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THIAMINE-TETRAPHENYL BORATE SENSORS FOR DETERMINATION OF THIAMINE HYDROCHLORIDE (VITAMIN B_1) IN ITS PHARMACEUTICAL FORMULATIONS AND BIOLOGICAL FLUIDS

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ABSTRACT

A new validated potentiometric method for determination of thiamine hydrochloride (TH) was developed. The developed method was based on the construction of two types of sensors; coated wire sensor (I) and coated graphite sensor (II). The fabricated sensors were performed using TH-tetraphenylborate (TH-TPB) as electroactive material. All performance characteristics of the fabricated sensors were investigated. The fabricated sensors exhibited Nernstain response (31.7±0.5 and 31.9±0.3 mV decade^{-1}) over concentration ranges of 1.0×10^{-6}-1.0×10^{-1} mol L^{-1} and 1.0×10^{-5}-1.0×10^{-1} mol L^{-1} with limits of detection of 2.7×10^{-7} and 2.5×10^{-6} mol L^{-1} for sensor I and II, respectively. The recorded pH range was 3.5-7.5 for both sensors. The interference of some cations, anions, sugars, amino acids and some pharmaceutical co-formulations were tested, also the effect of some pharmacological action related drugs were studied and no interferences were recorded. The fabricated sensors displayed good isothermal coefficients 0.00165 and -0.00061 V $^{\circ}$C^{-1}. The proposed method showed good results and these results were statistically treated and compared with those obtained from other reported methods. The analytical applications were performed using the fabricated sensors in the determination of TH in dosage forms and biological fluids.

Keywords: Potentiometric method, Thiamine hydrochloride, Coated wire sensor, Coated graphite sensor, Pharmaceutical formulations, Biological fluids.

INTRODUCTION

Ultraviolet (UV) spectroscopy is a physical technique Thiamine hydrochloride (Figure 1) is a vitamin, also called vitamin B_1. Vitamin B_1 is found in many foods, including yeast, cereal grains, beans, nuts, and meat. It is often used in combination with other B vitamins, and found in many vitamin B complex products. Vitamin B complexes generally include vitamin B_1 (thiamine), vitamin B_2 (riboflavin), vitamin B_3 (niacin/niacinamide), vitamin B_5 (pantothenic acid), vitamin B_6 (pyridoxine), vitamin B_{12} (cyanocobalamin), and folic acid. People take thiamine for conditions related to low levels of thiamine (thiamine deficiency syndromes), including beriberi and inflammation of the nerves outside the brain (peripheral neuritis) associated with pregnancy or with a vitamin-deficiency disease called pellagra. Thiamine is also used for digestive problems including poor appetite, ulcerative colitis, and ongoing diarrhea [1].

Several methods have been reported for determination of thiamine hydrochloride including high performance liquid chromatography [2-5], thin layer chromatography [6] spectrophotometry [7-10], potentiomerty [11,12], spectrofluorimetry [13, 14] and chemiluminescence [15].

In recent years, due to the great advantages of electrochemical sensors in monitoring and drug analysis rather than other spectroscopic methods, the researchers gave these sensors much attention. So the aim of the present study is the development of two fabricated sensors; coated wire sensor I and coated graphite sensor II.

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The fabricated sensors were performed using TH-tetraphenylborate as electroactive material and were used for the determination of the investigated drug in bulk form, its pharmaceutical preparations and biological fluids.

EXPERIMENTAL

Materials and reagents

All reagents were of analytical grade. Pure grade of thiamine hydrochloride B1 was purchased from Himedia Lab Co.India. Polyvinyl chloride (PVC) of high molecular weight was purchased from Aldrich, Germany. Methanol 99.0%, acetone 99.9% and tetrahydrofuran (THF) 97.0% were provided by Fluka, Switzerland. Sodium tetraphenylborate (TPB) was purchased from Aldrich. Carbon rod was obtained from Ultra Carbon Co. (Bay City, Mi, USA). The pharmaceutical dosage forms containing thiamine hydrochloride were purchased from local drug stores. Urine samples were collected from healthy volunteers and the human serum used was (Normal Serum HUMATROL N Control 5 mL, Germany).

Equipment

All electrochemical measurements were carried out using HANNA instruments, pH 211 microprocessor pH-meter (Italy). Saturated calomel electrode (SCE) was used as an external reference electrode. Heater with magnetic stirrer Ms-H-S Dragon Lab (USA) was employed for measurements of temperature.

Preparation of analytical solutions

Standard drug solution

A stock thiamine hydrochloride solution of 1.0×10^{-1} mol L^{-1} was prepared by dissolving 0.843 g of drug into 25 mL distilled water. Working solutions 1.0×10^{-7}-1.0×10^{-1} mol L^{-1} were daily prepared by serial dilution using distilled water.

Tablets treatment

To determine TH in its dosage forms, the standard addition method was employed. Not less than twenty tablets of Samavit B_1® 100 mg/tablet or New Rabion® 100 mg/tablet were finally powdered and mixed homogeneously. An accurate amount equivalent to 3.37 g of TH was dissolved in 50 mL distilled water, filtered (15cm, Schleicher & Schuell Germany) in 100-mL measuring flask and the volume was completed to the mark using distilled water. The investigated drug was determined by recording the potential of the fabricated sensors using concentration ranges of 1.0×10^{-6}-1.0×10^{-1} and 1.0×10^{-5}-1.0×10^{-1} mol L^{-1} for sensors I and II, respectively.

Preparation of spiked human urine and serum solutions

In order to determine TH in the human biological fluids, the human serum and urine samples were adjusted at pH 6 using phosphate buffer. 1.0 mL of the adjusted serum or 5.0 mL of urine was spiked by measuring aliquots of TH solutions. Deproteination of the serum samples was done by adding 1.0 mL acetonitrile, 0.1 mL NaOH (0.1 mol L^{-1}) and 1.0 mL of $ZnSO_4.7H_2O$ (5.0% w/v), then the solution was vortex for 5 min. Most interfering species (mainly proteins) were removed by precipitation [16]. Working solutions of ranges 1.0×10^{-6}-1.0×10^{-1} and 1.0×10^{-5}-1.0×10^{-1} mol L^{-1}were prepared after centrifugation for 30 min at 3500 rpm and the supernatant layer was filtered through 0.5 µm Milli-pore filter, by appropriate dilution with distilled water and then the analysis for samples carried out using general analytical procedures.

Preparation of thiamine-tetraphenylborate ion pair

The ion-pair TH-TPB was prepared by mixing 50 mL of equimolar 1.0×10^{-2} mol L^{-1} of sodium tetraphenyl borate (TPB) with TH. The resulting precipitate was left overnight to ensure complete precipitation and coagulation, then filtered and washed thoroughly with distilled water and left to dry for 24 h. Elemental analysis was carried out to confirm the composition of the ion-pair $[C_{12}H_{17}\ ClN_4OS][C_{24}H_{20}BNa]$. The calculated percentages of C, H and N are 67.22%, 5.80% and 8.72%, while, the found percentages are 67.76%, 5.84% and 8.69%, respectively. The obtained results revealed 2:1 [TH: TPB] ion pair.

Sensor construction

Preparation of coated wire sensor

Pure Copper wire of 4 cm length was tightly insulated by polyethylene tube leaving 1 cm at one end for the coating and 0.5 cm at the other end for connection. Prior to coating, the polished copper surface was washed with a detergent, thoroughly rinsed with distilled water and dried with acetone. Then the wire was rinsed with chloroform and allowed to dry. Afterwards, the copper wire was coated by quickly dipping it into the coating solution [10 mg ion-pair (TH-TPB), 190 mg polyvinylchloride (PVC) and 0.35 mL plasticizer di-butylphthalate (DBP)] several times and allowing the film left on the wire to dry for about 5 min. The fabricated sensor was preconditioned by soaking for 6 h in 1.0×10^{-3} mol L^{-1} TH solution. All potentiometric measurements were performed using the following cell assembly: Cu/membrane/test solution//KCl salt bridge//SCE.

Preparation of coated graphite sensor

The coated graphite sensor was prepared by insulating a pure graphite rod 4.0 cm length and 4.0 mm diameter using a tight polyethylene tube. The polished sensor surface was coated with the active membrane by

dipping the exposed end into the coating solution that was described in (2.5.1) for ten times and allowing the film left on a graphite rod to dry in air for 1 min each time. The prepared electrode was preconditioned by soaking for 10 h in $1.0x10^{-3}$ mol L^{-1} TH solution [17]. All potentiometric measurements were performed using the following cell assembly: Graphite rod/ membrane / test solution // SCE.

Sensor calibration

All potentiometric measurements were carried out using the proposed sensors in conjunction with SCE reference. The potential of each sensor was recorded over the concentration range of 1.0×10^{-6}-1.0×10^{-1} mol L^{-1}. The calibration graphs were plotted using the recorded potential readings against the logarithm of drug concentrations.

Standard addition method

The standard addition method was employed for determination of TH in its dosage forms. The method based on adding small increments of the investigated drug test solution vs. the sensor potential. Each fabricated sensor was immersed into 50 mL drug test solution with unknown concentration and the equilibrium potential of E_1 was recorded. Then 0.1 mL of the standard drug solution was added into the testing solution and the equilibrium potential E_2 was recorded. The concentration of the testing sample can be obtained from the change of potential

($\Delta E = E_2$-E_1).

RESULTS AND DISCUSSION

Nature and response characteristics of the fabricated sensors

Thiamine hydrochloride was reacted with sodium tetraphenylborate to form TH-TPB as water insoluble electroactive material. The prepared ion-pair was soluble in several organic solvents such as tetrahydrofuran (THF). The nature and performance characteristics of the fabricated sensors were investigated. As shown in Figure 2, the investigated sensors displayed Nernstian responses of 31.7 ± 0.5 and 31.9 ± 0.3 mV $decade^{-1}$ at 25˚C over drug concentration ranges of 1.0×10^{-6}-1.0×10^{-1} and 1.0×10^{-5}-1.0×10^{-1} mol L^{-1} with lower detection limits of 2.7×10^{-7} and 2.5×10^{-6} mol L^{-1} for sensors I and II, respectively. The proposed sensors displayed dynamic responses of TH concentration ranges of 1.0×10^{-6} -1.0×10^{-1} and 1.0×10^{-5}-1.0×10^{-1} mol L^{-1} for coated wire and coated graphite sensors, respectively. The recorded dynamic responses for the fabricated sensors were ≥15 and ≤25 s for a lifetime of 31 and 25 days for sensors I and II, respectively, without significant change in the sensor parameters. Table 1 showed that the obtained results revealed better performance characteristics for TH-TPB coated wire than coated graphite sensor in the terms relevant to a wide linear concentration range, limit of detection, response time and life time.

Effect of plasticizers

The effect of plasticizers on the performance characteristics of fabricated sensors was studied. The effect of plasticizers was investigated by using four different types DOP with dielectric constant (ε = 5.1), DBP (ε = 6.4), DBS (ε = 4.5) and DOS (ε = 4.0) were examined. Table 2 showed the effect of plasticizers on the slopes of TH-TPB sensors. It is clear that the use of DBP as plasticizer provides good performance characteristics of the sensors. This can be attributed to the higher dielectric constant (ε = 6.4) of DBP than other plasticizers. On the other hand, the plasticizers play an important role in the improvement of sensor performance by increasing their permeable properties and improve their mechanical stability.

Effect of pH

The effect of pH on the fabricated sensors was examined using 1.0×10^{-3} mol L^{-1} TH solution. The selected drug solution was acidified using 0.1 mol L^{-1} hydrochloric acid and the pH was gradually increased using 0.1 mol L^{-1} sodium hydroxide. The potential readings were plotted vs. pH and the proposed sensors displayed safe pH range of 3.5-7.5 as Cleared in Figure 3. It was noticed that below pH 3.5 the potential reading was decreased due to the interference of H^+ ion. While, at higher pH more than 7.5 the sensors displayed a sharp decrease in potential due to the effect of OH^- on the test solution.

Selectivity of sensors

The effect of different possible interference on the response of the fabricated TH-TPB sensors were carefully investigated using a separate solution method [18]. Different common cations, sugars, amino acids and some additive substances were tested. The influence of any related pharmacological action drugs was also examined. The following equation was applied for the calculation of selectivity coefficients of the proposed sensors:

$$\text{Log } K^{pot}_{TH}\, J^{z+} = (E_2 - E_1)/S + \log [TH] - \log [J^{z+}]^{1/z}$$

Where, E_1 is the electrode potential in 1.0×10^{-3} mol L^{-1} TH solution, E_2 is the potential of the electrode in 1.0×10^{-3} mol L^{-1} solution of the interfering ion J^{z+} and S is the slope of the calibration plot. The results reported in Table 3 clarified the high selectivity of the fabricated sensors. The main mechanism of selectivity is dependent on the matching between the locations of lipophilic sites in the two competing species in the bathing solution side and those present in the receptor of ion pair. The obtained results showed an insignificant interference effect during the determination of TH. Also, the effect of riboflavin, vitamin B_5, nicotinamide, pyridoxine and vitamin B_{12} was tested and no interference was recorded.

Effect of temperature on the sensors performance

The effect of temperature of the TH test solution on sensor performance was studied by plotting the

calibration graphs (sensor potential vs. p^{TH}) at different test solution temperatures (25, 30, 40, 50, 60 and 70˚C) for both sensors. Table 4 summarized the slopes, usable concentration ranges and the standard sensor potential (E°) at each temperature.

To determine the isothermal coefficients (dE°/dt) of the sensors, the standard sensor potentials (E°) against the normal hydrogen electrode at the different temperatures were measured. This can be carried out by plotting the calibration graphs as the intercepts at $p^{TH} = 0$ (after subtracting the values of the standard sensor potential of a calomel electrode at these temperatures) and plotting vs, (*t-25*) where *t* is the temperature of test solution in $^{\circ}$C. The obtained straight line was according to the following equation [19]:

$$E^{\circ} = E^{\circ}(25) + (dE^{\circ}/dt)\,(t-25)$$

The isothermal coefficients were represented from the slopes of the straight lines obtained. They were amounted to 0.00165 and - 0.00061V$^{\circ}$C^{-1} for sensors I and II, respectively. These low values were revealed that the TH-TPB sensors have high thermal stability within the studied temperature range (25-70°C).

Effect of soaking time

The optimum soaking time for the fabricated sensors was tested by immersing each sensor in 1.0×10^{-3} mol L^{-1} drug solution for 24 h. The optimum soaking time was found to be 6 and 10 h, at which the slopes of the calibration curves were 31.7±0.5 and 31.9±0.3 mV decade^{-1} at 25 ºC for sensors I and II, respectively. Upon prolonged soaking for different intervals 7, 15, 25, 30, and 35 days the slopes were slightly decreased to be 30.8 and 29.7 mV decade^{-1} after 15 days. The continuous soaking for 35 days caused a sharp decrease in sensors potential to reach 28.5 and 27.3 mV decade^{-1} for TH-TPB coated wire and coated graphite, respectively. The obtained results revealed that the coated wire sensor has a longer life span than coated graphite sensor.

The regeneration of TH-TPB sensors was successfully carried out by soaking the exhausted sensor for 24 h in 1.0×10^{-2} mol L^{-1} TPB, followed by 3 h in 1.0×10^{-2}mol L^{-1} TH solution. After regeneration the fabricated TH-TPB sensors displayed a potential response of 30.6 and 29.5 mV decade^{-1} for sensors I and II, respectively. It was found that the life span of the regenerated sensor is limited to 4 and 2 h for the previously mentioned sensors (Figure 4).

Analytical applications

Quantification of thiamine hydrochloride

Thiamine hydrochloride was determined using a direct calibration method in its bulk form. The obtained results were calculated as the mean % recoveries using the fabricated TH-TPB coated wire and coated graphite sensors. As shown in Table 5 the calculated results were 99.8±0.7 and 99.3±0.5 for sensors I and II, respectively. Furthermore, to encourage the obtained results, the proposed method was applied for the determination of TH in its pharmaceutical preparations; the results were 99.6±0.8 and 99.4±0.8 for the above mentioned sensors.

The proposed method was successfully employed for the determination of TH in biological fluids. The results obtained for determination of TH in urine were 99.2±0.4 and 98.8±0.7, while in human serum the recorded results were 99.3±0.7 and 98.9±0.6 for sensors I and II, respectively.

The evaluation of the proposed method was carried out by applying statistical analysis of the obtained results using student's *t*- and *F*- tests at the 95.0% confidence level [20]. Table 6 showed that the obtained results were in good agreement with those obtained from a reported spectrophotometric method [7].

Content uniformity assay of tablets

The fabricated TH-TPB coated wire and coated graphite sensors were used to study the content uniformity assay of TH in its tablets. Ten individual tablets (Samavit ® 100 mg/tablet) were placed in separate 100-mL beakers and dissolved in 100 mL distilled water. The prepared solutions were subjected directly to the fabricated sensors to measure the investigated drug. The mean potential was used to evaluate the content uniformity from the calibration graph. The obtained results obtained as % recoveries and standard deviations were 98.6±0.9 and 98.3±1.3 for sensors I and II, respectively.

Dissolution test

The dissolution test of TH in its tablets (Samavit B_1® 100 mg/ tablet) was carried out according to USP [21] using Type II dissolution apparatus (Erweka, Germany). The dissolution medium was 900 mL distilled water and the temperature was adjusted at 37.0±0.5˚C. 50 rpm rotation was used for 1 h. Each TH-TPB coated wire and coated graphite in conjunction with standard calomel reference electrode was used as a detection system for recording the potential at different time intervals. After 45 min, the amounts of TH released were calculated from the calibration curve and represented by the dissolution profile as shown in Figure 5. The results obtained were 88 and 85%. These results meet the requirements of pharmaceutical manufacturer that require not less than 75% of the drug should be dissolved within 45 min.

Method validation

According to ICH guidelines [22], the proposed method was validated for linearity, lower limit of detection, accuracy, precision, robustness and ruggedness.

Linearity and lower limit of detection

In order to verify the method linearity, TH test solutions ranging from $1.0X10^{-6}$ -1.0×10^{-1} mol L^{-1} were subjected to TH-TPB sensors detection system. The

linearity was determined by plotting the potential readings against –log concentration of TH. The results obtained clarified that the constructed sensors displayed Nernstian response over linear concentration ranges of 1.0×10^{-6}-1.0×10^{-1} and 1.0×10^{-5}-1.0×10^{-1} mol L^{-1} for sensors I and II, respectively. The detection limit was calculated according to IUPAC recommendation which stated 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 detection limits were found to be about 2.7×10^{-7} and 2.5×10^{-6} mol L^{-1} for sensors I and II, respectively.

Accuracy and precision

To evaluate the accuracy of the proposed method, the investigated drug was determined in the presence of its coformulated substance starch using the standard addition method. The obtained results were calculated in terms of % recovery values. The calculated % recoveries were 99.4±0.7 and 99.2±0.9 for sensors I and II, respectively indicating good accuracy.

The precision of the proposed method was tested by intra-day and inter-day terms. The studies were performed by repeating the determination to nine replicates. The calculated %RSD values were 0.92 % and 0.37% for determination of TH in Samavit $B_1^{®}$ 100 mg/tablet using TH-TPB coated wire and coated graphite sensors. The above %RSD values are less than 2% indicating good precision.

Robustness and ruggedness

In order to study the robustness of the proposed method, phosphate buffer pH 7.5±1 was used to introduce small changes in pH. The pH of the investigated drug solutions was adjusted at pH 7.5±1 and the percentage recoveries were calculated. The obtained results were 99.7±0.9 and 99.1±0.7 for sensors I and II, respectively. These results were closely in agreement with those obtained from standard drug solutions.

The investigation of the ruggedness of the proposed method was carried out using another pH-meter (Jenway 3510). The recorded results as % recoveries were 99.4±0.6 and 99.2±0.4 for the previously mentioned sensors, respectively which indicates that the proposed method is rugged.

CONCLUSION

New electrochemical sensors for determination of thiamine hydrochloride have been proposed. The fabrication of these sensors was mainly based on the use of TH-TPB as electroactive material. Thiamine hydrochloride in bulk form, its pharmaceutical dosage forms and in biological fluids was subjected to investigation using TH-TPB coated wire and coated graphite sensors. The obtained results revealed good sensitivity and reproducibility for determination of thiamine hydrochloride. The recorded data were statistically treated and compared with those obtained from other reported methods.

Table 1. Critical response characteristics of TH-TPB coated wire and coated graphite sensors

Parameter[a]	**TH-TPB coated wire Sensor I**	**TH-TBP coated graphite Sensor II**
Slope (mV decade^{-1})	31.7	31.9
Intercept	14.33	350.7
Regression equations	E_{mV} = (31.7±0.5) log[TH] + 14.33	E_{mV} = (31.9±0.3) log[TH] + 350.7
Correlation coefficient (r)	0.9997	0.9995
Linear range (mol L^{-1})	1.0×10^{-6}-1.0×10^{-1}	1.0×10^{-5}-1.0×10^{-1}
Lower limit of detection	2.7×10^{-7}	2.5×10^{-6}
Response time/s	≤ 15	≤ 25
Working pH range	3.5-7.5	3.5-7.5
Life time/day	31	25
Temperature ˚C	25˚C	25˚C
Accuracy (%)	99.4±0.7	99.2±0.9
Robustness[b]	99.7±0.9	99.1±0.7
Ruggedness[c]	99.4±0.6	99.2±0.4

[a]Mean of six measurements [b]A small variation in method parameters were carried out at pH of phosphate buffer (pH 7.5±1). [c]Comparing the results with those obtained by different sensors assemblies using Jenway 3510 pH meter

Table 2. Effect of plasticizers on slopes with TH-TPB coated wire and coated graphite sensors

Slope mV decade^{-1}		
Plasticizer	**TH-TPB coated wire Sensor I**	**TH-TPB coated graphite Sensor II**
DOS	28.4	27.9
DBS	29.7	28.7
DBP	31.7*	31.9*
DOP	30.2	30.1

**The optimum value for the studied sensors*

Table 3. Selectivity coefficients for TH-TPB coated wire and coated graphite sensors by a separate solution method using (1.0×10^{-3} mol L^{-1} TH solution)

Interferent	$K^{Pot}_{TH^+}$	
	TH-TPB coated wire Sensor I	TH-TPB coated graphite Sensor II
Na^+	6.7×10^{-3}	5.4×10^{-3}
Cu^{2+}	5.3×10^{-3}	2.6×10^{-4}
Ba^{2+}	0.3×10^{-2}	1.9×10^{-2}
PO_4^{3-}	5.1×10^{-2}	1.6×10^{-3}
Ag^+	5.0×10^{-3}	2.1×10^{-4}
Riboflavin	8.4×10^{-2}	2.1×10^{-3}
Vitamin (B_5)	1.0×10^{-3}	5.2×10^{-4}
Nicotinamide	1.6×10^{-3}	8.1×10^{-3}
Pyridoxine	5.2×10^{-4}	0.9×10^{-4}
Vitamin B_{12}	2.4×10^{-2}	1.2×10^{-3}
Sod. diclofenac	2.1×10^{-3}	4.1×10^{-3}
Fructose	4.6×10^{-3}	3.3×10^{-3}
Lactose	1.3×10^{-3}	1.3×10^{-3}
Sucrose	1.6×10^{-3}	2.3×10^{-3}
Glucose	2.1×10^{-3}	5.9×10^{-3}
Aspartic acid	3.4×10^{-4}	4.4×10^{-3}
Histadine	2.8×10^{-4}	6.3×10^{-4}
Glycine	4.0×10^{-3}	2.3×10^{-4}
Cystine	3.4×10^{-4}	1.7×10^{-3}
Starch 0.1%	1.0×10^{-4}	2.1×10^{-4}

Table 4. Performance characteristics of TH-TPB coated wire and coated graphite sensors at different temperatures

Type of Sensors	Temperature °C	Slope/mV decade^{-1}	Usable concentration range	E°/mV*
TH-TPB coated graphite Sensor I	25	31.7	1.0×10^{-6}-1.0×10^{-1}	180
	30	32.3	5.0×10^{-6}-1.0×10^{-2}	188
	40	35.2	5.0×10^{-6}-1.0×10^{-2}	204
	50	36.2	5.0×10^{-6}-1.0×10^{-2}	220
	60	36.9	5.0×10^{-6}-1.0×10^{-2}	239
	70	38.0	1.0×10^{-5}-1.0×10^{-3}	257
TH-TPB coated wire Sensor I	25	31.9	1.0×10^{-5}-1.0×10^{-1}	-117
	30	32.8	5.0×10^{-6}-1.0×10^{-1}	-120
	40	34.4	5.0×10^{-6}-1.0×10^{-2}	-125
	50	35.9	5.0×10^{-6}-1.0×10^{-2}	-131
	60	37.2	5.0×10^{-6}-1.0×10^{-2}	-135
	70	40.5	1.0×10^{-5}-1.0×10^{-3}	-142

**E°: Standard sensor potential against the normal hydrogen electrode (NHE)*

Table 5. Statistical treatment of data obtained from determination of TH in bulk form, dosage forms and biological fluid samples using TH-TPB sensors

Samples	TH-TPB coated wire Sensor I			TH-TPB coated graphite Sensor II		
	Taken -log conc. mol L^{-1}	Found -log conc. mol L^{-1}	Recovery %	Taken -log conc. mol L^{-1}	Found -log conc. mol L^{-1}	Recovery %
Pure drug	6.0	6.01	100.2	5.0	4.99	99.8
	5.0	4.99	99.8	4.3	4.26	99.1
	4.0	3.97	99.3	4.0	3.96	99.0
	3.0	2.96	98.7	3.0	2.97	99.0
	2.0	2.01	100.5	2.0	2.00	100.0
	1.0	1.00	100.0	1.0	0.99	99.0

*%Mean±SD	99.8±0.7			99.3±0.5		
n	6			6		
Variance	0.49			0.25		
%SE	0.28			0.20		
%RSD	0.70			0.50		
Samavite B_1® tablets (100mg/tablet)	5.0	4.94	98.8	5.0	4.96	99.2
	4.3	4.28	99.5	4.3	4.25	98.8
	4.0	3.95	98.8	4.0	3.93	98.3
	3.3	3.29	99.7	3.3	3.29	99.7
	3.0	2.99	99.7	3.0	3.02	100.7
	2.0	2.02	101.0	2.0	1.99	99.5
*%Mean±SD	99.6±0.8			99.4±0.8		
n	6			6		
Variance	0.64			0.67		
%SE	0.33			0.33		
%RSD	0.80			0.82		
Serum sample	5.0	4.96	99.3	5.0	4.98	99.6
	4.3	4.22	98.2	4.3	4.26	99.1
	4.0	3.97	99.3	4.0	3.93	98.3
	4.3	4.31	100.2	4.3	4.28	99.5
	3.0	2.97	99.1	3.0	2.97	99.0
	2.0	1.99	99.6	2.0	1.96	98.0
*%Mean±SD	99.3 ± 0.7			98.9± 0.6		
n	6			6		
Variance	0.49			0.36		
%SE	0.29			0.24		
%RSD	0.70			0.60		
Urine sample	5.0	4.96	99.2	5.0	4.98	99.6
	4.3	4.25	98.8	4.3	4.24	98.6
	4.0	3.99	99.8	4.0	3.96	99.0
	4.3	4.28	99.5	4.3	4.27	99.3
	3.0	2.96	98.7	3.0	2.97	99.0
	2.0	1.98	99.0	2.0	1.95	97.5
*%Mean±SD	99.2 ± 0.4			98.8± 0.7		
n	6			6		
Variance	0.16			0.49		
%SE	0.16			0.29		
%RSD	0.40			0.70		

**Mean of five measurements*

Table 6. Determination of Thiamine HCl in pure form and pharmaceutical formulations in comparison with reference method [7] using TH-TPB coated wire and coated graphite sensors

Sample	TH-TPB coated wire Sensor I	TH-TPB coated graphite Sensor II
Pure form		
*%Mean±SD	98.8±0.7	99.3±0.5
t-test	1.58 (2.23)*	0.75 (2.23)*
F-test	2.94 (5.05)*	4.80 (5.05)*
Samavite B_1® tablets (100mg/tablet)		
*%Mean±SD	99.6±0.8	99.4±0.8
t-test	1.79 (2.23)*	1.43(2.23)*
F-test	1.89 (5.05)*	1.89 (5.05)*
Reported method [7]		
Pure form	98.9±1.2	
Pharmaceutical formulations	98.6±1.1	

***The figures between parentheses are the theoretical values of " t" and "F" at P=0.05*

Figure 1. Chemical structure of thiamine hydrochloride

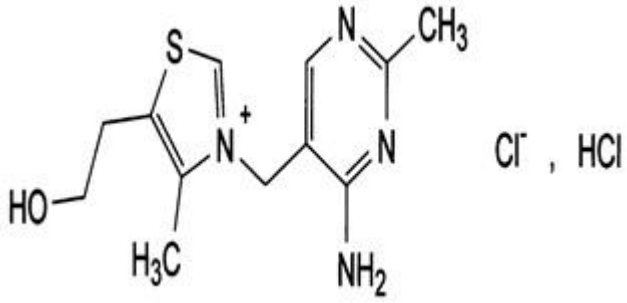


Figure 2. Typical calibration graph of TH-TPB Coated wire and coated graphite sensors

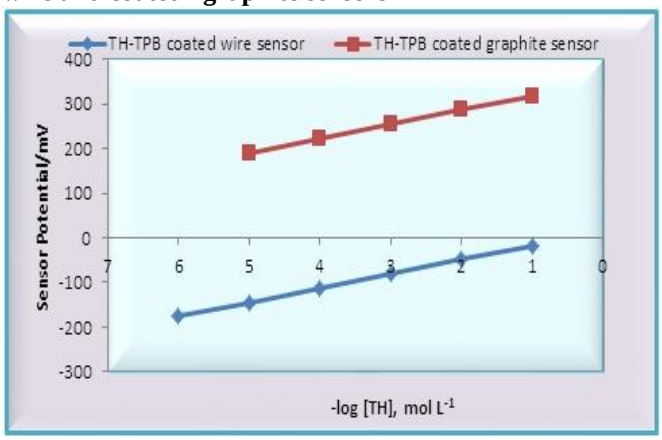


Figure 3. Effect of pH on the potentials of TH-TPB coated wire and coated graphite using $1.0x10^{-2}$ and $1.0x10^{-4}$ mol L^{-1} TH solutions

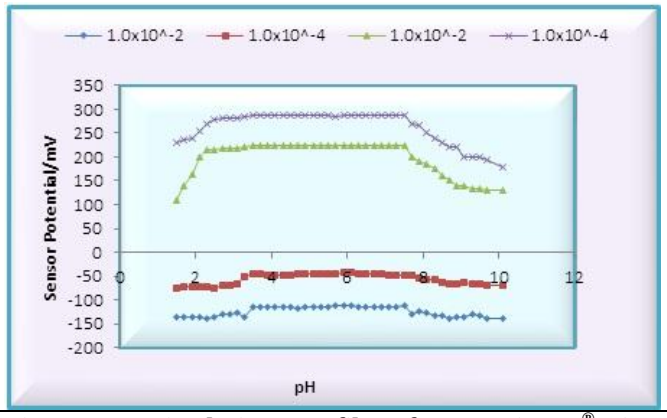


Figure 4. Regeneration of TH-TPB coated wire and coated graphite sensors

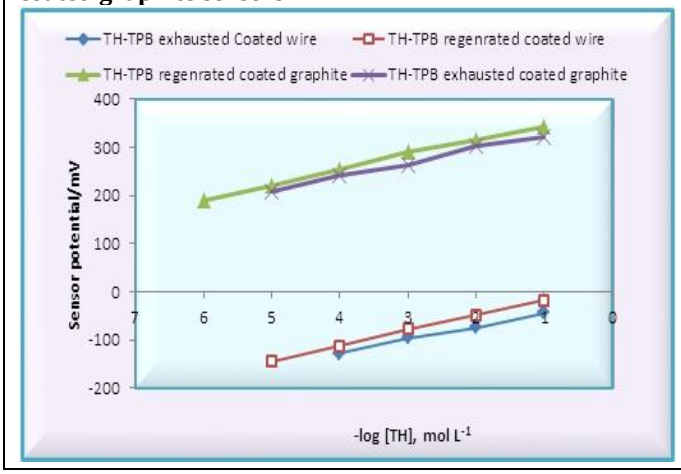
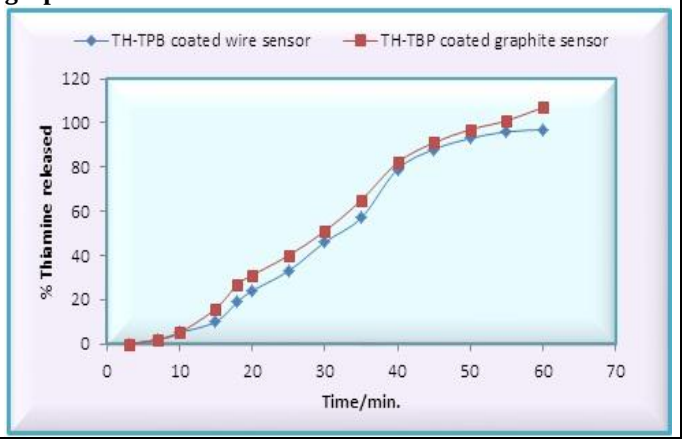


Figure 5. Dissolution profile of Samavit B_1® (100 mg/tablet) using TH-TPB coated wire and coated graphite sensors



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