Voltammetric Determination of Pyridoxine (Vitamin B<sub>6</sub>) in Drugs using a Glassy Carbon Electrode Modified with Chromium(III) Hexacyanoferrate(II)

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A Cr<sup>III</sup> hexacyanoferrate(II) (CrHCF)-modified glassy carbon electrode was used to determine pyridoxine (vitamin B<sub>6</sub>) 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<sup>-6</sup> mol L<sup>-1</sup> to 1.32 × 10<sup>-5</sup> mol L<sup>-1</sup> of vitamin, with r = 0.9990 and desvio padrão relativo of 4.2%. The limits of detection and quantification were 3.46 × 10<sup>-7</sup> mol L<sup>-1</sup> and 1.05 × 10<sup>-6</sup> mol L<sup>-1</sup>, respectively. The voltammetric proposed method for determination of vitamin B<sub>6</sub> 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<sup>III</sup> hexacyanoferrate(II), Voltammetry, Vitamin B<sub>6</sub>

Introduction

Vitamins are small organic molecules whose lack or excess may result in several diseases to the organisms that need them. Vitamin B<sub>6</sub> belongs to the hydrophilic 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<sub>6</sub> 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<sup>III</sup> hexacyanoferrate(III) has been proposed.⁹
Voltammetric response of PN with a glassy carbon electrode chemically modified with carbon nanotubes has also been evaluated.

The combined use of glassy carbon electrodes modified with hexacianoferate complexes may be instrumental in the determination of PN. Thus, the present work sought to use a glassy carbon electrode modified with Cr\textsuperscript{III} hexacyanoferate(II)\textsuperscript{11} (CrHCF) to determine vitamin B\textsubscript{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\textsuperscript{-3} mol L\textsuperscript{-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,\textsuperscript{11} 20 mL KCl 0.10 mol L\textsuperscript{-1}, pH 3.0 with 1.0 × 10\textsuperscript{-2} mol L\textsuperscript{-1} CrCl\textsubscript{3}·6H\textsubscript{2}O and 5.0 × 10\textsuperscript{-3} mol L\textsuperscript{-1} K\textsubscript{6}[Fe(CN)\textsubscript{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\textsuperscript{-1} and NaOH 0.10 mol L\textsuperscript{-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\textsuperscript{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\textsuperscript{-1} (pH 3.0) solution containing 1.0 × 10\textsuperscript{-2} mol L\textsuperscript{-1} CrCl\textsubscript{3}·6H\textsubscript{2}O and 5.0 × 10\textsuperscript{-3} mol L\textsuperscript{-1} K\textsubscript{6}[Fe(CN)\textsubscript{6}]. The electrodeposition scan rate was 50 mV s\textsuperscript{-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\textsuperscript{-1} and pH 3.0.\textsuperscript{11}

**Preparation and analysis of drug samples**

The following drug samples were used in the experiment: Seis-B\textsuperscript{®} - APSEN, Dramin\textsuperscript{®} B\textsubscript{6} - ALTANA and Citoneurin\textsuperscript{®} - 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 collected in a volumetric flask. The volume of Seis-B\textsuperscript{®} 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.\textsuperscript{8}

**Results and Discussion**

**Modification of the Glassy Carbon Electrode**

The voltamograms obtained on modified and non-modified glassy carbon electrodes can be seen in Figure 1. According to the potential values obtained, the resulting complex of CrCl\textsubscript{3}·6H\textsubscript{2}O and K\textsubscript{6}[Fe(CN)\textsubscript{6}] deposition onto the glassy electrode is Cr\textsuperscript{III} hexacyanoferate (II) (K[Cr(Fe(CN)\textsubscript{6})])\textsuperscript{2+} which is deposited in the proportion of 2:1 of Cr\textsuperscript{III}/[Fe(CN)\textsubscript{6}]\textsuperscript{3-}\textsuperscript{11} that presents blue color, which is in agreement with data present in the literature.\textsuperscript{12}

Cyclic voltammogram of KCl solution 0.10 mol L\textsuperscript{-1} at pH 3.0 as electrolyte on CrCl\textsubscript{3} modified glassy carbon electrode (Figure 1a) shows that there is no peak, indicating the absence of electroactive species. Cyclic voltammogram of the precursor potassium hexacyanoferate (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 \([\text{Fe}^{II}(\text{CN})_6]^{3-}/[\text{Fe}^{III}(\text{CN})_6]^{3-}\).\textsuperscript{11} Peaks 1 and 2 at 0.22 V and 0.88 V, observed after the electrode surface modification (Figure 1c), corresponds to the \(K_2\text{Cr}^{III}[\text{Fe}^{II}(\text{CN})_6]\) 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 \([\text{Cr}^{III}-\text{CN}-\text{Fe}^{II}]\)/\([\text{Cr}^{II}-\text{CN}-\text{Fe}^{III}]\) and \([\text{Cr}^{III}-\text{CN}-\text{Fe}^{II}]\)/\([\text{Cr}^{II}-\text{CN}-\text{Fe}^{III}]\) with and with two degeneracy states.\textsuperscript{13} The small peaks (5 and 6) may be attributed to the inclusion and exclusion of potassium ions during the redox process.\textsuperscript{13}

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

\[
\begin{align*}
    \text{K}_2\text{Cr}^{III}[\text{Fe}^{II}(\text{CN})_6](s) & \rightarrow \text{KCr}^{II}[\text{Fe}^{II}(\text{CN})_6](s) + \text{K}^{+}(aq) + e^- \\
    \text{KCr}^{III}[\text{Fe}^{II}(\text{CN})_6](s) & \rightarrow \text{Cr}^{III}[\text{Fe}^{III}(\text{CN})_6](s) + \text{K}^{+}(aq) + e^- 
\end{align*}
\]

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

The applied conditioning reduction potential of -0.2 V resulted in the Cr\textsuperscript{II} and Fe\textsuperscript{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\textsuperscript{+} (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\textsubscript{3} by Cr(NO\textsubscript{3})\textsubscript{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 \(\times\) 10\textsuperscript{-2} mol L\textsuperscript{-1} CrCl\textsubscript{3}•6H\textsubscript{2}O and 5.0 \(\times\) 10\textsuperscript{-3} mol L\textsuperscript{-1} K\textsubscript{3}[Fe(CN)\textsubscript{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 electrolyte (Figure 2a).

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

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**Figure 1.** Cyclic voltammograms for: (a) CrCl\textsubscript{3}; (b) potassium hexacyanoferrate(III); (c) Cr\textsuperscript{II} hexacyanoferrate(II) on glassy carbon electrode in an electrolyte solution containing 0.10 mol L\textsuperscript{-1} KCl. Scan rate 50 mV s\textsuperscript{-1}. 
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 $3.65 \times 10^{-6}$ 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, 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) 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.

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. However, the voltammetric response of the CrHCF-modified electrode, after the addition of $3.65 \times 10^{-6}$ 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 $3.65 \times 10^{-6}$ 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...
the degradation of the vitamin, as reported in literature,\textsuperscript{12} besides the conversion of Cr\textsuperscript{III} hexacyanoferrate(II) into a form of chromium gel.\textsuperscript{11}

**Analytical curve**

The curve was linear for vitamin concentrations between 1.33 \times 10^{-6} \text{ mol L}^{-1} and 1.32 \times 10^{-5} \text{ 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:

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

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

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

It was observed the repeatability of the analytical signal for the concentration of 3.65 \times 10^{-6} \text{ 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) \times 10^{-6} \text{ 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\textsubscript{1}), riboflavin (vitamin B\textsubscript{2}), cyanocobalamin (vitamin B\textsubscript{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 \times 10^{-6} \text{ 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\textsubscript{2}; a twofold larger concentration of mannitol; a tenfold larger concentration of vitamin B\textsubscript{12}, L-lysine, and vitamin B\textsubscript{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 \times 10^{-6} \text{ mol L}^{-1} pyridoxine (PN)**

| Interferant                                      | Level of Tolerance / (mol L\textsuperscript{-1})\textsuperscript{a} |
|------------------------------------------------|-------------------------------------------------|
| Vitamin C                                      | 1.01 \times 10\textsuperscript{6}               |
| Caffeine                                       | 1.78 \times 10\textsuperscript{6}               |
| Sodium citrate, sodium benzoate, Vitamin B\textsubscript{2} | 3.65 \times 10\textsuperscript{6}               |
| Mannitol                                       | 7.37 \times 10\textsuperscript{6}               |
| Vitamin B\textsubscript{12}, L-Lysine, Vitamin B\textsubscript{1} | 3.65 \times 10\textsuperscript{5}               |
| Fructose                                       | 1.83 \times 10\textsuperscript{4}               |

\textsuperscript{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® B6  | 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. Comparison of the linear dynamic 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$^{18}$ | $2.4 \times 10^{-6}$-$1.7 \times 10^{-4}$ | $1.3 \times 10^{-4}$ |
| Spectrofluorimetry$^{19}$ | $4.9 \times 10^{-5}$-$1.9 \times 10^{-3}$ | $3.7 \times 10^{-7}$ |
| Cyclic Voltammetry$^{11}$ | $4.5 \times 10^{-6}$-$3.3 \times 10^{-7}$ | $3.7 \times 10^{-7}$ |
| Cyclic Voltammetry$^{11}$ | $1.2 \times 10^{-6}$-$6.9 \times 10^{-7}$ | $4.1 \times 10^{-7}$ |
| Proposed Method         | $1.3 \times 10^{-6}$-$1.3 \times 10^{-5}$ | $3.5 \times 10^{-7}$ |

Conclusions

The determination of PN in drugs was possible due to the modification in the glassy carbon electrode with Cr$^{III}$ hexacyanoferate (II). The concentration range was linear between $1.33 \times 10^{-6}$ mol L$^{-1}$ and $1.32 \times 10^{-5}$ 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 $3.46 \times 10^{-7}$ mol L$^{-1}$ and $1.05 \times 10^{-6}$ 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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