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ELECTROCHEMICAL SENSORS FOR DIRECT DETERMINATION OF CYANOCOBALAMIN USING SEPARATED AND MIXED ELECTROACTIVE MATERIALS

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ABSTRACT

A new, simple and highly sensitive coated wire sensors for direct determination of cyanocobalamin (CYN) were developed. The developed sensors were based on the fabrication of three different kinds of sensors, using cyanocobalamin-phosphomolybdate (CYN-PM) and cyanocobalamin-phosphotungstate (CYN-PT) as electroactive materials for sensor I and sensor II. While, sensor III was fabricated using mixed electroactive material (CYN-PM/PT). The fabricated sensors were employed for detection of the investigated drug in its pure form, dosage forms and biological fluids such as human serum and urine. The performance characteristics of the fabricated sensors were investigated and optimized. Under optimum conditions the developed senors were displayed Nernstian slopes of (54.0±0.4, 55.4±0.6 and 57.4±0.8 mV decade^{-1} at 25°C) at safe pH range 3-8 and concentration ranges of $1.0x10^{-5}$-$1.0x10^{-2}$, $5.0x10^{-6}$-$1.0x10^{-2}$ and $1.0x10^{-7}$-$1.0x10^{-2}$ mol L^{-1} for sensor I, II and III, respectively. The selectivity coefficientsof the fabricated sensors towards possible interfering species such as cations, amino acids, sugars and some different related pharmacological action drugs. The obtained results were statistically treate4d and compared in terms of student's *t*-test and *F* test with those obtained from other reported methods.

Keywords: Potentiometry, Cyanocobalamin, Coated wire membrane sensor, Pharmaceutical preparations,Biological fluids.

INTRODUCTION

Cyanocobalamin (CYN), is chemically known as 5,6-dimethyl-benzimidazolyl cyanocobamide (Figure 1). It is used for the treatment of vitamin B_{12}deficiency, anemia, to the lack of vitamin B_{12} absorption through the intestine and in permanent damage of the nerves [1]. The literature survey was reported several methods for determination of cyanocobalamin including high performance liquid chromatography [2-6], liquid chromatography coupled with mass spectrometry [7], spectrophotometry [8, 9], voltammetry[10, 11]. As revealed from the literature review no published electrochemical sensors have been reported for determination of cyanocobalamin yet. The present study aims to develop three eletrochemical sensors for determination of cyanocobalamin. The developed method based on the fabrication of three different coated wire sensors using CYN-PM, CYN-PT and CYN-PM/PT as electroactive materials.

EXPERIMENTAL

Reagents and Materials

All chemicals used were of analytical grade and no further purification were done. Pure grade of cyanocobalamin was kindly supplied from (Himedia Lab Co., India). Cyanocobalamin® 10000 µg tab.$^{-1}$/ tablets,was purchased from local drug stores.Methanol 99.0 %, Acetone 99.9%, ortho-nitrophenyloctyl ether (*o*-NPOE) 99.0 %, hydrochloric acid 36.5%, tetrahydrofuran (THF) 97.0 % were provided by (Fluka, Switzerland).Sodium hydroxide 98.0%, Zinc sulphate≥ 99.0%and hydrochloric acid 36.5% were purchased from (BDH laboratory supplies, England). Phosphomolybdic acid

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(PMA) 99.9%, phosphotungstic acid (PTA) 99.9%, high molecular weight polyvinyl chloride (PVC) was purchased from (Sigma-Aldrich, Germany). Healthy volunteers were provided the urine samples, while, human serum samples (Normal Serum HUMATROL N Control 5 mL, Germany) were supplied from commercial sources.

Instrumentation

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

Preparation of analytical solutions

Standard drug solution

A stock solution 1.0×10^{-1} mol L^{-1} of CYN was prepared by dissolving 6.78 g of CYN in 50 mL distilled water. The working solutions were prepared daily in the concentration range of 1.0×10^{-8}-1.0×10^{-1} mol L^{-1} were prepared by serial dilutions using the same solvent.

Preparation of cyanocobalamin® tablets solutions

The content of20 cyanocobalamin® tablets 1000 µg/tablet werecrushed and dissolved in 100 mL volumetric flask and mixed well the finalCYN solutionwas found to be 1.5×10^{-4} mol L^{-1}.The working solutions in the range of1.0×10^{-5}-1.5×10^{-4} mol L^{-1}were prepared by serial dilutions using distilled water.

Preparation of spiked serum and urine solutions

The spiking technique, method was employed for detection of CYN in human serum and urine.Phosphate buffer pH 6.0 was used to adjust the human serum and urine. 1.0 mL of the previously adjusted serum or 5.0 mL of urine were transferred into a centrifuge tube, spiked with astandard drug solution to obtain 1.0×10^{-2} mol L^{-1} and the main possible interfering substances specially protein was removed by precipitation using 1.0 ml acetonitrile, 0.1 mL of NaOH (0.1 mol L^{-1}), 1.0 mL of $ZnSO_4.7\ H_2O$ (5.0% w/v) [12]. After centrefugation at 3500 rpm for 30 min and filtration serial dilution were carried out to obtain working solutions in the ranges of 1.0×10^{-5}-1.0×10^{-2}, 5.0×10^{-6}-1.0×10^{-2} and 1.0×10^{-7}-1.0×10^{-2} mol L^{-1} for sensor I, II and III, respectively.

Preparation of CYN-PM and CYN-PT ion pairs

The ion pairs of CYN-PM and CYN-PT were prepared by the incorporation of 50 mL of equimolar 1.0×10^{-2} mol L^{-1} acidified CYN solution using 1.0 mol L^{-1} hydrochloric acid with 150 mL phosphomolybdic acid (PMA) or phosphotungstic acid (PTA) solution. The prepared ion pairs were filtered and the precipitates were left to dry at room temperature for 24 h. Elemental analysis was employed to confirm the composition of the formed ion-pairs. The obtained results were found to be$[C_{63}H_{89}CoN_{14}O_{14}P]_3[P(Mo_3O_{10})_4]$ and $[C_{63}H_{89}CoN_{14}O_{14}P]_3[P(W_3O_{10})_4]$ indicating 3:1 for both CYN-PM and CYN-PT ion pairs. The percentages of C, H and N were calculated and were found to be 38.52%, 4.62% and 9.98% for sensorI, 32.92%, 3.87% and 8.66% for sensor II. On the other hand, the found percentages were found to be 38.50%, 4.51% and 9.95% for sensor I, 32.65%, 3.92% and 8.40% forsensor II.

Membrane composition

In the present study the membrane composition was studied with respect to thetype ofion pair used. The developed sensors were fabricated using 190 mL of PVC, 0.35 mL of plasticizer (*o*-NPOE) and 10 mg of ion pair CYN-PM and CYN-PT for sensor I and II. While, in sensor III a mixture of 5.0 mg CYN-PM and 5.0 mg CYN-PT was used. All membrane compositions were dissolved in 10 ml tetrahydrofuran (THF) and poured in a petri dish, left aside to evaporate and dried slowly at room temperature.

Sensor fabrication

The developed sensors were fabricated using pure aluminum wire of 4 cm. The wire was tightly insulated using polyethylene tube leaving one end for coating and about 0.5 cm on the other end for connection. Prior to coating the wire with coating solution, the surface of the wire was polished and washed with detergent and water and dried with acetone. Then the wire rinsed with chloroform and immersed for different times in coating solution which previously prepared in membrane composition. The formed coating film was left to dry for 24 h at room temperature. Then the fabricated sensors were preconditioned for about 10 h in 1.0×10^{-3} mol L^{-1} CYN solution.

Selectivity

Separate solution method [13] was used to determine the selectivity of the proposed sensors towards some possible interfering cations, sugars, amino acids, additives and other related pharmacological action drugs. The following equation was applied

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

Where $K^{Pot}_{CYN}J^{z+}$ is the selectivity coefficient, $E_1$is the electrode potential in 1.0×10^{-3}mol L^{-1}CYN solution, E_2 is the potential of the electrode in 1.0×10^{-3} mol L^{-1} solution of interferent $J^{z+}$and S is the slope of the calibration graph in mV.

RESULTS AND DISCUSSION

Effect of membrane composition

In the membrane composition we should obtain

reasonable ionic exchange at the gel layer / test solution interface. So we should adjust the amount of ion pair which responsible for the membrane potential. The good physical properties of the membrane were achieved by adding suitable amount of plasticizer, but we should take into our consideration that the increase of the amount of plasticizer will improve the adhesion properties of the membrane, but it also aids in the deterioration of the membrane and this may depend on the properties of the properties of the ion pair and the matrix [14].In the present study, the fabricated sensors were prepared using separate and mixed ion pairs. To adjust their composition 5-10 mg of each CYN-PM or CYN-PT and CYN-PM/PT was tested. The obtained results revealed that the addition of 5 mg of each ion pair to the third sensor increases its sensitivity by the low %RSD values of slopes which were found to be 0.4, 0.6 and 0.1% for the three developed sensors. The optimum values of the ion pair were used for all further studies.

Nature and response characteristics of the sensors

Phosphomolybdic acid or phosphotungstic acid were reacted with CYN to produce water insoluble ion-pair, but soluble with organic solvent such as tetrahydrofuran (THF). The critical response characteristics were tested and the recorded resultswere summarized inTable 1. The fabricated sensors were exhibited Nernstian responses of (54.0±0.4, 55.4±0.6 and 57.4±0.8 mV decade^{-1} at 25°C) over concentration ranges of 1.0×10^{-5}-1.0×10^{-2}, 5.0×10^{-6}-1.0×10^{-2} and 1.0×10^{-7}-1.0×10^{-2} mol L^{-1}for sensor I, II and III, respectively (Figure 2). The choice of THF 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.

Effect of pH

The influence of pH on the potential readings of the proposed sensors were studied using 1.0×10^{-3} mol L^{-1} CYN test solution. This investigation was carried out by acidifying the tested drug solution using 0.1 mol L^{-1} hydrochloric acid, then the pH was gradually increased using 0.1 mol L^{-1} sodium hydroxide. The potential readings were plotted against each pH valueas shown in Figure 3. The obtained results indicated that the developed sensors were displayed stable responses at pH values 3-8. This can be attributed to at pH less than 3 sensor potential was increased with the high concentration of only acidity, which may be ascribed to extraction of H^+ ions by a membrane. While,at pH higher than 8 the sensor potential was decreased by increasing OH^- concentration [15].

Effect of soaking

The developed sensors should be preconditioned in 1.0×10^{-3} mol L^{-1} drug solution to produce an active thin gel layer at which the ion exchange occurs. The time required for the precondition was depended on the diffusion and equilibrium at the sensor interface. The developed sensors the preconditioned time was found to be 10 h for all sensors. The fabricated sensors displayed Nernstian responses 54.0±0.4, 55.4±0.6 and 57.4±0.8 mV decade^{-1} at 25°C over concentration ranges of 1.0×10^{-5}-1.0×10^{-2}, 5.0×10^{-6}-1.0×10^{-2} and 1.0×10^{-7}-1.0×10^{-2} mol L^{-1} for sensor I, II and III, respectively. The slopes of the fabricated sensors were nearly constant for about 30 days in case of sensors I and II, 45 days for sensor III. While, the continuous immersing of the sensors in 1.0×10^{-3} mol L^{-1}drug solution causes a negative effect on their response and the slopes were found to be 52.0±0.5, 53.8±0.4 and 55.4±0.3 mV decade^{-1} for the three mentioned sensors. The slopes were stated to decrease gradually and were found to be 50.3±0.6, 51.2±0.6 and 53.6±0.2 mV decade^{-1}. The regeneration of the exhausted sensors was carried out by immersing each sensor in 1.0×10^{-2} mol L^{-1} PMA or PTA for 24 h, then immersed for 6 h in 1.0×10^{-2} mol L^{-1} CYN solution. The regenerated sensors were displayed Nernastian responses of 51.5±0.3, 52.6±0.5 and 56.2±0.1 mV decade^{-1} for sensors I, II and III, respectively. The lifespan of the regenerated sensors was limited to 6 h for both sensors I and II, while, sensor III the lifespan was found to be 10 h (Figure 4).

Analytical Applications

This work was introduced the application of the developed CYN-PM, CYN-PT and CYN-PM/PT sensors in the determination of the investigated drug in its pure drug. The obtained results were found to be98.9±0.7, 98.7±0.9 and 99.5±0.4 for sensors I, II and III, respectively (Table 2). The good results encouraged us to apply the developed sensors to determine the selected drug in its dosage forms such as cyanocobalamin tablets. The recorded results were 99.1±0.8, 98.6±0.9 and 99.2±0.7for the above mentioned sensors. Statistical treatment in terms of t-student's test and F-test were used to compare the obtained results with those obtained from other reported method [8] as shown in Table 3.

Application of serum and urine

Spiked technique, method was used to facilitate the application of the developed sensors in biological fluids such as human serum and urine. Table 4, presented the results obtained from the determination of the investigated drug in human serum and urine. They found to be 98.8±0.9, 99.1±0.5 and 99.3±0.4 in human serum while, in human urine the obtained results were 98.8±0.6, 98.7±0.8 and99.1±1.1 for sensors I, II and III, respectively.

Content uniformity assay of cyanocobalamin® tablets

The content of ten tablets was individually placed in a separate 100-mL volumetric flask and dissolved in 100 mL distilled water. The fabricated sensor was directly immersed in each test solution for five times and should be washed with distilled water between each measurement. The potential readings were recorded and the content of each tablet was evaluated from calibration graph. The fabricated sensors displayed good results in the content uniformity assay and the mean recoveries were found to be 98.9±0.2, 98.7±0.5 and 99.6±0.4 for sensors CYN-PM, CYN-PTand CYN-PM/PT, respectively.

Method Validation

ICH guidelines [16] were applied to validate the proposed method to ensure its suitability for the intended use. The proposed method was validated with respect to specificity, linearity, lower limit of detection, accuracy, precision, robustness and ruggedness.

Specificity

The selectivity of the fabricated sensors towards some cations, amino acids, sugars and other related pharmacological action drugs was investigated. The mechanism can be simply based on the stereospecificity and electroststic environment. It does not depend on the matching between the lipophilic sites in the competing species in the tested solution and those present in the receptor of the ion-pair [17]. The selectivity coefficient was calculated and the obtained results were presented in Table 5. As indicated from the obtained results, no interferences were recorded from the cations, sugars, amino acids. Also the fabricated sensors exhibited good tolerance towards the thiamine hydrochloride and pyridoxine hydrochloride.

Linearity and detection Limit

Under the optimal experimental conditions the proposed sensors were employed to detect the investigated drug CYN and the linear relationships were plotted for sensors potential vs. logarithm of the corresponding drug concentrations. The recorded results were found to be 1.0×10^{-5}-1.0×10^{-2}, 5.0×10^{-6}-1.0×10^{-2} and 1.0×10^{-7}-1.0×10^{-2} mol L^{-1}. According to IUPAC recommendation [18] 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 be5.0×10^{-6}, 2.5×10^{-6} and 5.0×10^{-8} for sensors I, II and III, respectively.

Accuracy and precision

The accuracy of the developed method was studies using the standard addition method and the fabricated sensors were employed for determination of the tested drug. The obtained data were calculated in terms of percentage recoveries and standard deviation. It was found to be 98.6±0.3, 99.2±0.8 and 99.5±0.6 for sensors I, II and III, respectively.

The precision of the proposed method was investigated using intra-day and inter-day terms. To carry out the precision studies nine replicates of the investigated drug were employed.The calculated %RSD values were found to be 0.5, 0.7 and 0.2% for intra-day detection, 0.7, 0.3 and 0.1 % for inter-day determination of CYN in cyanocobalamin®tablets using CYN-PM, CYN-PT and sensors. These lower values of the above %RSD values are less than 2% indicating good precision.

Robustness and Ruggedness

According to ICH guidelines [16] the robustness of the developed method was tested to ensure the capacity of the method to remain unaffected by a small variation in the method parameters. To investigate the robustness of the proposed method small change in pH was carried out using phosphate buffer pH 8±1and the results were calculated as percentage recoveries. They were found to be 99.2± 0.3, 98.9±0.9 and 99.7±0.2for sensor I, II and III. While, to investigate the ruggedness of the proposed method the same tested samples were investigated under different laboratories, analysts and instruments. The recorded data were obtained using Jenway 3510 pH meter and were found to be 98.4± 0.6, 98.1±0.5 and 99.2±0.1 for the previously mentioned sensors, respectively.

Table 1. Electrochemical response characteristics of CYN-PM and CYN-PT sensors

Parameter[a]	CYN-PM	CYN-PT	CYN-PM/PT
Slope (mV decade^{-1})	54.0±0.4	55.4±0.6	57.0±0.8
Intercept	389.5	479.9	669.3
Regression equations	E_{mV} = (54.0±0.4) log [CYN] + 389.5	E_{mV} = (55.4±0.6) log [CYN] + 479.9	E_{mV} = (57.0±0.8) log [CYN] + 669.3
Correlation coefficient , r	0.9999	0.9997	0.9999
Linear range (mol L^{-1})	1.0×10^{-5}-1.0×10^{-2}	5.0×10^{-6}-1.0×10^{-2}	1.0×10^{-7}-1.0×10^{-2}
Lower limit of detection	5.0×10^{-6}	2.5×10^{-6}	5.0×10^{-8}
Response time/s	35	35	20
Working pH range	3-8	3-8	3-8
Life time/day	30	30	45
Temperature ˚C	25	25	25
Accuracy (%)	98.6±0.3	99.2±0.8	99.5±0.6
Robustness[b]	99.2±0.3	98.9±0.9	99.7±0.2
Ruggedness[c]	98.4±0.6	98.1±0.5	99.2±0.1

[a]*Mean of three measurements;* [b] *A small variation in method parameters were studied at pH of buffer (phosphate buffer 6)*
[c]*Comparing the results with those obtained by different sensor assemblies using Jenway 3510 pH meter.*

Table 2. Statistical treatment of data obtained by determination CYN in pure drug and Cyanocobalamin® tablets (1000µg/tablet) using CYN-PM, CYN-PT and CYN-PM/PT sensors

Sample	CYN- PM Sensor I			CYN-PT Sensor II			CYN-PM/PT Sensor III		
Pure drug	Taken -log [CYN] molL^{-1}	Found	% Recovery	Taken -log [CYN] molL^{-1}	Found	% Recovery	Taken -log [CYN] molL^{-1}	Found	% Recovery
	5.0	4.99	99.8	5.3	5.26	99.2	7.0	6.99	99.9
	4.3	4.29	99.8	5.0	4.98	99.6	6.0	5.98	99.7
	4.0	3.95	98.8	4.0	3.97	99.3	5.0	4.96	99.2
	3.3	3.25	98.5	3.3	3.25	98.5	4.0	3.99	99.8
	3.0	2.96	98.7	3.0	2.96	98.7	3.0	2.98	99.3
	2.0	1.96	98.0	2.0	1.94	97.0	2.0	1.98	99.0
*% Mean±SD	98.9±0.7			98.7±0.9			99.5±0.4		
n	6			6			6		
Variance	0.49			0.81			0.16		
% SE	0.29			0.37			0.16		
% RSD	0.71			0.91			0.40		
	5.0	4.88	97.6	5.0	4.87	97.4	5.0	5.0	100.0
	4.5	4.48	99.6	4.5	4.45	98.9	4.5	4.49	99.7
Cyanocobalamin®	4.3	4.29	99.8	4.3	4.27	99.3	4.3	4.24	98.6
Tablets1000µg/tablet	4.2	4.17	99.3	4.2	4.15	98.8	4.2	4.16	99.0
	4.05	4.03	99.5	4.05	3.95	97.5	4.05	3.98	98.3
	4.0	3.94	98.5	4.0	3.98	99.5	4.0	3.99	99.8
*% Mean±SD	99.1±0.8			98.6±0.9			99.2±0.7		
n	6			6			6		
Variance	0.64			0.81			0.49		
%SE	0.33			0.37			0.29		
%RSD	0.81			0.91			0.71		

**Mean of six measurements*

Table 3. Statistical treatment of data obtained for the determination of CYN in Cyanocobalamin® (1000 µg/tablet) by proposed and a reported method [8] using the standard addition method

Type of sensor	Taken - log conc, mol L^{-1}	Mean%±SD	n	Variance	SE	%RSD	*t*-test	*F*-test
CYN-PM sensor I	5.0-2.0	99.1±0.8	6	0.64	0.3	0.8	1.94 (2.228)*	2.56(5.05)*
CYN-PT sensor II	5.3-2.0	98.6±0.9	6	0.81	0.4	0.9	2.68 (2.228)*	3.24 (5.05)*
CYN-PM/PT sensor III	7.0-2.0	99.2±0.7	6	0.49	0.3	0.7	1.66 (2.228)*	1.96(5.05)*
Reported method [8]	6.0-2.0	99.8±0.5	6	0.25	0.2	0.5		

**Figures in parentheses are the tabulated values of t-and F- testes at 95% confidence limit [19]*

Table 4. Statistical treatment of data obtained by determination CYNin human serum and urine usingCYN-PM, CYN-PT and CYN-PM/PT sensors

Sample	CYN-PM Sensor I			CYN-PT Sensor II			CYN-PM/PT Sensor III		
	Taken -log[CYN] molL^{-1}	Found	% Recovery	Taken -log[CYN] molL^{-1}	Found	% Recovery	Taken -log[CYN] molL^{-1}	Found	% Recovery
	5.0	4.97	99.6	5.3	5.24	98.9	7.0	6.95	99.3
Human serum	4.3	4.23	98.4	5.0	4.95	99.0	6.0	5.96	99.3
	4.0	3.95	98.8	4.0	3.98	99.5	5.0	4.99	99.8
	3.3	3.30	100.0	3.3	3.24	98.2	4.0	3.96	99.0
	3.0	2.96	98.7	3.0	2.98	99.3	3.0	2.99	99.7
	2.0	1.95	97.5	2.0	1.99	99.5	2.0	1.95	98.5
*% Mean±SD	98.8±0.9			99.1±0.5			99.3±0.4		
n	6			6			6		
Variance	0.81			0.25			0.16		
%SE	0.37			0.20			0.16		
%RSD	0.91			0.50			0.40		

Human urine	5.0	4.98	99.6	5.3	5.26	99.2	7.0	6.99	99.9
	4.3	4.25	98.8	5.0	4.98	99.6	6.0	5.97	99.5
	4.0	3.95	98.7	4.0	3.96	99.0	5.0	4.98	99.6
	3.3	3.25	98.4	3.3	3.24	98.2	4.0	3.96	99.0
	3.0	2.98	99.3	3.0	2.96	98.7	3.0	2.99	99.7
	2.0	1.96	98.0	2.0	1.95	97.5	2.0	1.94	97.0
*% Mean±SD	98.8±0.6			98.7±0.8			99.1±1.1		
n	6			6			6		
Variance	0.36			0.64			1.21		
%SE	0.24			0.33			0.45		
%RSD	0.61			0.81			1.11		

**Mean of six measurements*

Table 5. Selectivity coefficients ($K^{Pot}_{CYN^+}$) for CYN-PM and CYN-PT and CYN-PM/PT sensors using a separate solution method (1.0×10^{-3} mol L^{-1}cyanocobalamin)

$K^{pot}_{CYN\text{-}J^+}$			
Interference	**CYN-PM**	**CYN-PT**	**CYN-PM/PT**
Na^+	1.8×10^{-5}	2.2×10^{-3}	3.1×10^{-5}
K^+	3.3×10^{-5}	3.8×10^{-3}	6.1×10^{-3}
NH_4^+	7.2×10^{-4}	2.2×10^{-3}	4.5×10^{-3}
Ca^{2+}	1.5×10^{-5}	8.0×10^{-4}	1.3×10^{-4}
Mg^{2+}	2.8×10^{-4}	5.0×10^{-4}	8.8×10^{-5}
Zn^{2+}	1.9×10^{-4}	3.3×10^{-4}	4.5×10^{-4}
Cu^{2+}	1.7×10^{-4}	1.8×10^{-6}	6.1×10^{-5}
Fe^{3+}	2.8×10^{-4}	1.7×10^{-3}	3.1×10^{-5}
Al^{3+}	6.5×10^{-3}	5.9×10^{-3}	6.7×10^{-4}
Glucose	1.4×10^{-5}	8.4×10^{-3}	4.0×10^{-3}
Lactose	5.6×10^{-4}	5.6×10^{-4}	7.5×10^{-4}
Sucrose	1.7×10^{-5}	2.2×10^{-5}	1.4×10^{-3}
Starch	2.9×10^{-5}	3.6×10^{-4}	1.4×10^{-3}
Serine	3.2×10^{-5}	3.7×10^{-4}	4.2×10^{-4}
Glycine	7.8×10^{-4}	5.8×10^{-4}	1.8×10^{-4}
Histadine	8.7×10^{-3}	1.4×10^{-4}	8.2×10^{-4}
Thymine	9.0×10^{-3}	2.2×10^{-3}	4.4×10^{-3}
Ornithine	5.5×10^{-3}	2.7×10^{-3}	5.0×10^{-5}
Glutamine	4.0×10^{-4}	3.4×10^{-3}	8.8×10^{-4}
Thiamine hydrochloride	6.6×10^{-3}	8.7×10^{-4}	7.9×10^{-3}
Pyridoxine hydrochloride	7.2×10^{-3}	5.7×10^{-3}	8.7×10^{-3}

Figure 1. Chemical structure of cyanocobalamin

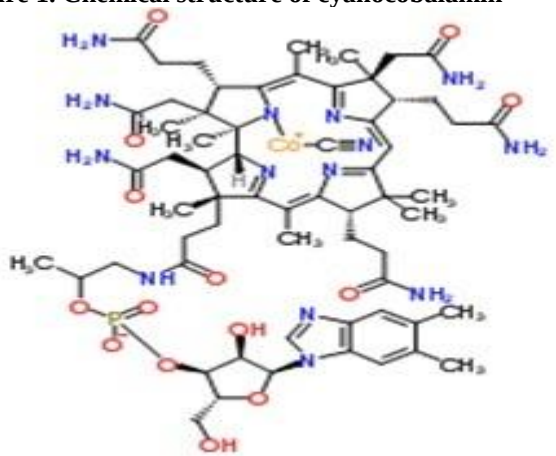


Figure 2. Typical calibration graphs of CYN sensors using CYN-PM, CYN-PT and CYN-PM/PT

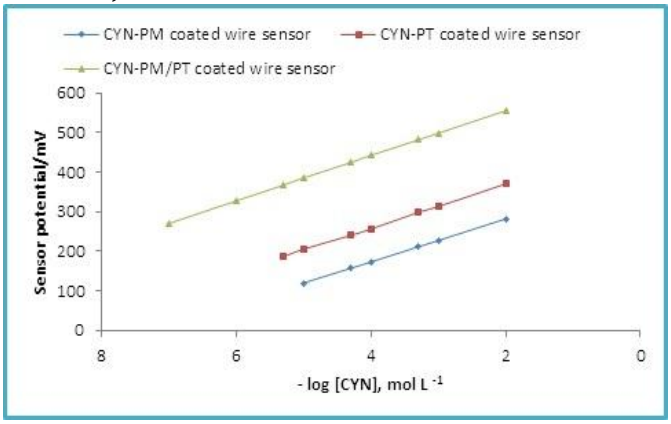


Figure 3. Effect of pH on sensors potential using 1.0×10^{-3} mol L^{-1} CYN drug solution

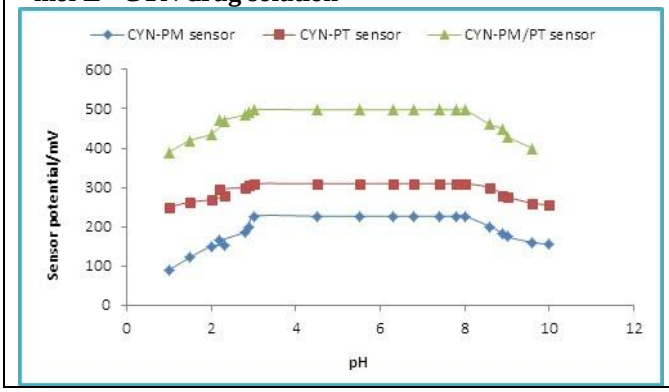
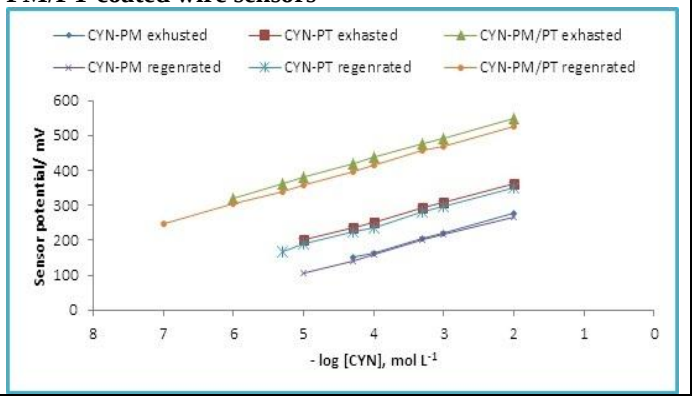


Figure 4. Regeneration of CYN- PM, CYN-PT, and CYN-PM/PT coated wire sensors



CONCLUSION

Three different fabricated sensors for determination of CYN were described in the present study.The described sensors (I and II) were based on the incorporation of CYN with phosphomolybdic acid or phosphotungstic acid to introduce CYN-PM or CYN-PT as electroactive materials.While, sensor III was fabricated using mixed electroactive material (CYN-PM/PT). The obtained results revealed a useful analytical tool for detection of the investigated dug in its pure form, dosage forms and biological fluids such as human serum and urine. The introduced sensors showed a high sensitivity and selectivity towards the tested drug, with no interferences were recorded from the additive materials, impurities and matrix components. Also, from the obtained results we can notice that the use of mixed electroactive material leads to a significant increase in the sensor performance characteristics in terms of the linear concentration range, response time and lower detection limit.

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