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Ultrasensitve Modified Carbon Paste Inclusion β -Cyclodextrin and Carbon Nanotubes Sensors for Electrochemical Detection of Anticancer Nimustine Hydrochloride

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In this study, novel carbon paste and modified carbon paste inclusion β- cyclodextrin and carbon nanotubes sensors for determination of nimustine hydrochloride was introduced. Three different carbon paste sensors were fabricated based on the incorporation of nimustine hydrochloride with sodium tetraphenylborate to produce nimustine-tetraphenylborate as electroactive material. Ortho-nitrophenyloctyl ether (*o*-NPOE) was added as plasticizer. Sensor I was a conventional carbon paste type, while sensor II and III were modified carbon sensors using β-cyclodextrin and carbon nanotubes, respectively. The fabricated sensors were displayed Nernstian responses 55.8±0.8, 57.7±0.3 and 58.2±0.1 mV decade^{-1} over concentration ranges of 1.0×10^{-5}-1.0×10^{-2}, 1.0×10^{-7}-1.0×10^{-2} and 1.0×10^{-8}-1.0×10^{-2} mol L^{-1} for sensors I, II and III, respectively. The detection limits were 4.8×10^{-6}, 5.0×10^{-8} and 7.9×10^{-9} mol L^{-1}, respectively. The critical characteristics of the fabricated sensors were investigated. The developed method was validated according to ICH guidelines and the recorded data were statistically assessed and compared with those obtained from other reported methods.

Keywords: Nimustine hydrochloride; Potentiometry; Carbon paste sensors; Modified carbon nanotubes sensors; 2-Hydroxypropyl β-cyclodextrin

1. INTRODUCTION

Nimustine hydrochloride (NMT) is a nitrosourea compound with antineoplastic activity, used as alkylating agent which crosslinked with DNA leading to DNA fragmentation. The main target of this agent is the inhibition of the protein synthesis and hence causes cell death [1]. It is effective against malignant brain tumors and used as a cytostatic agent in cancer therapy, also used in

combination with other neoplastic agents in the treatments of various neoplasms [2]. It is chemically known as N'-[4-amino-2-methyl-5-pyrimidinyl) methyl-N-(2-chloroethyl)-N-nitrosourea hydrochloride (Figure 1).

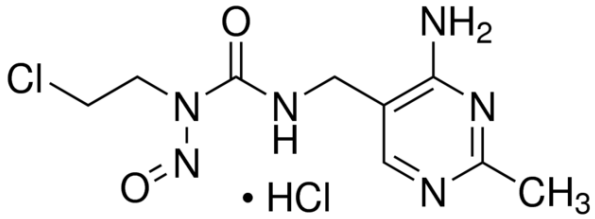

Figure 1. Chemical structure of nimustine hydrochloride

The literature survey reported few analytical methods for determination of NMT. The chromatographic separation techniques were reported using high performance liquid chromatography [3, 4]. Also, other methods have been reported such as X-ray powder diffraction and solid-state NMR [5] and spectrophotometry [6].

The remarkable ability of oligosaccrides to produce inclusion complexes with various molecules may be attributed to that cyclic oligosaccraide compounds posses different cavity size [7]. The literature survey revealed many analytical applications such as drug analysis [8], catalysis [9], food analysis [10] and medicine [11].

The extraordinary properties including chemical, electrical, mechanical and structural properties of carbon paste (CP) sensors and modified carbon nanotubes (MCNTs) sensors gave us great attention in recent years [12].

Many applications of these sensors have been reported in electrochemical analysis [13], sensors [14] and medicinal applications [15].

The present study aims to introduce novel, simple, highly sensitive carbon paste sensors for determination of NMT. The developed sensors based on the modification of the conventional carbon paste sensor with β-cyclodextrin and carbon nanotubes. Method optimization and the critical performance were studied using the bulk form of the selected drug, its pharmaceutical formulations and biosamples.

2. EXPERIMENTAL

2.1. Materials and reagents

Pure grade of NMT was provided by (Tokyo chemical industry Co., Japan). 2-hydroxypropyl β-cyclodextrin (β-CD), sodium tetraphenylborate (TPB) 99.9%, high molecular weight of polyvinyl chloride (PVC), high purity graphite powder with particle size (1-2 μm) and multi-wall carbon nanotubes powder (carbon >95.0%, O.D. × L 6-9 nm × 5 μm) were purchased from (Sigma-Aldrich, Hamburg, Germany). Different kinds of plasticizers such as *o*-nitrophenyloctyl ether (*o*-NPOE)

99.0%, di-butyl phthalate (DBP) with purity 99.0%, di-octylphthalate (DOP) 99.5%, di-butyl sebacate (DBS) ≥ 97.0%, di-octyl sebacate (DOS) ≥ 97.0% and tetrahydrofuran (THF) were provided by (Fluka, Switzerland). Other chemicals such as acetonitrile, hydrochloric acid 36.5% and sodium hydroxide pellets 98.0% were purchased from (BDH laboratory supplies, Philadelphia, USA). Phosphate buffer was prepared using potassium monobasic and potassium dibasic phosphates as well as Zinc sulfate ≥ 99.0% were purchased from (WinLab suppliers, East Midlands, UK). Nimustine hyrochloride® 50 mg/vial were obtained from local drug stores. The urine samples were provided by healthy volunteers. While, serum samples were obtained from commercial sources (Multi-Serum Normal, Randox Laboratories, UK). Finally, all the above chemicals were of analytical grade and used without further purifications.

2.2. Instruments

HANNA instruments model-211 microprocessor pH-meter (Romania) was used for potentiometric and pH measurements. AREX heating magnetic stirrer connected with a circulator thermostat was used to control the temperature of the test solutions. Ag/AgCl electrode was connected to the system as an external reference electrode. Ivyman distiller system -AC-L8 was used for providing distilled water. The surface structure of carbon nanotubes sensor was examined using scanning electron microscope (SEM), JEOL JSM-6060 LV-(Japan).

2.3. Preparation of analytical solutions

2.3.1. Standard drug solution

A daily freshly prepared stock solution of 1.0×10^{-1} mol L^{-1} NMT was applied for the analytical uses. This solution was prepared by dissolving 0.3 g in 10 mL distilled water. Serial dilutions using distilled water were carried out to obtain working solutions in the range of 1.0×10^{-8}-1.0×10^{-1} mol L^{-1}.

2.3.2. Preparation of nimustine hydrochloride injection solution

The content of 10 vials (Nimustine hydrochloride®50 mg/vial) was mixed well. The molar concentration of NMT solution was prepared by transferring an accurate amount of the vial contents to a 10-mL volumetric flask and dissolved in 10 mL distilled water. The prepared solution was centrifuged at 2500 rpm for 10 min and then filtered to obtain 1.0×10^{-1} mol L^{-1} NMT solution. Working solutions were prepared by serial dilutions using distilled water to obtain NMT concentration ranges of 1.0×10^{-5}-1.0×10^{-2}, 1.0×10^{-7}-1.0×10^{-2} and 1.0×10^{-8}-1.0×10^{-2} mol L^{-1} for sensors I, II and III, respectively.

2.3.3. Preparation of human serum and urine samples

The fabricated NMT-TPB sensors were applied for the detection of NMT in human serum and urine using the spiking technique method. The pH of human serum and urine samples was adjusted to pH 6 using phosphate buffer and spiked with an accurate amount of the investigated drug. 1.0 mL of the previously spiked human serum was treated with 1.0 mL of acetonitrile, 0.1 mL of NaOH (0.1 mol L^{-1}) followed by 1.0 mL of $ZnSO_4.7\ H_2O$ (5.0% w/v) for deprotination. Centrifugation was done at 3500 rpm for 25 min. The clear layer was filtered using membrane filter 0.5 Milli-pore. No further treatment was required after dilution of human urine with distilled water and the prepared samples were subjected to analysis without further treatment.

2.3.4. Preparation of nimustine-tetraphenylborate ion pairs

To prepare the electroactive material NMT-TPB, 50 mL of 1.0×10^{-2} mol L^{-1} NMT solution was mixed with 50 mL of TPB solution. The obtained ion-pair was filtered, washed using distilled water and then left to dry for 24 h at room temperature. The prepared electroactive material was confirmed with respect to the % of C, H and N using elemental analysis. From the obtained data it was found that the ion pair was $[C_9H_{13}ClN_6O_2]$ $[(C_6H_5)_4B]$ which indicated that the formed ion pair NMT: TPB was found to be 1:1 and the percentages of C, H and N were calculated to be 66.94%, 5.62% and 14.19%, respectively. The found percentages of the previously mentioned elements were 66.89%, 5.63% and 14.06% for C, H and N, respectively.

2.5. Sensor fabrication

2.5.1. Fabrication of carbon paste, modified carbon nanotubes and B-cyclodextrin carbon paste sensor

A carbon paste sensor was simply fabricated using about 60% of pure graphite powder (1-2 μm), 30.0% *o*-NPOE as liquid plasticizer and 10.0% of NMT-TPB as ion pair. To obtain a homogenous paste the above materials were mixed well and carefully packed in a Teflon holder (3.0 mm in diameter). A copper rod was connected to the electrical contact. Before employing the sensor for potentiometric measurements, Tissue paper was used to polish its surface. A shiny, smooth and reproducible surface was obtained and the sensor was preconditioned in 1.0×10^{-3} mol L^{-1} NMT solution for 8 h. Carbon paste/test solution/Ag/AgCl reference electrode was used as the cell assembly for all potentiometric measurements. The modification of the sensor using carbon nanotubes was done by adding a small amount of multi-wall carbon nanotube particles and the paste was homogeneously mixed and the procedure as previously mentioned was used. As illustrated in Figure 2, the surface structure of carbon paste sensors was investigated using scanning electron microscope (SEM). The modified β-CD carbon paste was prepared by adding 50 mg of 2-hydroxypropyl β-CD with the content of carbon paste and also, the previously mentioned procedure was followed.

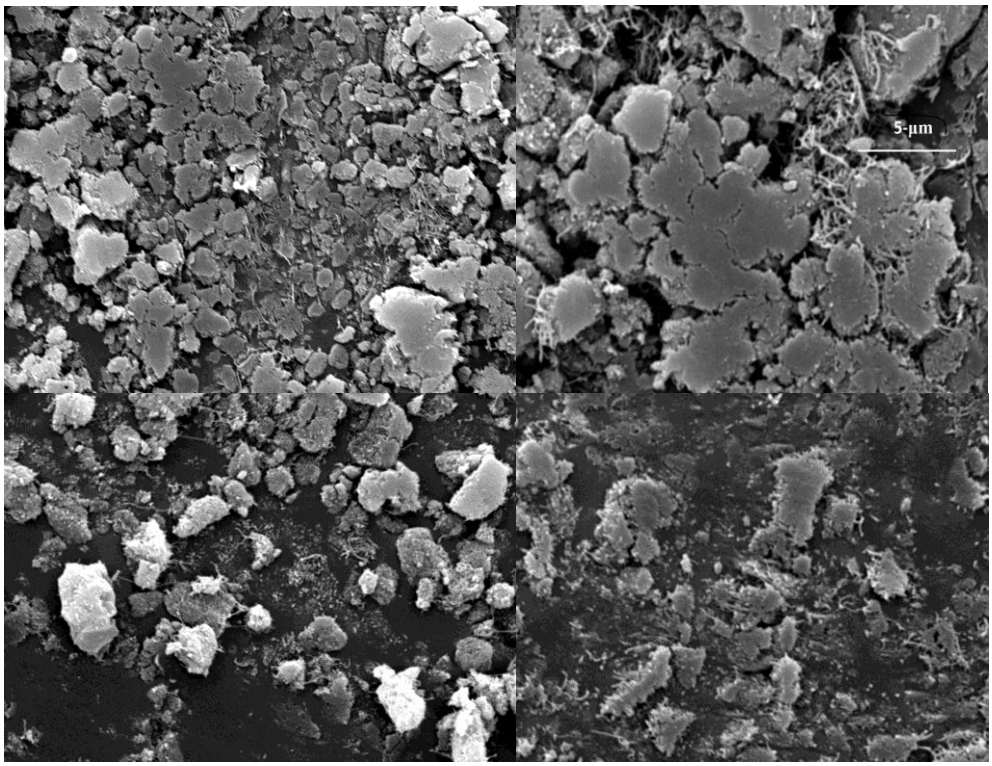

Figure 2. The surface structure of carbon paste sensors using SEM

2.6. Sensor calibration

Sensor calibration curves were plotted for all potential measurements as a function of -logarithm of drug concentrations using the fabricated NMT-TPB carbon paste sensors. Working solutions containing 1.0×10^{-8}-1.0×10^{-1} mol L^{-1} of NMT were employed.

2.7. Standard addition method

To investigate the selected drug in its dosage forms, standard addition method [16] was employed. Small increments of NMT solution were added and the potential of each fabricated sensor was recorded. The concentration of the testing sample can be obtained from the change of potential ($\Delta E = E_2$-E_1). Where, E_1 is the sensor potential of the sample and E_2 is the sensor potential after adding the drug.

3. RESULTS AND DISCUSSION

3.1. Performance characteristics of nimustine sensors

The introduced sensors were fabricated using the electroactive material NMT-TPB which is insoluble in water, but soluble in organic solvents such as THF. The performance characteristics were

investigated with respect to responses and linear relationships, Figure 3, showed that the fabricated sensors were displayed Nernstian responses of 55.8±0.8, 57.7±0.3 and 58.2±0.1 mV decade^{-1} at 25˚C over drug concentration ranges of 1.0×10^{-5}-1.0×10^{-2}, 1.0×10^{-7}-1.0×10^{-2} and 1.0×10^{-8}-1.0×10^{-2} mol L^{-1} for sensors I, II and III, respectively. The lower detection limits for the constructed sensors were found to be 4.8×10^{-6}, 5.0×10^{-8} and 7.9×10^{-9} mol L^{-1} for sensors I, II and III, respectively. The critical performance characteristics data were listed in Table 1.

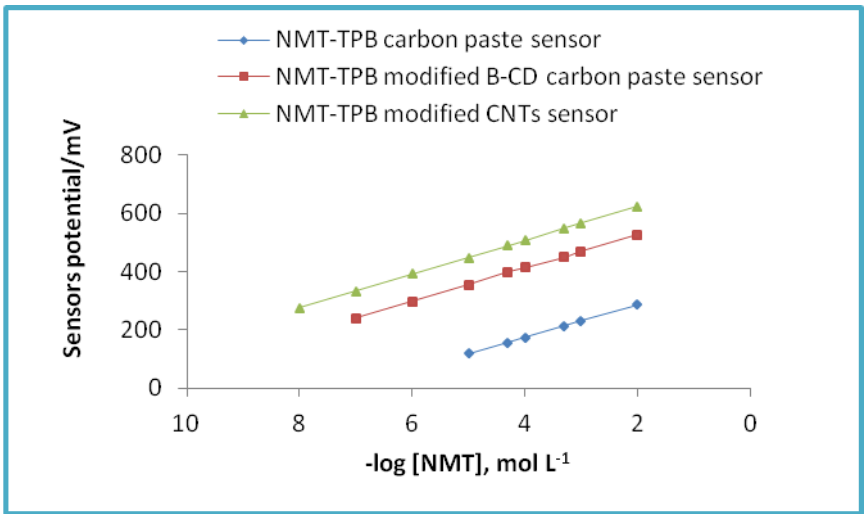

Figure 3. Typical calibration graph of NMT-TPB carbon paste, modified 2- hydroxypropyl β-CD and modified CNTs carbon paste sensors

Table 1. Electrochemical response characteristics of NMT-TPB carbon paste, modified β-cyclodextrin and modified carbon nanotube carbon paste sensors

Parameter [a]	NMT-TPB Carbon paste (sensor I)	NMT-TPB Modified β-CD (sensor II)	NMT-TPB Modified CNTs (sensor III)
Slope (mV decade^{-1})	55.8±0.8	57.7±0.3	58.5±0.1
Intercept	398.3	644.0	740.5
Regression equations	E_{mV} = (55.8±0.8) log [NMT] -398.3	E_{mV} = (57.7±0.3) log [NMT] +644.0	E_{mV} = (58.5±0.1) log [NMT] +740.5
Correlation coefficient, r	0.9997	0.9998	0.9999
Linear range (mol L^{-1})	1.0×10^{-5}-1.0×10^{-2}	1.0×10^{-7}-1.0×10^{-2}	1.0×10^{-8}-1.0×10^{-2}
Lower limit of detection	4.8×10^{-6}	5.0×10^{-8}	7.9×10^{-9}
Response time/s	40	35	30
Working pH range	4-8	4-8	4-8
Lifetime/day	35	45	60
Temperature ˚C	25˚C	25˚C	25˚C
Accuracy (%)	99.2± 0.5	99.7± 0.4	99.9± 0.2
Robustness[b]	98.9± 0.5	99.3± 0.6	99.7± 0.2
Ruggedness[c]	99.2±0.8	99.5±0.3	99.8±0.1

[a]Mean of six measurements [b]A small variation in method parameters were carried out at pH of phosphate buffer (pH 8.0±1) [c]Comparing the results with those obtained by different sensor assemblies using Jenway 3510 pH meter

From the recorded data it was found that the performance characteristics of the modified β-CD (sensor II) was better than conventional carbon paste (sensor I). This can be attributed to the high stability of the complex formed between the cationic drug and the chelating agent of high OH^- donation group. Thus, high selectivity and sensitivity were achieved when using the modified β-CD sensor for determination of NMT. While, in the case of using about 3.0 w% of CNTs for modification of sensor III the performance characteristics were improved. This can be indicated due to the chemical stability and good conductivity of the modified CNTs sensor. Additionally, this type of modified sensor has a porous surface structure and large surface area of CNTs that facilitated a sensor interface wetting property with solvents [17].

3.2. Effect of plasticizers:

The influence of plasticizers on the performance characteristics of NMT-TPB carbon past sensors were tested and optimized. The suitable plasticizer was selected by adding varying content ratios (25, 35, 45, 55 and 65%) of different types of plasticizers such as DOP with dielectric constant (ε = 5.1), DBP (ε = 6.4), DBS (ε = 4.5), DOS (ε = 4.0) and *o*-NPOE (ε =24.0) to the carbon paste and the fabricated sensors were tested.

Table 2. Effect of plasticizers on the slopes of the fabricated NMT-TPB sensors

Plasticizer	NMT-TPB CP (sensor I)	NMT-TPB modified β-CD (sensor II)	NMT-TPB MCNTs (sensor III)
DOS	49.8	52.4	54.9
DBS	51.7	54.5	55.3
DBP	53.5	54.9	56.8
DOP	54.6	56.2	57.2
0-NPOE	55.8*	57.7*	58.5*

*The optimum value for the studied sensors

As clarified from the recorded data in Table 2, it was found that the most suitable plasticizer was *o*-NPOE. The high performance characteristics of the sensors can be attributed to the dielectric constant (ε =24.0) of *o*-NPOE compared to the other types of plasticizers. Also, it gave high permeable properties of sensors and improved their mechanical stability.

3.3. Response time

According to IUPAC recommendation the response time is defined as the first instant at which the potential reading of the sensor equal to its steady-state value within 1 mV. In the present study the response time was determined over the concentration range of $1.0x10^{-8}$-$1.0x10^{-2}$ mol $L^{-1}$of NMT solution. The obtained dynamic response time was found to be 55, 40 and 30 s for sensors I, II and III, respectively, over 45, 50 and 65 days. The developed NMT-TPB carbon paste sensors displayed no significant change in performance characteristics within such periods.

3.4. Effect of pH

The fabricated NMT-TPB sensors were tested with respect to the effect of pH on their potential readings. 1.0×10^{-3} mol L^{-1} NMT test solution was firstly acidified using 0.1 mol L^{-1} hydrochloric acid and then the pH was gradually increased using 0.1 mol L^{-1} sodium hydroxide. The recorded potential readings were plotted against the logarithm of the drug concentration.

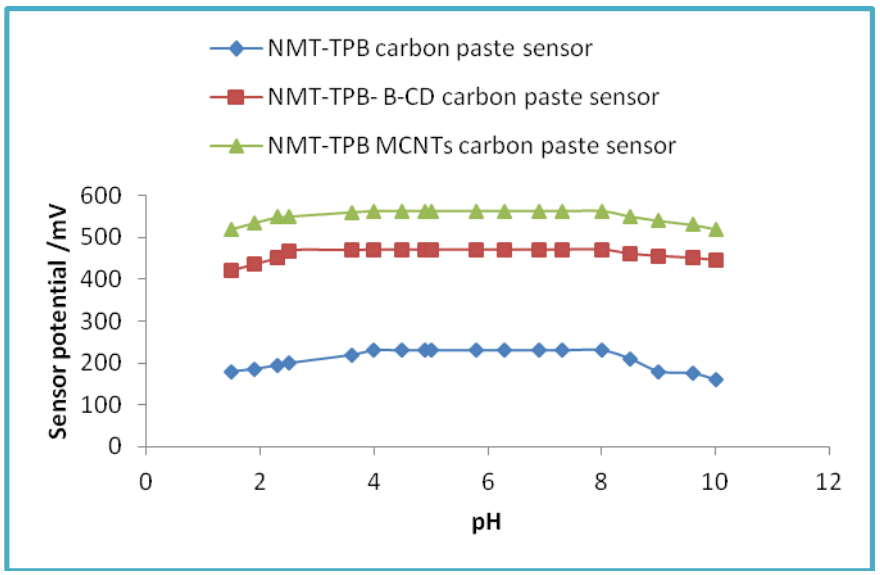

Figure 4. Effect of pH on NMT-TPB carbon paste, modified β-CD and modified CNTs carbon paste sensors using 1.0×10^{-3} mol L^{-1}

Figure 4, showed that the safer pH range of the fabricated sensors was at 4-8. We can attribute the gradual increase of the potential readings for the sensors until reaching pH 4 to the increase of the H^+ in the test solution, while, an increasing the OH^- ions in the solution above pH 8, leads to a significant decrease in the potential readings of the sensors.

3.5. Selectivity of sensors

The selectivity of the fabricated sensors considered as one of the most important parameters that should be studied. The main mechanism of the selectivity was explained by the matching between the locations of lipophilic sites in the two competing species in the test solution side and those present in the active surface of ion pair. To ensure the high selectivity of the developed sensors, they were employed for determination of the investigated drug in the presence of many possible interferences such as the coformulated additives, common cations, some sugars and amino acids. Separate solution method [18] was applied using the following equation:

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

Where, E_1 and E_2 are the sensor potentials in $1.0\text{x}10^{-3}$ mol L^{-1} of both NMT and the interfering ion J^{z+} solution, respectively. S is the slope of the calibration plot.

Table 3. Selectivity coefficients ($K^{Pot}_{NMT^+}$) for NMT-TPB sensors using a separate solution method (1.0×10^{-3} mol L^{-1} nimustine hydrochloride)

Interferent	$K^{Pot}_{NMT^+}$		
	NMT-TPB CP (sensor I)	NMT-TPB modified β-CD (sensor II)	NMT-TPB MCNTs (sensor III)
Na^+	1.5×10^{-3}	5.1×10^{-3}	2.9×10^{-4}
K^+	2.9×10^{-3}	4.2×10^{-4}	6.2×10^{-4}
NH_4^+	5.1×10^{-3}	4.5×10^{-4}	2.2×10^{-4}
Ca^{2+}	6.3×10^{-4}	8.2×10^{-5}	4.9×10^{-4}
Mg^{2+}	7.8×10^{-4}	7.0×10^{-5}	1.3×10^{-5}
Zn^{2+}	6.9×10^{-4}	5.3×10^{-4}	6.8×10^{-4}
Cu^{2+}	2.7×10^{-3}	2.2×10^{-5}	2.6×10^{-4}
Fe^{3+}	6.8×10^{-4}	1.2×10^{-4}	4.8×10^{-5}
Al^{3+}	3.5×10^{-3}	5.3×10^{-4}	8.4×10^{-5}
Glucose	4.5×10^{-5}	2.0×10^{-4}	2.5×10^{-5}
Lactose	5.9×10^{-4}	4.8×10^{-4}	5.9×10^{-4}
Sucrose	4.8×10^{-5}	3.3×10^{-5}	9.4×10^{-4}
Starch	1.7×10^{-4}	6.1×10^{-4}	7.4×10^{-4}
Serine	5.0×10^{-5}	8.9×10^{-4}	6.9×10^{-5}
Glycine	4.1×10^{-4}	4.5×10^{-5}	4.4×10^{-5}
Histadine	8.2×10^{-5}	5.2×10^{-4}	5.6×10^{-5}
Thymine	4.2×10^{-4}	3.8×10^{-4}	8.9×10^{-4}
Ornithine	7.5×10^{-4}	5.7×10^{-5}	4.8×10^{-4}
Glutamine	8.6×10^{-5}	6.8×10^{-4}	2.2×10^{-5}

Table 3 presented the recorded data and it was found that no interferences were displayed by the fabricated sensors in the presence of common cations, sugars, amino acids and some additive compounds.

3.6. Effect of temperature

To study the impact of temperature on the potentials of the fabricated NMT-TPB sensors, the fabricated sensors were examined under the effect of different temperatures (25, 30, 40, 50, 60 and 70˚C). The isothermal coefficients (dE°/dt) of the sensors were determined by measuring the standard sensor potentials (E°) and the intercepts at $p^{NMT} = 0$ (after subtracting the values of the standard sensor potential of an SCE electrode at these temperatures) were plotted *vs*, (*t-25*) where *t* is the temperature of test solution in ˚C. The following equation was used to calculate the isothermal coefficients [19]:

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

Table 4, presented the slopes, the standard sensor potential (E°) at each temperature, and the usable concentration ranges of the NMT test solutions.

Table 4. Performance characteristics of NMT-TPB sensors at different temperatures

Type of sensors	Temperature °C	Slope/mV decade^{-1}	Usable concentration range	E°/mV[a]
NMT-TPB carbon paste (sensor I)	25	55.8*	1.0×10^{-5}-1.0×10^{-2}	120
	30	56.2	1.0×10^{-5}-1.0×10^{-3}	129
	40	57.5	1.0×10^{-5}-1.0×10^{-3}	137
	50	58.4	5.0×10^{-4}-1.0×10^{-4}	140
	60	60.0	5.0×10^{-4}-1.0×10^{-4}	148
NMT-TPB modified β-CD carbon paste (sensor II)	25	56.0	1.0×10^{-6}-1.0×10^{-2}	240
	30	57.7*	1.0×10^{-7}-1.0×10^{-2}	254
	40	58.2	5.0×10^{-5}-1.0×10^{-3}	263
	50	60.5	5.0×10^{-5}-1.0×10^{-3}	270
	60	62.8	5.0×10^{-5}-1.0×10^{-4}	281
NMT-TPB modified CNTs (sensor III)	25	58.5*	1.0×10^{-8}-1.0×10^{-2}	275
	30	59.8	9.0×10^{-7}-1.0×10^{-3}	281
	40	61.2	9.0×10^{-7}-1.0×10^{-3}	287
	50	64.5	5.0×10^{-6}-1.0×10^{-4}	293
	60	65.7	5.0×10^{-6}-1.0×10^{-4}	301

[a]*E°: Standard sensor potential against normal hydrogen electrode (NHE)*

The obtained results were found to be 0.000013, 0.000018 and 0.000011 V°C^{-1} for sensors I, II and III, respectively. These results revealed the high thermal stability throughout the investigated temperature ranges.

3.7. Effect of soaking time

The fabricated NMT-TPB carbon paste, modified β-CD and modified CNTs sensors were investigated with respect to the effect of soaking time. To determine the optimum immersing time, each fabricated sensor was immersed in 1.0×10^{-3} mol L^{-1} standard NMT solution. The recorded data were found to be 8 h for sensor I and about 6 h for both sensors II and III. The previously mentioned sensors displayed Nernstian responses of 55.8±0.8, 57.7±0.3 and 58.2±0.1 mV decade^{-1}for sensors I, II and III, respectively. After prolonged immersing of the fabricated sensors upon different intervals 15, 25, 35, 45, 55 and 65 days, the employed NMT-TPB sensors displayed a slight decrease of responses to be 54.5, 56.3 and 57.6 mV decade^{-1} when the sensors soaking for 35 days. The sensors potential were sharply decreased when soaking for 65 days. It was found that after 45 days the potential reading for sensor I was found to be 51.8 mV decade^{-1}, while after 65 days the potential reading of sensor I and II were dropped to be 54.6 and 56.3 mV decade^{-1}. The recorded data indicated that the use of modified β-CD and carbon nanotube sensors improved the performance characteristics of the sensors. The exhausted sensors were regenerated using 1.0×10^{-2} mol L^{-1} of TPB for one day and the same concentration 1.0×10^{-2}mol L^{-1} of the investigated drug for about 6 h. The potential readings of the regenerated sensors were recorded and the obtained data revealed higher Nernastian responses of

53.6, 55.2, and 57.3 mV decade^{-1} for sensors I, II and III, respectively (Figure 5). The lifespan of the regenerated sensors was limited to 3, 6 and 10 h for the previously mentioned sensors.

3.8. Analytical Applications

3.8.1. Quantification of nimustine hydrochloride

The fabricated NMT-TPB carbon paste, modified β-CD and modified CNTs sensors were employed for determination of the investigated drug in its bulk drug. As summarized in Table 5, the obtained results were evaluated as % recoveries of 98.6±0.9, 99.3±0.5 and 99.5±0.6 for sensors I, II and III, respectively, and the statistical interpretation of data was carried out with respect to student's t-test and F-test [20] and compared with those obtained from the reported method [6] which is based spectrophotometric determination of nimustine hydrochloride using hydrogen bromide and acetic acid followed by the reaction with griess reagent (0.2 % naphthylethylenediamine dihydrochloride and 2 % sulphanilamide in 5 % phosphoric acid) the absorbance was recorded at 530 nm. It was found that the described sensors were highly sensitive and selective for determination of the selected drug. Therefore, this technique can be employed for determination of NMT by a direct and clear way without the need for pretreatment of samples as required in other chromatographic and spectroscopic techniques. The developed sensors were also used to determine the investigated drug in its dosage forms, the calculated results were 99.1±0.8, 99.3±0.8 and 99.8±0.5 for the three previously mentioned sensors, respectively and presented in Table 6.

Table 5. Analytical results of the determination nimustine hydrochloride in pure form using NMT-TPB carbon paste, modified β-cyclodextrin and modified carbon nanotube carbon paste sensors

Samples	NMT-TPB CP (sensor I)			NMT-TPB modified β-CD (sensor II)			NMT-TPB MCNTs (sensor III)			Reference method [6]
	Taken -log conc. mol L^{-1}	Found mol L^{-1}	% Recovery	Taken -log conc mol L^{-1}	Found mol L^{-1}	% Recovery	Taken -Log conc. mol L^{-1}	Found mol L^{-1}	% Recovery	
Pure drug	5.0	4.99	99.8	7.0	6.99	99.9	8.0	7.89	98.6	
	4.3	4.25	98.8	6.0	5.96	99.3	7.0	7.00	100.0	
	4.0	3.89	97.3	5.0	4.94	98.8	6.0	5.96	99.3	
	3.3	3.24	98.2	4.0	3.95	98.7	5.0	4.95	99.0	98.9±0.8
	3.0	2.98	99.3	3.0	2.99	99.7	4.0	4.00	100.0	6
	2.0	1.96	98.0	2.0	1.99	99.5	3.0	2.99	99.7	0.6
							2.0	2.00	100.0	0.3
Mean±SD	98.6±0.9			99.3±0.5			99.5±0.6			
n	6			6			7			
Variance	0.8			0.3			0.4			
%SE**	0.4			0.2			0.2			
t-test	0.600(2.228)*			1.114(2.228)*			1.664(2.201)*			
F-test	1.33(5.05)*			2.00(5.05)*			1.50(4.93)*			

** Figures in parentheses are the tabulated values of t-and F- testes at 95% confidence limit* [20]

** %SE= SD/$\sqrt{n}$

Table 6. Analytical results of the determination nimustine hydrochloride in pharmaceutical injection using NMT-TPB carbon paste, modified β-cyclodextrin and modified carbon nanotube carbon paste sensors

Samples	NMT-TPB CP (sensor I)			NMT-TPB modified β-CD (sensor II)			NMT-TPB MCNTs (sensor III)			Reference method [6]
	Taken -log conc. mol L^{-1}	Found mol L^{-1}	% Recovery	Taken -log conc mol L^{-1}	Found mol L^{-1}	% Recovery	Taken -Log conc. mol L^{-1}	Found mol L^{-1}	% Recovery	
NMT	5.0	4.94	98.8	7.0	7.01	100.1	8.0	7.99	99.9	
injection®	4.3	4.31	100.2	6.0	5.99	99.8	7.0	7.01	100.1	
50 mg/vial	4.0	3.96	99.0	5.0	5.00	100.0	6.0	5.99	99.8	
	3.3	3.24	98.2	4.0	3.96	99.0	5.0	4.98	99.6	99.5±0.9
	3.0	2.99	99.7	3.0	2.97	99.0	4.0	3.96	99.0	6
	2.0	1.97	98.5	2.0	1.96	98.0	3.0	2.99	99.7	0.8
							2.0	2.01	100.5	0.4
Mean±SD	99.1±0.8			99.3±0.8			99.8±0.5			
n	6			6			7			
Variance	0.6			0.6			0.3			
%SE**	0.3			0.3			0.2			
t-test	0.800(2.228)*			0.400(2.228)*			0.671(2.201)*			
F-test	1.33(5.05)*			1.33(5.05)*			2.67(4.93)*			

* *Figures in parentheses are the tabulated values of t-and F- testes at 95% confidence limit* [20]

** %SE= SD/$\sqrt{n}$

Table 7. Analytical results of the determination nimustine hydrochloride in biological fluids using NMT-TPB carbon paste, modified β-cyclodextrin and modified carbon nanotube carbon paste sensors

Samples	NMT-TPB CP (sensor I)			NMT-TPB modified β-CD (sensor II)			NMT-TPB MCNTs(sensor III)		
	Taken -log conc. mol L^{-1}	Found mol L^{-1}	% Recovery	Taken -log conc mol L^{-1}	Found mol L^{-1}	% Recovery	Taken -Log conc. mol L^{-1}	Found mol L^{-1}	% Recovery
Human serum	5.0	4.92	98.4	7.0	6.99	99.9	8.0	7.97	99.6
	4.3	4.28	99.5	6.0	5.98	99.7	7.0	7.00	100.0
	4.0	3.99	99.8	5.0	4.98	99.6	6.0	5.98	99.7
	3.3	3.26	98.8	4.0	3.99	99.8	5.0	4.98	99.6
	3.0	2.89	96.3	3.0	2.95	98.3	4.0	3.99	99.8
	2.0	1.96	98.0	2.0	1.98	99.0	3.0	3.00	100.0
							2.0	1.99	99.5
Mean±SD	98.5±1.3			99.4±0.6			99.7±0.2		
n	6			6			7		
Variance	1.7			0.4			0.4		
%SE*	0.5			0.2			0.1		
	5.0	4.96	99.2	7.0	6.98	99.7	8.0	8.00	100.0
	4.3	4.26	99.1	6.0	5.99	99.8	7.0	7.01	100.1
Human urine	4.0	3.89	97.3	5.0	4.95	99.0	6.0	5.98	99.7
	3.3	3.28	99.4	4.0	3.99	99.8	5.0	4.99	99.8
	3.0	2.95	98.3	3.0	3.01	100.3	4.0	3.98	99.5
	2.0	1.96	98.0	2.0	1.96	98.0	3.0	2.99	99.7
							2.0	2.00	100.0
Mean±SD	98.6±0.8			99.4±0.8			99.8±0.2		
n	6			6			7		
Variance	0.6			0.6			0.04		
%SE*	0.3			0.3			0.07		

* %SE= SD/$\sqrt{n}$

Furthermore, the developed NMT-TPB sensors were successfully employed for determination of the selected drug in human serum and urine.

As indicated in Table 7, the obtained results in human serum were found to be 98.5±1.3, 99.4±0.6 and 99.7±0.2 for sensors I, II and III, respectively. While, in human urine were found to be 98.6±0.8, 99.4±0.8 and 99.8±0.2 for the three investigated sensors.

3.8.2. Content uniformity assay of injection vials

The fabricated carbon paste sensors were employed for determination of the content uniformity assay of the investigated drug in its injection vials. The detection was carried out by dissolving the content of 10 individual vials in 100 mL distilled water. The calculated content of each vial was recorded using the fabricated sensors and the % recoveries ± standard deviations were found to be 99.2±0.3, 99.4±0.1, and 99.7±0.6 for sensors I, II and III, respectively.

3.9. Method Validation

ICH guidelines were used to ensure the method validation [21]. The suitability of the proposed method was tested with respect to the specificity, linearity, accuracy, precision etc.

3.9.1. Linearity and lower limit of detection

The fabricated NMT-TPB carbon paste, modified β-CD and CNTs sensors were applied using the drug concentration range of 1.0×10^{-8} -1.0×10^{-1} mol L^{-1}. It was found that the fabricated sensors displayed Nernstian response over linear concentration ranges of 1.0×10^{-5}-1.0×10^{-2}, 1.0×10^{-7}-1.0×10^{-2} and 1.0×10^{-8}-1.0×10^{-2} mol L^{-1} for the sensors I, II and III, respectively. The obtained data revealed that the addition of 2-hydroxypropyl- β-CD increased the sensitivity of modified carbon paste sensor than the conventional carbon paste sensor. On the other hand, owing to the porous large surface area and the best sensor electrolyte interface of the modified CNTs sensor, we can see the high performance characteristics of the sensor rather than sensor I and II. The calculated lower detection limits were found to be 4.8×10^{-6}, 5.0×10^{-8} and 7.9×10^{-9} mol L^{-1} for the sensors I, II and III, respectively.

3.9.2. Accuracy and precision

The accuracy of the proposed method was examined using the standard addition method. The obtained results were calculated as percentage recoveries and they were found to be 99.2±0.5, 99.7±0.4 and 99.9±0.2 for sensors I, II and III, respectively. The precision of the developed method was investigated in the terms of intra-day and inter-day. Nine replicates of the tested drug solution were used and the evaluated % RSD values were recorded as 0.8, 0.5 and 0.2 % for intra-day detection and 0.6, 0.4 and 0.2 % for inter-day determination of NMT in nimustine hydrochloride® injection using NMT-TPB sensors. The calculated results revealed good precision with %RSD less than 2%.

3.9.3. Robustness and ruggedness

The developed method was validated with respect to the robustness by introducing a small change in pH value 8±1 using phosphate buffer. The recorded data were calculated as percentage recoveries ± standard deviations. The calculated robustness were found to be 98.9±0.5, 99.3±0.6, and 99.7±0.2 for sensors I, II and III, respectively. While, the ruggedness of the proposed method was evaluated using another pH-meter (Jenway 3310). The obtained percentage recoveries were found to be 99.2±0.8, 99.5±0.3 and 99.8±0.1 for the three mentioned sensors, respectively, indicating good agreement with those recorded from standard drug solutions.

4. CONCLUSION

In the described study, novel electrochemical sensors for determination of NMT were fabricated based on the incorporation of NMT with tetraphenylborate as electroactive material. Sensor I was fabricated as conventional carbon paste, while sensors II and III were modified using β-cyclodextrin and carbon nanotubes. The fabricated sensors exhibited excellent Nernstian responses with wide linear concentration ranges and low limits of detection. As indicated from the obtained results, the use of β-cyclodextrin was improved the sensitivity and selectivity of sensor II than that displayed by sensor I. On the other hand, the modificated carbon nanotube sensor showed a significant sensitivity for the detection of the investigated drug. The recorded data were assessed statistically and displayed excellent agreement with those obtained from the reported methods.

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