

ELECTROANALYSIS OF VITAMIN B6

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Vitamin B6 is a significant member of the B-vitamin complex. It has an important role especially in brain development and in the nervous system's health. A sensor based on graphite nanopowder and chitosan was developed, characterized, and validated for the quantification of vitamin B6 in pharmaceutical products and orange juice. Differential pulse voltammetry was used for all measurements. Vitamin B6 was determined on a linear concentration range between 1.69×10^{-16} and 1.69×10^{-12} g mL⁻¹, with a sensitivity of 37227 A g⁻¹mL. Recovery values higher than 99.00% and a relative standard deviation lower than 1.50l were obtained when vitamin B6 was determined from pharmaceutical compounds and orange juice.

Keywords: Vitamin B6; electroanalysis; juice; pharmaceutical compounds

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1. Introduction

Vitamin B6, known as pyridoxine, is one of the most important vitamins from the B-class. Vitamin B6 has an important role in brain development and in the nervous system's health. It also contributes to immunity, keeping the system healthy. An association between vitamin B6 intake and cardiovascular diseases was determined [1]. Atherosclerosis was also associated with a deficiency in vitamin B6 [2]. Vitamin B6 intake can contribute to the remediation of post-stroke depression [3]. A deficiency in vitamin B6 in the body can influence core metabolic pathways, driving oncogenic programs in both solid tumors and haematologic malignancies [4].

There are several sources of vitamin B6, such as potatoes, orange juice, fish, poultry, bananas, cereals, and chickpeas. Fast remediation of vitamin B6 in the body

can be achieved by drinking one of its pharmaceutical formulations. Adults should consume 1.3 and 1.7 mg of vitamin B6 daily.

Many methods have been proposed to date for the quantification of vitamin B6, such as high-performance liquid chromatography (HPLC), liquid chromatography, and chemiluminescence [5-8]. Nevertheless, the majority of the previously mentioned techniques require skill-intensive operations, costly instrumentation, and laborious procedures involving sample pre-treatments and processing, which may also yield insensitivity in results. Chromatographic methods were used especially for the quantification of vitamin B6 in mixtures [9, 10].

Electrochemical methods of analysis are the most used, especially for the quantification of vitamin B6 in pharmaceutical formulations [11]. For example, linear sweep voltammetry [12], square wave voltammetry [13], and differential pulse voltammetry [14] are electrochemical techniques that can be used to quantify vitamin B6. Fast development of electrochemical methods was facilitated by the introduction of nanomaterials in the obtaining of electrochemical sensors as well as of composite materials in the obtaining of the electrochemical sensors [15, 16]. Automated acquisition of data was also considered, especially for on-site analysis using differential pulse voltammetry [17]. The accuracy and reproducibility of the electrochemical methods of analysis were recognized, especially when determinations were done on pharmaceutical compounds and food samples [18]. There are many electrochemical sensors and methods proposed for the quantification of vitamin B6 from different matrices [19-21]. Fluorescence [22] and optical, spectrometric, and immunoassay methods were also used for the determination of vitamin B6 [9, 19].

A new electrochemical sensor employing the immobilization of chitosan within graphite nanopowder was proposed for the quantification of vitamin B6 in pharmaceutical compounds. Chitosan has proven electrocatalytic activity [23]. Graphite nanopowder provides a high surface area for the sensor, enhancing sensitivity.

2. Materials and Methods

2.1 Materials and reagents

Vitamins B1, B2, B5, B6, B7, B9, B12, and E, as well as graphite nanopowder, ascorbic acid, sucrose, magnesium (Mg^{2+}) and chitosan were bought from Sigma-Aldrich. Paraffin oil was purchased from Fluka (Buchs, Switzerland). Starting with a 0.01 g mL^{-1} vitamin B6 solution, serial dilutions were made up to a concentration of $1.69 \times 10^{-21} \text{ g mL}^{-1}$. All solutions were made in a 1:1 (v/v) mixture of distilled water: phosphate buffer solution (0.1 mol L^{-1} , pH 6.74).

The monosodium phosphate and disodium phosphate solutions were mixed to create the phosphate buffer solution (PBS, 0.1 mol L^{-1}). Various pH values were

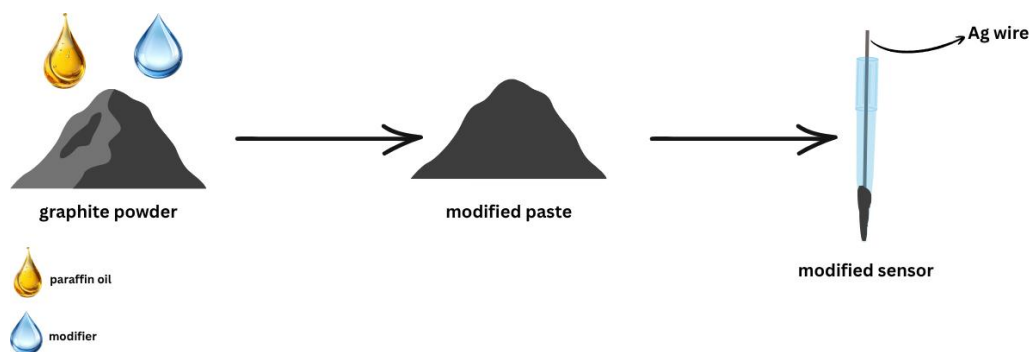
achieved by adjusting the buffer solution with varying amounts of 0.1 mol L⁻¹ NaOH or HCl solutions.

2.2 Equipment

A potentiostat/galvanostat AUTOLAB/PGSTAT 302 (Metrohm, Utrecht, The Netherlands) linked to a personal computer with GPES software was utilized for all measurements. The pH was adjusted using a pH meter made by Mettler Toledo. The HPLC UltiMate 3000 (DIONEX – U.S.A.) was also used for comparison.

2.3 Construction of the electrochemical sensor

100 mg of graphite nanopowder was incorporated with paraffin oil until a paste was formed. The paste was modified with 100 μ L of a chitosan solution at a concentration of 10⁻³ mol L⁻¹ to attain the modified paste.



Scheme 1. Schematic representation of the proposed electrochemical sensor.

The modified paste was placed in a tub with a diameter of 20 μ m (Scheme 1). The connection between the modified paste and the external circuit was facilitated by an Ag wire.

2.4 Procedure

All measurements were conducted using differential pulse voltammetry (DPV). Ag/AgCl and Pt wires served as reference and auxiliary sensors for the measurements. The scanning range was between -1.0 V and +1.5 V, with a scan rate of 80 mV s⁻¹. The $E_{1/2}$ was 297 mV. The linear regression method was used to determine the parameters of the calibration equation. The intensity values of the current obtained after the measurements of the pharmaceutical samples and the orange juice were interpolated into the calibration equation to determine the concentration of vitamin B6. The general operation conditions were: $E_{\text{begin}} = -1.0$ V, $E_{\text{end}} = +1.5$ V, $E_{\text{step}} = 0.01$ V, scan rate = 0.08 V s⁻¹, temperature = 25 °C, humidity = 50 %.

To determine the working concentration range, which is the linear concentration range, the concentration range was between 1.69×10^{-21} and 1.69×10^{-2} g mL⁻¹.

A reverse-phase C-18 octadecylsilyl (C₁₈H₃₇) bonded-phase column packing was employed for HPLC analysis. The pyridoxine analyte consistently eluted at 1.6 minutes. Detection was achieved using an ultraviolet detector calibrated to 290 nm, with a rise time of 0.1 and an AUFS range set to 1.0. The HPLC pump was configured to 1900 psig with a flow rate of 1 mL min⁻¹. Vitamin B6 reliably elutes at 1.6 minutes. The actual volume introduced into the column is around 20 µL. The dead time for eluting non-retained species is 1.5 minutes, computed using the formula: dead time (t_0)=volume/flow rate = 1.5 mL/1.0 mL/min [24].

2.5 Samples

The Optisana and Vitamax samples were purchased from a local pharmacy. The Optisana and Vitamax tablets were properly crushed with a mortar and a pestle. An equivalent of one tablet (containing 1.4 mg of vitamin B6) was put into a volumetric flask and dissolved in a mixture of distilled water and phosphate buffer solution (0.1 mol L⁻¹, pH 6.74). The solution was diluted to a final volume of 1 L and stirred until fully dissolved. The solution was filtered using a filter paper of 90 mm in diameter to exclude insoluble excipients. The fresh orange juice was extracted by physically pressing oranges. The juice was filtered to eliminate the pulp and suspended solids to avoid electrode fouling. The purified sample was further diluted with a phosphate buffer solution (0.1 mol L⁻¹, pH 6.74) at a ratio of 1:10 (v/v) to lessen matrix effects.

For HPLC determinations, the following step was taken: 9 mL from each sample used for electrochemical measurements was combined with 1 mL of 10% ethanol (v/v) solution. The filtering was done using a Whatman 6900-25502 GD/X 25 Sterile Syringe Filter.

3. Results and Discussion

3.1 Electrochemical characterization of the sensors

The electrochemical characterization was conducted employing two approaches, cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS), on the bare (graphite) and modified (chitosan/graphite) electrodes. The CV approach was utilized to evaluate the electrochemical response of the chitosan/graphite sensor. The CVs presented in Fig. 1a were obtained in a solution of 5.0×10^{-3} mol L⁻¹ K₃[Fe(CN)₆] (0.1 mol L⁻¹ KCl) across a potential range of -0.6 V to 1.0 V, at a scan rate of 0.1 V s⁻¹, utilizing unmodified (graphite) and modified (chitosan/graphite) sensors as working electrodes. The modified sensor shows a higher conductivity compared to the bare sensor. The bare sensor exhibited well-

defined quasi-reversible redox peaks, with anodic and cathodic peak currents (I_{pa} and I_{pc}) of $1.541 \mu\text{A}$ and $-1.198 \mu\text{A}$, respectively, while the chitosan-modified sensor showed increased peak currents ($I_{pa} = 3.462 \mu\text{A}$ and $I_{pc} = -2.871 \mu\text{A}$), indicating an enlarged electroactive surface area and improved electron transfer kinetics.

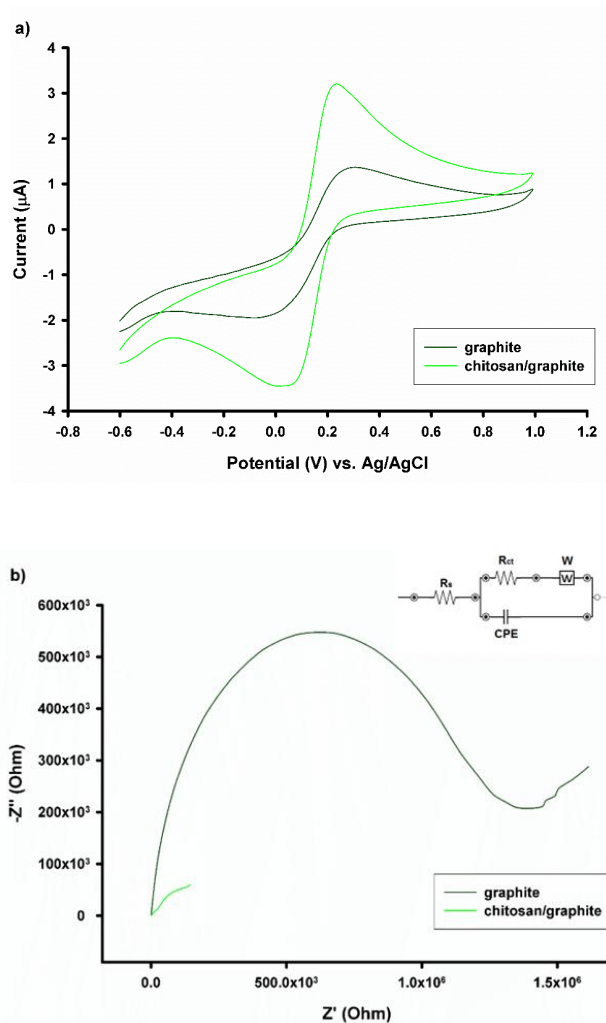


Figure 1. a) CVs comparing the current versus the potential (working conditions: step potential 0.025 V ; scan rate 0.1 V s^{-1}) in a $5.0 \times 10^{-3} \text{ mol L}^{-1} \text{ K}_3[\text{Fe}(\text{CN})_6]$ ($0.1 \text{ mol L}^{-1} \text{ KCl}$) solution utilizing graphite (dark green line) and chitosan/graphite (light green line); b) EIS for graphite (dark green line) and chitosan/graphite (light green line) in a $5.0 \times 10^{-3} \text{ mol L}^{-1} \text{ K}_3[\text{Fe}(\text{CN})_6]$ ($0.1 \text{ mol L}^{-1} \text{ KCl}$) solution (working conditions: a frequency range of 10^5 to 10^{-1} Hz). Inset: the equivalent circuit diagram of the electrochemical interface utilized to fit the EIS, where R_s denotes the solution resistance; CPE represents the constant phase element; W signifies the Warburg diffusion resistance; R_{ct} indicates the electron-transfer resistance.

The analysis of the interfaces of both the unmodified and modified electrodes using EIS was conducted within a frequency range of 10^5 to 10^{-1} Hz. The studies were conducted in a solution of 5.0×10^{-3} mol L⁻¹ K₃[Fe(CN)₆] (0.1 mol L⁻¹ KCl). Fig. 1b presents the Nyquist plots for graphite (depicted in dark green) and chitosan/graphite (shown in light green). The Nyquist plot indicates that graphite displays a high electrical resistance ($R_{ct}=6454.0 \Omega$). After the modification of graphite with chitosan, the R_{ct} value decreased to 5617.0Ω , due to the higher conductivity of the material, leading to a better electron transfer rate at the chitosan/graphite interfaces. The EIS results correspond accurately with the CV values.

The calculation of the electroactive surface area facilitated the analysis of the electrocatalytic activity of the sensors, conducted using the Randles-Sevick equation [25, 26] for quasi-reversible redox processes subject to diffusion. The maximum current intensity at both the anodic and cathodic peaks is precisely proportional to the square root of the scan rate, as illustrated below:

$$I_p = (2.69 \times 10^5) n^{3/2} A C_0 D_R^{1/2} \nu^{1/2}$$

where I_p represents the peak current (μA), n denotes the number of transferred electrons ($n=1$ in this instance), A signifies the electrode active surface area (cm^2), C_0 indicates the concentration of 5.0×10^{-3} mol L⁻¹ K₃[Fe(CN)₆] (mol cm⁻³), D_R is the diffusion coefficient (7.60×10^{-6} cm² s⁻¹), and ν represents the scan rate (V s⁻¹). The linear dependence between the anodic and cathodic peaks, I_{p_a} (black dots) and I_{p_c} (red dots), and the square root of the scan rate (Fig. 2 a, b) across a scan rate variation of 0.010 to 0.100 V s⁻¹ signifies that the redox process is diffusion-controlled.

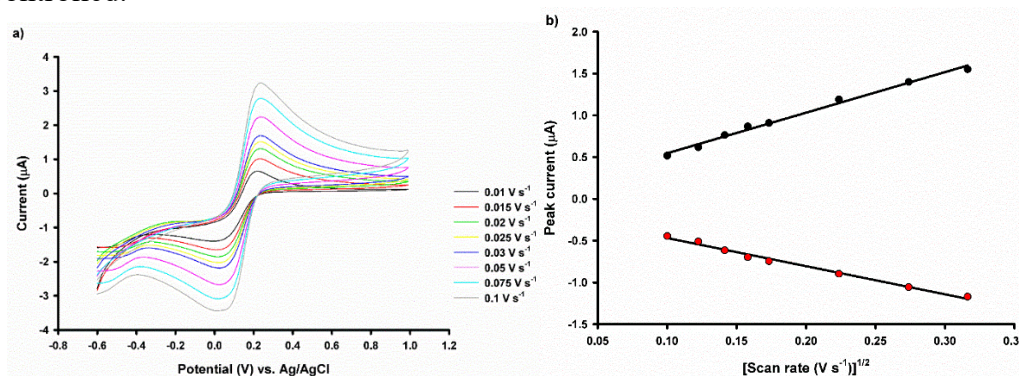


Figure 2. a) CVs in a 5.0×10^{-3} mol L⁻¹ K₃[Fe(CN)₆] (0.1 mol L⁻¹ KCl) solution at scan rate between 0.010 and 0.100 V s⁻¹ with the chitosan/graphite sensor (working conditions: potential range from -0.6 to 1.0 v; step potential 0.025 V); b) Dependence between the peak current and the square root of the scan rate for the chitosan/graphite sensor.

Fig. 2a depicts the pattern that appears with the increasing scan rate and current intensity, while Fig. 2b shows the linear dependences of the two peaks, I_{pa} vs $v^{1/2}$ and I_{pc} vs $v^{1/2}$. The chitosan/graphite-based sensor shows a high active area of $2.72 \times 10^{-3} \text{ cm}^2$, in contrast to the unmodified graphite sensor, which has an active area of $1.33 \times 10^{-3} \text{ cm}^2$.

3.2 Optimization of the pH values

According to Fig. 3, the pH value of the solution determines the rate of electrooxidation of vitamin B6. Consequently, the DPV approach was used to learn about the impact of pH.

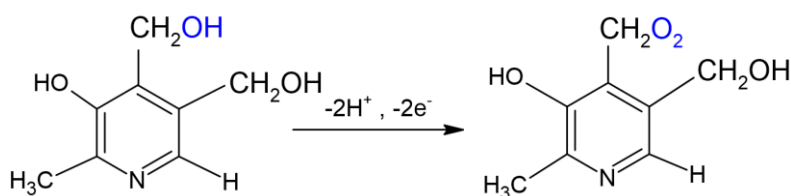
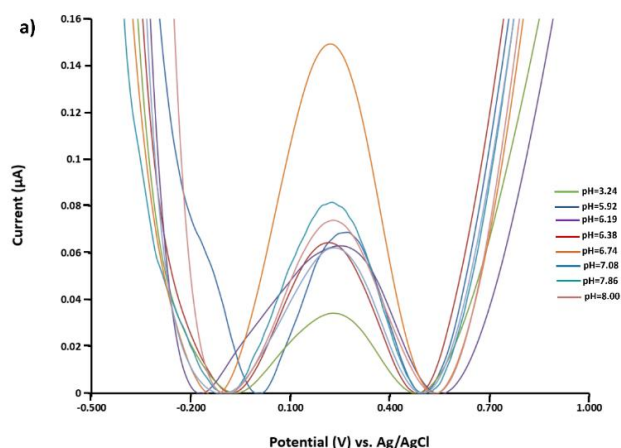


Figure 3. Possible mechanism of vitamin B6 electrooxidation reaction.

Solutions of PBS containing $1.69 \times 10^{-9} \text{ g mL}^{-1}$ vitamin B6 at pH values ranging from 3.0 to 8.0 were examined for the pH influence investigation (Fig. 4 a). The DPV parameters were: $E_{\text{begin}} = -0.5 \text{ V}$, $E_{\text{end}} = 1 \text{ V}$, $E_{\text{step}} = 0.01 \text{ V}$, scan rate $= 0.08 \text{ V s}^{-1}$. As can be seen from Fig. 4 b, from pH 3.0 to pH 6 a plateau is present, followed by a slight decrease and a high increase at around a pH value of 6.7. Accordingly, the pH of choice was 6.7 to further proceed with the investigations.



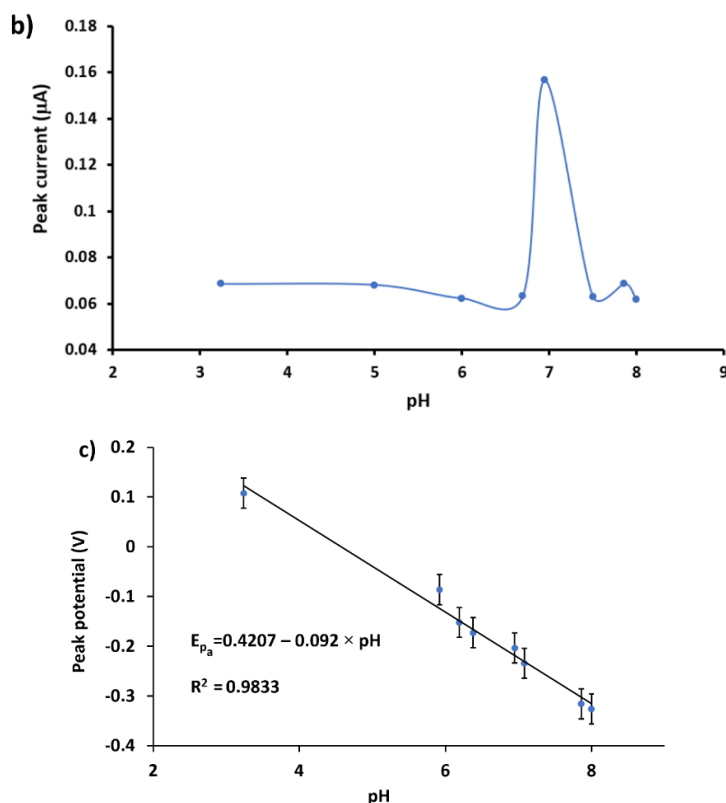


Figure 4. a) Differential pulse voltammograms of $1.69 \times 10^{-9} \text{ g mL}^{-1}$ vitamin B6 using the proposed sensor in PBS at pH values 3.0 – 8.0. b) The effect of the pH on the peak current and c) the linear dependence of the peak potential with the pH

The following equation describes a linear relationship between pH and the potential peak: $E_{p_a} = 0.4207 - 0.092 \times \text{pH}$ with a good $R^2 = 0.9833$ (Fig. 4 c). It is shown that the anodic peak potential is pH dependent using a proton-transfer step in the electro-oxidation of vitamin B6. One possible explanation for the discrepancy between the ideal Nernstian slope (0.059 V pH^{-1}) at 25°C and the observed slope (-0.092 V pH^{-1}) is the intricacy of the oxidation mechanism, which causes an imbalance in the number of transported electrons and protons.

3.3 Response characteristics of the electrochemical sensor

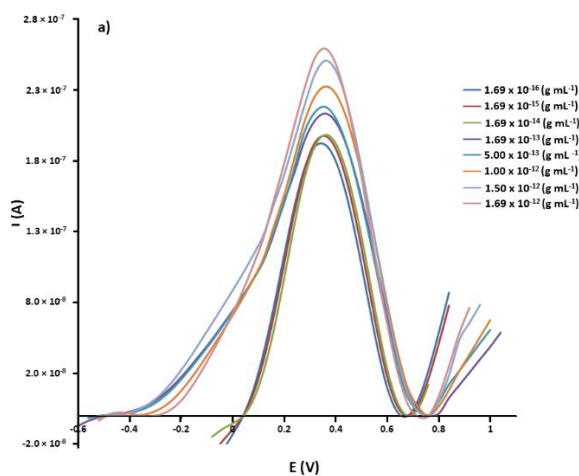
The response characteristics were determined using the DPV method at 25°C . The sensor was used on a linear concentration range: $1.69 \times 10^{-16} - 1.69 \times 10^{-12} \text{ g mL}^{-1}$, with a sensitivity of $37227 \text{ A g}^{-1} \text{ mL}$. The limit of determination was $1.69 \times 10^{-16} \text{ g mL}^{-1}$ while the limit of detection was $1.30 \times 10^{-18} \text{ g mL}^{-1}$. The equation of calibration was:

$$I = 2.00 \times 10^{-7} (\pm 0.98 \times 10^{-7}) + 37227 (\pm 2.09) C_{\text{Vitamin B6}}$$

where I is the intensity of the current ($\langle I \rangle = A$) and $C_{\text{vitamin B6}}$ is the concentration of vitamin B6 ($\langle C_{\text{vitamin B6}} \rangle = g\ mL^{-1}$). The values of the terms of the equation of calibration are the results of three consecutive calibrations of the sensor on the range mentioned above. The standard deviation from the mean values of the terms is shown in the equation. The correlation coefficient was 0.9945. To determine the LOD and LOQ, the IUPAC requirements were used [27].

The voltammograms obtained for the calibration of the sensor as well as the calibration equation are shown in Fig. 5. The working concentration range made possible the assay of vitamin B6 in both pharmaceutical compounds and in fruit juices, like orange juice.

Electrochemical analysis makes extensive use of carbon paste electrodes because of their exceptional characteristics. The carbon graphite powder and mineral oil, silicon, or paraffin oil are combined to form a uniform and versatile paste, which is then used to make these electrodes. With their wide surface area, carbon paste electrodes provide fast electrochemical reactions and efficient electron transport between the analyte and the electrode. More accurate detection and quantification of the target analytes can be achieved by modifying the electrodes with chitosan, which increases their sensitivity and selectivity [28].



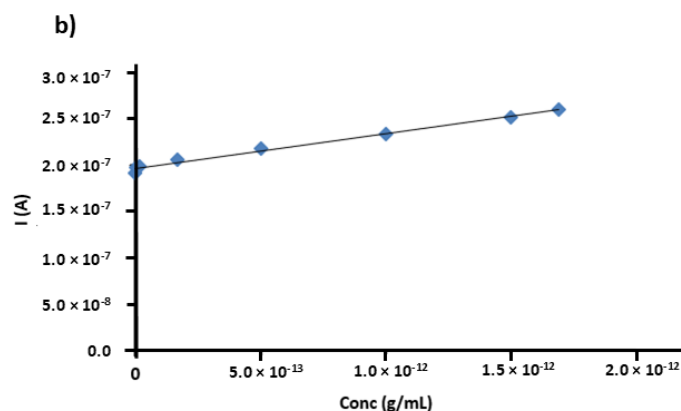


Figure 5. Differential pulse voltammograms obtained for the calibration of the proposed sensor (a) and the calibration graph of the proposed sensor (b).

A comparison of other approaches developed thus far for the quantification of vitamin B6, such as DPV [29-31], square wave voltammetry (SWV) [32, 33] and square wave anodic stripping voltammetry (SWASV) [34], and CV [35], can be found in Table 1.

Table 1. Various types of electrochemical methods used for the determination of vitamin B6

Method	Sensor	Linear concentration range	LOD	LOQ	Sensitivity	Ref
DPV	ZnFe ₂ O ₄ /SPGE ^{a)}	$8.00 \times 10^{-7} - 5.85 \times 10^{-4} \text{ mol L}^{-1}$	$1.70 \times 10^{-7} \text{ mol L}^{-1}$	$8.00 \times 10^{-7} \text{ mol L}^{-1}$	$0.0501 \text{ A mol L}^{-1}$	29
DPV	CuNP/CPE ^{b)}	$8.88 \times 10^{-7} - 1.00 \times 10^{-1} \text{ mol L}^{-1}$	$32.12 \times 10^{-6} \text{ mol L}^{-1}$	$8.88 \times 10^{-7} \text{ mol L}^{-1}$	-	30
DPV	CuO-GA ₂ /BDDE ^{c)}	$5.96 \times 10^{-6} - 3.75 \times 10^{-4} \text{ mol L}^{-1}$	$1.99 \times 10^{-7} \text{ mol L}^{-1}$	$5.96 \times 10^{-6} \text{ mol L}^{-1}$	-	31
SWV	poly(phenylalanine)/F-MWCNT/GCE ^{d)}	$5.00 \times 10^{-7} - 2.00 \times 10^{-5} \text{ mol L}^{-1}$; $2.00 \times 10^{-5} - 2.00 \times 10^{-4} \text{ mol L}^{-1}$	$3.80 \times 10^{-8} \text{ mol L}^{-1}$	$5.00 \times 10^{-7} \text{ mol L}^{-1}$; $2.00 \times 10^{-5} \text{ mol L}^{-1}$	-	32
SWV	Fe ₃ O ₄ NPs-ePADs ^{e)}	$2.00 \times 10^{-4} - 2.00 \times 10^{-3} \text{ mol L}^{-1}$	$2.95 \times 10^{-5} \text{ mol L}^{-1}$	$2.00 \times 10^{-4} \text{ mol L}^{-1}$	$2.14 \times 10^{-2} \text{ A L mol}^{-1}$	33
SWASV	N,S-GQD-CS/ GCE ^{f)}	$1.00 \times 10^{-7} - 1.80 \times 10^{-5} \text{ mol L}^{-1}$	$3.01 \times 10^{-5} \text{ mol L}^{-1}$	$1.00 \times 10^{-7} \text{ mol L}^{-1}$	-	34
CV	CrHCF/GCE ^{g)}	$1.33 \times 10^{-6} \text{ mol L}^{-1} - 1.32 \times 10^{-5} \text{ mol L}^{-1}$	$3.46 \times 10^{-7} \text{ mol L}^{-1}$	$1.33 \times 10^{-6} \text{ mol L}^{-1}$	-	35
DPV	Chit/nGr	$1.00 \times 10^{-16} - 1.70 \times 10^{-12} \text{ g mL}^{-1}$	$1.30 \times 10^{-18} \text{ g mL}^{-1}$	$1.00 \times 10^{-16} \text{ g mL}^{-1}$	$37227 \text{ A g}^{-1} \text{ mL}$	This work

a) zinc ferrite nano-particles modified screen-printed graphite electrode, b) adapted carbon paste with copper nanoparticles, c) copper (II) oxide-glutaraldehyde modified boron doped diamond electrode, d) poly(phenylalanine) and functionalized multi-walled carbon nanotubes modified glassy carbon electrode, e) electrochemical paper-based analytical devices were developed from a conductive ink using graphite powder, automotive varnish and magnetite nanoparticles, f) glassy

carbon electrode was modified with nitrogen and sulfur co-doped graphene quantum dots, g) glassy carbon electrode modified with Cr^{III} hexacyanoferrate(II).

Compared to the values presented in Table 1, the proposed method shows higher sensitivity and lower determination and detection limits. Sensor-to-sensor variation was determined as following: 10 sensors were designed and used for the determination of vitamin B6 in the standard solutions, on the working concentration range given above. The sensitivities obtained were compared, and the standard deviation from the mean value was 1.27, showing that the proposed design is reliable. For the inter-day-reliability, these sensors were tested in the same way, day by day, and the sensitivities were determined and compared; the standard deviation from the mean was 5.21, showing a good reliability of the sensors, when used for 30 days.

3.4 The selectivity of the electrochemical sensor

The mixed solution method was used for the selectivity studies of the proposed electrochemical sensor. The mixed solution method uses a mixed solution containing vitamin B6 and the supposed interferent in a ratio of 1:10 (mol/mol). The intensity of the current obtained for the mixed solution is compared with the intensity of the current measured for a solution containing the same concentration of vitamin B6 as the mixed solution.

$$K_{i,j}^{amp} = \left(\frac{\Delta I_t}{\Delta I_i} - 1 \right) \times \frac{c_i}{c_j}$$

The above equation was used for calculating the amperometric selectivity coefficients ($K_{i,j}^{amp}$). In the equation, c_i and c_j denote the concentrations of vitamin B6 and of the interferent. ΔI_t and ΔI_i represent the current variations recorded for the mixed solution and for the solution containing only vitamin B6, respectively, compared to the background current.

Vitamins B1, B2, B5, B7, B9, B12, and E, as well as sucrose, Mg²⁺ and ascorbic acid, were checked as possible interferences. The amperometric selectivity coefficients are given in Table 2.

Table 2. Amperometric selectivity coefficients.

Interferent	$K_{i,j}^{amp}$
Vitamin B1	2.00×10^{-3}
Vitamin B2	1.91×10^{-3}
Vitamin B5	1.87×10^{-4}
Vitamin B7	1.70×10^{-3}
Vitamin B9	1.20×10^{-4}
Vitamin B12	1.50×10^{-3}

Vitamin E	1.21×10^{-4}
Mg^{2+}	1.53×10^{-4}
Ascorbic acid	3.20×10^{-3}
Sucrose	1.00×10^{-4}

The values obtained for the amperometric selectivity coefficients have shown that the substances tested do not interfere with the determination of vitamin B6.

3.5 Electroanalysis of vitamin B6 in pharmaceutical formulations and orange juice

Two different vitamin tablets, Optisana and Vitamax, and orange juice obtained following the procedure described in the “Samples” section were analyzed.

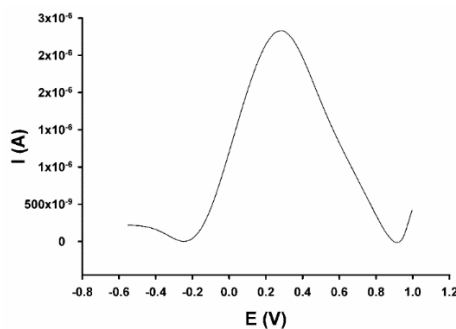


Figure 6. Voltammogram recorded for the determination of vitamin B6 in Optisana.

The sampling of the pharmaceutical tablets was reduced to their dissolution in a mixture of distilled water and buffer solution (phosphate buffer solution pH 6.74). The orange juice sample was analyzed after being filtered, as described in the “Samples” section. Fig. 6 presents a voltammogram obtained for the assay of vitamin B6 in Optisana.

To validate the proposed sensor for the determination of vitamin B6 in pharmaceuticals and orange juice, the results obtained for the determinations using the electrochemical sensor were compared with those obtained using the HPLC method reported earlier [24].

Table 3. Determination of vitamin B6 in pharmaceutical and food samples (N=10).

Sample	Electrochemical sensor	HPLC [24]	t-test
Optisana*	1.39 ± 0.02	1.30 ± 0.12	2.11
Vitamax*	1.38 ± 0.04	1.41 ± 0.09	2.76
Orange juice**	0.83 ± 0.03	0.80 ± 0.10	1.97

*mg vitamin B6 per tablet; **ng mL⁻¹ vitamin B6 in orange juice

High recovery values obtained for the determination of vitamin B6 showing the good accuracy achieved using the proposed sensor are shown in Table 4. Moreover, the values obtained for the standard deviation indicate good precision for the determination of vitamin B6, as well as good reliability.

Table 4. Recovery of vitamin b6 in different samples (N=3).

Sample	%, Recovery	Standard deviation
Optisana	99.96	1.12
Vitamax	99.14	1.07
Orange juice	99.08	1.27

No differences in standard deviation and recovery % values were detected when the highest, lowest, or middle concentrations were tested as spiking levels.

The correlation between the results obtained using the two methods of analysis was done using the Student t-test at a 99.00% confidence level. At this level, the tabulated value is 4.13. The t-test values from Table 3 are lower than 4.13. Accordingly, there is no significant difference between the results obtained using the electrochemical sensor and the HPLC method. Also, the recovery of vitamin B6 in Optisana was 99.96%, while in Vitamax it was 99.14% versus the amount of vitamin B6 written on the package of vitamins (1.4 mg per tablet). Different amounts of vitamin B6 were added to the orange juice, and a comparison of the recovered amount with the added amount was performed; a recovery of 99.08% was obtained for the quantification of vitamin B6 in orange juice. These recovery values are higher than those reported for previous methods proposed for the quantification of vitamin B6 [15].

4. Conclusions

The proposed electrochemical sensor, used by employing the DPV method, exhibited a good analytical performance for the quantification of vitamin B6, including a high sensitivity of $37227 \text{ A g}^{-1} \text{ mL}$, a low detection limit of $1.30 \times 10^{-18} \text{ g mL}^{-1}$, a low quantification limit of $1.69 \times 10^{-16} \text{ g mL}^{-1}$ and a wide linear concentration range of $1.69 \times 10^{-16} - 1.69 \times 10^{-12} \text{ g mL}^{-1}$. CV and EIS were performed to verify the electrochemical characteristics of the chitosan-modified sensor. The proposed sensor exhibited good reproducibility and selectivity, even in the presence of potential interferences, such as vitamins B1, B2, B5, B7, B9, B12, and E, as well as sucrose, Mg^{2+} and ascorbic acid, and sucrose. Moreover, the developed method was successfully applied for the assay of real samples, such as pharmaceutical tablets and orange juice, where high recovery values validated the accuracy of the proposed approach.

Author Contributions

Conceptualization, E.-Y.R., R.-I.S.-v.S., D.-C.G., and R.-M.I.-M.; methodology, E.-Y.R., R.-I.S.-v.S., D.-C.G., and R.-M.I.-M.; validation, E.-Y.R., R.-I.S.-v.S., D.-C.G., and R.-M.I.-M.; investigation, E.-Y.R.; writing—original draft preparation, E.-Y.R., R.-I.S.-v.S., D.-C.G., and R.-M.I.-M.; writing—review and editing, E.-Y.R., R.-I.S.-v.S., D.-C.G., and R.-M.I.-M.; supervision, R.-I.S.-v.S.; funding acquisition, R.-I.S.-v.S. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

The data will be made available upon reasonable request and ethics committee approval.

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Conflicts of Interest

The authors declare no conflict of interest.

Artificial Intelligence Disclosure

No artificial intelligence-based or generative tools were used in the preparation of this manuscript.

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