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Construction of different types of ion-selective electrodes and validation of direct potentiometric determination of phenytoin sodium

Research Article

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Abstract: The construction and performance characteristics of phenytoin sodium selective electrodes are detailed. Two types of electrodes: plastic membrane I and coated wire II, were constructed based on the incorporation of phenytoin sodium with tungstosilicic acid. The influence of membrane composition, kind of plasticizer, pH of the test solution, soaking time and the electrodes' foreign ions were investigated. The electrodes showed a Nernstian response with a mean calibration graph slope of 30.9±0.1 and 28.9±0.1 mV decade^{-1} at 25ºC for electrode I and II respectively, over a phenytoin sodium concentration range of 5×10^{-3}-5×10^{-6} M and 1×10^{-3}-1×10^{-6} M with a detection limit 1.3×10^{-6} M and 2.5×10^{-7} M for electrode I and II, respectively. The electrodes gave average selective precision and were usable within the pH range 6-10. Interference studies from common cations, alkaloids, sugars, amino acids and drug excipients are reported. The results obtained by the proposed electrodes were also applied successfully for the determination of the drug in pharmaceutical preparations and biological fluids.

Keywords: *Plastic membrane • Coated wire electrode • Ion-selective electrode • Phenytoin sodium • Potentiometric titration*

1. Introduction

The structure of phenytoin sodium salt, 5, 5-diphenylimidazolidine-2,4-dione is depicted in Fig.1. Phenytoin acts to dampen the unwanted, runaway brain activity seen in seizures, by reducing electrical conductance among brain cells by stabilizing the inactive state of voltage gated sodium channels. Aside from seizures, it is an option in the treatment of trigeminal neuralgia as well as certain cardiac arrhythmias. There are some indications that phenytoin has other effects, including anxiety control and mood stabilization, although it has never been approved for those purposes by the FDA [1].

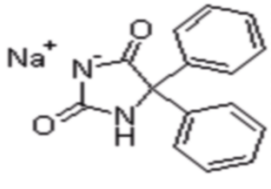

Figure 1. Chemical structure of phenytoin

Several methods have been reported for the determination of phenytoin sodium using HPLC [2-7], LC-MS [8,9], GC [10-12], spectrophotometry [13,14], capillary zone electrophoresis [15], polarography [16], potentiometry [17]. This work describes two different implementation of a new and selective type of membrane sensors: plastic membrane and coated wire electrodes for the determination of phenytoin sodium in pure solutions, pharmaceutical preparations and biological fluids.

2. Experimental Procedure

2.1. Standard Drug Solution

Fresh stock of phenytoin sodium solution (1×10^{-1} M) was prepared on daily basis by dissolving an appropriate amount of the drug in double distilled water followed by addition of a few drops of ethanol to the clear solution. More dilute solutions were prepared by appropriate dilution.

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2.2. Recommended procedures

2.2.1. Preparation of phenytoin-tungstosilicate ion-pair

The ion-pair was prepared by mixing stoichiometric amounts of 1×10^{-2} M tungstosilicic acid with an equimolar solution of phenytoin sodium followed by stirring for 10 min. The resulting white precipitate of the ion-pair was filtered through a G_4 sintered glass crucible and washed thoroughly with deionized water, then dried at room temperature for 24 hours. The product was stored in a desiccator.

2.2.2. Membrane composition

The membrane composition was studied by varying the percentages (w/w) of the ion pair, poly(vinyl chloride) PVC and plasticizer *o*-nitrophenyl octyl ether, until the optimum composition that exhibits the best performance characteristics was obtained. The membranes were prepared by dissolving the required amount of the ion-pair, PVC and *o*-nitrophenyl octyl ether, in 5 mL tetrahydrofuran (THF). The solution was poured into a petri dish (3 cm in diameter), covered with a filter paper and the solvent was allowed to evaporate slowly at room temperature. To obtain uniform membrane thickness, the amount of (THF) was kept constant, and evaporation carried out for 24h.

2.2.3. Electrode construction

Plastic membrane electrode: A punched circular membrane was attached to a poly-ethylene tube of 8 mm diameter in an electrode configuration by means of PVC-THF solution. A mixture containing equal volume of 1×10^{-3} M phenytoin sodium and potassium chloride was used as internal reference solution in which the Ag/AgCl reference electrode was dipped. The electrode was pre-conditioned after preparation by soaking it for at least 9 h in 1×10^{-3} M phenytoin sodium and stored in the same solution. All potentiometric measurements were performed using the following cell assembly: Ag/AgCl/internal solution /membrane/test solution//KCl salt bridge//SCE.

Coated wire electrode: A pure aluminum wire was polished, washed with a detergent and water, thoroughly rinsed with water, and dried with acetone. Then the wire was rinsed with chloroform and allowed to dry. A piece of this aluminum wire of 4.0 cm length was tightly insulated by polyethylene tube leaving 1.0 cm at one end for the coating and 0.5 cm at the other end for connection. The coating on the electrode was carried out as described previously under membrane composition. The aluminum wire was coated by dipping it quickly into the coating solution several times, and after each coat allowing the film on the wire to dry for about 3 min. The process was repeated until a plastic membrane of approximately 1.0 mm thickness was formed. The electrode was conditioned by soaking for 4 h in 1.0×10^{-3} M phenytoin sodium solution [18]. All potentiometric measurements were performed using the following cell assembly: Al/membrane/test solution// KCl salt bridge//SCE.

2.3. Determination of phenytoin sodium in the dosage forms

2.3.1. Phenytoin sodium ampoules

The solutions with phenytoin sodium in the concentration range from 5×10^{-3}-1×10^{-5} M and 1×10^{-3}-5×10^{-6} M were prepared by dilution of the stock solution with deionized water. These solutions were transferred into 50 mL beakers, adjusted to pH 6 using 0.1 N sodium hydroxide. The phenytoinelectrode(s) (electrode I / electrode II) was immersed in the solutionand the system allowed to equilibrate with stirring. The e.m.f. was recorded and compared with the calibration graph.

2.3.2. Phenytoin sodium capsules

The solutions with phenytoin sodium concentration range from 5×10^{-3}-1×10^{-5} M and 1×10^{-3}-5×10^{-6} M, for electrode I and II respectively, were prepared using appropriate amount of capsules content and solutions diluted with deionized water. The solutions were adjusted to pH 6 using 0.1 N dilute sodium hydroxide. The phenytoin-electrode(s) was immersed in the solution. The electrode(s) system was allowed to equilibrate with stirring and the e.m.f. recorded and compared with the calibration graph. The standard addition (spiking technique) was also applied by recording the electrode(s) potential after addition of 0.1 mL of stock 1×10^{-1} M phenytoin sodium solution to the above drug test solutions.

2.3.3. Content uniformity assay of phenytoin sodium ampoules

Ten individual ampoules of 250 mg mL^{-1} phenytoin sodium were placed in separate 100 mL beakers and dissolved in 90-100 mL of distilled water. The electrode(s) was directly immersed into 10 mL of each sample for five times and washed with deionized water to reach steady potential between the individual measurements. The mean potential was used to evaluate the content uniformity from the calibration graph.

2.4. Application to serum and urine

Added phosphate buffer to urine or serum samples dropwise until a pH of 6 is obtained. Transferred 5 mL of the pH-adjusted urine or serum into four small separatory funnels, and then to each was added 5 mL 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} M standard drug solution, followed by the addition of 20 mL toluene for urine and 20 mL diethyl ether for serum samples, respectively. After shaking each funnel for 5 minthe aqueous layer was transferred to a centrifuge tube. Centrifuged for 2 min at 1500 rpm, then

transferred to a 50 mL volumetric flask and the solution diluted with deionized water to the appropriate level. Apply the procedure described under electrode calibration [19].

3. Results and discussion

3.1. Optimization of Membrane Composition

From the previous experimental investigations [20], it is obvious that both, the kind of plasticizer selected and the membrane composition used, can influence the response performances such as the sensitivity, linear concentration range, the detection limit, the response time *etc*. of PVC membrane sensors, if other properties of the sensor, *e.g.* selectivity or pH response, are omitted. In this study, four membrane compositions were investigated, the results were summarized in Table 1. The results showed that the electrode(s) made by membrane of type (c) with 2.5 wt% phenytoin-tungstosilicate ion pair, 31.5 wt% PVC and 66.0 wt% plasticizer *o*-NPOE exhibits the best performance characteristics with slopes 30.9±0.1 and 28.9±0.1 mV decade^{-1} at 25ºC for electrode I and II, respectively, over the phenytoin sodium concentration range of 5×10^{-3}-5×10^{-6} M and 1×10^{-3}-1×10^{-6} M for electrode I and II, respectively.

3.2. Nature and response characteristics of the electrode(s)

Phenytoin sodium reacts with tungstosilicic acid to form a stable phenytoin-tungstosilicate ion-pair complex which is water insoluble but readily soluble in an organic solvent such as tetrahydrofuran. The complex was prepared and tested as active material with *o*-NPOE as a solvent mediator in a poly(vinylchloride) membrane for studying its response to phenytoin sodium. The critical response characteristics of plastic membrane and coated wire electrodes were determined and results are summarized in Table 2. The electrode(s) exhibits a Nernstian response over the concentration range from 5×10^{-3}-5×10^{-6} M and 1×10^{-3}-1×10^{-6} M for phenytoin sodium with electrode I and II, respectively. A slope of 30.9±0.1 and 28.9±0.1 mV decade^{-1} was observed for change in concentration with electrode I and II, respectively, as shown in Fig. 2. The choice of membrane solvent to achieve the required selectivity is based on its electric permittivity and its immiscibility with aqueous phase, high viscosity, low solubility of the matrix in the membrane and ability to dissolve ion-pair complex.

Table 1. Optimization of membrane composition (wt/wt%).

Type of sensor	m	PVC wt%	o-NPOE wt%	Ion-pair wt%	Slope	RSD%	r	Linear conc. range
Plastic membrane electrode	(a)	39.0	60.0	1.0	26.2	0.9	0.9992	1.0×10^{-3}-5.0×10^{-5}
	(b)	35.0	64.0	1.0	27.5	1.0	0.9987	1.0×10^{-3}-9.0×10^{-5}
	(c)	31.5	66.0	2.5	30.9	0.3	0.9995	5.0×10^{-3}-5.0×10^{-6}
	(d)	20.0	70.0	2.0	28.3	0.7	0.9997	1.0×10^{-3}-5.0×10^{-6}
Coated wire electrode	(a)	39.0	60.0	1.0	24.7	1.1	0.9998	1.0×10^{-3}-1.0×10^{-5}
	(b)	35.0	64.0	1.0	26.3	1.2	0.9993	1.0×10^{-3}-1.0×10^{-5}
	(c)	31.5	66.0	2.5	28.9	0.3	0.9998	1.0×10^{-3}-1.0×10^{-6}
	(d)	20.0	70.0	2.0	28.2	1.1	0.9997	1.0×10^{-3}-5.0×10^{-6}

Table 2. Critical response characteristics of phenytoin-tungstosilicate sensors.

Parameters	Phenytoin-tungstosilicate plastic membrane electrode	Phenytoin-tungstosilicate coated wire electrode
Slope (mV per decade)	30.9±0.1	28.9±0.1
Intercept	396.76	475.8
Correlation coefficient r.	0.9995	0.9998
Linear range (M)	5.0×10^{-3}-5.0×10^{-6}	1.0×10^{-3}-1.0×10^{-6}
Detection limit (M)	1.3×10^{-6}	2.5×10^{-7}
Response time for 10^{-3} M (s)	≤35	≤30
Working pH range	6-10	6-10
Lifetime /day	30	37
Accuracy (%)	99.55	99.45
Standard deviation (%)	0.8	0.5
Repeatability (CV_w%)	0.9	0.6
Between day variability (CV_b %)	0.9	0.8
Robustness[b]	99.84±0.5	100.31±0.2
Ruggedness[c]	99.75±0.1	100.11±0.1

[a] Mean of three measurements.
[b] A small variation in method parameters were studied as pH of buffer.
[c] Comparing the results by those obtained by different sensors assemblies using -Orion 420 A.

3.3. Life time

The response time of the electrode(s) was tested for 1×10^{-1}-1×10^{-6} M phenytoin sodium solutions. The sequence of measurements was always from low to high concentrations. The electrode(s) stabilizes its response of ≤35 s and ≤30 s, for electrode I and II, respectively. The electrode(s) was used for a period of 30 and 37 days for electrode I and II, respectively, without significant change in the electrode(s) parameters.

3.4. Effect of plasticizer

In this study, four plasticizers, di-butyl sebacate (DBS), di-octylphthalate (DOP) and di-butylphthalate (DBP) and *o*-nitrophenyl octyl ether (*o*-NPOE) were used to examine the optimization of the membrane with plasticizer which entailed the use of plasticizer content ratio of 60.0, 64.0, 66.0 and 70.0 wt%, and the use of PVC contents of 39.0, 35.0, 31.5, and 28.0 wt%. The electroactive compound (phenytoin-tungstosilicate) content of 1.0, 1.0, 2.5 and 2.0 wt%. The results obtained showed that the response performances of the membranes prepared were rather different depending on the use of plasticizer, the proportion of the plasticizer toward PVC and of the electroactive compound. The typical potential responses of the electrodes constructed with four plasticizers are given in Fig. 3. As shown in Fig. 3, the *o*-NPOE-PVC electrodes were superior to DBS-, DOP-, DBP-PVC electrodes in both, the response slope and linear concentration range. Hence, *o*-NPOE was selected as the plasticizer of the membranes. The best membrane composition of the *o*-NPOE-PVC electrode(s) was 31.5 wt % PVC, 66.0 wt % *o*-NPOE and 2.5 wt % ion-pair.

3.5. Effect of soaking

The performance characteristics of the phenytoin-tungstosilicate electrode(s) was studied as a function of soaking time. For this purpose the electrode(s) was soaked in 1×10^{-3} M solution of phenytoin sodium and the calibration graphs were plotted after 1, 2, 3, and 24h, respectively. The optimum soaking time was found to be 9 h and 4 h at which the slope of the calibration curve was 30.9±0.1 and 28.9±0.1 mV decade^{-1}, at 25 ºC for electrode I and II, respectively. The influence of prolonged soaking on the lifetime of phenytoin-tungstosilicate electrode(s) was followed by constructing calibration plots. The electrode(s) was soaked continuously in 1×10^{-3} M solution of phenytoin sodium for 10, 15, 20, 25, 30 and 37 days, respectively. The slopes of the calibration plot decreased slightly to 26.17, 25.91 mV decade^{-1} after 25 days and continued to decrease reaching 25.98, 25.89 mV decade^{-1} after 30 days for electrode I and II, respectively. Figs. 4, 5 show

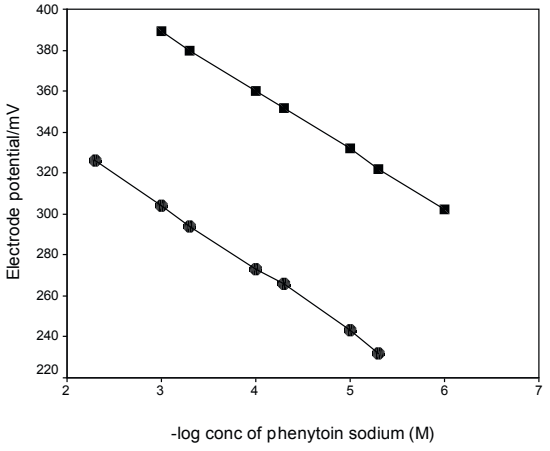

Figure 2. Typical calibration graph of phenytoin sodium sensors: (●) phenytoin-tungstosilicate-plastic membrane electrode, (■) coated wire-phenytoin-tungstosilicate-electrode

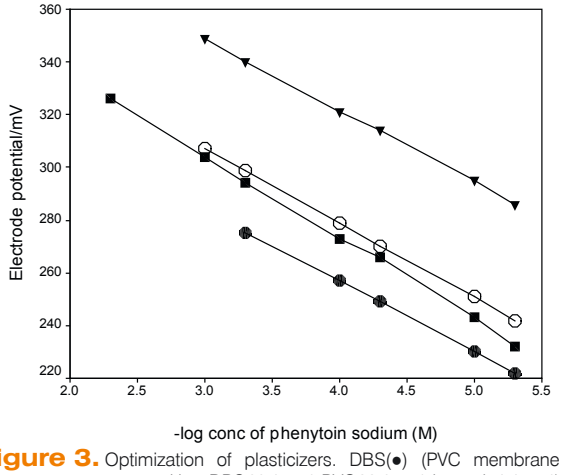

Figure 3. Optimization of plasticizers. DBS(●) (PVC membrane composition: DBS 60.0 wt%, PVC 39.0 wt%, ion-pair, 1.0 wt%), o-NPOE(■) (PVC membrane composition: o-NPOE 66.0 wt%, PVC 31.5 wt%, ion pair, 2.5 wt%), DBP (○) (PVC membrane Composition: (DBP 64.0 wt%, PVC 35.0 wt%, ion-pair, 1.0 wt%), (▼) DOP (PVC membrane composition: (DOP 70.0 wt%, PVC 28.0 wt%, ion-pair, 2.0 wt %

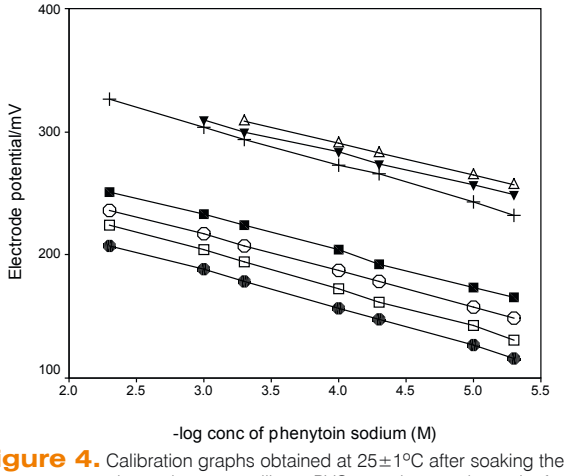

Figure 4. Calibration graphs obtained at 25±1ºC after soaking the phenytoin-tungstosilicate-PVC membrane electrode for (●) 9 h, (□) 24 h, (○) 7 days,(■) 12 days, (+) 18 days, (▼) 25 days, (Δ) 30 days

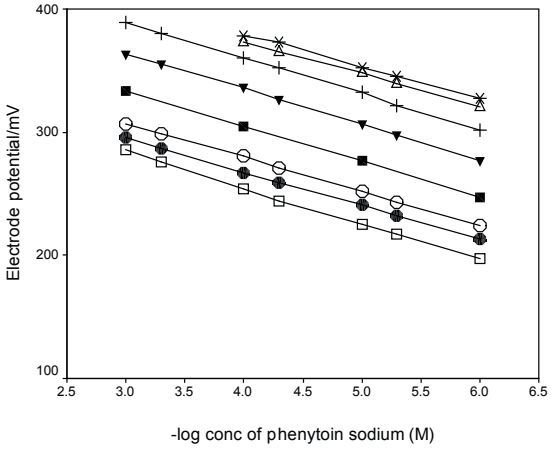

Figure 5. Calibration graphs obtained at 25±1°C after soaking the phenytoin-tungstosilicate- coated wire electrode for (□) 4 h, (●) 24 h, (○) 7 days,(■) 10 days, (▼) 20 days, (+) 25 days, (Δ) 30 day, (*) 37 days

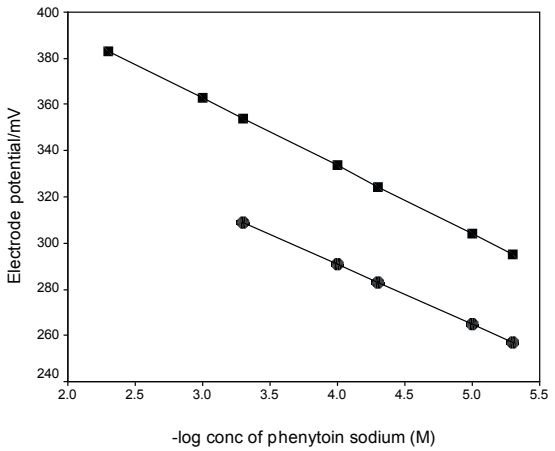

Figure 6. Regeneration of phenytoin-tungstosilicate-PVC membrane electrode (●) calibration graph of the exhausted electrode, (■) calibration graph of a regenerated electrode

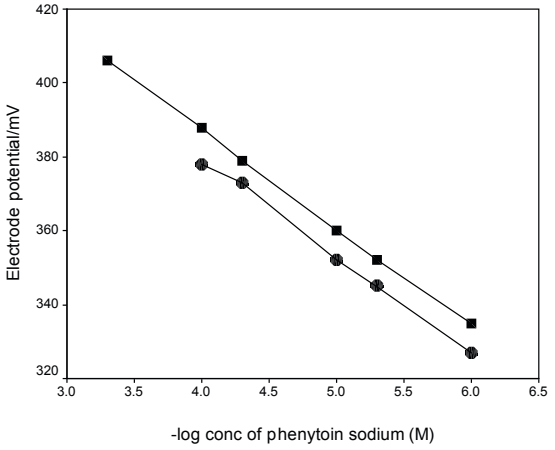

Figure 7. Regeneration of phenytoin-tungstosilicate-coated wire electrode (●) calibration graph of the exhausted electrode, (■) calibration graph of a regenerated electrode

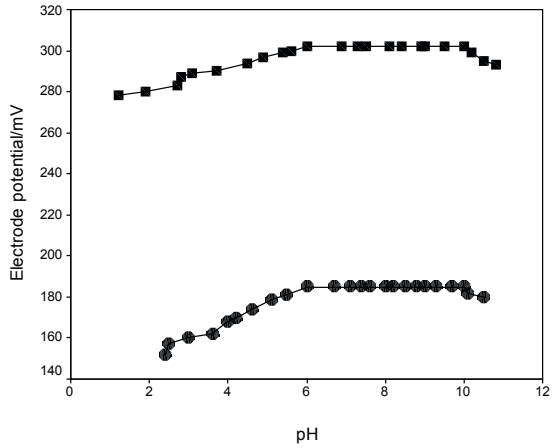

Figure 8. Effect of pH on potential/mV of 1.0×10^{-3}M, (■) Plastic phenytoin-tungstosilicate-PVC electrode, (●) Coated wire-phenytoin-tungstosilicate electrode

the effect of prolonged soaking time and the life span of the phenytoin-tungstosilicate electrodes.

3.6. Regeneration of the electrode

The above discussion reveals that soaking of the electrode(s) in the drug solution for a long time has a negative effect on the response of the membrane towards the phenytoin sodium. The same effect appears after working with the electrode(s) for a long time. The regeneration of the electrode(s) was tried by a simple reformation with ion-exchange on the external gel layer of membrane [21]. The regeneration of the phenytoin-tungstosilicate membrane was successfully achieved by soaking the exhausted electrode(s) for 24 h in a solution of 1×10^{-2} M tungstosilicic acid, followed by soaking for 3 h in 1×10^{-2} M phenytoin sodium solution. Figs. 6 and 7 show the calibration graphs for an exhausted electrode(s) with slopes of 25.98, 25.89 mV decade^{-1} for electrode I and II, respectively, and the same electrode(s) after regeneration showed slopes of 29.40, 27.03 mV decade^{-1} for electrode I and II, respectively. It was found that the lifespan of the regenerated electrode(s) is limited to 5 h due to the ease of leaching of the lipophilic salts from the gel layer at the electrode(s) surface compared with those that are attached homogeneously to the PVC network through the solvent mediator.

3.7. Effect of pH

The effect of pH of the phenytoin sodium solution using 1×10^{-3} M phenytoin sodium on the electrode(s) potential was investigated. The solution was acidified by the addition of very small volumes of 0.1 N hydrochloric acid then the pHwas increased gradually using 0.1 N

sodium hydroxide. For each pH value, the potential was recorded and thus the potential-pH curves for phenytoin sodium concentration were constructed as in Fig. 8. At pH<6, calibration graphs deviated from linearity due to increased response to protons. Above pH>10, phenytoin sodium possibly exists as the base form with lower activity. Thus, the optimum pH range for measuring the drug under investigation is 6-10.

3.8. Selectivity of the electrode

The influence of various basic substances on the response of phenytoin sensors was investigated by measuring the potentiometric interference from many sugars, inorganic cations, certain alkaloids and amino acids. The selectivity coefficients were determined by the separate solution method. Table 3, showed that the proposed phenytoin-tungstosilicate membrane electrode(s) is highly selective toward phenytoin. The electrode(s) showed no response to a number of potentially interfering ionic excipients such as starch and lactose that are usually used in the manufacturing of the pharmaceutical preparations. The inorganic cations did not interfere due to the differences in their mobilities and permeabilities as compared with phenytoin cation. With respect to amino acids, the high selectivity is mainly attributed to the difference in polarity and lipophilic character of their molecules relative to phenytoin.

3.9. Quantification of phenytoin sodium

Direct potentiometric determination of phenytoin sodium using phenytoin-tungstosilicate electrode(s) type I and II was performed and calculated from the calibration curve. The direct potentiometric determination of phenytoin sodium in pure form using the proposed electrodes gave average recovery of 99.55±0.9 % and 99.45±0.5 % for electrode I and II respectively. Furthermore, the results obtained were compared with the official method [22] ofpotentiometric titration using 0.1 M sodium hydroxide, for determination of phenytoin sodium, and the results are listed in Table 4.

Table 3. Selectivity coefficients of the phenytoin-tungstosilicate sensors calculated by the separate solution method (1×10^{-3} M of both phenytoin sodium and interferent at 25ºC).

Interferent	K^{pot}_{Phen}	
	Phenytoin-tungstosilicate plastic-membrane electrode	Phenytoin-tungstosilicate coated-wire electrode
Sodium chloride	5.0×10^{-4}	5.3×10^{-4}
Potassium chloride	3.4×10^{-4}	6.4×10^{-4}
Magnesium sulphate	1.9×10^{-3}	1.2×10^{-3}
Calcium chloride	2.2×10^{-4}	1.4×10^{-3}
urea	7.9×10^{-3}	6.9×10^{-3}
Glucose	4.9×10^{-5}	5.0×10^{-3}
Lactose	2.6×10^{-4}	3.9×10^{-3}
Starch	8.2×10^{-5}	7.1×10^{-4}
Quinidine	7.2×10^{-5}	7.4×10^{-4}
Caffeine	2.6×10^{-6}	1.7×10^{-5}
L-systin	2.9×10^{-4}	3.6×10^{-4}
L-leucin	1.5×10^{-3}	2.5×10^{-4}
Pseudoephedrine hydrochloride	1.8×10^{-4}	2.9×10^{-4}
Gabapentin	4.7×10^{-3}	1.4×10^{-5}
Paroxetine hydrochloride	2.1×10^{-3}	8.1×10^{-3}
Sulfathiazole	1.7×10^{-5}	3.2×10^{-5}

Table 4. Determination of phenytoin sodium in pure form using phenytoin-tungstosilicate sensors in comparison with official method [21].

Statistical parameter	Phenytoin-tungstosilicate plastic membrane electrode			Phenytoin-tungstosilicate coated wire electrode		
	Official method [22]	Direct potentiometry		Official method [22]	Direct potentiometry	
		Calibration method	Standard addition method		Calibration method	Standard addition method
Mean%	98.90	99.38	99.55	99.70	99.56	99.45
N	6	6	6	6	6	6
Variance	0.278	0.064	0.769	0.334	0.835	0.247
SD	0.527	0.253	0.877	0.236	0.914	0.497
SE	0.215	0.103	0.358	0.580	0.345	0.203
RSD	0.533	0.255 (2.013)	0.881 (1.557)(2.228)*		0.918 (0.335)	0.499 (0.803)(2.228)*
"t"		(2.228)*	(2.77)(5.05)*		(2.228)*	(1.35)(5.05)*
F		(4.34)(5.05)*			(2.50)(5.05)*	

*Theoretical Values of "t" and F at P=0.05

4. Validation of the proposed ISE method

4.1. Accuracy

The accuracy of the proposed ISE method was investigated by the determination of phenytoin sodium in spiked placebo samples prepared from serial concentrations of phenytoin sodium reference standards. The results summarized in Table 5, show that the proposed ISE method is an accurate one for the determination of phenytoin sodium in their pharmaceutical preparations without interferences from the coformulated adjuvants as indicated by the percentage recovery values.

4.2. Linearity

Under the optimal experimental ISE conditions, linear relationships exist between the electrode potential/mV and the logarithm of corresponding concentration of the drug. The regression data, correlation coefficients (r) and other statistical parameter are previously listed in Table 2.

Table 5. Determination of phenytoin sodium in phenytin-spiked placebo samples using phenytoin-tungstosilicate sensors.

Type of electrodes	Statistical parameters	Sample of placebo capsules	Sample of placebo ampoules
Phenytoin-Tungstosilicate plastic membrane electrode	Mean±SD	99.52±0.622	99.60±0.516
	N	9	9
	RSD	0.625	0.518
	SE	0.207	0.172
Phenytoin-Tungstosilicate coated wire electrode	Mean±SD	99.63±0.467	99.70±0.564
	N	9	9
	RSD	0.469	0.566
	SE	0.156	0.188

4.3. Precision

The precision of the proposed ISE method, measured as percentage relative standard deviation (RSD%) was tested by repeating the proposed method for determination of the investigated drug in its pharmaceutical preparations of "three batches" to nine replicates. The RSD% values for the repeated determinations were 0.490%, 0.793% and 0.519% for determination of phenytoin sodium in Phenytin® ampoule using phenytoin-tungstosilicate plastic membrane electrode and 0.652%, 0.740% and 0.544% in Phenytin® ampoule using electrode phenytoin-tungstosilicate coated wire electrode. The above RSD values are less than 2% indicating good precision.

4.4. Robustness and Ruggedness

The robustness of the proposed ISE method was tested by investigating the capacity of the method to remain unaffected by a small but a deliberate variation in method parameters thereby provide an indication of its reliability during normal usage [23]. While the ruggedness of the proposed method was by investigating the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions such as different laboratories, analysts and instruments. The results obtained using another model of pH-meter (Orion 420 A) were compared with those obtained using

Table 6. Comparative analytical results of the proposed and official method for the tested drug in some pharmaceutical preparations.

Sample	Statistical parameter	Phenytoin-tungstosilicate plastic membrane electrode			Phenytoin-tungstosilicate coated wire electrode		
		Official method [22]	Direct potentiometry		Official method [22]	Direct potentiometry	
			Calibration method	Standard addition method		Calibration method	Standard addition method
Phenytin®100 mg/Capsule Nile Co. for Pharmaceuticals	Mean%		99.23	99.53		99.43	99.20
	N	99.68	6	6	99.07	6	6
	Variance	7	0.766	0.530	7	0.293	0.920
	SD	0.375	0.875	0.728	0.667	0.541	0.959
	SE	0.612	0.357	0.297	0.817	0.221	0.392
	RSD	0.231	0.882	0.731 (0.399)	0.309	0.544	0.967
	"t"	0.614	(1.058)(2.201)*	(2.201)*	0.824	(0.948)(2.201)*	(0.260)(2.201)*
	F		(2.04)(4.39)*	(1.41)(4.39)*		(2.28)(4.39)*	(1.38)(4.39)*
Phenytin®250 mg/ampoule Nile Co. for Pharmaceuticals	Mean%		99.12	99.33		99.21	99.36
	N	99.73	6	6	99.48	6	6
	Variance	6	0.676	0.707	6	0.432	0.712
	SD	0.551	0.822	0.841	0.283	0.657	0.844
	SE	0.742	0.336	0.343	0.532	0.268	0.345
	RSD	0.303	0.829 (1.348)	0.847 (0.874)	0.217	0.662	0.849 (0.294)(2.228)*
	"t"	0.744	(2.228)*	(2.228)*	0.535	(0.783)(2.228)*	(2.52)(5.05)*
	F		(1.23)(5.05)*	(1.28)(5.05)*		(1.53)(5.05)*	

Table 7. Determination of phenytoin sodium in pure form "spiking technique" in human urine and serum using phenytoin-tungstosilicate sensors.

Sample	Statistical parameters	Phenytoin-tungstosilicate plastic membrane electrode		Phenytoin-tungstosilicate coated wire electrode	
		Calibration graphs	Standard addition method	Calibration graphs	Standard addition method
Human urine	Mean recovery%	99.14	98.82	99.28	99.38
	N	5	5	6	6
	Variance	0.949	0.518	0.408	0.801
	SD	0.974	0.720	0.639	0.895
	SE	0.436	0.322	0.261	0.365
	RSD	0.982	0.729	0.644	0.901
Human serum	Mean recovery%	98.94	99.02	99.16	99.06
	N	5	5	6	6
	Variance	0.371	0.870	0.966	0.591
	SD	0.609	0.933	0.983	0.769
	SE	0.272	0.417	0.401	0.314
	RSD	0.616	0.942	0.991	0.776

model of pH-meter (Jenway 3040). The results obtained are close and also reveal validity of the method. The results were previously listed in Table 2.

4.5. Detection limit

The detection limit of the investigated drug was calculated according to IUPAC recommendation which states that the detection limit is the concentration at which the measured potential differs from that predicted by the linear regression by more than 18 mV. The values previously reported in Table 2, indicate that the proposed ISE method is sensitive to detection of very small concentrations of phenytoin sodium.

5. Analytical applications of the proposed method

5.1. Application to pharmaceutical preparations

5.1.1.Phenytoin Sodium Ampoules:

The proposed ISE method was applied to determination of phenytoin sodium in their dosage forms. The mean % recovery and RSD%, indicate that the validated method could be adopted for the determination of the investigated drug in its pharmaceutical preparations without interference from the co-formulated adjuvants. Table 6, shows the results obtained from the determination of phenytoin sodium in its ampoules and comparison with official method [22].

5.1.2. Content uniformity assay of phenytin® ampoules

The proposed ISE method described good accuracy and precision for the quality control tests. The content uniformity assay showed that an R.S.D<2%, with mean standard deviation of 99.41±0.8 and 99.66±0.7 for electrode I, and II respectively.

5.1.3. Application to serum and urine

The proposed ISE method was applied to determination of phenytoin sodium in biological fluids such as human serum and urine. The results obtained are summarized in Table 7.

6. Conclusion

The potentiometric methods developed for the determination of phenytoin sodium have proved to be good and advantageous over the reported analytical methods due to their sensitivity, rapidity and accuracy. The good recoveries and low relative standard deviation reflect the high accuracy and precision of the proposed methods. Moreover, the procedures are simple, easy to operate and it is inexpensive to make the electrodes, therefore, an excellent tool for the routine determination of phenytoin sodium in quality control laboratories asa fast assay in its pharmaceutical preparations and biological fluids.

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