Potentiometric Study of Phenytoin – PVC Membrane Electrodes for Determination of Phenytoin in pharmaceutical preparations

Yehya K. Al-Bayati and Raad R. Karabat
Department of Chemistry, College of Science, Baghdad University, Al-Jaderia, Baghdad-Iraq.

Abstract

Ion selective electrodes for phenytoin were prepared using sodium tetraphenyl borate (NaTPB) ionophore and various plasticizers: di-n-butylphthalate (DBPH), tri-n-butylphosphate(TBP), o-nitrophenyloctyl ether (ONPOE) and di-octylphenylphosphonate (DOPH). 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 experimental results showed that the best electrode was based on DOPH and DBPH as plasticizers, displaying a linear range from1.00×10^{-1} M to 1.00×10^{-5} M and 1.00×10^{-1} M to 2.00×10^{-5} with a Nernstian slope of 26.6 mV/decade and 26.2 mV/decade, correlation coefficient of 0.9993 and 0.9989, The detection limit was 6.00×10^{-6} M and 9.00×10^{-6}, the lifetime was around 30 and 28 days respectively. The proposed electrodes were successfully applied to the determination of phenytoin in a pharmaceutical preparation.

Keywords: Ion-Selective Electrode; PVC Membrane; Phenytoin Electrodes.

Introduction

In recent years, the potentiometric membrane sensors have been widely used in pharmaceutical analysis. [1-3]. This is mainly because of low cost, simple design, wide linear concentration range, low detection limit, adequate selectivity, high accuracy, and applicability of the selective electrodes to colored and turbid solutions [4].Ion selective electrodes (ISEs) are one of the most used poentiometric sensors in laboratory analysis as well as in industry, process control, physiological measurement, environmental monitoring and drug analysis [5].

Phenytoin has the molecular formula $C_{15}H_{12}N_2O_2$ and the chemical name 5,5-diphenylimidazolidine-2,4-dione with molecular weight of 252.268 g mol^{-1}. Phenytoin is an anticonvulsant drug, which is useful in the treatment of epilepsy. The primary site of action appears to be the motor cortex where spread of seizure activity is inhibited [6] .Phenytoin is also used to control arrhythmias(irregular heartbeat) and to treat migraine headaches and facial nerve pain. Phenytoin (diphenylhydantoin) was first synthesized by German physician Heinrich Biltz in 1908. In 1938, outside scientists including. Houston Merritt and Tracy Putnam discovered phenytoin's usefulness for controlling seizures, without the sedative effects associated with phenobarbital. There are some indications that phenytoin has other effects, including anxiety control and mood stabilization [7].

Other methods for the estimation of phentoin in pharmaceutical preparation and-or biological fluid [8], spectrophotometry using orthogonal function [9], thin layer chromatography [10], and high performance liquid chromatography (HPLC) [11] In this work, new phenytoin selective electrode sodium tetra phenyl borate (PHT-NaT) as an ionophore in PVC plastic membranes with different plasticizers. The study was carried out for determination, selectivity coefficients, pH range, and electrode parameters and used for determination of phenytoin in pharmaceutical drugstores were prepared based on.

Experimental

Equipment

1. An expandable ion analyzer (WTW model, Germany), a pH meter (WTW model pH 720, Germany), and a saturated calomel electrode (Gallenkamp, USA) were used in this work.
2. The electrode used for phenytoin was home constructed according to reference (12), as follows: The Ag-AgCl electrode and 0.1 M phenytoin solution were used as the reference electrode and the internal filling solution of the electrode, respectively. One

side of a piece of PVC tube (1-2 cm long) was flattened and smoothed by placing it on a glass plate moistened with THF. A disk of the membrane was cut equal to the external diameter of the PVC tubing and mounted on the polished end. The other side of the PVC tubing was then connected to the electrode body. The assembled electrodes were conditioned by soaking in 0.1 M phenytoin solution for at least 3 h before the use of the electrodes

Chemicals and Reagents

1- A pure phenytoin was a gift from the State Company of Drug Industries and Medical Appliances (Samera IRAQ-SDI).

2-Phenergan tablets (100 mg Phenytoin Sodium) (Park-Davis Company, Germany, and Pfizer Company,turkey) were purchased locally .

2. Plasticizers, di-n-butyl phthalate (DBPH), tri-butyl phosphate (TBP), o-nitro phenyl octyl ether (NPOE) and di-octyl phthalate (DOP) were obtained from Fluka AG. Other chemicals and reagents of analytical grade quality were obtained from Fluka, BDH and Aldrich.

3-- Stock solutions of 0.1 M for each of LiCl, NaCl, $CaCl_2$, $MgCl_2$, $FeCl_3$ and $AlCl_3$ were prepared. More diluted solutions were prepared by subsequent dilution of the stock solutions.

4- A solution of 0.1 M phenytoin was prepared by dissolving 0.6306 g of standard and making the solution up to 25 mL with deionized water.

5-A 0.05 M potassium hydrogenphthalate buffer solution (pH 4.00) was prepared by dissolving 10.21 g of solid potassium hydrogen phthalate in 1 L of deionized water after adjusting the pH.

Selectivity coefficient determination

Separate solution method (SSM) and the Matched Potential Method (MPM) [13] were employed to determine the selectivity Coefficients K^{pot} of the potentiometric sensor towards different species. In the SSM, the potential of a cell comprising a working electrode and a reference electrode is measured in two separate solutions, one containing the drug ions, E_A, and the other containing the interferent ions (J), E_B, and S is the slope of the calibration graph.

These values were used to calculate the selectivity coefficient K^{pot} from the following equation:

$$\log K^{pot} = [(E_B - E_A)/(2.303RT/zF)] + (1 - Z_A/Z_B)\log a_A \quad \text{.....(1)}$$

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.

The selectivity coefficients were also measured by the match method according to the equation (2).

$$K^{pot} = \Delta a_A / a_B, \quad \Delta a_A = a_A' - a_A \quad \text{.....(2)}$$

Where (a_A') is Known activity of drug ion A and (a_A) is fixed activity of drug ion, (a_B) is the activity of the interfering ion .

Results and Discussion

Phenytoin-sodium tetra phenyl borate is a stable ion-pair complex which is water insoluble but readily soluble in an organic solvent such as tetrahydrofuran. The obtained complex was incorporated into a PVC membrane with the following plasticizers: di-n-butylphthalate (membrane no.I), tri-n-butyl phosphate (membrane no.II), o-nitro phenyl octyl ether (membrane no.III) and di-octyl phthalate (membrane IV). The working characteristics for investigating the electrodes were assessed on the basis of the calibration curves. The physical properties of these prepared menbranes are white, flexible, clear and transparent (non crystalline). Response characteristics of prepared phenytoin are summarized in Table (1).

Table (1)
Response characteristics of PHT – NaT selective electrodes using different plasticizers.

Membrane Composition	*PHT-NaT +DBPH*	*PHT-NaT +TBP*	*PHT-NaT+ONPOE*	*PHT-NaT +DOP*
Slope mV/decade	26.2	28.2	22.6	26.6
Linearity Range/M	$1\times10^{-4} - 1\times10^{-1}$	$1\times10^{-5} - 1\times10^{-1}$	$4\times10^{-4} - 1\times10^{-1}$	$2\times10^{-4} - 1\times10^{-1}$
Correlation coefficient	0.9989	0.9999	0.9978	0.9993
Detection Limit/M	3×10^{-5}	2×10^{-5}	3×10^{-4}	2×10^{-5}
Life time/day	28	3	26	30

Non Nernstian slopes were obtained for electrodes based on ONPOE (membranes III). The slope are 22.6 mV/decade with correlation coefficients 0.9978. The linear range for electrode was 4 x 10^{-4} – 1 x 10^{-1} M and the detection limit of 3 x 10^{-4}. This behavior of non-Nernstain slopes can be attributed to the low viscosity of ONPOE (11.44 cST) or incompatibility of the plasticizer with the complex in PVC. Near Nernstian slopes were obtained for the electrodes based on DBPH, TBP and DOP (membranes I,II and IV) in which the electrode based on DBPH which gave a slope of 26. 2 mV/decade with a correlation coefficient equal to 0.9989, a linear concentration range 1 x 10^{-1} – 1 x 10^{-4} M and a detection limit of 3 x 10^{-5} M. While the electrode based on TBP gave a slope 28.2 mV/decade with a correlation coefficient around 0.9999, a linear concentration range 1 x 10^{-5} – 1 x 10^{-1} M and the detection limit was 2 x 10^{-5} M. But the life time of the electrode (II) was around 3 days. This short time may be due to low viscosity of TBP (3.11 cSt) which cause a leaching of the complex to the external solution during the measurements or incompatibility of the plasticizer with the complex in PVC. While the electrode based on DOP which gave a slope of 26.6 mV/decade with a correlation coefficient equal to 0.9993, a linear concentration range 2 x 10^{-4} – 1 x 10^{-1} M and a detection limit of 2 x 10^{-5} M. A typical calibration plot for electrodes I and IV is shown in Fig.(1).

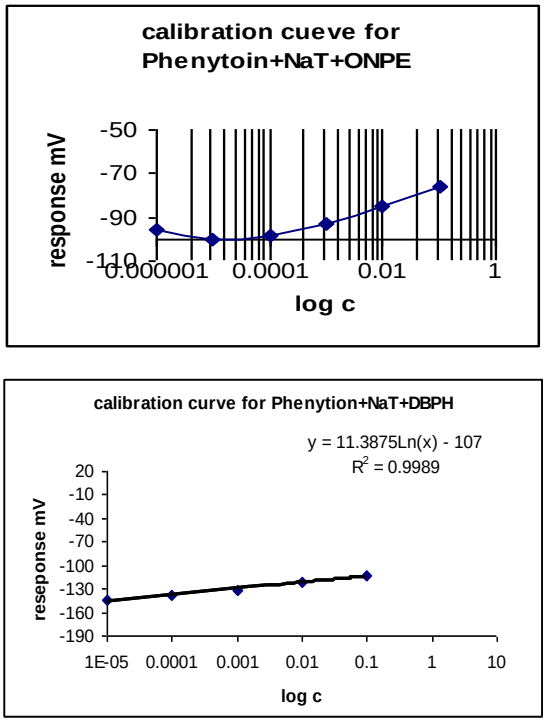

Fig.(1) Calibration curves of phenytoin selective electrodes. DBPH and ONPOE.

One of the excellent electrodes parameters were obtained for the electrode based on DBPH as a plasticizer which gave a good response and stability and was used for the quantitative determination of phenytoin in its pharmaceutical forms.

Effect of pH

The effect of pH on the electrode response of Phenytoin was examined by measuring the electro motive force (e.m.f) of the cell for phenytoin solution at three different concentrations 10^{-2}, 10^{-3} and 10^{-4} M. The pH

was measured from 1.0 to 11.5 and was adjusted by adding few drops of ammonia or hydrochloric acid solutions. The results of the working pH ranges of the Phenytoin selective electrodes are listed in Table (2).

Typical plot of pH effect on electrode response for electrode based on DBPH is shown in Fig.(2).

Table (2)
Working pH ranges for phenytoin selective electrodes.

Number	*Membrane Composition*	*pH range*		
		1×10^{-2}	*1×10^{-3}*	*1×10^{-4}*
I	PHT- NaT+ DBPH	3.5 – 8.5	3.6 – 8.5	3.4– 7.0
II	PHT - NaT + TBP	2.9– 8.1	2.0– 8.5	2.0 – 9.0
III	PHT - NaT+ ONPOE	2.0 – 8.6	1.5 – 6.0	2.6 – 8.0
IV	PHT - NaT+ DOP	2.5 – 8.1	3.0 – 8.6	2.5 – 9.0

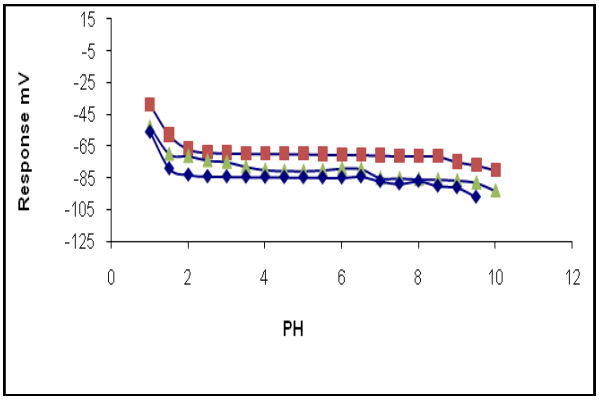

Fig.(2) Effect of pH on the potential of the Phenytoin electrode at concentrations (■ 10^{-2}, ▲ 10^{-3} and ♦ 10^{-4}) M.

Response time

Dynamic response time is an important factor for an ion-selective electrode. In this study, the practical response time was recorded by changing solution with different Phenytoin concentrations from 1.0×10^{-5} to 1.0×10^{-1} M.

The response time for the electrode is to reach a steady state potential ± 1 mV from the final equilibrium value. The response time can be measured directly after immersing the electrode in the Phenytoin solutions. The values at t_{95} for all the electrodes of Phenytoin concentrations ranging from 10^{-1} to 10^{-5} M as calculated from the plot of electrode response with time are listed in Table (3).

Table (3)
Response time of Phenytoin electrodes.

Conc. (M)	*Electrode I(sec)*	*Electrode II(sec)*	*Electrode III(sec)*	*Electrode IV(sec)*
10^{-1}	13	12	15	12
10^{-2}	15	16	17	14
10^{-3}	18	19	20	16
10^{-4}	21	22	25	20
10^{-5}	24	27	29	23

As shown, the longer response time reached around 29 s at 10^{-5} M. All the electrodes gave the same range of response times.

Selectivity

The potentiometric selectivity coefficient of an electrode, as one of the most important characteristics, is defined by its relative response for the primary ion over the other ions present in the solution [14]. The separate solution method (SSM) is recommended by IUPAC to determine the selectivity coefficient of the ISE. SSM is based on Nickolsky-Eisenman equation. However, it has been shown that this method suffers some limitations in terms of the values for ions of unequal charges, a non-Nernstain behavior of interfering ions [15]. The influence of some interfering inorganic cations, Li^+ , Na^+ , Mg^{2+} , Ca^{2+}, Al^{3+}, and Fe^{3+} on the electrode response was studied. The selectivity of the electrodes based on DBPH and ONPOE was measured by the separate solution method for a concentration range from 10^{-5} to 10^{-1} M. The potentiometric selectivity coefficients were calculated using equation (1) at cation concentrations ranging between (10^{-5} and 10^{-1}) M. A typical plot is shown in Fig.(3) for the interference of Al^{3+} on the DBPH electrode. The values of the selectivity coefficients for DBPH and ONPOE electrodes are listed in Table (4).

Table (4)
Selectivity Coefficients for (PHT-NaT+DBPH)I electrodes at different concentrations by separation method.

Interfering ions	*Concentrations of Phenytoin (M)*				
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
	$K_{A,B}$	$K_{A,B}$	$K_{A,B}$	$K_{A,B}$	$K_{A,B}$
Li^+	6×10^{-1}	27×10^{-2}	2.77	8.09	8.99
Na^+	3.9×10^{-2}	33×10^{-2}	52×10^{-2}	1.5	8.24
Ca^{2+}	6×10^{-2}	25×10^{-3}	13×10^{-3}	3.9×10^{-3}	4.7×10^{-4}
Mg^{2+}	4.2×10^{-2}	1.6×10^{-2}	6.1×10^{-3}	2.1×10^{-3}	2.5×10^{-3}
Al^{3+}	8.1×10^{-3}	1.7×10^{-3}	9.6×10^{-5}	4.4×10^{-5}	4.1×10^{-5}
Fe^{3+}	7.3×10^{-2}	4.4×10^{-2}	3.5×10^{-3}	7.4×10^{-3}	3.5×10^{-5}

Table (5)
Selectivity Coefficients for (PHT-NaT+ONPOE) III electrodes at different concentrations by separation method.

Interfering ions	*Concentrations of Phenytoin (M)*				
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
	$K_{A,B}$	$K_{A,B}$	$K_{A,B}$	$K_{A,B}$	$K_{A,B}$
Li^+	6.5×10^{-3}	5.3×10^{-2}	6.9×10^{-2}	5.6×10^{-1}	1.5×10^{-1}
Na^+	12.5×10^{-2}	3.1×10^{-2}	6.2×10^{-2}	3.7×10^{-1}	3.2×10^{-1}
Ca^{2+}	7.5×10^{-3}	3.6×10^{-3}	7.2×10^{-4}	1.9×10^{-4}	3.9×10^{-4}
Mg^{2+}	9.2×10^{-3}	4×10^{-3}	1×10^{-3}	3.5×10^{-4}	2.8×10^{-4}
Al^{3+}	7.8×10^{-3}	3.3×10^{-3}	4.5×10^{-4}	5.3×10^{-6}	1.7×10^{-6}
Fe^{3+}	13.7×10^{-2}	5.7×10^{-2}	6.3×10^{-3}	3.9×10^{-6}	1.5×10^{-6}

The results in Tables (4 and 5) showed that the selectivity coefficients for monovalent interfering ions is in the order mono> di> trivalent. This may be attributed to the difference in ionic size, mobility and permeability. A typical plot is shown in Fig.(3) for the interference of Al^{+3} on the DBPH electrode.

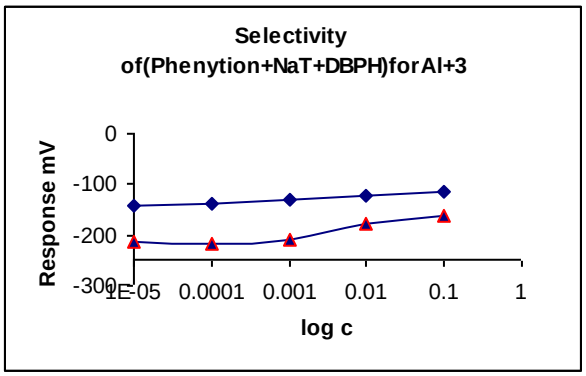

Fig.(3) Selectivity of (PHT– NaT + DBPH) and the interfering cation(Al^{+3}) by separation method, ♦ phenytoin ▲ Solution of interfering cation(Al^{+3}).

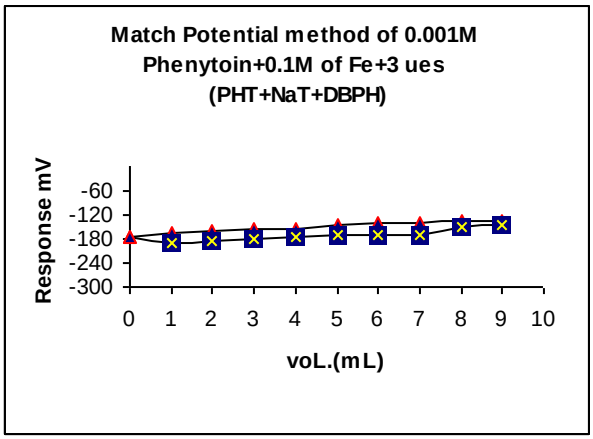

Fig.(4) Selectivity of electrode I for (10^{-3}) M based on DBPH, for Fe^{+3} interfering by mach method , ▲solutions of Fe^{+3} interfering solutions., ■ Phenytoin solutions.

Sample Analyses:

Potentiometric techniques were used for the determination of Phenytoin including: direct, standard addition, Gran plot and titration method. Synthetic solutions of Phenytoin at concentrations ranging from 10^{-3} to 10^{-5} M were used for a standard addition method using electrodes based on ONPOE and DBPH. The change in potential at (25±0.1oC) was recorded after each increment and these data were used to calculate the concentration of Phenytoin in the drug samples using the following equation.

$$C_X = \frac{C_S x V_S}{(V_X + V_S)10^{\Delta E/S} - V_X} \quad \text{.....(3)}$$

where Cx is Phenytoin concentration in the testing sample, Cs is the concentration of the standard, Vx and Vs are the corresponding volumes, S is the slope of the electrode response, and ΔE is the change in potential [16]. The results of % recover (RC), % relative standard deviation RSD and relative errors (RE) were calculated and listed in Table (6).

Table (6)
Analysis of Phenytoin samples by potentiometric techniques.

Elect. No.	*Sample*	*Measurements by potentiometric methods*			
		Direct	*SAM*	*MSA*	*Titration*
(I)	1×10^{-3}	1.013×10^{-3}	0.995×10^{-3}	1.016×10^{-3}	1.04×10^{-3}
	%RSD*	0.456	0.089	-	-
	%RC	101.3	99.5	101.6	100.4
	%RE	1.3	-0.5	1.6	4.0
(III)	1×10^{-3}	1.0204×10^{-3}	1.034×10^{-4}	1.03×10^{-3}	1.06×10^{-3}
	%RSD*	0.242	2.69	-	-
	%RC	102	103.4	103.0	106.0
	%RE	2	3.4	3.0	6.0
(IV)	1×10^{-3}	0.98×10^{-3}	1.003×10^{-3}	1.00×10^{-3}	1.01×10^{-3}
	%RSD*	2.4	0.096	-	-
	%RC	98.0	100.3	100.0	101.0
	%RE	-2.0	0.3	0.5	1.0

**** Each measurement was repeated for three times.***

The relative recovery was calculated for five additions of the standard Phenytoin concentration of 10^{-1} M. A typical plot of antilog (E/S) versus the volume of the addition of standard Phenytoin using electrode based DBPH and the concentration of synthetic promathezine 10^{-3} M is shown in Fig.(5). Gran plot paper with 10% volume correction was used in which the potentials can be directly plotted against the volume of standard Phenytoin.

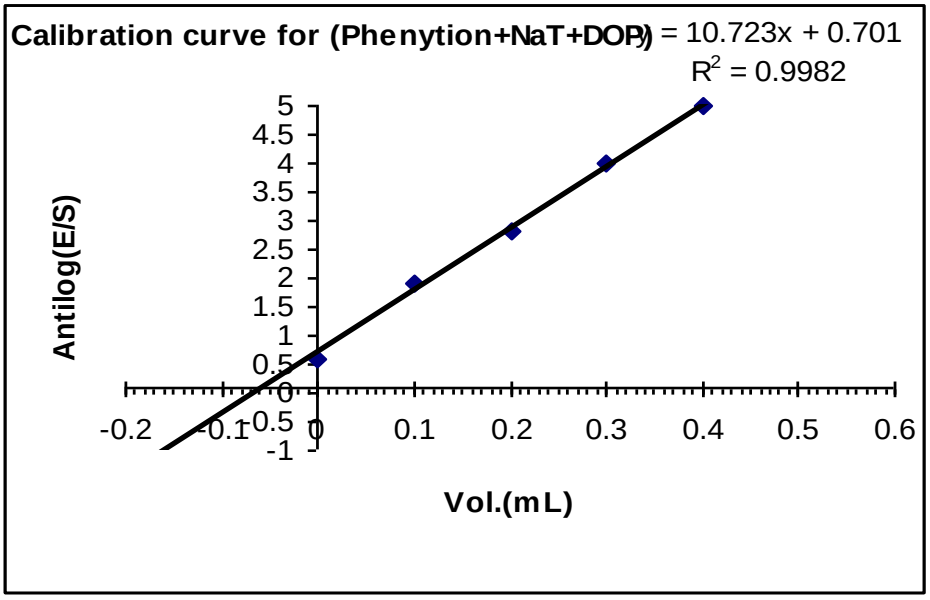

Fig.(5) Plot of antilog (E/S) versus the volume of standard Phenytoin (10^{-3} M) by MSA using electrode based on DOP.

The results in Table (6) indicate that the electrode based on DBPH as a plasticizer is the best electrode which can be used for a quantitative determination of Phenytoin in its pharmaceutical Forms. Fig.(6) shows a typical plot for the titration curve of 0.001 M phenytoin standard solution with 0.001 M sodium tetraphenyl borate as a titrant using the phenytoin electrode based on membrane containing DBPH plasticizer.

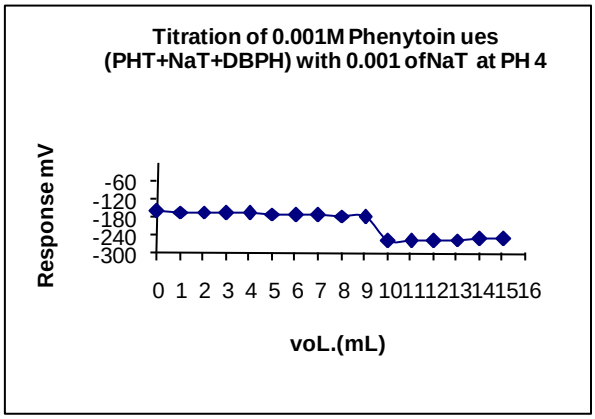

Fig.(6) Titration curve of electrode (PHT-NaT +DBPH) for drug solution containing 0.001 M Phenytoin with 0.001 M of NaT as titrant solution.

The direct potentiometric method was applied for the determination of Phenytoin in pharmaceutical tablets (Epanutin from samara, Germany and Turkey) as listed in Table (7) using the electrode based on membrane (I). The average recovery for phenytoin determination in tablets was around 99.03% with astandard deviation of about 0.1, based on an average of 3 measurements for each sample.

Table (7)
Sample analysis for tablets using the phenytoin selective electrode based on DBPH plasticizer using the direct potentiometric method.

Pharmaceuticl	***Epanutin (Germany)***	***Epanutin (Turkey)***
Concentration of phenytoin(prepared)/M	1×10^{-3}	1×10^{-3}
Concentration of phenytoin(found)/M	1.020×10^{-3}	1.027×10^{-3}
%recovery	102.0	102.7
%RE	2.0	2.7
%RSD	0.476	2.8

Reference

[1]. Abu Shawish MH, "Potentiometric Response of Modifi ed Carbon Paste Electrode Based on Mixed Ion-Exchangers", Electroanalysis, Vol. 20 Issue (5), 491-497 (2008).

[2]. Kormosh Z, Hunka I, Bazel Y, "Preparation and Characterization of a Diclofenac Sensitive Electrode Based on a PVC Matrix Membrane", Acta Chim. Slov, Vol 55(2), 261-267, (2008).

[3]. Dimeski G, Badrick T, John AS "Ion Selective Electrodes (ISEs) and interferences—A review", Clinica Chimica Acta, Vol 411 Issue (5-6), 309-317, (2010).

[4]. Bakhtiarzadeh F, AbGhani S, "Anion selective electrode formercury (II) basedon mercury (II) complex of poly (4-vinyl

pyridine)", J Electroanal Chem, Vol 624 Issue (1-2), 139-143, (2008).
[5]. Abdul-Muhsin A. AL-Haideri, Nabil S Nassory, Souhaila K. Sayhood, "Construction of Phenytoin Selective Electrodes and its Applaction to Pharmaceutical Preparation", Baghdad Science. Journal, Vol 7(1),1-7, (2010).
[6]. ROY S. M. N, YETAL S.M, VAIDYA V.V, JOSHI S.S, "Determination and Quntifiction of Phenytoin in Human Plasma by Liquid Chromatography with Electroscopy Ionization Tandem Mass Spectrometry", E-Journal Chemistry, Vol. 5 (1), 169-176, (2008).
[7]. Ashnagar A, Gharib Naseri N, Amini M, "Synthesis of 5,5 –dipheny l-2,4-imidazolidinedione (Phenytoin) from Almond", International Journal of Chem Tech Research, Vol 1(1),47-52, (2009).
[8]. Yehya K.AL-bayati, "Preparation and potentiometric study of phenytoin selective electrodes based on a PVC matrix membrane", Basrah Journal of Science (C), Vol 30 (1), 42-53, (2012).
[9]. Andrea Bereczkia, Antal Tolokan, George Horvai, Viola Horvath, Francesca Lanza, Andrew John Hall, Bo¨rje Sellergren, "Determination of phenytoin in plasma by molecularly imprinted solid-phase extraction", Journal of Chromatography A, Vol 930 Issue (1-2), 31–38, (2001).
[10]. Vendelin olesen .O, "Determination by-Thin-Layer chromatography of phenytoin in serum in the presence of Barbiturates and other Antiepileptic and Various Drugs", Acta Pharmacological Toxicologica, Vol 23 (1),41-48, (2012).
[11]. Alaa Khedr, Mohamed Moustafa, Ashraf B. Abdel-Naim, Abdulrahman Alahdal and Hisham Mosli, "High-Performance Liquid Chromatographic Method for Determination of Phenytoin in Rabbits Receiving Sildenafil", Analytical Chemistry Insights, Vol 1(3),61-67, (2008).
[12].Moody G.J, Oke R.B, and Thomas J.D.R, Analyst, "Acalcium-sensitive electrode based on ionexchanger in poly (Vinyl Chloride) Matrix", Vol 95 Issue (1136), 910-918, (1970).
[13]. Umezawa Y, Buhlmann P, Umezawa K, Tohda K, Amemiya S, "Selectivity coefficients for ion-selective electrodes: Recommended methods for reporting *Kpot* values", Pure Appl Chem, Vol 72(45), 1851- 2082, (2000) .
[14].Singh A K, Mehtab S, "Calcium (II)-selective potentiometric sensor based on α-furildioxime as neutral carrier", Sens Actuators, Vol 123 Issue (1), 429-436, (2007).
[15]. Chandra S, Agarwal H, Singh K. "highly selective and sensitive thorium (IV) PVC membrane electrode based on a dithio-tetraaza macrocyclic compound", Anal Sci, Vol 23 (4),469-473, (2007).
[16]. Buck R P, Lindner E, "IUPAC recommendation for nomenclature of ionselective electrodes", Pure Appl Chem, Vol 66 Issue(12), 2527-2536, (1994).

الخلاصة

الاقطاب الانتقائية لدواء الفينتون تم تحضيرها في هذا البحث من خلال استخدام الليكاند صوديوم تترا فنيل بوريت وتكوين معقد المزدوج الايوني (فينتون–تترافنيل بوريت) ومن ثم تحضير الاغشية البولميرية بوجود بولي فانيل كلورايد وبأستخدام الملدنات الاتية الاتية

di-n-butylphthalate (DBPH)−1
tri-n- butylphosphate(TBP)-2
o- nitrophenyloctyl ether (ONPOE)-3
di-octylphenylphosphonate (DOPH)-4

على التوالي

وتم دراسة زمن الاستجابة وعمر القطب والدالة الحامضية ووجد ان الاقطاب المحضرة الحاوية على الملدن

di-n-butylphthalate (DBPH)−
di-octylphenylphosphonate (DOPH)-

تمتلك مى الخطية

from1.00×10^{-1} M to 1.00×10^{-5} M and 1.00×10^{-1} M to2.00×10^{-5}

على التوالي والميل 26.6 and 26.2 ملي فولت/حقبة وعمر القطب 30,28 يوم وعلى التوالي وتم تقدير الأدوية الصيدلانية باستخدام الاغشية المحضرة.