ISSN : 0974 - 7540 Volume 2 Issue 2

Trade Science Inc.

Research & Reviews On Electrochemistry

Full Paper

RREC, 2(2), 2010 [78-87]

Membrane electrodes for the simultaneous determination of vitamins B_1, B_6 and B_{12}

Nesreen K.Ramadan, Marianne Nebsen*, Eman S.El Zanfaly

Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr-El-Aini, 11562, Cairo, (EGYPT)

E-mail : Mariannenebsen@hotmail.com

Received: 13th October, 2010 ; Accepted: 23rd October, 2010

ABSTRACT

Three poly(vinyl chloride) matrix membrane electrodes responsive to some vitamins, thiamine hydrochloride (B_1), pyridoxine hydrochloride (B_6) and cyanocobalamin (B_{12}) were developed, described and characterized. Precipitation based technique with ammonium reineckate (R) anion as electroactive material in poly(vinyl chloride) (PVC) matrix with B_1 and B_6 cations was used for electrodes 1 and 2 fabrication respectively. β-Cyclodextrin (β-CD)-based technique with ammonium reineckate as fixed anionic site for B_{12} in PVC matrix was used for fabrication of electrode 3. Fast and stable Nernstian responses near $1x10^{-6}$-$1x10^{-2}$ M for vitamin B_1, $1x10^{-5}$-$1x10^{-2}$ M for vitamin B_6 and $1x10^{-7}$-$1x10^{-4}$ M for vitamin B_{12} over pH range 1-3 for the three electrodes revealed the performance characteristics of these electrodes which were evaluated according to IUPAC recommendations. The three proposed electrodes were successfully applied for the determination of vitamins in some pharmaceutical formulations and in human spiked plasma samples. Validation of the method according to the quality assurance standards showed the suitability of the proposed electrodes for the use in the quality control assessment of these drugs.

KEYWORDS

Thiamine hydrochloride (B_1);
Pyridoxine hydrochloride (B_6);
Cyanocobalamin (B_{12});
β-Cyclodextrin;
Ammonium reineckate;
Pharmaceutical formulations.

INTRODUCTION

Vitamins are commonly used in the treatment and prevention of vitamins deficiency. Thiamine deficiency leads to the development of a syndrome known as beriberi[1].

Pyridoxine deficiency leads to sideroblastic anaemia, dermatitis, cheilosis, and neurological symptoms such as peripheral neuritis, and convulsion. Pyridoxine has also been used to treat seizures due to hereditary syndromes of pyridoxine deficiency or dependency in infants[1].

Vitamin B_{12} deficiency leads to the development of megaloblastic anaemias and demyelination and other neurological damage.

Determination of vitamins is described in the British Pharmacopoeia[2] by titrimetric method for B_1 and B_6 and spectrophotometric method for B_{12}. The three vitamins were simultaneously determined by several methods including HPLC[3-7], TLC[8,9], micellar electrokinetic

capillary chromatography MECC[10], derivative spectrophotometry[11] and mass spectrometry[12].

In the last decade modern ion-selective electrodes have been recommended for several applications in various fields, such as direct measurements of various analytes in food industry[13] and environmental samples[14].

Also biosensors have been used for solving many biochemical problems related with enzymes, urea and creatinine[15]. Also microelectrodes have been used in highly resistive biological media[16] and intracellular measurements for ionized calcium, sodium and potassium[17]. The combination between ISEs and molecular recognition[18], inclusion complexation[19] and molecular imprinting[20] are of current interest in host–guest chemistry and supramolecular chemistry.

Vitamins B_1, B_6 and B_{12} are co formulated together in the Egyptian market in various dosage forms (ampoules and tablets). In all these dosage forms vitamin B_{12} was found to have very small concentration which was about 100-1000 times less than the other two vitamins, the condition that provided a problem in their simultaneous determination.

The aim of this work was to develop a new, simple, accurate and precise method, which can be applied in routine quality control for the simultaneous determination of B_1, B_6 and B_{12} in their ternary mixture. This was achieved by developing sensitive and highly selective polymeric membrane electrodes for the potentiometric determination of the vitamins in some pharmaceutical formulations and in plasma without the need of preliminary extraction and separation steps. Three electrodes were investigated for this purpose; the first and second electrodes depended on the formation of ion pair association complexes using R with B_1 and B_6, while the third electrode depends on the use of β-CD-based technique with ammonium reineckate as fixed anionic site for B_{12}.

EXPERIMENTAL

Apparatus

1. Jenway digital ion analyzer model 3330 (Jenway, Essex, UK) with Ag/AgCl double junction reference electrode no. 924017-LO3-Q11C.
2. pH glass electrode (Jenway, UK) no. 924005-BO3-Q11C.
3. Magnetic stirrer, Bandelin Sonorox, Rx510S (Budapest, Hungary).

Reference samples

Pure samples of thaimine hydrochloride, pyridoxine hydrochloride and cyanocobalamin were kindly supplied by Glaxo SmithKline pharmaceutical company and assayed for their purity according to the pharmacopoeial method[2] to contain 101.0 %, 101.1% and 99.9 % respectively.

Pharmaceutical formulations

Neurovit tablets: Manufactured by Amriya pharmaceutical Ind. (Cairo, Egypt), batch no. 7033291 labeled to contain 250 mg, 100 mg and 250 μg of B_1, B_6 and B_{12} respectively in each tablet.

Neurovit ampoules: Manufactured by Amriya pharmaceutical Ind. (Cairo, Egypt), batch no. 866507 labeled to contain 150 mg, 100 mg and 1 mg of B_1, $B_6$and B_{12} respectively in 3ml ampoule.

Tri - B – ampoules: Manufactured by The Nile Co. for Pharmaceuticals and Chemical Industries (Cairo, Egypt), batch no. 91056B labeled to contain 100 mg and 40 mg of B_1 and B_6 respectively in 1 ml ampoule No. 1 and 1000 μg of B_{12} in 1 ml ampoule No. 2.

Neurobion – ampoules: Manufactured by Merck, GlaxoSmithKline (Cairo, Egypt), batch no. 082705A labeled to contain 100 mg, 100 mg and 1000 μg of B_1, B_6 and B_{12} respectively in 3ml ampoule.

Reagents

All chemicals and solvents used were of analytical grade (water used was bi-distilled).

1. 2- nitrophenyl octyl ether (oNPOE); Sigma,Aldrich.
2. Tetrahydrofuran (THF); BDH.
3. Ammonium reineckate (R); BDH.
4. Poly(vinyl chloride) (PVC) of high molecular weight; BDH.
5. Potassium chloride, $1x10^{-2}$ and 10^{-4}M aqueous solution; Prolabo.
6. Dioctyl phthalate (DOP); Sigma.
7. 2-nitrophenyl phenyl ether (oNPPE); Aldrich.
8. β-Cyclodextrin (β-CD), Fluka Chemie GmbH, Germany.

9. Human plasma was kindly supplied by VACSERA.
10. Citrate buffer pH 3.0[21].

9.77 gm of citric acid monohydrate, and 1.03 gm of trisodium citrate monohydrate were dissolved in water and diluted to 1 liter, the pH was adjusted with citric acid.

Standard solutions

Vitamins stock solutions (1 x 10^{-1}M)

Stock solutions were prepared by dissolving 3.37, 2.06 and 13.55 g of B_1, B_6 and B_{12} respectively, in 100 ml citrate buffer pH 3. Few drops of dilute HCl were added in case of B_{12} to enhance its solubilization.

Vitamins working solutions (1 x 10^{-7} to 1 x 10^{-2}M)

They were prepared by suitable dilution from their stock solutions using citrate buffer pH 3.

Procedures

Precipitation based technique for the preparation of vitamins B_1 and B_6 ion exchangers (electrodes 1,2)

R-drug ion pair complexes were prepared by mixing 10 ml of 10^{-2} M solution of vitamins (B_1 and B_6) with 20 ml 10^{-2} M R solution.

The resultant precipitates formed were filtered using whatman filter paper no. 42, washed with cold water, dried at room temperature (about 20°C) and ground to fine powder.

A portion (10 mg) of vitamins-ion exchanger was thoroughly mixed with 0.19 g PVC and 0.35 g oNPOE in a glass petri dish (5 cm diameter) then dissolved in 5 ml THF. The petri dish was covered with filter paper and left to stand overnight to allow solvent evaporation at room temperature. Master membranes with thickness of 0.1 mm were obtained and used for the construction of the sensors[22,23].

From these master membranes, a disk (about 8 mm diameter) was cut using a cork borer and pasted, using THF, to an interchangeable PVC tip that was clipped into the end of the electrode glass body.

Equal volumes of 10^{-2} M vitamin solution (B_1 and B_6) and 10^{-2} M KCl were mixed and these solutions were used as internal reference solutions. Ag/AgCl wires (1mm diameter) were immersed in the internal reference solutions as internal reference electrodes.

The electrodes (1 and 2) were conditioned by soaking in 1 x 10^{-2} M vitamins B_1 and B_6 solutions respectively for one day and were stored in the same solutions when not in use.

β-CD- based technique for the preparation of PVC-membrane electrode (electrode 3)

In a glass petri dish (5 cm diameter) 0.04 g β-CD was mixed with 0.4 g oNPOE and with 0.01 g R for the preparation of sensor 3. 0.18 g PVC previously dissolved in 6 ml THF were added, the petri dish was covered with filter paper and left to stand overnight to allow solvent evaporation at room temperature. Master membrane with thickness of 0.1 mm was obtained and used for the construction of electrode 3.

From this master membrane, a disk (about 8 mm diameter) was cut using a cork borer and pasted, using THF, to an interchangeable PVC tip that was clipped into the end of the electrode glass body.

Equal volumes of 10^{-4} M B_{12} and 10^{-4} M KCl were mixed and this solution was used as internal reference solution. Ag/AgCl wire (1mm diameter) was immersed in the internal reference solution as internal reference electrode.

Electrode 3 was conditioned by soaking in 10^{-4} M vitamin B_{12} solution for 2h, and stored in the same solution when not in use.

Direct potentiometric determination of vitamins in their pure samples

The conditioned sensors were calibrated by separately transferring 50 ml aliquots of solutions covering the concentration range of (1 x 10^{-7} to 1 x 10^{-2} M) vitamins, into a series of 100 ml beakers. The electrode system was immersed in each solution, with constant stirring in conjunction with a double junction Ag/AgCl reference electrode.

The electrodes were washed with buffer solution between measurements. The electrode potential was plotted versus each negative logarithmic concentration of vitamins standard solutions. The calibration plots obtained and the regression equations were used for subsequent measurements of unknown samples.

Identification of the slope, response time and operative life of the studied electrodes

The electrochemical performance of the three proposed sensors was evaluated according to the IUPAC

recommendations data[24].

The dynamic response times for the electrodes in the discussion to reach values ± 1 mV of the final equilibrium potential after increasing the drug concentration 10 folds were measured.

Effect of pH

The effect of pH on the potential values of the three electrodes was studied over pH range of 1 to 11 by adding drops of 1 N HCl and 1 N NaOH, the potentials obtained at each value were recorded.

Effect of temperature

The effect of the temperature was studied, the potential response displayed by the investigated electrodes as a function of temperature in the range of 20°- 40°C at 5°C interval was monitored. The potentials obtained at each temperature were recorded.

Effect of interfering compounds on the electrode selectivity

The influence of various basic substances and a number of pharmaceutical additives and diluents commonly used in drug formulation (e.g. urea, glycine, hydroxylamine, starch, sodium chloride, potassium chloride, ammonium chloride, cupper chloride, glucose, sucrose and lactose) and other B-vitamins drugs were examined for their effect on the assay method. The selectivity coefficients were calculated by the separate solution method (SSM)[23], where potentials were measured for 10^{-4} M drug solution and then for 10^{-4}M interferent solution, separately, then potentiometric selectivity coefficients were calculated

Direct potentiometric determination of vitamins in their laboratory- prepared mixtures containing different ratios of B_1, B_6 and B_{12}

A series of laboratory-prepared mixtures of B_1, B_6 and B_{12} were prepared in the ratios 100:100:1, 150:100:1, 100: 25: 1 and 100:40:1 in citrate buffer of pH 3 for determination of B_1, B_6 and B_{12}, respectively, using the specified electrode sensors for each drug. In conjunction with double junction Ag/AgCl reference electrode. The membrane sensor was washed between measurements with citrate buffer of pH 3 in the determination of B_1, B_6 and B_{12}. The e.m.f. produced by the three proposed electrodes was recorded for each drug and the concentration of B_1, B_6 and B_{12} was calculated from the corresponding regression equations.

Direct potentiometric determination of B_1, B_6 and B_{12} in their pharmaceutical formulations

Analysis of Neurovit tablets

Ten tablets of the drug formulations were weighted and finely powdered in a small dish. An accurately weighed portion of the powder, equivalent to one tablet, was accurately transferred to a 100-ml volumetric flask; few drops of HCl were added to enhance the solubilization of B_{12}. The volume was completed to the mark with citrate buffer pH 3. The potential readings produced by immersing the prepared electrodes in conjunction with the double junction Ag/AgCl reference electrode in the prepared solution were recorded. The concentrations of B_1, B_6 and B_{12} were calculated from their corresponding regression equations.

Analysis of ampoules

One ampoule (3 ml) of Neurovit, One ampoule (3 ml) of Neurobion and two mixed ampoules No.1 and No.2 (1 ml + 1 ml) of Tri-B drug formulations were separately transferred into three 50-ml volumetric flasks and completed to the mark with citrate buffer pH 3. The potential readings produced by immersing the prepared electrodes in conjunction with the double junction Ag/AgCl reference electrode in the prepared solutions were recorded. The concentrations of B_1, B_6 and B_{12} were calculated from their corresponding regression equations.

Direct potentiometric determination of B_1, B_6 and B_{12} in plasma samples

4.5 ml of plasma samples were placed into three stoppered tubes then 0.5 ml of 10^{-2} M, 10^{-2} M and 10^{-4} M of B_1, B_6 and B_{12} respectively in citrate buffer pH 3 were added separately and shaken. The potential readings produced by immersing the prepared electrodes in conjunction with the double junction Ag/AgCl reference electrode in the prepared solutions were recorded. The concentrations of B_1, B_6 and B_{12} were calculated from their corresponding regression equations.

RESULTS AND DISCUSSION

I.S.Es are electrodes containing membranes having a selective response for a particular ion[22].

Selective membranes in ion selective electrodes (ISEs) have shown both ion exchange and perm-selectivity of the sensor ions[25].

The present work originates from the fact that the studied vitamins behave as cations, this fact suggested the use of ion exchangers of anionic types. It was found that ammonium reineckate was an optimum anionic exchanger for the studied vitamins for its complexes low solubility product and suitable grain size[26,27]. B_1 react with ammonium reineckate to form water insoluble 1: 2 ion association complex while B_6 form 1: 1 drug ammonium reineckate ion association complex . The complexes were prepared, characterized, and incorporated with a suitable solvent mediator in poly(vinyl chloride) matrix membranes.

Molecular recognition at the surface of solid materials has attracted the interest of researches who are trying to realize functional materials for chemical sensors. Uses of selective inclusion complexation and complementary ionic or hydrogen bonding are two main strategies for preparing synthetic host molecules that recognize the structure of guest molecules[28-30]. The molecular recognition and inclusion complexation are of current interest in host-guest chemistry or supramolecular chemistry[31]. Molecular recognition of analytes by host- guest chemistry is a promising approach to chemical sensing[32-35]. Natural and chemically modified cyclodextrins can be viewed as molecular receptor because their chemical structure provides well defined inclusion cavities with a specific receptor function[36]. They can accommodate a wide variety of organic, inorganic, and biological guest molecules to form stable host-guest inclusion complexes or nanostructure supramolecular assemblies in their hydrophobic cavity, showing high molecular selectivity and enantioselectivity[37]. Cyclodextrins were previously applied as sensor ionophores to potentiometric ion selective electrodes for determination of onium ions[38], chiral molecules incorporating aryl rings[39], and protonated amines[40].

In the present work, the possibility of using R as anionic exchanger and β-CD as sensor ionophore in the preparation of a B_{12} selective electrode (sensor 3) with PVC as the polymeric matrix to immobilize the sensor and to attain the formation of highly stable complex was evaluated. The effect of using β-CD as an ionophore that provides high stability complex between that molecule and the cationic drug present in the solution, the membrane selectivity and sensitivity were enhanced.

Sensors fabrication

The electrochemical performance characteristics of the sensors were systematically evaluated according to IUPAC recommendations[33]. The results are given in TABLE 1.

In the present work, it has been found that B_1, B_6 and B_{12} vitamins can behave as cations, which suggests the use of ion exchangers of anionic types. It has been found that ammonium reineckate was optimum anionic exchanger for the studied drugs, for its low solubility product and suitable grain size[26,27].

Also oNPOE has been used in the fabrication of the three studied sensors. oNOPE, a nitroaromatic mediator and a polar plasticizer with high dielectric constant facilitated the inclusion of organic molecules by competitive inclusion. It was found to be the optimum available mediator for PVC membrane sensors. It plasticized the membranes and adjusted both permittivity and the mobility of the ion exchanger sites to give the highest possible selectivity and sensitivity. Polar plasticizers were reported to make membranes more selective compared with nonpolar plasticizers[41]. The improved selectivity is also attributed to the increase in the acidity of the R in the presence of polar solvents and hence, interaction with the drug. The use of non polar plasticizer as DOP leads to insufficient selectivity differences in the response of sensor to foreign cations.

For B_{12} sensor trials to fabricate it using R directly as the other two sensors revealed bad response therefore addition of β cyclodextrin which is optically active oligosaccharide that form inclusion compounds in the aqueous and in solid state with organic molecules was used. β-CD based sensor showed accurate results in both response and selectivity.

Sensors calibration and response time

The potential displayed by the three proposed electrodes for constructive measurements of standard drugs solutions in the same day and from day-to-day did not vary by more than ± 1 mV. Calibration slopes did not change by more than ± 0.3 mV/decade concentration

over a period of 5-6 weeks for electrodes (1-3) (TABLE 1). The dynamic response time for the electrodes to reach values within ± 1 mV of the final equilibrium potential after increasing the drug concentration 10-folds were found to be 30, 20 and 10s for electrodes 1, 2 and 3 respectively, (TABLE 1).

The slopes of the calibration plots were 28, 57.4 and 58.2 mV/concentration decade for electrode1, electrode 2, and electrode 3 respectively. Slope of sensor 1 was about 30 mV; the typical value of divalent substance as B_1 behaves as divalent anion via its two amino groups. On the other hand, slopes values of sensors 2 and 3 were about 60; the typical value of monovalent substances as these drugs behave as monovalent cations via their cationic groups. Figure 1 shows a decrease in negative potential as the concentration increases due to the decrease in negative charge on the membrane. The response characteristics of the three electrodes are summarized in TABLE 1.

Deviation from the ideal Nernstian slope (30 mV/decade) for sensor 1, and (60 mV/decade) for sensors 2 and 3, stems from the fact that the electrodes respond to the activities of the drugs rather than their concentrations.

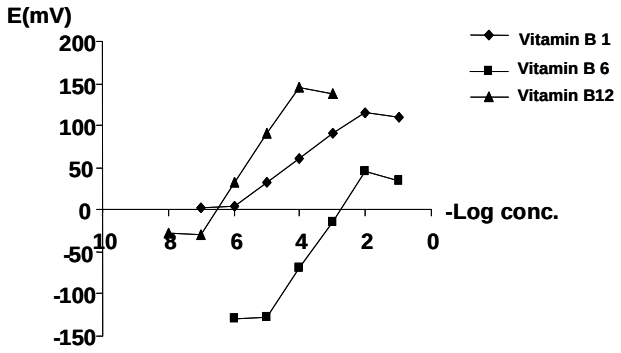

Figure 1 : Profile of the potential of vitamins selective electrodes

TABLE 1 : Validation of the response characteristics of the three investigated electrodes.

Parameter	Electrode 1	Electrode 2	Electrode 3
Validation of the regression equations	28 ± 0.1	57.4 ± 0.3	
Slope (mV/decade)[a]	172.7 ± 0.4	158.9 ± 0.2	58.2 ± 0.2
Intercept (mV)[a]	0.9993	0.9997	379.6 ± 0.1
Correlation coefficient (r^2)			0.9990
Validation of the responses	$1x10^{-6}$-$1x10^{-2}$	$1x10^{-5}$-$1x10^{-2}$	
Concentration range (M)	30	20	$1x10^{-7}$-$1x10^{-4}$
Response time (s)	1-3	1-3	10
Working pH range	$3.5x10^{-7}$	$3x10^{-6}$	1-3
LOD (M)*	5-6	5-6	$2x10^{-8}$
Stability (weeks)	101.1	100.0	5-6
Average accuracy (%)	0.7	0.6	99.7
Standard deviation (precision)	0.7	0.6	0.5
Relative standard deviation (precision %)	0.3	0.7	0.5
Repeatability[*a] (RSD%)	0.9	0.5	0.5
Reproducibility[*b] (RSD%)	99.1	100.8	0.7
Robustness [b]	99.7	99.8	98.6
Ruggedness [c]			98.9

[a]Results of five determinations

[b]Average recovery percent of determining 10^{-5} and 10^{-4} M solutions of the three studied electrodes preparing the membrane using oNPPE as plasticizer instead of oNPOE.

[c]Average recovery percent of determining 10^{-5} and 10^{-4} M solutions of the three studied electrodes using Jenway 3310 digital ion analyzer instead of 3330.

***The lower detection limit (LOD) defined as drug concentration obtained at the intersection of the extrapolated high concentration (linear segment) with the low concentration (zero slope segment) of the calibration plot.**

***[a] n = 3x3, *[b] n = 3x3**

The fabricated I.S.Es. gave a Nernstian response within 10^{-6}-10^{-2} M, 10^{-5}-10^{-2} M and 10^{-7}-10^{-4} M concentration range for electrodes 1, 2 and 3 respectively, (TABLE 1).

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Effect of pH and temperature on sensors

The pH effect was studied to be optimized from the point of view of both sensors function and chemical form of the test substances. This was done by immersing the electrodes in solutions of B_1 and B_6 and B_{12} of different pH values ranging from 1 to 11 obtained by adding drops of 1N HCl and 1N NaOH. The investigated electrodes gave a useful pH range from 1-3. Above this range, the potential displayed by the electrodes was noisy and the potential showed a sharp decrease due to the formation of non protonated group of vitamins, (Figure 2).

The effect of temperature was also studied by monitoring the potential response displayed by the electrodes as a function of – log the drug concentration at 20, 30 and 40°C. It was found that the suggested electrodes exhibited a gradual increase in their potentials as the temperature increased in the range of 20-40°C, however, the calibration graphs obtained at different temperatures were parallel. In spite of this, the limit of detection and response-time did not significantly vary with variation of temperature; indicating reasonable thermal stability of vitamins membranes up to 40°C.

Statistical analysis of the results for analysis of the pure vitamins by the proposed electrodes and the reference methods[2] showed no significant difference in terms of accuracy and precision; (TABLE 2).

Sensors selectivity

The potentiometric selectivity coefficient, -log ($K^{Pot}_{Primary\ ion,\ interferernt}$) was used to evaluate the extent to which a foreign ion would interfere with the response of an electrode to its primary ion. The selectivity coefficients were calculated from the equation:

$$- \log (K^{pot}_{primary\ ion\ interferent}) = E_1 - E_2 / S^{[22]}$$

Where E_1 and E_2 are the potential readings recorded after exposing the electrode to the same concentration of the studied vitamin and the interferent; respectively, and S is the slope of the investigated electrode. The results in TABLE 3 showed no interference from these additives and other B-vitamins in the studied concentration which reveal that the investigated sensors have reasonable selectivity.

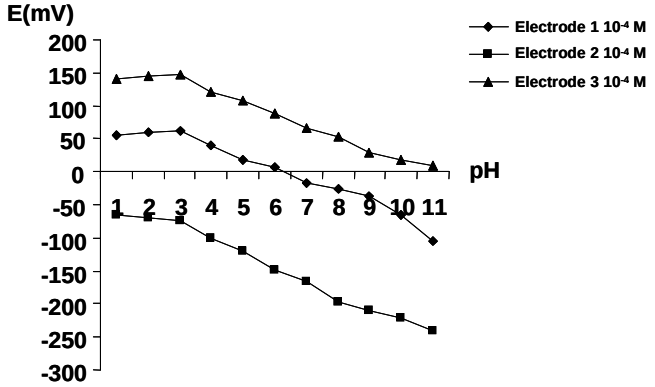

Figure 2 : Effect of pH on the responses of the electrodes (1-3)

TABLE 2 : Statistical analysis between the results obtained for the determination of studied vitamins in pure samples by the proposed method and those by the official methods.

Item	Electrode 1	Official method BP[2]	Electrode 2	Official Method BP[2]	Electrode 3	Official method BP[2]
Mean	101.1	101.0	100.0	100.1	99.7	99.9
S.D.	0.7	0.8	0.6	0.4	0.5	0.6
RSD%	0.7	0.8	0.6	0.4	0.5	0.6
Variance	0.5	0.6	0. 4	0.2	0.3	0.4
n	5	6	5	6	5	5
F test	1.2(6.26)		2(6.26)		1.3(6.39)	
Student's t test	0.7(2.262)		0.9(2.262)		1.5(2.306)	

Figures between parenthesis are the corresponding tabulated values (P=0.05)

The proposed electrodes were used for determination of the studied vitamins in laboratory prepared mixtures. Good recoveries were obtained for each vitamin in the presence of the other two vitamins indicating the selectivity of each electrode (TABLE 4).

The proposed electrodes were also used for determination of B_1, B_6 and B_{12} in some pharmaceutical dosage forms, (TABLE 5). Diluents and exipients present did not show any interference. Thus, analysis was carried out without prior treatment or extraction.

On application to the biological fluids, it was found that the three electrodes gave stable results as revealed

by high precision and accuracy of the recoveries of the spiked human plasma samples, (TABLE 6).

To evaluate precision and accuracy, three concentrations within the linear range (10^{-5}, 10^{-4} and 10^{-3} M solutions of B_1 and B_6 and 10^{-6},10^{-5} and 10^{-4} M solutions of B_{12}) were chosen. Three solutions of each concentration were prepared and analyzed in triplicate (repeatability assay). This assay was repeated on three different days (reproducibility assay), (TABLE 1).

As for the robustness, determining 10^{-5} and 10^{-4} M solutions of vitamins drugs for the three studied electrodes preparing the membrane in oNPPE as plasticizer instead of oNPOE was studied; the methods proved to be robust. Also to study the methods ruggedness, 10^{-5} and 10^{-4} M solutions of vitamins drugs were analyzed by the three studied electrodes using Jenway 3310 digital ion analyzer instead of 3330 Model. The results showed no significant difference upon change of the instrument. Results obtained are depicted in TABLE 1. These data render the proposed method applicable for the quality control of the drug formulations.

TABLE 3 : Potentiometric selectivity coefficients (average of five determinations) ($-\log K^{Pot}_{D,I}$) of vitamins proposed sensors by separate solution method

Interferent* 10^{-4} M	Selectivity coefficient		
	Sensor 1	Sensor 2	Sensor 3
Urea	4.0×10^{-4}	4.7×10^{-4}	8.6×10^{-4}
K^+	3.5×10^{-4}	3.8×10^{-4}	3.3×10^{-4}
Glycine	5.3×10^{-3}	2.8×10^{-4}	6.5×10^{-3}
Starch	2.1×10^{-4}	5.4×10^{-4}	0.6×10^{-4}
Na^+	8.2×10^{-4}	4.0×10^{-4}	7.4×10^{-4}
NH_4^+	0.4×10^{-2}	8.4×10^{-3}	9.1×10^{-3}
Glucose	3.3×10^{-3}	3.9×10^{-2}	8.2×10^{-3}
Sucrose	1.4×10^{-4}	1.7×10^{-4}	0.6×10^{-4}
Lactose	2.2×10^{-4}	6.3×10^{-4}	1.5×10^{-4}
Cu^{2+}	2.4×10^{-4}	4.4×10^{-4}	2.0×10^{-4}
Hydroxylamine	0.6×10^{-3}	2.1×10^{-3}	0.6×10^{-2}
Vitamin B_1	-	6.3×10^{-2}	8.1×10^{-3}
Vitamin B_6	1.2×10^{-2}	-	6.4×10^{-3}
Vitamin B_{12}	2.5×10^{-2}	1.7×10^{-2}	-

***Average of five determinations.**

TABLE 4 : Determination of intact vitamin B_1, vitamin B_6 and vitamin B_{12} in laboratory prepared mixtures by the proposed potentiometric method

Concentration M			Ratio Vitamin B_1 : Vitamin B_6 : Vitamin B_{12}	Recovery % of vitamin B_1	Recovery % of vitamin B_6	Recovery % of vitamin B_{12}
Vitamin B_1	Vitamin B_6	Vitamin B_{12}				
1.0×10^{-3}	1.0×10^{-3}	1×10^{-5}	100 : 100 : 1	98.5	100.3	99.3
1.5×10^{-3}	1.0×10^{-3}	1×10^{-5}	150 : 100 : 1	101.8	100.9	100.4
1.0×10^{-3}	0.4×10^{-3}	1×10^{-5}	100 : 25 : 1	99.2	98.9	99.9
2.5×10^{-3}	1.0×10^{-3}	1×10^{-5}	100 : 40 : 1	100.3	99.5	101.3
		Mean		99.9	99.9	100.2
		R.S.D.%		1.4	0.9	0.8

TABLE 5 : Determination of vitamin B_1, vitamin B_6 and vitamin B_{12} in some pharmaceutical formulations by the suggested potentiometric method

Product	Found * % ± R.S.D.		
	Sensor 1	Sensor 2	Sensor 3
Neurovit tablets batch no. 033291	99.9 ± 0.3	101.4 ± 0.5	98.1 ± 0.7
Neurovit ampoule batch no. 866507	98.8 ± 0.4	99.7 ± 0.8	100.5 ± 0.4
Tri – B ampoule batch no. 91056B	100.7 ± 0.6	98.4 ± 0.3	99.6 ± 0.1
Neurobion ampoule batch no. 082705A	101 ± 0.2	100.5 ± 0.8	101 ± 0.1

***Average of three determinations.**

TABLE 6: Determination of vitamin B_1, vitamin B_6 and vitamin B_{12} in spiked human plasma by the proposed electrodes

Drug	Concentration (M)	Recovery* % ± R.S.D
Vitamin B_1	1×10^{-3}	101.5 ± 0.6
Vitamin B_6	1×10^{-3}	100.9 ± 0.4
Vitamin B_{12}	1×10^{-5}	98.7 ± 0.1

***Average of three determinations.**

CONCLUSION

In this work, three poly(vinyl chloride) matrix membrane electrodes responsive to some vitamins, thiamine

(B_1), pyridoxine hydrochloride (B_6) and cyanocobalamin (B_{12}) were developed, described and characterized. The proposed electrodes are simple and sufficiently specific for quantitative determination of the studied vitamins. in pure powders, dosage forms and spiked plasma samples. The electrodes offer the advantages of ease of construction, rapid manipulation, low cost, fast response, wide concentration range, good selectivity, elimination of drug pretreatment or separation steps and applicability to turbid and coloured solutions. Further advantages offered by using ammonium reineckate and β-CD based membrane sensors are low detection limit ($2x10^{-8}$-$3x10^{-6}$M), extending working concentration range (10^{-2}-10^{-7}M), wide pH range (1-3), stability (5-6 weeks), repeatability (0.3-0.7 %) and reproducibility (0.5-0.9 %). The proposed method is precise, accurate, simple, rapid and does not require any expensive or sophisticated apparatus. Also it is more sensitive and selective if compared with the official titrimetric methods of the vitamins. So, the proposed method can be used for the routine quality control of vitamins either in pure powder form or in pharmaceutical formulations.

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