Construction of ion selective membrane electrodes for the potentiometric determination of enalapril maleate in its pure form and pharmaceutical preparations.

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(NJC)

(Recevied on 15/4 /2012) **(Accepted for publication 1/7/2012)**

Abstract

Two novel enalapril maleate (ENM) ion selective electrodes were constructed and used for the determination of ENM in its pure form and pharmaceutical formulations. The electrodes were based on the use of the ion association complexes of either phosphotungstic acid (PTA) or phosphomolybdic acid (PMA) as anions with Enalapril cation in a PVC matrix plasticizers with di-butyl phthalate (DBP). The results of these electrodes showed stability (life time 33 and 51 days), near Nernstian response (slope 57.2 and 52.2 mV/decade), low detection limit (1.3×10^{-7} and 4.2×10^{-7} M) for DBP-ENM-PTA and DBP-ENM-PMA electrodes respectively. For both electrodes the best concentration for internal filling solution was 1×10^{-3} M ENM and the linear range was 1×10^{-5}- 1×10^{-1} M. The electrodes were found to be usable within the pH range 2.2 – 3.8 and 2.5 – 3.0 for the above mentioned electrodes. This study also included the measurements of selectivity of these electrodes in the presence of common cations, anions and some drug excipients. The $K_{i,J}^{Pot}$ found to be less than 1. The electrodes were successfully applied for determination of ENM in pure form and in tablet pharmaceutical preparation with recovery of not less than 98 %.

Keywords: Enalapril, potentiometric determination, ion selective membrane electrodes.

الخلاصة

تم في هذا البحث تصنيع قطبين انتقائيين لتقدير الأينالابريل في شكله النقي وفي مستحضراته الصيدلانية بالاعتماد على معقد الترابط الأيوني المتكون بين أنيونات أما حامض الفوسفوتنكستك (PTA) أو حامض الفوسفومولبيدك (PMA) وكاتيونات الأينالابريل باستخدام ثنائي بويتيل الفثالات (DBP) كملدن ومتعدد كلوريد الفينايل(PVC) ركيزة لها. بينت النتائج ثباتية الأقطاب (العمر 33 و 51 يوم) واستجابة قريبة من القيمة النيرنستية (الميل 57.2 و 52.2 mV/decade) وحد كشف واطئ (1.3×10^{-7} و 4.2×10^{-7} مولاري) لكل من قطب DBP–ENM–PTA و DBP–ENM–PMA على التوالي . كذلك بينت النتائج ان التركيز الأفضل لمحلول الملئ الداخلي هو 1×10^{-3} مولاري لمحلول ENM ومدى الخطية هو (1×10^{-5} – 1×10^{-1}) مولاري

ومدى الدالة الحامضية المناسب هو 2.2–3.8 و 2.5–3.0 ولكلا القطبين على التوالي. وتضمنت الدراسة أيضاً قياس انتقائية هذه الأقطاب بوجود كاتيونات وأنيونات شائعة وبعض مسوغات الدواء ووجد ان قيمة $K_{i,j}^{Pot}$ لجميع الأصناف المدروسة هي أقل من 1. تم تطبيق القطبين المصنعين لتقدير الـ ENM بشكله النقي وكذلك في مستحضراته الصيدلانية وباسترجاعية لا تقل عن 98%.

الكلمات الدالة: اينالابريل, التقدير الجهدي, أقطاب الأغشية الانتقائية الأيونية.

Introduction

Enalapril (ENM) (**fig.1**), (N-[(1S)-1-(Ethoxy carbonyl)-3-phenyl propyl]-1-proline hydrogen maleate) [1]

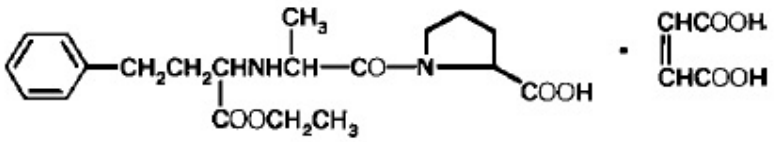

Figure (1): Structure of Enalapril Maleate

ENM is an angiotensin – converting enzyme inhibitor used in the treatment of hypertension and heart failure [2]. High-performance liquid chromatography (HPLC) and potentiometric titration are the official methods of ENM analysis [3,4]. Owing to the importance of ENM many methods have been developed for the determination of this drug they are HPLC [5-12], UV spectrophotometry [7-9] and [13-15] and membrane selective potentiometry [16,17]. Other methods such as flow injection [18,19], Gas Chromatography (GC) [20], capillary electrophoresis [21-23], cardio-enzymatic assay [24] and fluorimetric procedure were also developed [25]. The present paper describes a new potentiometric ion selective electrode method for determination of enalapril based on the use of the ion association complexes of either PTA or PMA as anion with Enalapril cation in a PVC matrix plasticizers with DBP.

Experimental

Instrumentations

The following instruments were used:

1- JENWAY pH /mV meter 3310.
2- Reference Calomel Electrode (Fisher Scientific Company cat. No. 13-639-52).
3- (Silver- Silver chloride Electrode) as working electrode (Orion 90-02) .
4- Magnetic Stirrer with Hot Plate BIOSAN MSH 300.
5- Drying Oven / Soyokaze Isuzu Seisakusho Com. Ltd .
6- Ultrasonic with water bath UNISONICS model Fxp12.
7- Sartorius balance Model BL 210S.

Reagents

All reagents were of analytical-reagent grade supplied by Fluka, BDH and MUMBI companies and deionised water was used throughout.

Solutions

0.1M Phosphomolybdic acid (PMA): prepared by dissolving 22.5760 gm of (PMA) in 100 ml of deionised water and solutions of concentrations from 1×10^{-7} – 1×10^{-2} M were prepared by appropriate dilution.

0.1M Phosphotungstic acid (PTA): prepared by dissolving 28.81 gm of (PTA) in 100 ml of deionised water

and solutions of concentrations from 1×10^{-7} – 1×10^{-2} M were prepared by appropriate dilution.

0.1M Enalapril (ENM): prepared by dissolving 4.9250 gm of ENM in 100 ml of deionised water). Solutions of KCl, NaOH, HCl, NaCl, KBr, $CuSO_4.5H_2O$, Na_2HPO_4, magnesium stearate, sodium hydrogen carbonate, ferric oxide, Lactose, Glucose, Mannitol and Fructose with concentrations ranged from 1×10^{-2} - 1×10^{-1} M were also prepared.

Preparation of ionic pair for ENM-PTA or PMA electrode membrane

90 ml of 0.1M ENM were dropped wisely, mixed with 90 ml of 0.1M PTA or PMA and the mixture was continuously stirred for 15 min. and then filtered (Whatman filter paper No.42) and washed several times by deionised water and left for drying at room temperature (25 ºC) for two days. A white and bluish green precipitate insoluble in water was obtained and ground to a fine powder for each electrode respectively.

Preparation of liquid membranes for ENM drug

The liquid membrane was prepared by mixing 0.1 gm of ENM-PTA or -PMA ion pair with 0.45 gm of PVC(dissolved in 4 ml acetone + 4 ml THF). After this step of dissolution, 0.45 gm of DBP (as plasticizer) was added and mixed until a homogenous mixture was formed. The resulting solution was gradually poured into a Petri dish [26] of 10 cm diameter and covered with a filter paper. This solution was then allowed to evaporate for two days at room temperature. The membrane was carefully lifted by a tong and kept in a refrigerator. The resulting membrane is of 0.3 mm thickness and is sufficient to provide about 8 membranes.

Construction of ion selective electrodes

One end of PVC tube of 3 – 4 cm length was softened by a circular motion on a glass on which few drops of THF were added. A circular part of the membrane of larger diameter than the PVC tube was cut and glued onto the soft end of PVC tube (**fig. 2**) using adhesive prepared from PVC + THF.

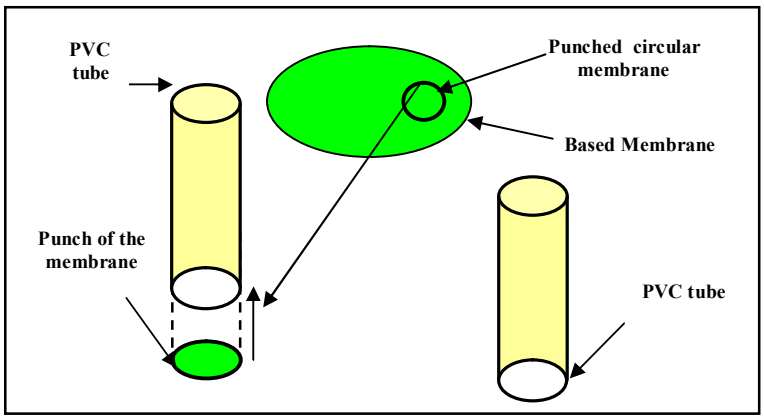

Figure (2): Construction of the membrane onto the PVC tube

The other end of PVC tube is attached to an empty and opened Ag/ AgCl electrode. This new electrode was filled by Enalapril as internal solution and connected with the saturated calomel as a reference electrode (**fig.3**). Before starting any measurements, this manufactured electrode was conditioned by immersing for 10 hours in 1×10^{-4} M ENM solution and kept in the same solution when not in use.

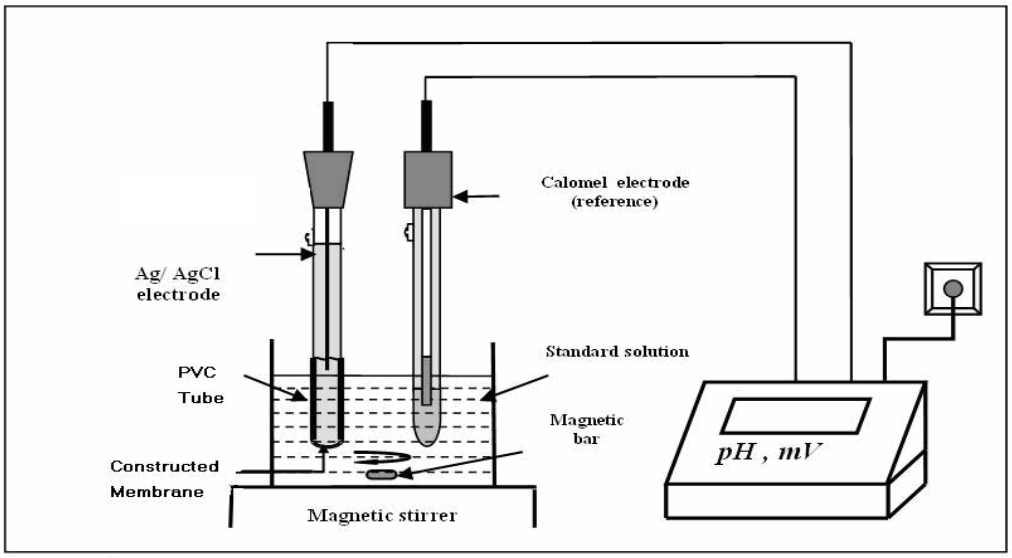

Figure (3): Parts of the constructed electrode and the electric cell

Results and Discussion

Selective membrane of PVC containing a complex was prepared by reaction of Enalapril with an active materials of either PTA or PMA, using DBP as a plasticizer. The scheme of the manufactured electric cell is as follow:

internal reference electrode (Ag/AgCl)	internal filling solution	manufactured ISE membrane	external filling solution (analyte)	external reference electrode (SCE)

Polymeric ISE membrane

The properties of these electrodes including concentration of internal filling solution, pH range, effect of temperature and life time were investigated.

Concentration of internal filling solution

The concentration of 1×10^{-3} M ENM as internal filling solution showed the nearest value to the theoretical Nernestian slope thus it is adopted to be the suitable concentration (**fig.4**).

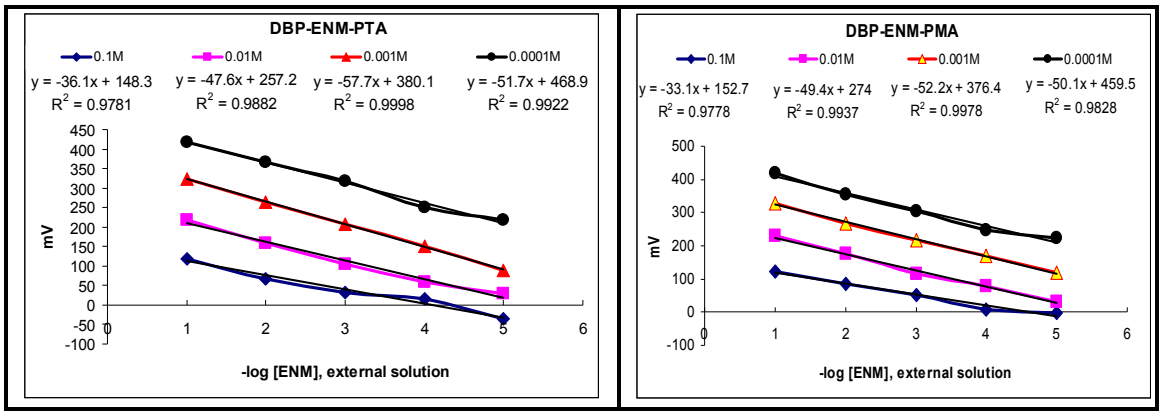

Figure (4): Effect of internal filling solution for DBP-ENM-PTA and DBP-ENM-PMA electrodes.

Standard curve

The results (**fig.5**) showed that the plot of potential (mV) versus –log [ENM] gives a linear regression relationship. The linear ranges, slopes and detection limit were 1×10^{-1}-1×10^{-5} M and 1×10^{-1}-1×10^{-5} M, 57.2 and 52.2 mV/decade, 1.3×10^{-7} and 4.2×10^{-7} M for DBP-ENM-PTA and DBP-ENM-PMA electrodes respectively.

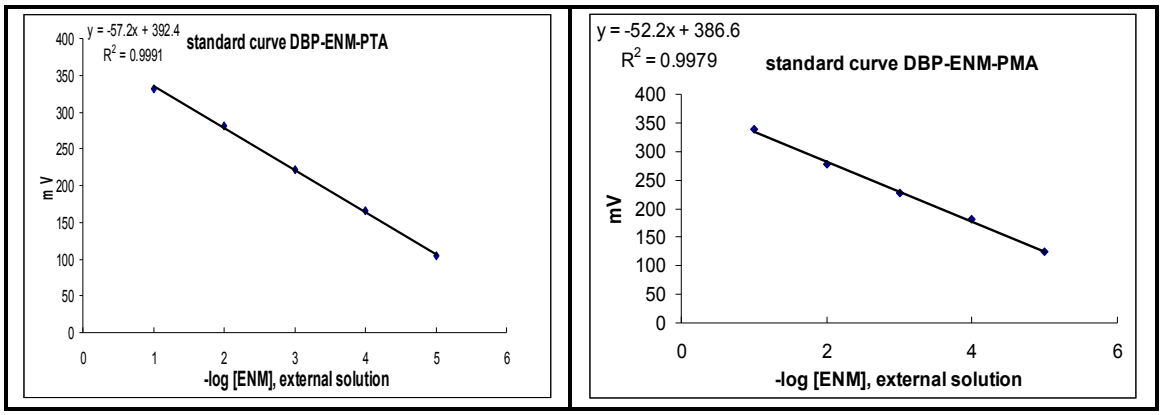

Figure (5): Standard curve of ENM using DBP-ENM-PTA and DBP-ENM-PMA electrodes.

Effect of pH

A standard 1×10^{-1} M and 1×10^{-3} M of aqueous ENM solution were prepared and adjusted to the desired pH values with dilute HCl or NaOH solutions. The suitable pH range for electrodes were 2.2 – 3.8 and 2.5 – 3.0 for DBP-ENM-PTA and DBP-ENM-PMA electrodes respectively (**fig.6**). The increase of potential at pH of less than 2 can be due to the penetration of H^+ into the membrane surface [27]. At higher pH values (pH>4), free base precipitates and cause an increase in the concentration of unprotonated species resulting in a decrease of mV readings [28] and may also a result of penetration of OH^- ions into the membrane [29]. It is

worthy of note that at pH's more than 3 and less than 1.5 a fluctuated mV readings were observed accompanied with hardening of the membranes.

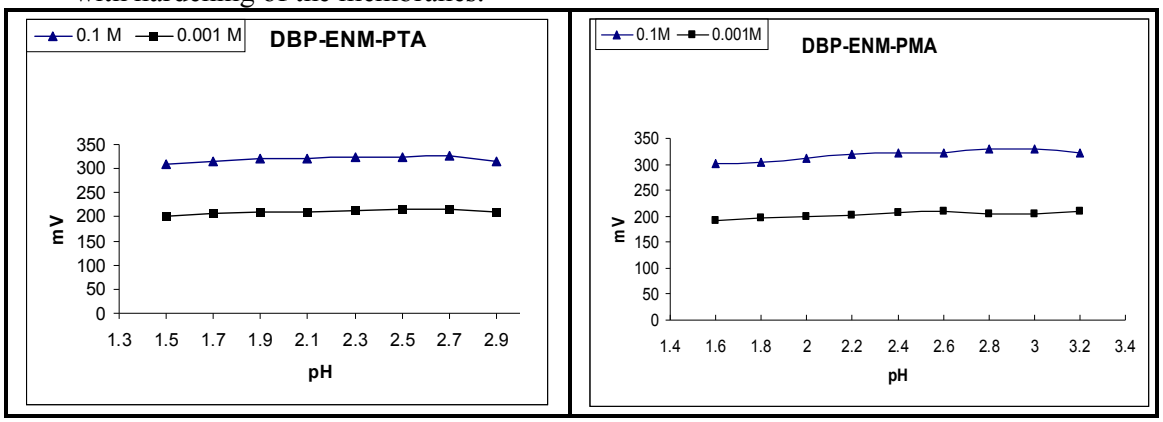

Figure (6): Effect of pH on the response of DBP-ENM-PTA and DBP-ENM-PMA electrodes using two series of ENM solutions 1×10^{-3} and 1×10^{-1}M.

Effect of temperature

The results showed that the appropriate working temperature is 25 °C for both electrodes (**fig.7**). The increase in potential with the increase of temperature is may due to the increase in surface area of the membrane resulting in easier permeation and equilibration.

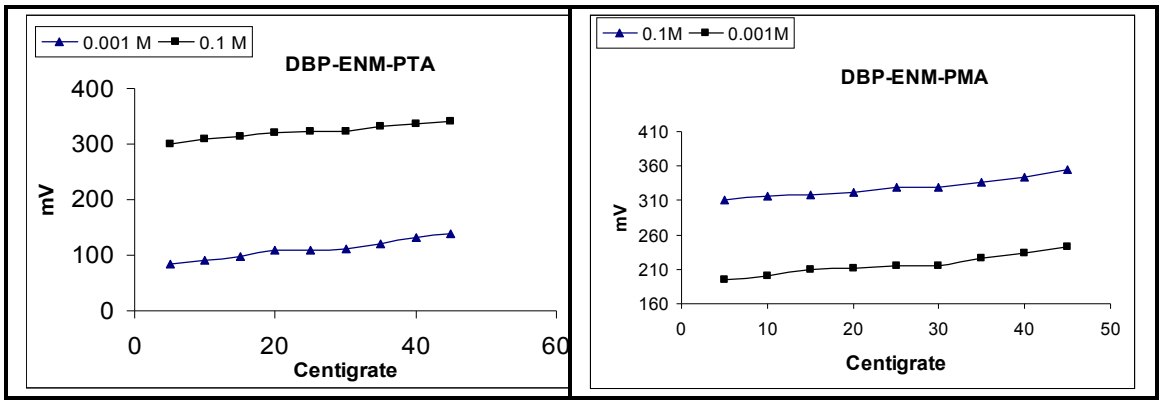

Figure (7): Effect of temperature on the response of DBP-ENM-PTA and DBP-ENM-PMA electrodes.

Response time

This time is defined as the time required for the electrode to reach a steady value within ± 1 mV of the final equilibrium value. The present study showed that the response time vary with the concentration of ENM and type of formulation (table **1**).

Table (1): Response time of DBP-ENM-PTA and DBP-ENM-PMA electrodes.

ENM Electrodes / Tablet	Conc. (M)	Response time (Sec.)
DBP-ENM-PTA Enapril 20mg	1×10^{-2} 1×10^{-3}	22 40
DBP-ENM-PTA Enalapril Maleate 20mg	1×10^{-2} 1×10^{-3}	50 55
DBP-ENM-PTA EnaHEXAL 20mg	1×10^{-2} 1×10^{-3}	52 66
DBP-ENM-PMA Enapril 20mg	1×10^{-2} 1×10^{-3}	35 45
DBP-ENM-PMA Enalapril Maleate 20mg	1×10^{-2} 1×10^{-3}	43 50
DBP-ENM-PMA EnaHEXAL 20mg	1×10^{-2} 1×10^{-3}	55 65

These short response times indicated the fast equilibrium of the permeation process of the solution species with membrane ingredients.

Life time

The electrodes displayed a constant potential readings ±1-5 mV from day to day and the calibration slopes almost did not alter over a period of 33 and 51 day for PTA and PMA electrodes respectively (**fig. 8**). This short life time is due to the loss of plasticizer and the active material from the polymeric layer of the membrane [30].

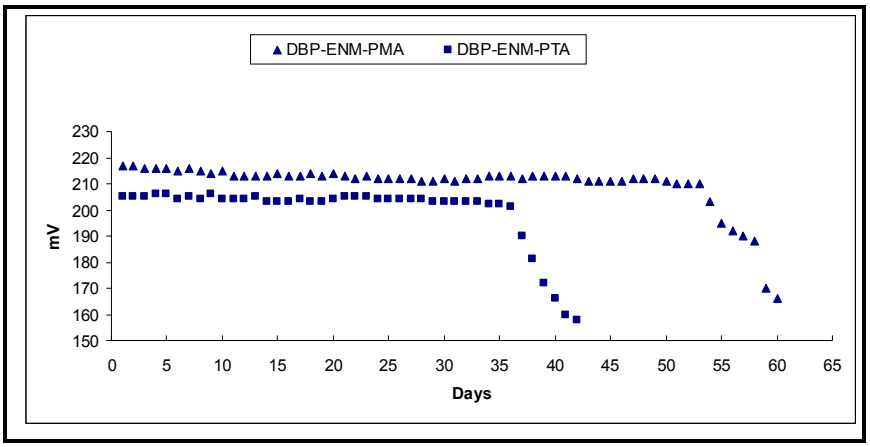

Figure (8): life time of DBP-ENM-PTA and DBP-ENM-PMA electrodes

Selectivity

The potentiometric selectivity coefficient $K^{Pot}_{i,J}$ of the two proposed electrodes were calculated in the presence of related substances using mixed solutions method [31] at 0.01M ENM and concentration range between 0.01- 0.1M for the investigated species. The selectivity coefficient $K^{Pot}_{i,J}$ was calculated from the following equation :

$$K^{Pot}_{ij} = \frac{a_i \cdot 10^{(Eij - Ei)/S} - a_i}{a_j^{Zi/Zj}}$$

Where $K^{Pot}_{i,J}$ is the potentiometric selectivity coefficient; $\mathbf{a_i}$ is the activity of **(i)** ion where the interfering ion is not present; $\mathbf{E_{ij}}$ is the potential of selective electrode for the **(i)** ions in a solution containing **(j)** ions; $\mathbf{E_j}$ is the potential of selective electrode for **(i)** ions when the interfering ions **(j)** are not present; $\mathbf{a_j}$ is the activity of interfering ions **(j)**. The following substances and species were investigated: *Glucose, Mannitol, Fructose,* magnesium stearate, sodium hydrogen carbonate, ferric oxide, Lactose, Na^+, K^+, Cl^-, Br^-, $SO_4^{=}$ *and* PO_4^{-3}.

The $K^{Pot}_{i,J}$ value represents the difference in potential in the presence of interfering ion (j) and when (j) is not present. When the value is less than 1 this indicates that the electrode shows low response to the interfering ions. The results of selectivity are shown on table (**2**).

The $K^{Pot}_{i,J}$ values shows a very high selectivity of the electrodes towards the Enalapril species (ENM^+).

Table (2): $K^{Pot}_{i,J}$ values

Ions	$K^{Pot}_{i,J}$ *values*			
	DBP-ENM-PTA		***DBP-ENM-PMA***	
	Conc. of Ions (M)		**Conc. of Ions (M)**	
	10^{-1}	10^{-2}	10^{-1}	10^{-2}
Na^{+1}	0.0523	0.0299	0.1495	0.0955
K^{+1}	0.1603	0.0704	0.1076	0.1033
Cl^{-1}	0.0098	0.0187	0.1944	0.0055
Br^{-1}	0.1103	0.0084	0.0068	0.0812
SO_4^{-2}	0.0819	0.1766	0.0709	0.0331
$PO4^{-3}$	0.0884	0.0521	0.1183	0.9103
Glucose	0.0392	0.0990	0.0422	0.0931
Mannitol	0.0903	0.1204	0.1665	0.0665
Fructose	0.0788	0.0755	0.1108	0.1008
magnesium stearate	0.1127	0.0331	0.1044	0.1371
sodium hydrogen	0.0720	0.0948	0.1306	0.0051
carbonate	0.0184	0.1995	0.0042	0.1711
ferric oxide	0.1072	0.0996	0.0503	0.0029
Lactose				

Application:
Determination of ENM in its pharmaceutical preparation.

Ten tablets of each of Enalapril formulations (Enapril-Asia, Syria 20 mg, Enalapril Maleate- Winthrop, UK 20 mg and EnaHEXAL-HEXAL, Germany 20mg) were weighted and finely crushed. An appropriate (1.9942, 1.7853 and 1.6348 gm) of this powder "equivalent to ten tablet" were separately dissolved in 5 ml of methanol and diluted to 25 ml with distilled water. These solutions were potentiometrically measured by the constructed ion selective electrodes using calibration method.

Table (3): Determination of ENM in its tablets pharmaceutical preparations using DBP-ENM- PTA and DBP-ENM-PMA electrodes.

ENM Electrodes / Tablet	**Conc. (M)**	**Measured Potential (mV)***	**Calculated Potential (mV)**	**RSD %**	**RE %**	**Recovery %**
DBP-ENM-PTA	1×10^{-2}	273.08	278.00	1.07	-1.77	98.23
Enapril 20mg	1×10^{-3}	218.50	220.80	0.77	-1.04	98.96
DBP-ENM-PTA	1×10^{-2}	280.75	278.00	0.94	0.99	100.99
Enalapril Maleate 20mg	1×10^{-3}	217.45	220.80	1.85	-1.52	98.48
DBP-ENM-PTA	1×10^{-2}	277.10	278.00	2.01	-0.32	99.68
EnaHEXAL 20mg	1×10^{-3}	218.95	220.80	0.09	-0.84	99.16
DBP-ENM-PMA	1×10^{-2}	278.56	282.20	1.07	-1.29	98.71
Enapril 20mg	1×10^{-3}	227.83	230.00	1.64	-0.94	99.06
DBP-ENM-PMA	1×10^{-2}	280.62	282.20	1.96	-0.56	99.44
Enalapril Maleate 20mg	1×10^{-3}	232.07	230.00	2.17	0.03	100.03
DBP-ENM-PMA	1×10^{-2}	283.15	282.20	1.06	0.34	100.34
EnaHEXAL 20mg	1×10^{-3}	227.50	230.00	0.98	-1.09	98.91

***Average of four determinations**

Table (**3**) shows that the constructed ion selective electrodes proved to be useful for the determination of ENM amount in tablet pharmaceutical preparations.

Validity of the proposed method:

As well as of good linear concentration range and low detection limit, the precision and the accuracy of the proposed methods were calculated for the analysis the ENM in its pharmaceutical preparations. The precision (RSD) is found to be not more than 2.17 and accuracy (Recovery %) is not less than 98 for both electrodes. Table (**4**) shows a comparison of some characteristics of the proposed electrodes with the literature .

Table (4): Comparison of some characteristics of the proposed electrodes with the literature

Parameter	**DBP-ENM-PTA (present study)**	**DBP-ENM-PMA (present study)**	**Ref. [16]**	**Ref. [17]**
Slope, mV/decade	57.2	52.2	55.8	55
Linear Conc. Range, M	1×10^{-5}- 1×10^{-1}	1×10^{-5}- 1×10^{-1}	5.2×10^{-5}- 1×10^{-2}	3.6×10^{-5}- 6.4×10^{-2}
Life time, day	33	51	-	-
Working pH	2.2 - 3.8	2.5 – 3.0	4.0 - 7.5	3.0 - 6.0
Response time, s	40-66 for 10^{-3} M	45-65 for 10^{-3} M	-	>1 min for 10^{-}6-10^{-5} M
Recovery %	not less than 98	not less than 98	> 98.8	99.96
Lower limit of detection, M	1.3×10^{-7}	4.2×10^{-7}	2.4×10^{-6}	1×10^{-5}

Conclusion

The proposed method introduced an ion selective electrodes for the determination of Enalapril based on PVC plasticized with DBP and using PTA or PMA as active materials. These electrodes showed a successful application with low limit of detection and good recovery. The electrodes also showed fast response, good selectivity and reasonable working concentration ranges.

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