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New ionophores for vitamin B1 and vitamin B6 potentiometric sensors for multivitaminic control

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Abstract

The construction, evaluation and analytical application of potentiometric sensors sensitive to vitamin B1 and vitamin B6 are reported. The solid contact electrodes were produced using β-cyclodextrins as ionophores in a carboxylated poly(vinyl chloride) support matrix. Near Nernstian slopes (mV/decade) of 51.7 ± 0.8, 60.6 ± 0.6 and 61.1 ± 1.4, within the intervals (M) of 1.0×10^{-4} to 1.0×10^{-1}, 5.8×10^{-5} to 1.0×10^{-1} and 4.3×10^{-5} to 1.0×10^{-1} were obtained, for thiamine and pyridoxine I and II prepared membranes, respectively. A pH operational range of 6.5–8.5 for thiamine and 2–4.5 for pyridoxine electrodes was found. Assessment of selectivity coefficients toward a large number of inorganic cations and organic cations usually present in multivitamin formulations revealed good performance. Analysis of vitamins B1 and B6 in complex multivitamin drugs was achieved with recoveries within the intervals of 95.1–99.6% for thiamine and 95.1–102% for pyridoxine. Furthermore, the results enabled by the proposed procedure revealed good agreement with those provided by HPLC.

Keywords: Cyclodextrins; Thiamine; Pyridoxine; Multivitamin formulations; Ion-selective electrodes

1. Introduction

Thiamine hydrochloride (vitamin B1) and Pyridoxine hydrochloride (vitamin B6) are included in a group of hydro soluble substances generically called Complex B Vitamins and act as coenzymes involved in the production of energy [1]. Additional effects of Vitamin B1 include the improvement of blood circulation, aid in the hydrochloride acid production and blood formation at the bone marrow. Severe deficiency leads to the development of a syndrome known as beri-beri. On the other hand, vitamin B6 performs not only an important role in the descarboxylation and transamination of amino acids but also in carbohydrate and fat metabolism. Pyridoxine deficiency may lead to sideroblastic anaemia, dermatitis, cheilosis and neurological symptoms such as peripheral neuritis and convulsions.

An equilibrated human diet provides all the vitamins necessary for body complete development and equilibrium maintenance. Physiological and physiopathological conditions in children, elderly and pregnant women may however require vitamin supplements, which are often consumed without prescription due to marketing and the abundance of preparations. The reference protocols [2] enable only isolated determinations of thiamine or pyridoxine in pharmaceutical formulations. Their application to multivitamin preparations renders inaccurate results. Thus, alternative methods to assure the security and quality of this kind of products have been proposed, involving UV–vis spectrophotometry [3,4], spectrofluorimetry [5], high performance liquid chromatography with spectrophotometric detection [6–9], voltammetry [10], turbidimetry [11], and flow injection with electrochemical [12] or optical detection [13–15]. Most of them present several disadvantages such as poor selectivity, requirement of tedious sample pretreatment (purification or preconcentration steps), use of toxic reagents, or even complex chemometric treatment of the analytical results.

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Potentiometry with ion-selective electrodes offers numerous advantages in analytical control of pharmaceutical organic species [16,17], once it enables simple and no time consuming procedures, resorts to less toxic reagents and is easily applicable to complex and turbid samples. Some potentiometric sensors for determination of vitamin B1, vitamin B6 or both have also been reported [18–21]. All of them are based on the use of classical ion exchangers, namely heteropolycyclic acids [19] or phenyl borate derivatives [18,20,21]. The limited selectivity enabled by these species is however a major drawback for samples where both vitamins are present.

Cyclodextrins are a family of cyclic oligosaccharides composed of α-(1,4) linked glucopyranose subunits, possessing a cage like supramolecular structure that allows to carry out chemical reactions of "host-guest" type, with specific receptor function [22]. This property turns out these ion-receptors in an interesting class of species to be studied as electrochemical sensors for organic molecules [23].

Several authors have reported the use of α, β, and γ-cyclodextrins as ionophores in construction of potentiometric ion-selective electrodes [24–26] and some published reviews summarize their use as analytical sensors. [22,23]. Those studies proved that when cyclodextrins are dissolved in the proper mediator solvent, they yield potentiometric detectors with good characteristics for organic amines [24,26] with reduced interference relatively to alkaline and alkaline earth metal ions [25].

In this work, the use of cyclodextrins is exploited in the construction and evaluation of novel potentiometric ion-selective electrodes for vitamins B1 and B6, with enhanced analytical capability.

2. Experimental

2.1. Apparatus

All potential measurements were performed at 25 ± 1 °C, with a 2002 Crison micro pH (sensitivity ±0.1 mV) coupled to an electrode commutator of the same brand.

A double junction Orion 90-02-00 silver chloride/silver reference electrode was used in conjunction with the proposed electrodes, containing 0.1 M lithium chloride in the outer compartment.

A Crison pH meter GLP 22 coupled to a combined glass electrode Crison Ref. 52-02 was used for pH determinations.

Spectrophotometric spectra of the sensor solutions and the respective vitamin B1 or B6 solutions were collected in a Perkin Elmer λ 45 double beam spectrophotometer.

The analysis of the samples by HPLC was carried out in a Merck Hitashi chromatographic system comprising a model 7100 pump, a Rheodyne 7725i injector (20 μl loop) and an Alltech ODS-2 C_{18} column (250 mm × 4.6 mm). A diode array system, model 7455 was used as detector and the data processed by means of software package of the same brand, model D7000.

2.2. Reagents and solutions

All solutions were prepared with distilled and double deionised water, with conductivity <0.1 μS/cm. Analytical grade chemicals without additional purification were used.

Stock solutions (0.1 M) of thiamine hydrochloride (Sigma), thiamine nitrate (Roche Pharmaceutics, USA) and pyridoxine hydrochloride (Roche Pharmaceutics, USA) were prepared by weighing and dissolving the right amount of pure powder in 0.1 M lithium chloride (Aldrich) solution, (used as an ionic strength adjuster), and stored protected from the light in the fridge. Solutions with the main ion (vitamin B1 or B6), used for the electrode characterization and analytical determinations (1×10^{-2} M/1×10^{-3} M/1×10^{-4} M), were daily prepared from the stock solution, diluting the required volume with LiCl 0.1 M and using 50 ml amber volumetric flasks, carefully kept apart from light and heat between measurements.

A 5×10^{-4} M silicotungstic acid solution in HCl 0.5 M was used as vitamin B1 precipitant.

Whenever necessary, concentrated lithium hydroxide (Merck) was used to adjust thiamine hydrochloride solutions to pH 7.

For HPLC method, the mobile phase constituted by sodium heptanesulphonate (Sigma), methanol HPLC grade (Merck), triethylamine (Aldrich) and orthophosphoric acid (Merck) was prepared, according to Ref. [2].

2.3. Sample preparation

Several multivitamin pharmaceutical preparations purchased on local drug stores were tested (ampoules, sachets and tablets). First, an amount of powder or liquid volume, corresponding to 5–200 mg of the vitamin B1 or B6 was dissolved in a LiCl 0.1 M solution in a 100 ml volumetric flask. For vitamin B1 assay, a volume of 20 or 25 ml of this solution was transferred to a beaker and the pH adjusted to 7, with concentrated LiOH, and afterwards transferred to a 50 ml volumetric flask, and the volume completed with LiCl (0.1 M).

Regarding the determination of vitamin B6, 20 ml of precipitant reagent was added to an aliquot of the previously reported solution (5 or 10 ml) for samples where the content of vitamin B1 was higher than vitamin B6. All the dilutions were prepared in order to fit the final concentration of both vitamins to the analytical concentration range of the electrodes.

To proceed with potentiometric results validation, samples were prepared and analysed following the methodology indicated in [2] in which previous filtration and degassing of the solutions were necessary.

2.4. Sensor preparation and electrode construction

Electrode membranes were obtained using β-cyclodextrin (Fluka), 2-hydroxipropyl-β-cyclodextrin (Fluka), potassium tetrakis (*p*-chlorophenyl) borate (NaTpClPB) (Fluka), 2-fluorophenyl-2-nitrophenyl ether (FNDPE) (Fluka), carboxylated poly(vinyl chloride) (PVC-COOH) (Janssen Qhimica) and tetrahydrofuran (THF) (Merck). General preparation of sensor

Table 1
Composition of VitB1 ISE, VitB6 ISE I and VitB6 ISE II membranes

Component	VitB1 ISE	VitB6 ISE I	VitB6 ISEII
β-Cyclodextrin	–	–	6.35
2-Hydroxypropyl β-cyclodextrin	6.45	6.35	–
2-Fluorophenyl-2-nitrophenyl ether (FNDPE)	64.5	63.5	63.5
Potassium tetrakis (*p*-chlorophenyl) borate (NaTpClPB)	–	1.59	1.59
Carboxylated poly(vinylchloride) (PVC-COOH)	29.05	28.56	28.56

Values are expressed in % (w/w).

solutions was carried out by mixing suitable amounts of the ionophore with the mediator solvent and by the addition of a previously prepared PVC-COOH solution in THF. A low percentage of a lipophilic additive was also included on Vit B6 sensor solutions.

Thus, VitB1 sensor was obtained by adding 0.04 g of the ionophore (2-hydroxipropyl-β-cyclodextrin) to 0.4 g of mediator solvent (FNDPE) and 0.18 g PVC-COOH (Table 1).

Two sensor membranes were prepared for vitamin B6 electrodes, one using a more lipophilic cyclodextrin (2-hydroxipropyl-β-cyclodextrin) and the other a less lipophilic one (β-cyclodextrin). For VitB6 ISE I an amount of 0.04 g of the ionophore (2-hydroxipropyl-β-cyclodextrin) and 0.01 g of additive (NaTpClPB) in 0.4 g of mediator solvent (FNDPE) and 0.18 g PVC-COOH were used. VitB6 ISE II was prepared with 0.04 g of the ionophore (β-cyclodextrin) and 0.01 g of additive (NaTpClPB) in 0.4 g of mediator solvent (FNDPE) and 0.18 g PVC-COOH (Table 1).

The sensor solutions were afterwards dropped over the surface of the solid contact of the electrode, formed by a support of epoxy resin (Araldite) mixed with powdered graphite, according to [27]. Successive applications of these solutions were done after the solvent evaporation (THF), to achieve a homogeneous membrane of about 1 mm thickness [27].

The electrodes were left on air during approximately 12 h, in order to completely dry their membranes, and then soaked in water for about 3 h, before being used.

2.5. Procedures

In order to evaluate the response characteristics of the constructed electrodes, several consecutive calibrations were daily performed. They were immersed together with the reference electrode in 50 ml beakers containing 20 ml of 0.1 M LiCl, as ionic strength adjuster. Under continuous stirring, the potentials were recorded when stabilized to ±0.2 mV, after the addition of different volumes of 1×10^{-3} M or 1×10^{-2} M standard solutions of VitB6 or VitB1. The electrodes were rinsed and stirred in distilled water between measurements and when not in use, they were left on air.

Gran's Plot method was performed on 20 ml of the pH-adjusted samples (for determination of vitamin B1), and on 25 or 30 ml of vitamin B6 samples. This method was carried out by adding volumes equivalent to 1 ml of the respective vitamin solution (1×10^{-2} M or 1×10^{-1} M) and the potential recorded after stabilization to ±0.2 mV.

3. Results and discussion

3.1. General working characteristics

Cyclodextrins are torus-shaped oligosaccharides that can form inclusion complexes by taking up the guest molecule (vitamin cation) into the central hydrophobic cavity. According to their complex formation constants, they can provide electrochemical ionophores giving a different selectivity pattern from those obtained with classical ion exchangers. The action mechanism of these last compounds is based on ion-interaction between a lipophilic ion (exchanger) and the analyte through electrostatic bonds. In this case, the selectivity pattern follows the Hofmeister series which are related with the hydration energy of the main ion.

In this study, cyclodextrins with different chemical characteristics (namely α, β, or γ cyclodextrins), several mediator solvents with different dielectric constants (2-nitrophenyloctyl ether (o-NPOE), dibutyl phthalate (DBP), 2-fluorophenyl-2-nitrophenyl ether (FNDPE), and also different immobilization support matrices (as PVC or PVC-COOH) were tested. Some sensor membranes incorporated NaTpClPB as an additive (see Table 1). For VitB1 ISE, the first approach consisted in using β-cyclodextrin as ionophore and FNDPE as mediator solvent in PVC-COOH. However, the slopes obtained were not satisfactory (40 mV/decade) and for that reason a more hydrophobic cyclodextrin was then tested. Concerning VitB6 I and VitB6 II ISEs the addition of a low percentage (1.59%, w/w) of lipophilic additive (NaTpClPB) improved the characteristics of the electrodes, contrarily to vitB1 ISE. As known, the addition of a lipophilic species as an additive can contribute for decreasing the membrane resistance and also for improving the slope and the selectivity of the membranes, Two different types of ionophores, including a more hydrophobic one (2-hydroxipropyl-β-cyclodextrin) and a more lipophilic (β-cyclodextrin), were used. Nevertheless, no significant difference concerning general calibration parameters was noticed from this fact (Table 2). Whatever the

Table 2
General working characteristics of thiamine (vitamin B1) and pyridoxine (vitamin B6) selective electrodes

Characteristics[a]	VitB1 ISE	VitB6 ISE I	VitB6 ISE II
Slope (mV/decade)	51.7 ± 0.8	60.6 ± 0.6	61.1 ± 1.4
LLLR (M)	1.0×10^{-4}	5.8×10^{-5}	4.3×10^{-5}
PDL (M)	2.1×10^{-5}	4.2×10^{-5}	3.5×10^{-5}
Linear range (M)	1.0×10^{-4}–1.0×10^{-1}	5.8×10^{-5}–1.0×10^{-1}	4.3×10^{-5}–1.0×10^{-1}
pH range	2–4.5/6.5–8.5	2–4.5	2–4.5
Response time (s)	<28	<30	<45

ISE: ion-selective electrode; LLLR: lower limit of linear response; PDL: practical detection limit.

[a] Average of two determinations with three units of each electrode proposed.

Table 3
Major characteristics of the electrodes referred in literature sensitive either to vitamin B1, vitamin B6 or both

Characteristics	Ref. [18]		Ref. [19]		Ref. [20]				Ref. [21]
	VitB1	VitB6	VitB6 I	VitB6 II	VitB1 I	VitB1 II	VitB6 I	VitB6 II	VitB1
Electrode construction	Ion association complex; without inner reference solution		Ion association complexes; with inner reference solution		Ion association complexes; with inner reference solution				Ion association complexes; with inner reference solution
Membrane composition	TCPB[a] + o-NPOE + PVC		Molybdophosphate (I)[a]/tungstophosphate (II)[a] + 2-NFFE + PVC		DNNS (I)[a]/ClTPB (II)[a] + o-NPOE + PVC				TFPB[a] + o-NPOE + PVC
Slope	32.6 ± 0.9[b]	62.6 ± 0.3	54.0 ± 0.5	54.5 ± 0.4	28.0 ± 0.4[c]	27.1 ± 0.5[c]	55.6 ± 0.7	55.3 ± 0.8	29.6 ± 0.7[b]
LLLR (M)	8.0×10^{-6}	2.0×10^{-5}	–	–	1×10^{-5}	1×10^{-5}	7.1×10^{-4}	1.2×10^{-4}	1.0×10^{-7}
PDL (M)	1.0×10^{-6}	3.9×10^{-6}	4×10^{-5}	4×10^{-5}	5×10^{-6}	5.6×10^{-6}	2.5×10^{-4}	6.3×10^{-5}	–
Response time	~15 s	~15 s	60 s	45 s	10–30 s in 1×10^{-1} to 1×10^{-4} M/ 3 min in more diluted				<2 min
pH	2–4	2–4	2–4	2–4	2–4.5	2–4	2–4.5	2–4	±4
Analytical application	was not performed on multivitamins for VitB6		Only two samples with very simple composition		Only two samples whose composition was not mentioned				Only one sample

LLLR: lower limit of linear response; PDL: practical detection limit; TCPB: tetra(2-clorophenyl)borate; O-NPOE: 2-nitrophenyl octyl ether; PVC: poly(vinyl chloride); DNNS: dinonylnaphthalene sulphonate; TFPB: 2-tetrakis [3,5-bis(2-methoxyhexafluoro-2-propyl)phenyl]borate.

[a] Molecule that forms the ion complex association with the respective vitamin.

[b] pH 4.

[c] pH 3.5.

sensor prepared poor responses in terms of slope and practical detection limits were obtained with the membrane formulations using PVC, instead of PVC-COOH and also mediator solvents of lower dielectric constants (o-NPOE and DBP) than FNDPE, thus these membranes were not further used. Once three final sensor membrane compositions were achieved (Table 1), the performance and characteristics of the corresponding electrodes was assessed, during the ISEs useful lifetime (Table 2).

Regarding VitB1 electrode, all tests were performed using thiamine solutions in hydrochloride or mononitrate salt forms, once they constitute the main raw materials in the preparation of multivitamin pharmaceuticals. In both solutions the electrodes present similar characteristics in what concerns the upper detection limit. This parameter is a consequence of a coextraction process of the primary cation and interfering anions from the sample into the ion-selective membrane and consequently related with the lipophilicity of the counter ions, which are similar in this case. Thiamine molecule Exhibits two pKa values of 4.8 and 9. A pure solution of thiamine mononitrate in lithium chloride shows a pH 7, and the molecule is then mono positively charged. On the other hand, the solution of the corresponding hydrochloride form shows a pH 4, and thus the double cationic form of thiamine predominates. The proposed electrodes showed a reproducible near Nernstian slope of 51.7 ± 0.8 mV/ decade, when calibrated with solutions of thiamine mononitrate salt form, or pH adjusted thiamine hydrochloride form (pH 7). A stable response of 31.8 ± 1.7 mV/decade was also obtained, when solutions of thiamine hydrochloride in LiCl 0.1 M (pH 4) without previous pH adjustment, were tested. In spite of the possibility of working in two different pH ranges, it was decided to choose the one that allows a higher slope value (pH 7), because of the higher precision of the results achieved. This difference in slope values was not reported before by other authors [18,20,21], as can be seen in Table 3, where the characteristics of VitB1 selective electrodes previously reported in literature are described. The possibility of working with a higher slope (51.7 ± 0.8 mV/decade) allows a better precision of the results, which is important mainly in the measurements where both vitamins are present in samples. Furthermore, the proposed electrodes present quicker response when compared with those reported before [20,21] and also a widened pH operational range [18,20,21].

In what concerns VitB6 electrodes, the two developed membranes, with slightly different compositions, showed similar characteristics (Table 2). As a consequence, they were both used in all the following evaluations. Comparing with those previously reported in literature (Table 3), some features were improved, namely the working slope and the response times considering those reported on [19,20], a widened pH range, and better LLLR and PDL regarding to [20].

The constructed units showed a period of useful lifetime superior to 3 months both for VitB1 and VitB6 membranes, and potential changes less than 2 mV were recorded between calibrations.

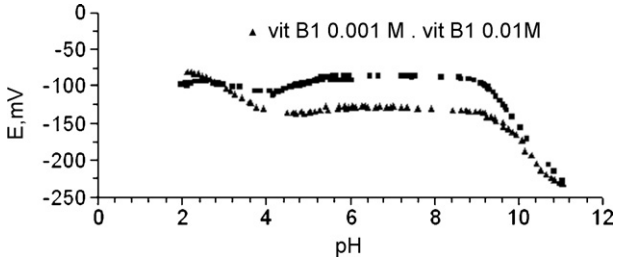

Fig. 1. Diagrams of Reilley for VitB1 ISE achieved at two concentration levels (10^{-3} M and 10^{-2} M) of the main ion.

3.2. Effect of pH in electrodes response

The effect of pH in the electrodes response was evaluated through Reilley's diagrams. The experiments were conducted with two electrodes of each type, for two concentration levels of the primary ion evolved (thiamine or pyridoxine)-1×10^{-2} M and 1×10^{-3} M, using sodium hydroxide or sulphuric acid to increase or decrease the pH, respectively, over the pH range 2–11 (Figs. 1 and 2).

The potential changes were recorded, and the operational pH range established, whenever potentials did not change by more than 5 mV.

Reilley diagrams for vitamin B1 electrode (Fig. 1) showed that two working pH ranges, namely 2–4 (in which thiamine is double charged) and 6.5–8.5 (thiamine molecule is single positively charged) were obtained.

In what concerns vitamin B6 ISEs I and II (Fig. 2A and B), an operational pH range between 2 and 4.5 was obtained for both type of electrodes.

3.3. Selectivity coefficients evaluation

The selectivity of the developed electrodes against several inorganic and organic species usually present on multivitamin formulations was evaluated. For the study of the interference

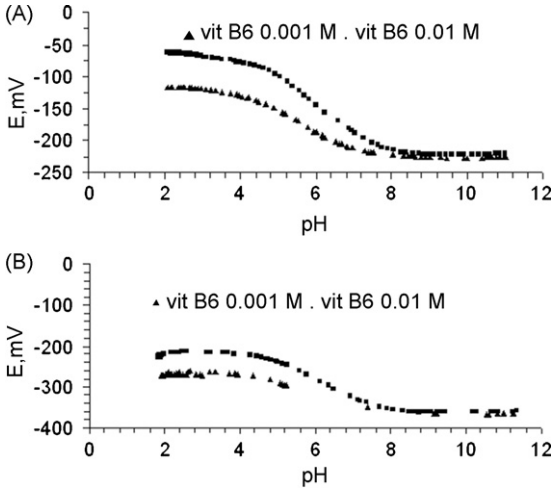

Fig. 2. Diagrams of Reilley for: (A) VitB6 ISE I and (B) VitB6 ISE II achieved at two concentration levels (10^{-3} M and 10^{-2} M) of the main ion.

Table 4
Potentiometric selectivity coefficients (log $K_{pot_{VitB1,interferent}}$) for VitB1 ISEs[a]

Interferent	vitamin B1 ISE		
	1.0×10^{-4}(M)	9.1×10^{-4} (M)	3.1×10^{-3} (M)
Na^{+b}	$-0.64 \pm 1 \times 10^{-2}$	$-1.54 \pm 4 \times 10^{-3}$	$-2.11 \pm 1 \times 10^{-2}$
K^{+b}	$-0.68 \pm 8 \times 10^{-2}$	$-1.1 \pm 7 \times 10^{-2}$	$-1.70 \pm 7 \times 10^{-2}$
Mg^{2+b}	$-2.89 \pm 8 \times 10^{-2}$	$-3.27 \pm 7 \times 10^{-2}$	$-3.54 \pm 4 \times 10^{-2}$
Fe^{3+b}	$-1.80 \pm 7 \times 10^{-2}$	$-1.39 \pm 5 \times 10^{-2}$	$-1.27 \pm 7 \times 10^{-2}$
Vitamin C[b]	$-0.80 \pm 6 \times 10^{-2}$	$-0.57 \pm 3 \times 10^{-2}$	$-0.48 \pm 8 \times 10^{-3}$
Pyridoxine (B6)[b]	$-0.51 \pm 9 \times 10^{-3}$	$-0.23 \pm 6 \times 10^{-3}$	$-0.12 \pm 5 \times 10^{-3}$
Pyridoxine(B6)[c]	-0.2 ± 0 a	-0.46 ± 0 b	-0.93 ± 0 c

[a] Mean of four results obtained with two units.
[b] Results obtained by separate solutions method [28].
[c] Results obtained by match potential method [28], for three concentration levels of vitamin B6 (M): (a) 2.5×10^{-4}; (b) 2.5×10^{-3}; (c) 2.5×10^{-2}.

Table 5
Potentiometric selectivity coefficients (log $K_{pot_{VitB1,interferent}}$) for VitB6 ISE I and VitB6 ISE II[a]

Interferent	Vitamin B6 ISE I			Vitamin B6 ISE II		
	1.0×10^{-4} (M)	9.1×10^{-4} (M)	3.1×10^{-3} (M)	1.0×10^{-4} (M)	9.1×10^{-4} (M)	3.1×10^{-3} (M)
Na^{+b}	$-0.22 \pm 3 \times 10^{-2}$	$-1.20 \pm 3 \times 10^{-2}$	$-1.70 \pm 1 \times 10^{-2}$	$-0.72 \pm 7 \times 10^{-2}$	$-1.48 \pm 4 \times 10^{-2}$	$-1.96 \pm 8 \times 10^{-2}$
K^{+b}	$-0.36 \pm 2 \times 10^{-1}$	$-1.03 \pm 1 \times 10^{-1}$	$-1.18 \pm 8 \times 10^{-2}$	$-0.48 \pm 1 \times 10^{-1}$	$-1.11 \pm 8 \times 10^{-2}$	$-1.28 \pm 1 \times 10^{-1}$
Mg^{2+b}	$-2.41 \pm 1 \times 10^{-1}$	$-3.0 \pm 1 \times 10^{-1}$	$-3.17 \pm 1 \times 10^{-1}$	$-2.66 \pm 2 \times 10^{-1}$	$-3.05 \pm 3 \times 10^{-2}$	$-3.40 \pm 7 \times 10^{-2}$
Fe^{3+b}	$-2.80 \pm 7 \times 10^{-2}$	$-3.05 \pm 2 \times 10^{-2}$	$-3.11 \pm 5 \times 10^{-2}$	$-2.89 \pm 9 \times 10^{-3}$	$-3.15 \pm 2 \times 10^{-2}$	$-3.37 \pm 2 \times 10^{-2}$
Thiamine(B1)[b]	$1.28 \pm 7 \times 10^{-2}$	$1.0 \pm 7 \times 10^{-2}$	$0.78 \pm 6 \times 10^{-2}$	$0.85 \pm 2 \times 10^{-2}$	$0.65 \pm 2 \times 10^{-2}$	$0.60 \pm 4 \times 10^{-2}$
Thiamine(B1)[c]	1.70 ± 0 a	1.52 ± 0 b	1.12 ± 0 c	1.49 ± 0 a	1.47 ± 0 b	0.86 ± 0 c

[a] Mean of 4 results obtained with two units.
[b] Results obtained by separate solution method [28].
[c] Results obtained by match potential method [28], for three concentration levels of vitamin B6 (M): (a) 2.5×10^{-4}, (b) 2.5×10^{-3}, (c) 2.5×10^{-2}.

caused by cations Na^+, K^+, Mg^{2+} and Fe^{3+}, separate solutions method was used [28], at three levels of similar concentrations of primary and interfering ion, namely 1.0×10^{-4}, 9.0×10^{-4} and 3.1×10^{-3} M (Tables 4 and 5). Regarding the vitamins usually present on multivitamin complexes, as vitamin B5, inositol, vitamin C, nicotinamide, riboflavin, cyanocobalamin and biotin, the evaluation of K_{pot} was performed using fixed interference method [28], as a way to approach the results to the real analytical conditions. Different proportions of vitamin B1 or B6 and interferent were used (Table 6), taking into account the limit values of these interferences found on the pharmaceuticals analysed.

Regarding the comparison of results for the interferences with those of previously reported electrodes [18–21], an improvement on selectivity for most of the cations studied was obtained, even in mutual interference of vitamin B1 and B6. This fact is related to the buffered activity of the free main ion in the membrane due to the host guest complex equilibrium, which repeals other ions of the same charge [18–21]. The molecular recognition has demonstrated to be more selective by using a macrocyclic compound, instead of that verified with classical ion exchanger type, whose action mechanism is based on electrostatic interactions [18–21].

Concerning the interference of thiamine on VitB6 electrode and of pyridoxine on VitB1 electrode, match potential method [28] was also carried out (Tables 4 and 5), at three levels

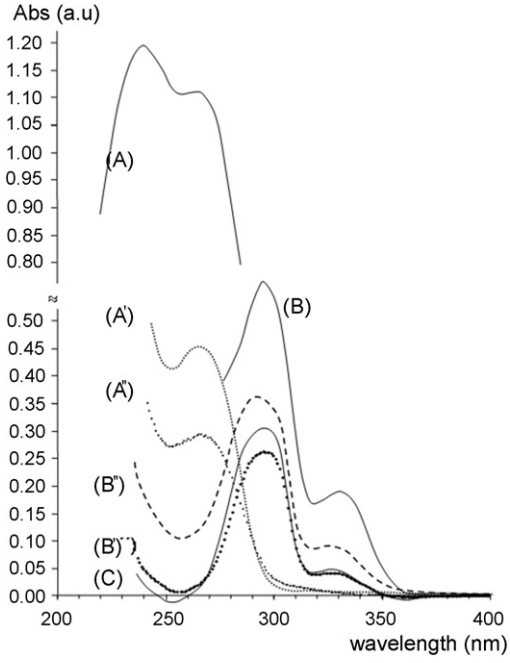

Fig. 3. UV spectra profiles obtained for two solutions with 3×10^{-4} mol L^{-1} in thiamine (A) and pyridoxine (B); after extraction with a similar volume of FNDPE (A′ and B′) or after extraction with cocktails used in the preparation VitB1 ISE and VitB6 ISE I (A″ and B″) or after extraction with cocktail used in the preparation VitB6 ISE II (C).

Table 6
Potentiometric selectivity coefficients (log $K_{pot_{VitB1\ or\ VitB6,\ interferent}}$) for VitB1 ISE, VitB6 ISE I and VitB6 ISE II (fixed interference method-ref. [28])

Interferent	VitB1 ISE			VitB6 ISE I			VitB6 ISE II		
	A	B	C	D	E	F	D	E	F
Nicotinamide	$-0.81 \pm 6 \times 10^{-3}$	$-1.80 \pm 9 \times 10^{-3}$	$-2.7 \pm 3 \times 10^{-3}$	$-1.39 \pm 8 \times 10^{-2}$	$-2.34 \pm 4 \times 10^{-3}$	$-3.0 \pm 2 \times 10^{-2}$	$-1.4 \pm 8 \times 10^{-2}$	$-2.37 \pm 5 \times 10^{-3}$	$-2.90 \pm 2 \times 10^{-2}$
Riboflavin	$-0.28 \pm 2 \times 10^{-2}$	$-1.44 \pm 3 \times 10^{-2}$	$-2.15 \pm 1 \times 10^{-2}$	$-1.34 \pm 2 \times 10^{-2}$	$-2.22 \pm 6 \times 10^{-3}$	$-3.07 \pm 8 \times 10^{-2}$	$-1.28 \pm 7 \times 10^{-2}$	$-2.23 \pm 9 \times 10^{-2}$	$-2.90 \pm 7 \times 10^{-2}$
Inositol	$0.15 \pm 5 \times 10^{-3}$	$-0.80 \pm 6 \times 10^{-2}$	$-2.02 \pm 2 \times 10^{-2}$	$-0.92 \pm 6 \times 10^{-2}$	$-1.92 \pm 9 \times 10^{-2}$	$-2.94 \pm 5 \times 10^{-2}$	$0.19 \pm 4 \times 10^{-2}$	-2.50 ± 0	$-1.64 \pm 2 \times 10^{-1}$
Cyanocobalamin	$-0.94 \pm 7 \times 10^{-3}$	$-0.0044 \pm 3 \times 10^{-2}$	$-1.16 \pm 9 \times 10^{-2}$	$0.73 \pm 6 \times 10^{-2}$	$-0.35 \pm 1 \times 10^{-2}$	$-1.25 \pm 3 \times 10^{-2}$	$0.74 \pm 9 \times 10^{-2}$	$-0.37 \pm 1 \times 10^{-1}$	$-1.33 \pm 1 \times 10^{-1}$
Biotine	$0.62 \pm 2 \times 10^{-2}$	[a]	[a]	$0.26 \pm 4 \times 10^{-2}$	$-0.70 \pm 5 \times 10^{-2}$	$-1.70 \pm 2 \times 10^{-2}$	$0.31 \pm 1 \times 10^{-1}$	$-0.68 \pm 5 \times 10^{-2}$	$-1.54 \pm 2 \times 10^{-1}$
Vitamin B5	$-2.19 \pm 6 \times 10^{-2}$	$-2.59 \pm 2 \times 10^{-2}$	$-2.95 \pm 2 \times 10^{-2}$	$-2.81 \pm 8 \times 10^{-2}$	$-3.42 \pm 1 \times 10^{-1}$	$-3.50 \pm 5 \times 10^{-3}$	$-2.85 \pm 6 \times 10^{-2}$	$-3.4 \pm 6 \times 10^{-2}$	$-3.82 \pm 5 \times 10^{-2}$
Vitamin C	[a]	[a]	[a]	-2.52 ± 0	$-4.00 \pm 4 \times 10^{-2}$	-4.45 ± 0	$-2.46 \pm 3 \times 10^{-1}$	$-3.37 \pm 1 \times 10^{-1}$	$-4.19 \pm 2 \times 10^{-1}$

Relative proportions of vitamin B1 or B6 vs. respective interferent (vitamin B1 or B6: interferent): (A) 1:0.3 nicotinamide; 1:0.1 riboflavine; 3:0.1 inositol; 125:0.1 cyanocobalamine; 33:0.1 biotine; 1:0.2 vitamin B5. (B) 1:3 nicotinamide; 1:1 riboflavine; 3:1 inositol; 125:1 cyanocobalamine; 33:1 biotine; 1:2 vitamin B5. (C) 1:30 nicotinamide; 1:10 riboflavine; 3:10 inositol; 125:10 cyanocobalamine; 33:10 biotine; 1:20 vitamin B5. (D) 1:0.5 nicotinamide; 1:0.3 riboflavine; 1:0.1 inositol; 125:0.1 cyanocobalamine; 11:0.1 biotine; 1:0.5 vitamin B5; 1:10 vitamin C. (E) 1:5 nicotinamide; 1:3 riboflavine; 1:1 inositol; 125:1 cyanocobalamine; 11:1 biotine; 1:5 vitamin B5; 1:100 vitamin C. (F) 1:50 nicotinamide; 1:30 riboflavine; 1:10 inositol; 125:10 cyanocobalamine; 11:10 biotine; 1:50 vitamin B5; 1:1000 vitamin C.

[a] No results were obtained for this concentration level.

of concentration of the main ion—2.5×10^{-4}, 2.5×10^{-3} and 2.5×10^{-2} M.

The obtained results are of the same order of magnitude, besides the method used for this evaluation, and only thiamine can be considered as an interferent to VitB6 ISEs. Taking into account the three types of electrodes, a maximum of $\log K_{pot}$ of 0.75 (Table 6) is obtained for inositol, biotin and cyanocobalamin, regarding the most unfavourable interferent proportion.

The thiamine interference is related with its more hydrophobic character. This observation could be corroborated through spectrophotometric experiments performed on aqueous solutions of both vitamins (Fig. 3). Extraction of the solutions with similar volumes of the mediator solvent lead to greater absorbance decrease in vitamin B1 spectrum compared to the obtained for vitamin B6 (Fig. 3 A-A′, B-B′, respectively). The presence of cyclodextrins increased the extraction yield into the organic mediator phase in both cases (Fig. 3 A″ and B″) less evident when β-cyclodextrin is considered as ionophore (Fig. 3C). Regarding the selectivity coefficients for VitB6 ISE I with VitB6 ISE II it was noticed that the former is generically more selective, which also can be related with better extraction observed for 2-hydroxypropyl-β-cyclodextrin.

3.4. Analytical applications

The characteristics obtained for the developed electrodes are adequate to analytical application on the determination of thiamine and pyridoxine in multivitamin pharmaceutical preparations (tablets, sachets, ampoules), where both drugs are present. The analytical usefulness of the aforementioned electrodes, whose construction, assessment and applications were described, was carried out by the analysis of complex pharmaceutical multivitamin formulations and the results are shown in Tables 7 and 8. The values reported correspond to the results obtained by Gran's plot method and also by HPLC method for the same products, in which both vitamins are present in different proportions. Mean recovery values of 98.8 ± 2.9 for VitB1 ISE, 96.5 ± 2.0 for VitB6 ISE I and 98.9 ± 2.1 for VitB6 ISE II were obtained. The best precision registered with VitB6 ISE I

Table 7
Vitamin B1 determination in several pharmaceuticals using the proposed electrodes, the corresponding recovery values and the results of the external reference method[a]

Pharmaceutical	Vitamin B1 content (mg)			
	Labelled	Gran's plot	Recovery (%)	HPLC
Neurobion ampoules	100.0	105.0 ± 15.1	95.1 ± 2.4	100.5 ± 6
Becozyme C tablets	15.0	15.3 ± 1.1	102.0 ± 3.3	15.5 ± 0
Dragavit B tablets	15.0	14.7 ± 0.8	98.6 ± 1.9	[b]
Varimine stress sachets	15.0	15.0 ± 0.4	99.6 ± 3.6	[b]

[a] Results obtained in two samples of the same product, with three electrodes and expressed in mg per dose unit (sachet, pill or ampoule).

[b] Separation of peaks was not achieved under the same experimental conditions.

Table 8
Vitamin B6 determination in several pharmaceuticals using the proposed electrode, the corresponding recovery values and the results of the external reference method[a]

Pharmaceutical	Vitamin B6 content (mg)					
	Labelled	Gran's plot (vB6I)	Recovery (%) vB6I	Gran's plot (VB6II)	Recovery (%) vB6II	HPLC
Neurobion tablets	200.0	173.0 ± 0	95.1 ± 0.5	181.8 ± 11.4	97.4 ± 4.4	164.4 ± 11
Neurobion ampoules	100.0	103.1 ± 0	99.3 ± 5.3	98.1 ± 10.5	102.0 ± 4.7	97.3 ± 6
Becozyme C tablets	5.0	5.6 ± 0.8	96.4 ± 0.3	5.2 ± 0.7	98.0 ± 4.0	5.0 ± 0
Dragavit B tablets	5.0	6.0 ± 0	95.0 ± 0.4	5.5 ± 0.6	98.0 ± 3.5	5.5 ± 0

[a] Results obtained in two samples of the same product, with three electrodes and expressed in mg per dose unit (sachet, pill or ampoule).

relatively to VitB6 ISE II is in accordance with its better selectivity, when compared with that one accomplished by VitB6 ISE II unit.

In Table 3, where the characteristics of the reported VitB1 and B6 electrodes are shown, it is possible to point out that any complex formulation was tested. In fact, for Ref. [18], no determinations were achieved for vitamin B6 on multivitamin formulations due to the interference of thiamine in pyridoxine selective membrane. This sensor proved to be able to quantify VitB6 only in pure pyridoxine tablets. In Ref. [19], analytical application was performed on two samples, with very simple composition (three active substances for both), and one of those did not include in its constitution the major VitB6 interference, which was VitB1. On references [20,21], only one or two samples were determined by using standard addition technique. However, with cyclodextrin-based electrodes it was possible to obtain good results even in samples with both vitamins, besides the enormous complexity of formulations. This fact was achieved due to the improvement on general characteristics of the electrodes, especially their selectivity performance.

4. Conclusions

The use of cyclodextrins as ionophores for construction of the proposed electrodes proved the assessment of good quality ISEs when compared with classical ion exchange materials [18–21], especially in what concerns slope, reproducibility and selectivity. This ionophore allowed obtaining electrodes for vitamin B1 with slopes corresponding to monoprotonated or diprotonated species, rendering their use in samples with different pHs. The more specific ionic recognition accomplished with this type of macrocycles, gave rise to less interference from alkaline and metal alkaline ions and also vitamins often present in complex matrix pharmaceutical preparations multivitamin-type. Therefore, analysis with good results of thiamine and pyridoxine were possible to obtain in formulations in which those substances are simultaneously present, contrarily to the previously reported works [18–21]. For the first time, the developed electrodes allowed successfully analytical application to real complex matrix pharmaceuticals, with a very simple, rapid and accurate method. Concerning the disposable official methods, the potentiometric one allows the possibility of determining both vitamins (thiamine and pyridoxine) on different pharmaceutical formulations, applying the same methodology, whatever the type of formulation analysed.

The proposed units seem to present excellent characteristics to further application into an automatic flow system.

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