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Article

Voltammetric Determination of Pyridoxine (Vitamin B_6) in Drugs using a Glassy Carbon Electrode Modified with Chromium(III) Hexacyanoferrate(II)

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Um eletrodo de carbono vítreo modificado com hexacianoferrato(II) de Cr^{III} foi utilizado para a determinação de piridoxina (vitamina B_6) em três diferentes fármacos, por voltametria cíclica. A influência de vários parâmetros na resposta voltamétrica do eletrodo foi analisada. A faixa linear encontrada foi de $1{,}33 \times 10^{-6}$ mol L^{-1} a $1{,}32 \times 10^{-5}$ mol L^{-1} da vitamina, com r = 0,9990 e desvio padrão relativo de 4,2%. Os limites de detecção e quantificação foram de $3{,}46 \times 10^{-7}$ mol L^{-1} e $1{,}05 \times 10^{-6}$ mol L^{-1}, respectivamente. O método proposto para a determinação voltamétrica da vitamina B_6 apresentou uma boa exatidão e os resultados experimentais demonstraram que o eletrodo de carbono vítreo modificado com hexacianoferrato(II) de Cr^{III} apresenta um grande potencial para análise de piridoxina em amostras reais. Além disso, apresenta vantagens como uma resposta rápida, um baixo limite de detecção, baixo custo e simplicidade no desenvolvimento e aplicação.

A Cr^{III} hexacyanoferrate(II) (CrHCF)-modified glassy carbon electrode was used to determine pyridoxine (vitamin B_6) in three drugs by cyclic voltammetry. The influence of several parameters on the voltammetric electrode response was analyzed. The linear range found was of 1.33×10^{-6} mol L^{-1} to 1.32×10^{-5} mol L^{-1} of vitamin, with r = 0.9990 and relative standard deviation of 4.2%. The limits of detection and quantification were 3.46×10^{-7} mol L^{-1} and 1.05×10^{-6} mol L^{-1}, respectively. The voltammetric proposed method for determination of vitamin B_6 presented good accuracy and the experimental results demonstrated that the CrHCF-modified glassy carbon electrode has a large potential for the analysis of pyridoxine in real samples. Furthermore, it has the advantages of a fast response, a low detection limit, low cost, and simple development and application.

Keywords: Modified glassy carbon electrode, Pyridoxine, Cr^{III} hexacyanoferrate(II), Voltammetry, Vitamin B_6

Introduction

Vitamins are small organic molecules whose lack or excess may result in several diseases to the organisms that need them. Vitamin B_6 belongs to the hydrosoluble vitamin group and is responsible mainly for the transference of amino acid groups, acting as a coenzyme.[1] Its lack results in skin and nervous system changes and certain types of anemia.[2]

Vitamin B_6 is found as either the corresponding aldehyde (pyridoxal) or as the primary amine (pyridoxamine) or even, the primary alcohol (pyridoxine) or its respective forms 5-phosphate derivatives,[3] being normally interconvertible in the organism.[4] Pyridoxine (PN) is the most stable form of these compounds, which results in its use in drug formulations.[2]

Several methods have been developed to determine PN, including high performance liquid chromatography,[5] chemiluminescence,[6] and flow injection spectrophotometry.[2] However, these methods are costly and require analyte derivatization. Additionally, voltammetric techniques have also been reported. The first study for the electrochemical behavior of PN was carried out by Söderhjelm and Lindquist in 1975.[7] Recently, several works involving the use of modified electrodes in voltammetry have been published.[8-10]

The working electrodes may be modified to improve the analytical signal, the detection range, the sensitivity, and the selectivity of this technique. The determination of PN in drugs using a carbon paste electrode modified with Cu^{II} hexacyanoferrate(III) has been proposed.[9]

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Voltammetric response of PN with a glassy carbon electrode chemically modified with carbon nanotubes has also been evaluated.[8]

The combined use of glassy carbon electrodes modified with hexacianoferrate complexes may be instrumental in the determination of PN. Thus, the present work sought to use a glassy carbon electrode modified with Cr^{III} hexacyanoferrate(II)[11] (CrHCF) to determine vitamin B_6 in drugs. The influence of parameters on the voltammetric electrode response, as well as pH, supporting electrolyte, scan rate, precursor reagent, and the interference of several compounds present in drugs containing PN have been evaluated.

Experimental

Solutions and reagents

All solutions were prepared using deionized water and analytical grade reagents. Stock solutions sensitive to light were stored in dark glass flasks.

The standard solution of PN 1.00×10^{-3} mol L^{-1} was carefully prepared by dissolution of pyridoxine hydrochloride (Aldrich) in 100 mL deionized water. The solutions used in the interference study were prepared by dissolution of appropriate amounts of the species.

To prepare the CrHCF-modified glassy carbon electrode,[11] 20 mL KCl 0.10 mol L^{-1}, pH 3.0 with 1.0×10^{-2} mol L^{-1} $CrCl_3 \cdot 6H_2O$ and 5.0×10^{-3} mol L^{-1} $K_3[Fe(CN)_6]$ were used.

Potassium chloride solution prepared by dissolving the appropriate amount of salt in deionized water was used as supporting electrolyte and the pH was adjusted with HCl 0.10 mol L^{-1} and NaOH 0.10 mol L^{-1} solutions.

Equipment

All voltammetric measurements were carried out by using an AUTOLAB PGSTAT-30 (Ecochemie) potentiostat coupled to a microcomputer to acquire and record data and to control the experiment. The glass electrochemical cell (20 mL) was equipped with the modified glassy carbon rotating disk electrode (Model 6a6, EG&G PARC) and a platinum wire served as counter electrode and Ag/AgCl electrode was used as reference. The cyclic voltammetry measurements were carried out after solution deaeration and rest.

Working electrode modification

The glassy carbon electrode, with diameter area of 12.6 mm^2, was polished before modification with alumina paste, washed and cleaned by sonication in deionized water for 10 min.

Electrodeposition to generate CrHCF was carried out applying potential cycles between -0.2 and +1.0 V for 30 min in a KCl 0.10 mol L^{-1} (pH 3.0) solution containing 1.0×10^{-2} mol L^{-1} $CrCl_3 \cdot 6H_2O$ and 5.0 x 10^{-3} mol L^{-1} $K_3[Fe(CN)_6]$. The electrodeposition scan rate was 50 mV s^{-1} under magnetic stirring at 400 rpm and the glassy carbon rotated disk at 30 rpm. After this step, the electrode was conditioned for 1 h in KCl solution 0.10 mol L^{-1} and pH 3.0.[11]

Preparation and analysis of drug samples

The following drug samples were used in the experiment: Seis-B® - APSEN, Dramin® B_6 - ALTANA and Citoneurin® - MERCK, all containing pyridoxine hydrochloride. The samples were macerated and the average mass corresponding to one pill was weighted and dissolved in deionized water. The drug insoluble excipient was removed by filtration with a 45 μm Millipore membrane. The filtered material was colleted in a volumetric flask. The volume of Seis-B® was completed with deionized water up to 200 mL and those of the other samples up to 100 mL.

The voltammetric determinations of PN were carried out by cyclic voltammetry. The current value related to the vitamin with modified glassy carbon electrode was that obtained by the difference of the current observed at 0.88 V in the presence and in the absence of PN. This potential value is close to the value found in the literature for the oxidation of PN in an unmodified glassy carbon electrode, 0.85 V. [8]

Results and Discussion

Modification of the Glassy Carbon Electrode

The voltamograms obtained on modified and non-modified glassy carbon electrole can be seen in Figure 1. According to the potential values obtained, the resulting complex of $CrCl_3 \cdot 6H_2O$ and $K_3[Fe(CN)_6]$ deposition onto the glassy electrode is Cr^{III} hexacyanoferrate (II) {$KCr[Fe(CN)_6]$} which is deposited in the proportion of 2:1 of $Cr^{III}/[Fe(CN)_6]^{3-}$)[11] that presents blue color, which is in agreement with data present in the literature.[12]

Cyclic voltammogram of KCl solution 0.10 mol L^{-1} at pH 3.0 as eletrolyte on $CrCl_3$ modified glassy carbon electrode (Figure 1a) shows that there is no peak, indicating the absence of eletroactive species. Cyclic voltammogram of the precursor potassium hexacyanoferrate (III) reagent on the glassy carbon electrode (Figure 1b) shows the appearance

of two peaks relative to this reagent at 0.22 and 0.34 V that correspond to the redox process of $[Fe^{II}(CN)_6]/[Fe^{III}(CN)_6]$.[11] Peaks 1 and 2 at 0.22 V and 0.88 V, observed after the electrode surface modification (Figure 1c), corresponds to the $K_2Cr^{II}[Fe^{II}(CN)_6]$ complex oxidation peaks, while their correspondent reduction peaks (3 and 4) were observed at 0.82 V and 0.19 V, respectively. Thus, we have a system composed by stages with mixed valence represented by $[Cr^{III}\text{-}CN\text{-}Fe^{II}]/[Cr^{II}\text{-}CN\text{-}Fe^{II}]$ and $[Cr^{III}\text{-}CN\text{-}Fe^{II}]/[Cr^{III}\text{-}CN\text{-}Fe^{III}]$ and with two degeneracy states.[13] The small peaks (5 and 6) may be attributed to the inclusion and exclusion of potassium ions during the redox process.[13]

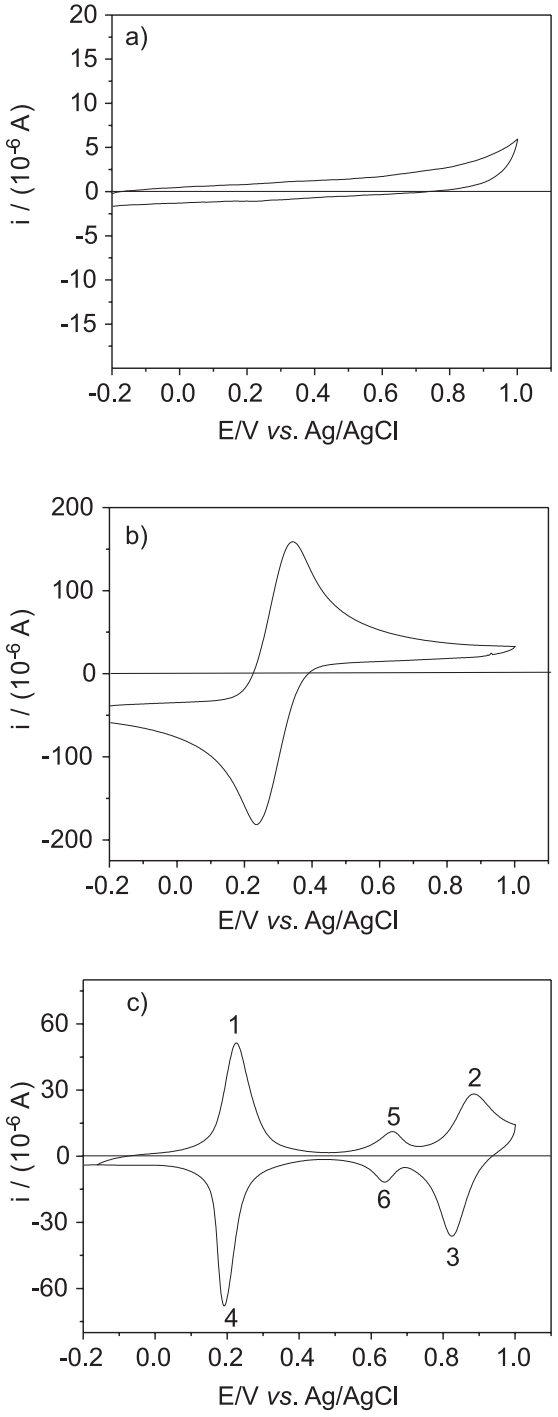

Figure 1. Cyclic voltammograms for: (a) $CrCl_3$; (b) potassium hexacyanoferrate(III); (c) Cr^{III} hexacyanoferrate(II) on glassy carbon electrode in an electrolyte solution containing 0.10 mol L^{-1} KCl. Scan rate 50 mV s^{-1}.

The probable electrode reaction is represented in Equation 1. The electrochemical oxidation/reduction process is followed by the cation flux (K^+) provided by the supporting electrolyte solution, which helps to keep the electroneutrality of the system and works as a counterion.

$$K_2Cr^{II}[Fe^{II}(CN)_6]_{(s)} \rightleftharpoons KCr^{III}[Fe^{II}(CN)_6]_{(s)} + K^+_{(aq)} + e^-$$
$$KCr^{III}[Fe^{II}(CN)_6]_{(s)} \rightleftharpoons Cr^{III}[Fe^{III}(CN)_6]_{(s)} + K^+_{(aq)} + e^- \quad (1)$$

Equation 1. Mechanism proposed for the voltammetric response of the Cr^{III} hexacyanoferrate(II)-modified electrode.

The applied conditioning reduction potential of -0.2 V resulted in the Cr^{II} and Fe^{II} complex and in the adsorbing of the CrHCF species at the electrode surface. As the scan progressed from the most negative to the most positive potentials, the metals oxidations occur and the oxidized species release the counterion $K^+_{(aq)}$ as well as electrons. On the other hand, from the most positive to the most negative potential, the species were reduced.

Studies were also carried out changing the $CrCl_3$ by $Cr(NO_3)_3$ as a precursor reagent along with potassium hexacyanoferrate (III) and the results were similar.

The precursor reagent concentration and electrodeposition time were investigated and it was observed a larger efficiency in the determination of PN with 1.0×10^{-2} mol L^{-1} $CrCl_3 \cdot 6H_2O$ and 5.0×10^{-3} mol L^{-1} $K_3[Fe(CN)_6]$ with deposition time of 30 min. The use of higher electrodeposition times results in excessively complex deposition on the electrode surface, leading to not reproducible current values. On the other hand, low precursor concentrations result in an electrode that cannot be applicable to the determination of PN due to generation of very low current values.

Supporting electrolyte

The electrochemical behavior of film of CrHCF changes when KCl, phosphate buffer and acetate buffer were used as supporting eletrolyte (Figure 2a).

The pyridoxine peaks appear at 0.80 V and the better voltammetric profile was obtained in the presence of KCl supporting eletrolite, which is in accordance with other

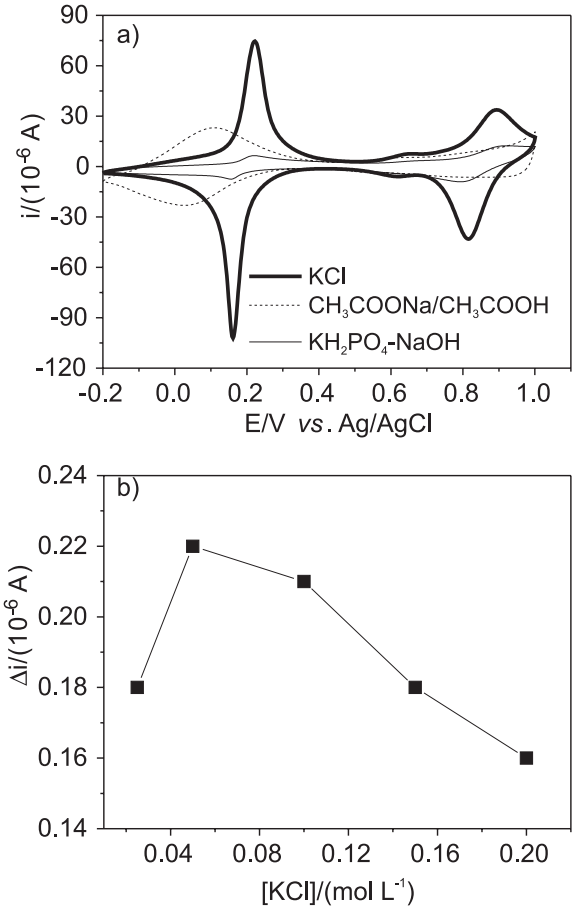

Figure 2. Cr^{III} hexacyanoferrate(II)-modified electrode at pH 5.0 and 50 mV s^{-1}. **a)** in different electrolytes at 0.10 mol L^{-1} and **b)** after the addition of 3.65 × 10^{-6} mol L^{-1} pyridoxine in different KCl concentrations.

works.[14, 15] The use of other salts instead of potassium chloride results in less defined voltammetric waves because the transport through the film is more difficult. This shows that the function of the supporting electrolyte, which is to reduce the resistance in the cell, is affected by the nature of the electrolyte.

The voltammetric response of the modified electrode for different KCl concentrations after the addition of 3.65 x 10^{-6} mol L^{-1} PN (Figure 2b) demonstrates that the better sensitivity, lower relative standard deviation and better voltammetric profile was attained when KCl was 0.050 mol L^{-1}. The concentration of the supporting electrolyte must be at least 100-fold larger than the concentration of the electroactive species so that the migration current becomes negligible,[16] and for this reason the lower KCl concentrations resulted in lower sensitivity.

Scan rate

The study of the effect of the scan rate on the voltammetric response of the CrHCF-modified electrode

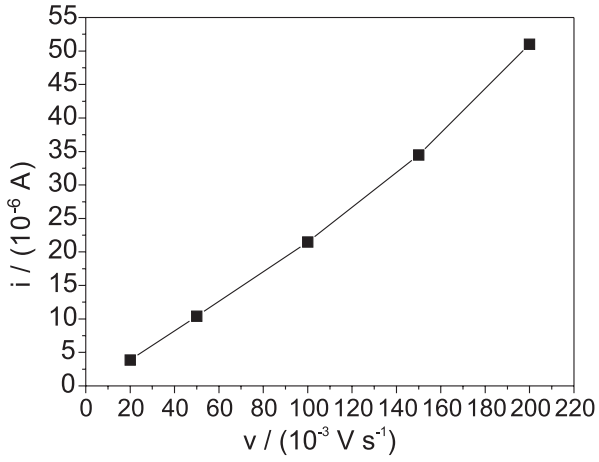

Figure 3. Variation of the anodic peak current at 0.88 V with the scan rate for the Cr^{III} hexacyanoferrate(II)-modified electrode in KCl 0.050 mol L^{-1} and pH 5.0.

(Figure 3) shows a proportional increase in the anodic peak current at 0.88 V with the the scan rate suggesting that there is an adsorption process on the electrode surface. Furthermore, the peak potential remained nearly unchanged with the variation of the scan rate, occurring a shift only at 20 mV towards the negative potential range when the rate was varied from 200 to 20 mV s^{-1} (data not presented). This indicates a reversible oxi-reduction process, according to Bard and Faulkner.[16]

The working electrode used presents a chemically active surface area of 12.6 mm^2, which classifies it as a microelectrode and suitable for use at high scan rates.[17] However, the voltammetric response of the CrHCF-modified electrode, after the addition of 3.65 x 10^{-6} mol L^{-1} PN, was evaluated and it was observed a better variation in the anode peak current of iron with the rate of 150 mV s^{-1}, which was used in the other studies. Although scan rates higher than 150 mV s^{-1} give higher analytical signal they result in much distorted profiles and in lower precision and, for these reasons, were not used.

Effect of pH

The electrochemical behavior of the CrHCF-modified electrode was evaluated for the pH range of 3.0 - 7.0 in a solution containing 3.65 x 10^{-6} mol L^{-1} PN (Figure 4).

It can be observed that the best pH to determine PN with the modified electrode, with the smallest relative standard deviation, was pH 5.5. The decrease in the current variation at a pH lower than 5.0 may occur due to the action of H^+ ions in the kinetics of the reaction between PN and the complex CrHCF on the electrode surface. Furthermore, the protonation of the pyridine could occur at a pH lower than 3.0. At more basic pHs, there may occur

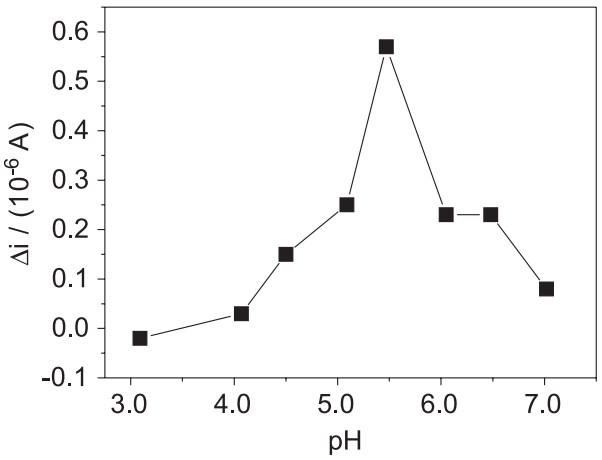

Figure 4. Variation of the current with pH for the Cr^{III} hexacyanoferrate(II)-modified electrode after the addition of 3.65×10^{-6} mol L^{-1} pyridoxine in KCl 0.050 mol L^{-1} and 150 mV s^{-1}.

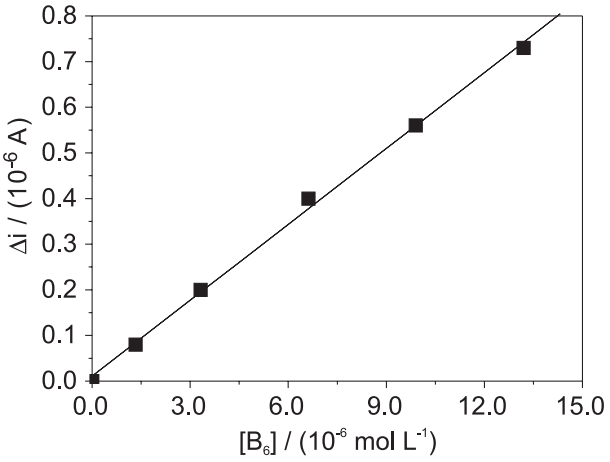

Figure 5. Analytical curve for the determination of pyridoxine in the linear concentration range in KCl 0.050 mol L^{-1}, pH 5.5, and 150 mV s^{-1}.

the degradation of the vitamin, as reported in literature,[12] besides the conversion of Cr^{III} hexacyanoferrate(II) into a form of chromium gel.[11]

Analytical curve

The curve was linear for vitamin concentrations between 1.33×10^{-6} mol L^{-1} and 1.32×10^{-5} mol L^{-1} (Figure 5). Four determinations in the same conditions established were made for each curve point in a total of five points, which resulted in the following linear regression Equation:

$$\Delta i_{pa}\ (\mu A) = 0.01091 + 0.05541\ [PN]\ (\mu mol\ L^{-1}) \quad (2)$$
$$(r = 0.9990,\ n = 5)$$

For values below 1.33×10^{-6} mol L^{-1} and above 1.32×10^{-5} mol L^{-1} PN, it was observed that the curve for the variation of the anode peak current as a function of the concentration of B_6 was not linear.

The limit of detection obtained with the CrHCF-modified glassy carbon electrode in the determination of PN was 3.46×10^{-7} mol L^{-1} and the limit of quantification was 1.05×10^{-6} mol L^{-1}.

It was observed the repeatability of the analytical signal for the concentration of 3.65×10^{-6} PN in the polarographic cell with 9 successive determinations. The average response of the current variation after the addition of PN was $(0.24 \pm 0.01)\ 10^{-6}$ A, with a relative standard deviation of 4.2%, indicating that the used electrode presents good repeatability and a low deviation between determinations. In addition, when it was used the CrHCF-modified glassy carbon electrode the voltammetric signal was 29.2% larger than that obtained with the non-modified glassy carbon electrode.

Study of interferences

The effect of several species such as thiamine hydrochloride (vitamin B_1), riboflavin (vitamin B_2), cyanocobalamin (vitamin B_{12}), sodium citrate, sodium benzoate, mannitol, fructose, L-lysine, ascorbic acid (vitamin C), and caffeine on the voltammetric response in the determination of PN was evaluated (Table 1). It was observed that in the determination of 3.65×10^{-6} mol L^{-1} PN using the CrHCF-modified electrode the method can tolerate up to the following interference concentrations without affecting the current response (for an error of 5%): an equal concentration of sodium citrate, sodium benzoate, and vitamin B_2; a twofold larger concentration of mannitol; a tenfold larger concentration of vitamin B_{12}, L-lysine, and vitamin B_1, and up to 50-fold larger concentration of fructose. Only vitamin C and caffeine presented interferences at low PN concentrations. This indicates that the voltammetric method developed has good selectivity for PN and that it may be used with

Table 1. Interference of compounds in the determination of 3.65×10^{-6} mol L^{-1} pyridoxine (PN)

Interferant	Level of Tolerance / (mol L^{-1})[a]
Vitamin C	1.01×10^{-6}
Caffeine	1.78×10^{-6}
Sodium citrate, sodium benzoate, Vitamin B_2	3.65×10^{-6}
Mannitol	7.37×10^{-6}
Vitamin B_{12}, L-Lysine, Vitamin B_1	3.65×10^{-5}
Fructose	1.83×10^{-4}

[a]5% error.

samples with interferant concentrations lower than the limit of tolerance.

Determination of pyridoxine in drugs

The CrHCF-modified glassy carbon electrode was used in the voltammetric determination of PN in three drugs. The PN content was determined by the standard addition method, the linear regression Equation, and confirmed by the recovery assay by the addition of known PN standard amounts to the analytical solution samples (Table 2).

Table 2. Determination of pyridoxine (PN) in drugs by the voltammetric method proposed

Sample	Informed PN content / (mg *per* pill)	PN content found / (mg *per* pill)	Recovery / (%)
Seis-B®	300	299.4	97.9
Dramin® B_6	10	10.3	101.1
Citoneurin®	200	198.7	97.4

The recoveries indicate that the proposed method is accurate and the experimental results demonstrate that the CrHCF-modified glassy carbon electrode presents a great potential for the analysis of PN in real samples. The results of the present method are not very different from other works devoted to determination of PN in drug samples (Table 3), but with the advantages of a fast response, a low detection limit, low cost, and simple development and application.

Table 3. Comparision of the linear dinamic range and limit of detection of different methods applied to pyridoxine (PN) determination in drug samples

Method	Linear dynamic range / (mol L^{-1})	Detection Limit / (mol L^{-1})
HPLC with electrochemical detection[18]	2.4×10^{-6}-1.7×10^{-5}	1.3×10^{-8}
Spectrofluorimetry[19]	4.9×10^{-7}-1.9×10^{-5}	3.7×10^{-7}
Cyclic Voltammetry[10]	4.5×10^{-4}-3.3×10^{-3}	3.7×10^{-5}
Cyclic Voltammetry[9]	1.2×10^{-6}-6.9×10^{-4}	4.1×10^{-7}
Proposed Method	1.3×10^{-6}-1.3×10^{-5}	3.5×10^{-7}

Conclusions

The determination of PN in drugs was possible due to the modification in the glassy carbon electrode with Cr^{III} hexacyanoferrate (II). The concentration range was linear between 1.33×10^{-6} mol L^{-1} and 1.32×10^{-5} mol L^{-1} of vitamin B_6, with r = 0.9990 and relative standard deviation of 4.2%. The limits of detection and of quantification obtained were 3.46×10^{-7} mol L^{-1} and 1.05×10^{-6} mol L^{-1} PN, respectively. These results were obtained with supporting electrolyte KCl 0.050 mol L^{-1}, pH 5.5, scan rate of 150 mV s^{-1}.

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