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Construction of Phenytoin Selective Electrodes and Its Application to Pharmaceutical Preparation

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Abstract

Phenytoin selective electrodes were constructed based on penytoin-phosphotungstate (Ph-PT) complex with different plasticizers; di-butyl phosphate (DBP), tri-butyl phosphate (TBP), di-butyl phthalate (DBPH),and o-nitro phenyl octyl ether (NPOE) phthalate. The electrodes based on DBPH, ONPOE plasticizers gave Narnistain slope which are, 56.4 and 55.3mV/decade with detection limit of $1.9x10^{-5}$ M , $1.8x10^{-5}$ and concentration range 10^{-1} to 10^{-4} M and pH range 3.0 – 8.0. The electrodes based on TBP and DBP showed non-Nernistain slopes, 40.2,40.5 mV/decade for both plasticizers. Interfering of some cations was investigated and shows no interfering with electrodes response. Potentiometric methods were used for measuring phenytion in pharmaceutical drugs (tablets) and the electrode based on DBPH was used for determination. The recovery obtained from measuring was in good agreements with that given in British Pharmacopeias.

Keywords: Phenytoin electrodes, Phosphotungstic acid ionophore,Potentiometric methods

Introduction

Ion –selective(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. Phenytoin 5,5 –diphenylimidazolidine-2,4-dione, is one of the most frequently prescribed anticonvulsant .It is considered as the drug of choice in treating all forms of epilepsy except absence seizures. It is also used to treat various psychchoses, trigeminal and related neuralgias and various cardiac arrhythmias [1]. Phenytoin is extensively metabolized in the liver to 5-(p-hydroxyphenyl)-5-phenylhydantion (PHPPH) and between 60 and 70% of the adiministered dose is excreted as free or as a glucuronide conjugate of PHPPH [2].Phentyion is a weakly absorbing compound ($A^{I\%}_{1cm}$ =27 at 258 nm) ,moreover ,the lack of a well defined UV absorption spectrum makes its determination in low concentration by direct UV spectrophotometery difficult ,and this problem is more aggravated if it needed to be estimated in biological fluids .

Phenytoin has been determined spectrophotometrically by a variety of methods ranging from a simple procedure based on measuring the absorbance at 235 nm [3] to more complicated ones that involve chemical derivatization .Wallace et al[4]. described a method determining phenytoin in blood based on hydrolysis of the hydantion ring in strong alkali followed by a Hofmann degradation of the resulting amide with bromine to yield benzophenone which was steam –dislilled and measured at 257 nm .At a later stage, Wallace[5] published a

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method for the assay of phenytoin in biological specimens consisting of hydrolysis of the hydantion ring in strong alkali ,then permanganate oxidation to benzophenone which was extracted into n-heptane after reflux for 30 min and measured at 247 nm. Amore recent method(6,7) , for the determination of phenytoin in plasma was based on extracting the sample with 1,2-dichloroethane ,followed by back extraction from the organic layer into alkali and oxidation by potassium permanganate with heating on a steam bath. The oxidation product was extracted into 2,2,4-trimethylpentane and measured at 247 nm. Other methods for the estimation of phention in pharmaceutical preparation and-or biological fluid include:colorimetery (8),spectrophotometry using orthogonal function(9) ,titrimetry (10) ,fluorimetry (11) ,thin layer chromatography (12) ,gas liquid chrmomatography (GLC) (13) and high performance liquid chromatography (HPLC) (!4)

. Ion selective electrodes (ISEs), which are applied for drug analysis due to their simplicity, fast response and easy to used. Kharitonov(15) reviewed a paper in using ion selective electrodes in organic medicinal drug determination, including the optimization of the selective electrodes and mechanism of the response. Several papers were published using phosphotungstic acid as an ionophore for drug complex formation and used for construction of drug electrodes. Al-Haideri et al.(!6) prepared and studied ampicilline selective electrodes on complexation of ampicilline with phosphotungstic acid as an active substances with different plasticizers. The best electrode was based on TBP plasticizer which gave a slope of 58.0 mV/decade and detection limit of 7.0 x 10^{-5} M and used for ampicilline determination in pharmaceutical drugs. Atenolol selective electrodes were prepared by Nassory et al.(!7) based on complex of atenolol-phosphotungstate as an active material using various plasticizers. The best electrode was based on DOPH plasticizer with slope 55.9 mV/decade and standard deviation of ± 0.1. Several amines and amiloride-selective electrodes were constructed by Nassory et al.(18). The amiloride electrode based on di-octyl phthalate plasticizer was excellently sensitive and was used for determination of amiloride in pharmaceutical drugs. A novel mebendazole PVC sensor was described by Kumar et al(19)) for fabrication, optimization and some possible applications of the mebendazole electrode. The membrane based on mebedazole-phosphotungstate complex with BEP plasticizer gave a slope of 55.8 mV/decade and detection limit of 6.3 x 10^{-7} M.

In this work, new phenytoin selective electrode phosphotungstic acid (PT) 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 part

Equipments

Orion EA-940 ion analyzer was used for measuring electrode response.

pH meter type pH M82 type Radio meter, Copenhagen.

Saturated calomel electrode type Gallenkam.

Silver wire coated with silver chloride used as internal reference electrode.

Chemicals and reagents

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

Phenytoin injection (250 mg Penytion in5 mL) was purchased locally (Nile Limited, Egypt).
PVC powder type Breon S110/10 B.P.
Di-butyl phosphate 98.9%, di-butyl phthalate, 99%, tri-buyl phosphate, 97%, o-nitro phenyl octyl ether, were obtained from Fluka AG, Switzerland.
Stock solutions of 0.1 M in each of LiCl, NaCl, KCl, $CaCl_2$, $MgCl_2$,$ZnCl_2$,$AlCl_3$, $CrCl_3$ and $FeCl_2$ were prepared. , diluted solutions were prepared by subsequent dilution of stock solutions.
Stock solution of 0.1 M phenytoin was prepared by dissolving 1.2651 g of pure drug in 15 mL of alcohol and dilute to 50 mL with water .
Phosphotungstic acid (PT), 0.1 M was prepared by dissolving 7.2 g in 25 mL water.
All solutions were prepared using doubly distilled water

Procedures

Preparation of ion pair complex

Ph-PT ion pair was prepared by mixing with stirring equal volumes of 0.1 M phosphotungstic acid (PT) and 0.1 M phenytoin The resultant precipitate was filtered on filter paper , washed with deionized water and dried in the room temperature for 5 days .

Assembly the electrode

The construction of the electrode body and immobilization were done according to the method described by Davis et al.[20]. The glass tube was ¾ filled with 0.1 M phenytoin solution as an internal filling solution. The membrane prepared by dissolving 0.04 g of phenytoin -phosphotungstate with 0.36 g plasticizer and 0.17g PVC in 6 mL THF. The mixture was poured into glass disc, 3.5 cm diameter .Then all of the contents were left for 2days to allow slow evaporation of the solvent and formation sensing membrane days .An Ag-AgCl electrode ,saturated calomel electrode (SCE) were used as internal refrence and reference electrode

Selectivity measurements:

A separate solution method(SSM) was used for the selectivity coefficient measurement, and was calculated according to the equation[21]:

$$\text{Log } K^{pot}_{A,B} = [(E_B - E_A) / (2.303 RT/zF)] + (1-z_A / z_B) \log a_A \text{ ....(1)}$$

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, respectively, at $a_A = a_B$.
Also the selectivity coefficients were measured by match potential method (MPM) according to equation [22].

$$K^{pot}_{A.B.} = \Delta a_A / a_B , \text{ with } \Delta a_A = a_A' - a_A$$

Sample preparation :

Tow phenytoin injection were mixed and then preparation of concentration 10^{-3} M and diluted to25 mL.

Results and discussion

phenytoin -phosphtungstate (Ph-PT) as an electro active complex was used to prepare new phenytoin selective electrodes. The characteristics of the electrode response based on Ph-PT) and different plasticizers, di-buyl phthalate (DBPH), di-butyl phosphate (DBP), tri-butyl phosphate (TBP),and o-nitro phenyl octyl ether (NPOE)) were investigated. All the membranes were soaked in 0.1 M phenytoin solution for 2 hours in order to conditioning the membrane before used. The results of electrode parameters measurements for phenytoin selective electrodes are listed in Table 1. The physical properties of the membranes prepared are colorless, flexible and transparent . Electrodes based on DBPH, DBP plasticizers gave slopes near to Nernstian slope are 56.4, 55.3 mV/decade, respectively. The slope values indicate that the complex(Ph-PT) formed is 3:1, three molecules of phenytoin interact with one mole phenytoin interact with phosphotungstic acid. Non-Nernistain slopes were obtained for electrodes based on DBP and TBP plasticizers was around 40.2,40.5 mV/decade.This may be attributed to the behaviors of the plasticizers with phenytoin complex, such as a weak interaction, incompatibility of the plasticizers with the complex or the viscosity of the plasticizers which cause a leaching of the complex to the external solution during the measurements. The linear concentration range was ranged from 10^{-1} to 10^{-4} M and with excellent detection limit of 1.9 x 10^{-5} M. A typical plot for electrode response with concentrations of phenytoin was shown in Figure 1 for electrode based on di-butyl phthalate (DBPH)plasticizer using Orion 7 cycle semilogarthmic paper for plot.

pH effect

phenytoin electrodes was studied for three concentrations of phenytion (10^{-2}, 10^{-3} and 10^{-4} M) by following the variation in potentials over pH range from 2.0 to 11.0 by addition dilute hydrochloric acid (and sodium hydroxide. Fixed potential (did not change in potentials) was noticed in the range 7.0- to 9.2at pH <7 phenytoin degradation and pH>9.2 phenytoin may be hydrolsis(23) The results are listed in Table 2. Representation curve for pH plot with potentials is shown in Figure 2 for phenytoin electrode based on DBPH plasticizer at 10^{-2} to 10^{-4} M penytion l solutions.

Response time and life time

The response times at t_{95} for the electrodes at concentrations ranging from 10^{-1} to 10^{-5} M were calculated from the response with time plot. The values of response time were ranged from 6 s at concentration 10^{-1} M phenytoin solution and the values increases when the concentration of phenytoin dincreases and reach to about 26.2 s at 10^{-5} M. The fast response time of the electrodes indicates the more stability of the electrodes and can be used for quantitative measurements of the drugs with very good values of standard deviations. The life time of the electrodes was measured from the calibration of the electrode continuously every 2 days and behaviors of the slopes were investigated. The life time of the electrode was ranged from 18 to 45 days. The short life time for electrode based on NPOE plasticizer is attributed to the leaching of the plasticizer (low viscosity 11.44 csT) to the external solution during the measurements or incompatibility of the plasticizer with the active complex.

Selectivity measurements

The influence of some interfering inorganic cations, Li^{+}, Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Zn^{2+}, Al^{3+}, Cr^{3+} and Fe^{3+} on the electrode response was studied. The selectivity coefficients for the electrodes were measured by the separate solution method(SSM) for the concentrations range from 10^{-1} M to 10^{-5} M. The values of the selectivity coefficients for electrodes based on DBP, TBP and NPOE plasticizers are listed in Table 3 for concentrations of phenytoin at 10^{-2} M and 10^{-4} M. As noticed from the Table 3 the interference was increases as the concentration of phenytoin decreased. None of the investigated cations interfere seriously with the electrode response. The match method(MPM) was used for the electrodes based on DBP and TBP plasticizers of the non-Nernstian slopes using the equation given in the experimental part. The selectivity coefficient can not be measured by this method because the cations show no interference with the electrode response. A typical plot for match method using electrode based on TBP and magnesium ion is shown in Figure 3.

Sample analysis

Potentiometric techniques were used for determination of phenytoin by using direct, standard addition and titration methods . Synthetic solutions of phenytion at concentrations $1x10^{-3}$, $1x10^{-3}$ M were used. The recoveries and linear equations for the electrodes obtained from the calibration curves are listed in Table 4. The results of direct, standard addition and titration methods for concentration of phenytion at $1x10^{-3}$ M using electrode based on DBPH plasticizer are listed in Table 5. A phosphotungstic acid was used as a titrant for potentiometric titration. Determination of phenytion in commercial drugs (injection) with electrode based on DBPH plasticizer by using potentiometrc methods was studied. 10^{-3} M of phenytion was taken from phenytion injection and the analysis showed that the recoveries and %RE obtained using direct, standard addition and titration methods are 98 , 99.2 and 103.2% and -2, -1 and 3.2.0%, respectively. The values of the recovery of phenytion in injection are in a good agreements with the results of British Pharmacopeias. A plot of standard addition method, antilog (E/S) versus volume (mL) of standard phenytoin addition is shown in Figure 4.

Conclusion

New phenytion selective electrodes were constructed based on phosphotungstic acid ionophore and different plasticizers. A good phenytion electrode was based on DBPH plasticizer and used for determination of phenytoin in pharmaceutical formulations.

Table1. Response characteristics of phenytion electrodes with different plasticizers.

Electrode No.	Plasticizer	Slope mV/decade	Detection limit/M	Conc. range/M	Response time/s	Life time/day
I	**DBPH**	**56.4 (0.9996)**	**1.9×10^{-5}**	**10^{-1} -10^{-4}**	**3.2-21.2**	**~45**
II	**DBP**	**40.5 (0.9995)**	**1.7×10^{-5}**	**10^{-1} -10^{-4}**	**4-26.2**	**~ 23**
III	**TBP**	**40.2 (0.9993)**	**1.5×10^{-5}-5**	**10^{-1} -10^{-4}**	**2.1-20.6**	**~ 18**
IV	**ONPOE**	**55.3 (0.9995)**	**1.8x 10-5**	**10^{-1} -10^{-4}**	**2.5-24.3**	**~ 35**

Values between the parentheses refer to correlation cofficinet (r)

Table 2 pH values for the electrodes at different concentrations of phenytion l solutions

Electrode number	**pH range**		
	10^{-2} M	**10^{-3} M**	**10^{-4} M**
I	**6.90-9.1**	**7.4-9.1**	**7.07-9.09**
II	**7.12-9.6**	**6.67-9.12**	**6.78-8.57**
III	**6.63-8.67**	**6.65-8.70**	**6.32-8.14**
IV	**7.1-9.5**	**6.87-8.5**	**6.65-8.51**

Table 3 Selectivity coefficient values for phenytoin electrodes at 10^{-2} and 10^{-4} M concentrations of Phenytion and some cations

Interfering cations	Selectivity coefficient $K_{ph.}$ DBP		TBP		DBPH	
	10^{-2} M	10^{-4} M	10^{-2} M	10^{-4} M	10^{-2} M	10^{-4} M
Li^{+}	0.58x10^{-2}	0.66x10^{-1}	0.15x10^{-2}	0.21x10^{-1}	0.808x10^{-2}	0.763x10^{-1}
Na^{+}	0.21x10^{-2}	0.16x10^{-2}	0.47x10^{-2}	0.87x10^{-2}	0.475x10^{-2}	0.195x10^{-1}
K^{+}	0.77x10^{-2}	0.87x10^{-1}	0.15x10^{-2}	0.27x10^{-1}	0.659x10^{-2}	0.431x10^{-1}
Mg^{2+}	0.27x10^{-2}	0.13x10^{-1}	0.29x10^{-2}	0.70x10^{-1}	0.124x10^{-1}	0.369x10^{-1}
Ca^{2+}	0.15x10^{-1}	0.35x10^{-1}	0.82x10^{-}	0.23x10^{-1}	0.105x10^{-1}	0.313x10^{-1}
Zn^{2+}	0.32x10^{-1}	0.62x10^{-1}	0.59$10^{-2}$	0.25x10^{-1}	0.623x10^{-2}	0.108x10^{-2}
Al^{3+}	0.27x10^{-2}	0.71x10^{-2}	0.47x10^{-2}	0.25x10^{-1}	0.124x10^{-1}	0.706x10^{-1}
Cr^{3+}	0.13x10^{-2}	0.20x10^{-1}	0.29x10^{-3}	0.13x10^{-1}	0.588x10^{-2}	0.181x10^{-1}
Fe^{3+}	0.22x10^{-2}	0.12x10^{-3}	0.92x10^{-3}	0.85x10^{-1}	0.762x10^{-3}	0.287x10^{-1}

Table 4 Recoveries and linear equations values for electrodes at different concentrations of Phenytion solutions.

Electrode No.	Conc. of Phenytoin taken/M	Conc. of Phenytoin found/M	%RE	%REC	Linear equation
I	1.0 x 10^{-2}	1.02 x 10^{-2}	2	102	Y=24.46LinX + 73.8 2
	1.0 x 10^{-3}	1.00 x 10^{-3}	---	100	
II	1.0 x 10^{-2}	99.1 x 10^{-3}	-0.9	99.1	Y=17.63LinX +
	1.0 x 10^{-3}	9.94 x 10^{-4}	-0.6	99.4	219.91
III	1.0 x 10^{-2}	1.01 x 10^{-2}	1	101	Y=17.46+ 25.53
	1.0 x 10^{-3}	1.01 x 10^{-3}	1	101	
IV	1.0 x 10^{-2}	1.01 x 10^{-2}	1	101	Y=24.04LinX +
	1.0 x 10^{-3}	9.91 x 10^{-3}	-0.9	99.1	116.5

Table 5 Analysis of Phenytion samples by potentiometric methods

Method	Conc. of p Phenytoin taken /M	Conc.of Phenytoin found /M	%RE	%REC	%RSD
Direct	1.0 x 10^{-3}	0.98x 10^{-3}	-2	98	3.27*
Standard addition	1.0 x 10^{-3}	0.99 x 10^{-3}	-1	99	1.28
Titration	1.0 x 10^{-3}	1.032 x10^{-3}	3.2	103.2	4.3

- Each value was an average of three measurements.

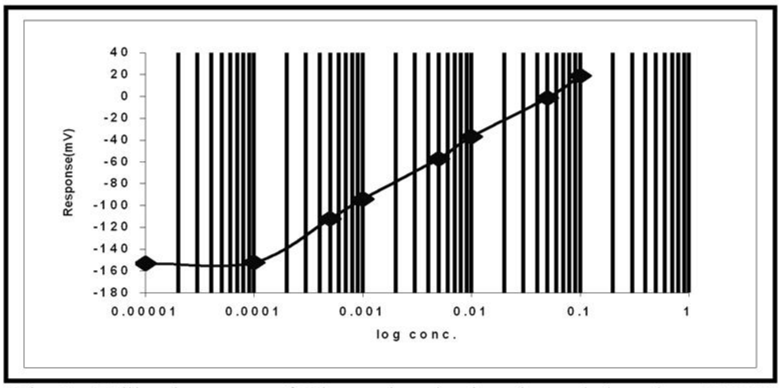

Fig. 1 Calibration curve of Phenytoin selective electrode based on DBPH plasticizer.

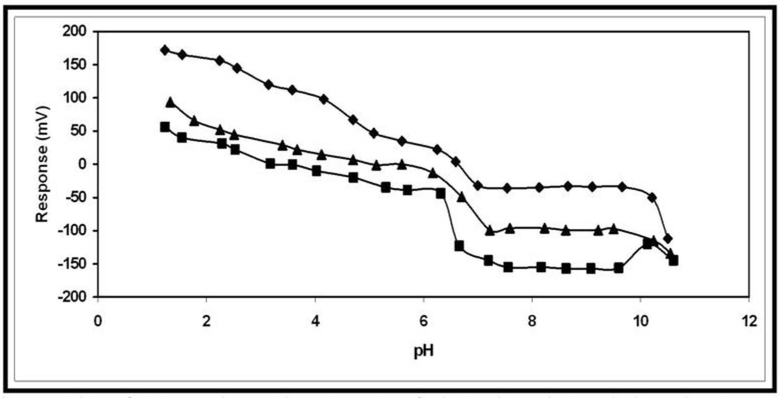

Fig. 2 Plot of pH vs. electrode response of Phenytion electrode based on DBPH plasticizer(◊ 10^{-2} ، ▲ 10^{-3} ، ■ 10^{-4}) M.

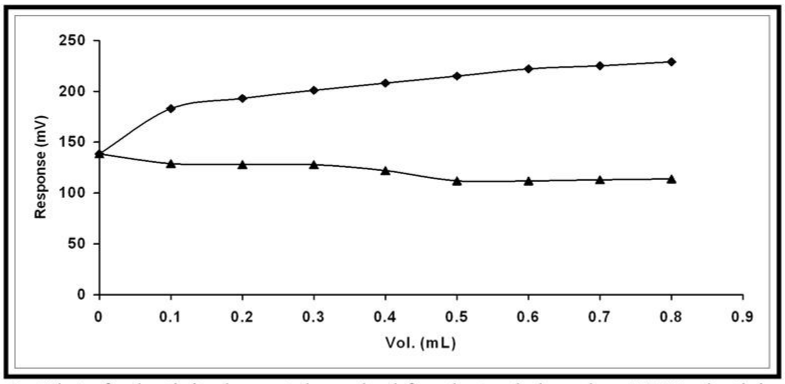

Fig. 3 Plot of selectivity by match method for electrode based on DBP plasticizer in present interfering Mg^{2+} ion.

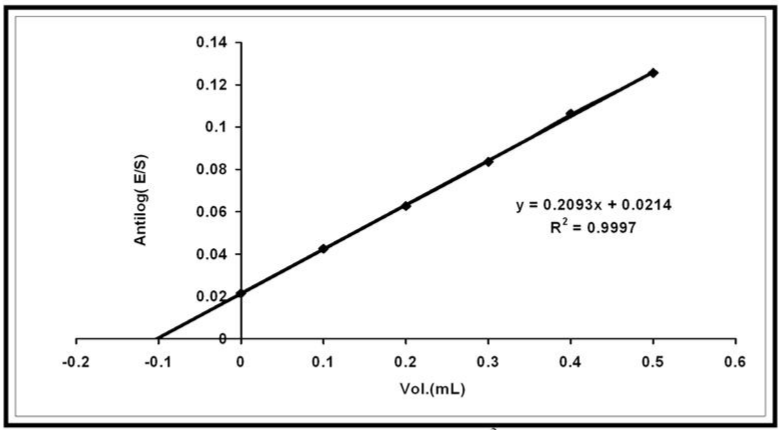

Fig. 4 Standard addition plot for determination 10^{-3}M Phenytion in injection using electrode based on DBPH plasticizer.

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بناء قطب الفنيتون الانتقائي لتقدير الفنيتون في المواد الصيدلانية

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الخلاصة

في هذه الدراسة حضرت اقطاب انتقائية للفنيتون معتمدة على معقد الدواء مع dodeca-Tungstophphsphoric acid ومجموعة من المواد الملدنة Di-butyl phthalate(DBPH),Di-bytyl phosphate (DBP),Tri-butyl phosphate (TBP),O-nitro phenyl octyl ether (ONOPE),

اظهرت النتائج ان اقطاب الفنيتون مع الملدنات (DBPH) و ONPOE اعطت انحدارا=56.4, 55.3 mV/decade على التوالي ومدى تركيز خطي $1x10^{-4}$ - $1x10^{-1}$ وحد تحسس $1.9x10^{-4}$. اما اقطاب المعتمدة على الملدنات TBP و DBP اعطت انحدارا لانرنستي 40.5, 40.2 mV/decade على التوالي .اظهرت الدراسة عدم وجود تداخل للعدد من الايونات الموجبة الاحادية والثنائية والثلاثية .

لدراسة الخواص العملية في محاليل قياسية محضرة مختبريا وكذلك في نماذج دوائية تم استعمال الاقطاب كاقطاب كاشفة في عملية التسحيح النرنستي واستعملت طريقة الاضافات القياسية لنماذج دوائية للفنيتون.