Ghoneim,E. &Ghoneim, M.," Electrochemical behavior and determination of amiloride drug in bulk form and pharmaceutical formulation at mercury electrodes", Journal of Pharmac - tical and Biomedical Analysis Journal of Pharmaceutical and Biomedical Analysis, 34, Issue 5:899-907(2004)
15. Nassory, N.S., Al-Haderi A.M,A.&Israa, K.," Preparation and Examination of Amineand Amiloride-Ion Selective Electrodes with PVC Matrix Membranes " Chem. Anal. (Warsaw), 52: 55-66(2007).
16. Lukasz,K.,Robert,S.,Agnieszka,G.&Agnieszka,O." Simultaneous quantitat -ion of amiloride hydrochloride and hydrochlorothiazide in tablets by UV spectrophotometry coupled with new chemometric regression techniques and artificial neural networks."Annales Universitatis Mariaecurie - Sklodow skal Ublin – Polonia, 2: 139-144(2007).
17. Ferraro, M.; Castellano,P. & Kaufman, T.," Chemometric determination of amiloride hydrochloride, atenolol, hydrochlorothiazide and timolol maleate in synthetic mixtures and pharmaceutical formulations.", J Pharm Biomed Anal., 34, (2):305-14(2004).
18. Buhlmann, P.; Pretsch, E. and Bakker, E.," Carrier-Based Ion-Selective Electrodes and Bulk Optodes. 2. Ionophores for Potentiometric and Optical Sensors", Chem. Rev., 98: 1593-1687(1998).
19. Richard, P. and Erno, L.," Tracing the History of Selective Ion Sensors, " Analytical Chemistry, 97:88-100(2001).
20. Kharitonov S.V.,"Ion-Selective in Medicing Drug Determinationinter" Russian Chemical Reviews, 76, 361 (2007).
21. Kumar K.G., John S., Popval P. and Saraswathyamma B.,"Mebendazole Selective Membrane Sensor and its Application to Pharmaceutical Analysis" Analytical Sciences, 23: 291-294 (2007).
22. Nassory N.S., Maki S.A.and Ali M.A., "Preparation and characterization of an Atenolol Selective Electrode Based on PVC Matrix Membrane" Turk. J. Chem., 31: 75-82 (2007).
23. Al-Haideri A.M., Nassory N.S. and Al-Saadi A.S.M., Ibn Al-Haitham, "Study the Polymeric Membrane Selective Electrodes for the Determination of Ampicillin Trihydrate", J. of Pure and Applied Sciences, 19: 101-115 (2006).
24. Al-Saidi, Kh. H. ; Nassory, N. S. and Maki, S. A.," Preparation and study of amoxicillin selective electrodes and Their application with the derivative spectrophotometer in pharmaceutical drugs" JNUS, 12 (1): 29-37, (2009).

25. Nassory N.S;Maki,S.A & AL-Phalahy,B.A.,"Preparation and Potentio - metric Study of Promethazine Hydrochloride Selective Electrodes and Their Use in Determining Some Drugs"Turk J Chem.,32:539– 548, (2008)
26. A.Craggs,G.J. Moody & J.D.R. Thomas," PVC Matrix Membrane Ione-Selective Electrodes: Construction and Laboratory Experiments"Chem. Educ. , 51(8):541- 544, (1974)
27. Y. Umezawa, K. Umezawa and H. Sato, "Selectivity Coefficients for Ion-Selective Electrodes Recommended Methods for Reporting $K^{pot}_{A,B}$ values", Pure Appl. Chem., 87(3): 507-518 (1995).
28. Y. Umezawa, P. Buhlmann, K. Umezawa, K. Tohda and S. Amemiya, "Potentiomtric Selectivity Coefficients of Ion-Selective Electrodes", Part 1. Inorganic cations (Technical Report)" Pure Appl.Chem.,72(10):1851-2082 (2000).
29. Lee Yook Heng, Loh Han Chern and Musa Ahmad," A Hydrogen Ion-Selective Sensor Based on Non-Plasticised Methacrylic-acrylic Membranes" Sensors, 2 :339-346(2002).
30. Susan Sadeghi , Mohammad Taghi Vardini, Hossein Naeimi, Copper (II) Ion Selective Liquid Membrane Electrode Based on New Schiff Base Carrier, Wiley Interscience journal, 96 Issue 1-2: 65-74(2006).
31. Carlo Maccà and Joseph Wang,"Experimental procedures for the determination of amperometric selectivity coefficients" Analytical Chemical Acta, Volume 303, Issues 2-3, 10 March, : 265-274(1995).
32. Gamal, M.."Potentiometric PVC Membrane Sensor for the Determination of Scopolamine in Some Pharmaceutical Formulations", Analytical Sciences, 18, (12):1335-1338(2002).
33. Gamal, M.; Mohamed, H. and Abdulrahman, A." PVC Membrane Sensors for Potentiometric Determination of Acebutolo"l, Sensors, 7: 3272- 3286 (2007).
34. Hassan,A.;Xian,X.and Cheng, J.," Ion Selective PVC Membrane Electrode for the Determination of Methacycline Hydrochloride in Pharmaceutical Formulation", Sensors, 2: 424-431(2002).
35. Simultaneous Determination of Paracetamol and Cephalexin Binary mixtures by Using Derivative Spectrophotometry and H-point Standard Addition Methods