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Study and Construction of New Polymeric Electrodes in PVC Matrix Membrane for Amiloride Hydrochloride Drug Determination

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ABSTRACT

Based on Amiloride hydrochloride-methyl orange, two electrodes of Amiloride hydrochloride were constructed in PVC matrix as ion pair complex. They were plasticized using Nitrobenzene (NB), and Di-butyl phosphate (DBP). Amiloride hydrochloride (AMH) electrodes (e1and e2) presented slopes (54.210 and 52.810 mV/decade) and linear ranges (10^{-5}-10^{-2}, and $2*10^{-5}$-10^{-2} M), respectively. (e1), which is the best electrode, was based on DBP plasticizer. It gave a detection limit of $4x10^{-6}$M, slope of 54.210 mV/decade, correlation coefficient 0.9998, lifetime 36 days. The proposed electrode displayed reproducibility and good stability and was used for determining Amiloride hydrochloride in pharmaceutical samples. The interference measurements using (Na^+, Cu^{+2}, K^+, Fe^{+3}, and Mn^{+2}) were studied for selectivity coefficient determination using the separated method, as well as the mixed method

Keywords: Ploymeric electrodeas, reproducibility.

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INTRODUCTION

Amiloride hydrochloride ($C_6H_8ClN_7O.HCl.2H_2O$) is 3,5-diamino-6-chloro-*N*-(diaminomethylidene) pyrazine-2-carboxamide hydrochloride dihydrate. The structural formula is presented in figure (1).[1]. It is a solid yellow crystal powder, odorless, and soluble in alcohol, dimethylsulfoxid (DMSO), and water [2]. It is practically insoluble in acetone, ether, and chloroform [3]. It has a molecular weight of 302.12. It is often added to diuretic treatment to minimize and limit the loss of excessive amounts of potassium. A few reports on the determination of Amiloride hydrochloride in tablets were made [4]. A digital derivative spectrophotometry presents a fast, simple method for the simultaneous determination of Amiloride hydrochloride. [5] Amiloride hydrochloride Analysis was conducted by HPLC [6]. Uses of ion selective electrodes are still drawing attention in pharmaceutical analysis.[7] The reason is that these electrodes have several advantages such as simple operation and design, fast response, low cost, applicability to colored and turbid solutions, and reasonable selectivity. [8,9] The sensor in this paper, is based on Amiloride hydrochloride –methyl orange as ion pair in PVC plasticized relying on different plasticizers for determining Amiloride hydrochloride in pharmaceutical samples. The prepared electrodes properties, selectivity and pH effect, were studied.

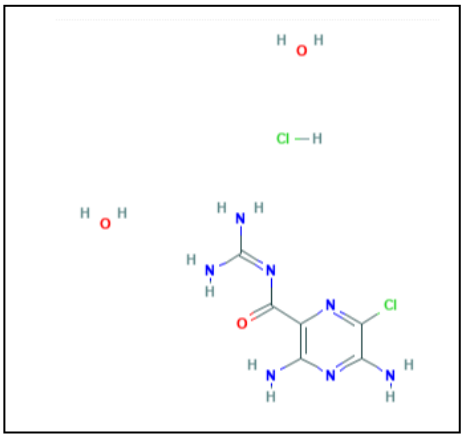

Fig 1: Structure formula of Amiloride hydrochloride

EXPERIMENTAL PART

2.1 Equipment

A digital pH/ion meter made in Germany, inoLab 740, with terminal 740 – WTW, was employed for measurements of pH and potentiometric. Hotplate Stirrer type LMS1003, Daihan Labtech, ultrasonic devise (ultrasonicator) were used for dissolving samples, (W. made in Germany). Sartorius Handy 4-digit analytical balance, pH combination electrodes (SenTix® 82 WTW) made in Germany, Saturated Calomel reference electrode and Silver-silver chloride wire were also used.

2.2 Solutions and Reagents

Standard Amiloride hydrochloride was offered by Samara-IRAQ-SDI (the State Company of Drug Industries, and Medical Appliances). Saluretic tablets (commercial drugs), made in Egypt, included 5 mg of Amiloride hydrochloride as well as 50 mg of Hydrochlorothiazide). A relatively high molecular weight Polyvinyl chloride (PVC), Molecular Weight 327.33 g·mol⁻¹, Methyl orange (M.O), were purchased from Fluka. DBP and NB were purchased from a Switzerland based company known as Fluka, E. Merck Tetrahydrofuran. In addition, BDH was the source for obtaining the rest of solvents and chemicals.

- M stock solutions of KCl, NaCl, $MnSO_4$, $Cu(NO_3)_2.3H_2O$ and $Fe_2(SO_4)_3.9H_2O$ were prepared by dissolving 0.3722, 0.2922, 0.7550, 1.2077, and 2.8100g in distilled water (50 mL), respectively.

- M Methyl orange (M.O) standard solution was obtained by distilled water to dissolve 0.3273 g of pure (M.O). A 0.01M Amiloride hydrochloride standard solution was obtained by dissolving 0.151g of standard Amiloride hydrochloride in distill water using (ultrasonicator) equipment in order to facilitate the dissolving of the drug and the obtaining of a 50ml solution. The rest of Amiloride hydrochloride standard solutions were obtained by subsequent dilution of the stock solution, ranged (10^{-7}-10^{-2} M).

2.3 Procedure

2.3.1 Ion pair Preparation:

The ion pair was made by mixing equal volume of 0.01 M Methyl Orange (M.O) solution dissolved in distilled water with an Amiloride hydrochloride (AMH) equimolar solution dissolved in distilled water. This process included the use of (ultrasonicator) equipment. 24 hr later, a precipitate formed.

2.3.2 Preparation of membrane and Construction of ISE

0.0400 g of ion pair was mixed with PVC powder (0.1700 g) and a plasticizer (0.3600 g). These ingredients were dissolved in THF (5 ml) and stirred until a viscous clear solution was gained. A cut membrane made from polyethylene in an electrode formation was attached to the last part of glass tube. Ag/ AgCl wire electrode was employed as an interior reference electrode. This electrode was attached to double junction Ag/AgCl electrode as an external reference electrode. [10]

2.3.3 Potential Measurements

The electrochemical cell is represented as: Ag/AgCl|internal filling solution||membrane||test solution|SCE. Using standard analyte solutions ranged from 10^{-7} to 10^{-2}M, a calibration curve was constructed for each electrode. Using a computer program (Microsoft office Excel 2010), these curves were constructed by plotting the concentration (M) log scale versus the potential E.

2.3.4 Pharmaceutical Samples Preparation

Ten tablets were crushed and weighted accurately. It was observed that the weight of Saluretic in average was equal to 0.2364g. Each tablet contained 0.005g to prepare 10^{-3} M Amiloride hydrochloride (0.7139 g) by dissolving it in distilled water and using ultrasonicator for 5min. Then, the precipitate was filtrated and washed, and the filtrate was collected in 50 mL volumetric flask.

2.3.5 Calculation of Selectivity Coefficient

A separate solution method [11] was used for the selectivity coefficient measurement. The calculations relied on equation (1): $\log K^{pot}_{A,B} = (E_B - E_A)/S + (1 - z_A/z_B) \log a_A$

For the primary A ion at $a_A = a_B$, a_A is the activities, z_A, z_B are charge numbers and E_A, E_B are the potentials. The mixed method (Fixed interference method) [12,13] was used to measure selectivity coefficients according to equation (2): $K^{pot}_{A,B} = a_A/(a_B)^{zA/zB}$ (2)

RESULTS AND DISCUSSION

Two plasticizers and two electrodes, (e1 and e2) of Amiloride hydrochloride (AMH) based on using AMH, M.O. DBP and NB were examined using PVC matrix. (e1) sensor gave a linear response ranged between (10^{-5}, 10^{-2}) M (AMH) and 54.210mV/decade Nernstian cationic slope. The detection limit was 4×10^{-6}M at the extrapolated segments intersection point of the of the two linear parts of AMH calibration curve. The slope value displayed by (e1) Electrode is due to a strong mixing between DBP and PVC because of the plasticizer compatibility with the electroactive compound as well as the structure. Figure (2) shows a typical plot for electrodes calibration curves based on two plasticizers DBP, and NB.

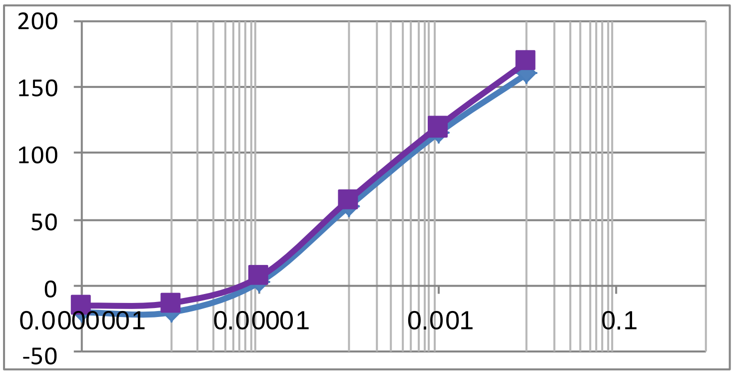

Fig. 2. Calibration curves of Amiloride hydrochloride selective electrodes using DBP and NB plasticizer

For the electrodes, based on DBP, and NB (e1, e2 membranes), the slopes were obtained. The values were (54.210 and 52.810mV/decade). The correlation coefficients were, respectively, 0.9998 and 0.9988. The linear range was

10^{-5}–$10^{-2}$and 2×10^{-5}–10^{-2}M. The limits of detection were 4×10^{-6} and 2×10^{-6}M, respectively. The parameters and results are shown in Table (1).

Table 1: Parameters of two electrodes of Amiloride hydrochloride.

Electrode	Slope (mV/Decade)	Correlation coefficient (r)	Linear concentration range (M)	Detection limit (M)	Response time (sec) 10^{-2} (M)	10^{-3}(M)	10^{-4}(M)	Lifetime (day)
e1 AMH+DBP + M.O	54.210	0.9998	10^{-5}-10^{-2}	4×10^{-6}	9	10	21	36
e2 AMH+NB+ M.O	52.810	0.9988	2×10^{-5}-10^{-2}	2×10^{-6}	19	27	32	27

3.1 pH Effect

The pH effect on the potentials of AMH, (e1) electrode, was analyzed by examining the cell potential in AMH solutions at concentrations of (0.01,0.001, 0.0001) M. The pH range was (0.6 -11.0). The level of pH was adjusted using small amounts of sodium hydroxide solution and/or hydrochloric acid. The results are illustrated in figure (3).

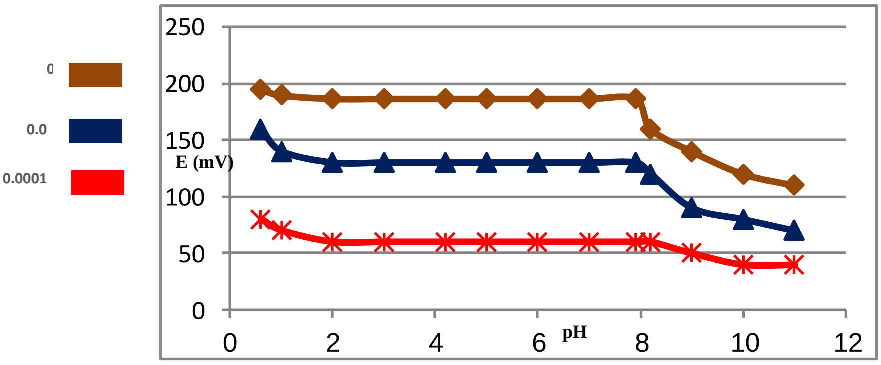

Fig 3: pH effect on the potential of (e1) electrode at concentrations 0.01, 0.001 and 0.0001M.

The electrodes' responses have increased in an irregular way in very high acidity, or at pH values lower than 1.0. The reason could be due to the electrode's responses to analyte ions and H+ activities. Table (2) illustrates the working pH.

Table 2: Working pH ranges for (e1) in (0.01, 0.001, 0.0001) M of AMH

Electrode	Composition of electrode (e1)	pH range		
		0.01 (M)	0.001 (M)	0.0001 (M)
e1	AMH+M.O+DBP	1.9-7.9	1.8-7.8	2.1-7.9

3.2 Interference Studies

To examine the selectivity of AMH in regard to different interfering ions towards (e1) ion selective electrode, this research employed the separate solution method, based on equation (1), and the mixed solution method, based on equation (2). Table (3) illustrates the selectivity coefficient values for the separate and mixed methods. The calibration curve of the fixed interfering method AMH (e1) selective electrode for (Na^+) is shown in figure (4).

Table 3: $K^{pot}_{A,B}$ values using the separate method, as well as FIM using (e1)

	Separate method		Mixed method
Interfering ions	$\log K^{pot}_{A,B}$	$K^{pot}_{A,B}$	$K^{pot}_{A,B}$
Na^+	-3.322	4.764×10^{-4}	1.362×10^{-3}
Fe^{+3}	-3.310	4.897×10^{-4}	4.997×10^{-5}
Cu^{+2}	-4.690	2.041×10^{-5}	2.381×10^{-4}
Mn^{+2}	-4.649	2.243×10^{-5}	1.995×10^{-4}
K^+	-2.669	2.142×10^{-3}	7.701×10^{-4}

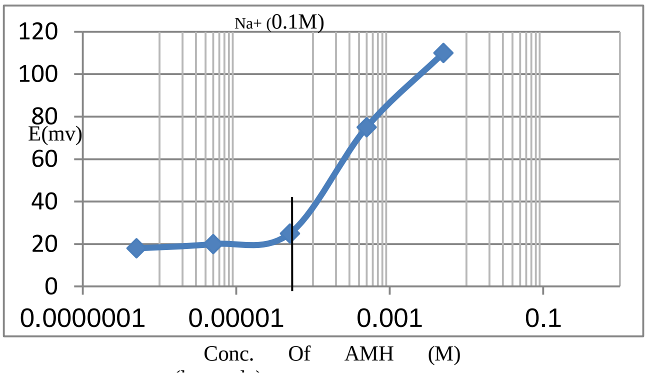

Fig 4: FIM calibration curve for (e1) electrode (AMH-M.O+DBP). Na$^+$ (5×10^{-2} M) as interfering ion a_A=7×10^{-5}M.

3.3 Sample analyses

For AMH determination, four potentiometric techniques were used. These techniques included direct method and (SAM); standard addition method, using the equation: $C_U = C_S/10^{\Delta E/S}[1+(V_U/V_S)]-(V_U/V_S)$

Where C_U, C_S are the concentrations, V_U, V_S are the volumes of unknown and standard solution, respectively. The (MSA); multiple standard additions, followed the equation: $C_U = V_S \times C_S/V_U$

Where V_S is the volume of standard solution and C_S, C_U are the concentrations of standard, and unknown, respectively. The results of the application of the method is shown in figure (5).

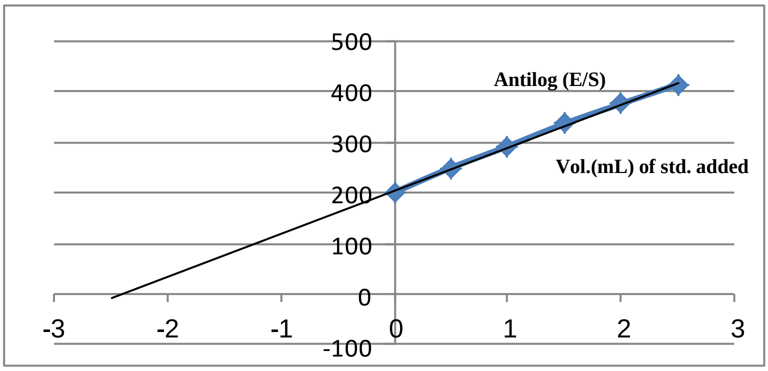

Fig 5: (E/S) antilog calibration curve versus the added volume of standard (10^{-2} M) for determining 25mL (AMH) solution 10^{-3} M using (MSA)

The plotting of the volume of the five additions of standard AMH versus antilog (E/S), using the concentrations mentioned above was compared with the curve of working range calibration for (MSA) and used for determining the concentration of AMH. The (E_r %); relative error, (Re %); recovery, and (RSD %); relative standard deviation, for each method were calculated. Table (4) illustrates the results.

Table 4: Analysis of AMH using potentiometric techniques for ISE (e1)

Methods	Conc.(M)	Found(M)	RSD%	Re%	Er%
Direct method	1.000×10^{-4}	0.998×10^{-4}	0.359%	99.8%	-0.2%
Multi SAM	1.000×10^{-3}	1.011×10^{-3}	---------	101.1%	1.1%
SAM	1.000×10^{-3}	0.996×10^{-3}	0.401%	99.6%	-0.4%

Potentiometric titration 15 mL of 0.01M Amiloride hydrochloride sample solution was mixed 0.01M molybdic acid as titrant solution. Figure (6) illustrates a typical titration plot.

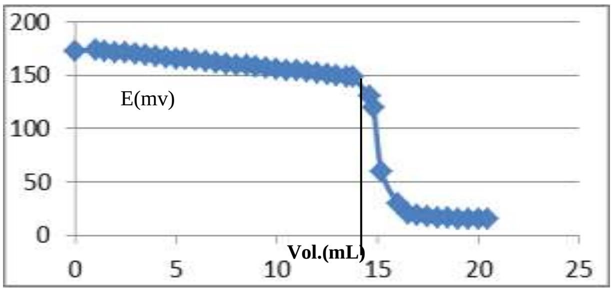

Fig 6: Electrode e1 titration curve of 15 mL sample solution 0.01 M Amiloride hydrochloride with a titrant solution of 0.01 M molybdic acid.

Relative error (E_r %), recovery (Re %), and relative standard deviation (RSD %) for the titration method were calculated. The results are shown in table (5).

Table 5: Analysis of AMH, ISE (e1), using the titration method.

Method	Conc.(M)	Found(M)	RSD%	Re%	Er%
Titration method	1.000×10^{-2}	0.997×10^{-2}	0.5%	99.7%	-0.3%

It was proved that (e1) electrode can be beneficial for the potentiometric determination of AMH in pharmaceutical preparations. Table (6) lists the pharmaceutical samples data.

Table 6: Analyses of AMH in pharmaceutical samples.

Parameter	Direct method	SAM	Multi SAM	Titration method
Concentration (M)	1.000×10^{-3}	1.000×10^{-3}	1.000×10^{-3}	1.000×10^{-3}
Found (M)	0.977×10^{-3}	0.967×10^{-3}	1.002×10^{-3}	0.991×10^{-3}
RSD* %	0.498%	0.484%	---------	0.688%
Re %	97.7%	96.7%	100.2%	99.1%
Er %	-2.3%	-3.3%	0.2%	-0.9%

CONCLUSION

Sensitive polymer electrodes (e1, e2) were prepared for the determination of Amiloride hydrochloride in pharmaceuticals based on the ionic complex (Amiloride hydrochloride-methyl orange) as an active substance with the PVC to form the membrane. These electrodes gave a linear concentration range (10^{-2}-10^{-5}) and (10^{-2}-2 *10^{-5}) and slopes (54.210 and 52.810mV/decade). The best electrode was (e1) with dibutyl phosphate as a plasticizer. It gave a correlation coefficient of 0.9998, a slope of 54.210, a detection limit of 4×10^{-6} and a lifetime of 36 days with good repeatability and stability. Selectivity coefficient of (e1) ion selective electrode in connection to Amiloride hydrochloride was also studied using two methods, the separate solution and the mixed solution methods with the following ions Fe^{+3}, Cu^{+2}, Mn^{+2}, K^{+}, Na^{+}.

CONFLICT OF INTEREST

None

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