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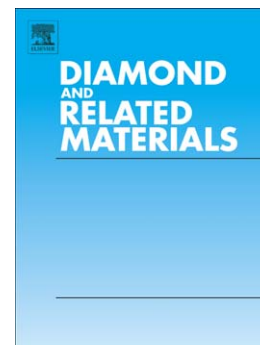
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PII: S0925-9635(16)30058-9
DOI: doi: [10.1016/j.diamond.2016.02.018](https://doi.org/10.1016/j.diamond.2016.02.018)
Reference: DIAMAT 6587

To appear in: *Diamond & Related Materials*

Received date: 7 November 2015
Revised date: 22 February 2016
Accepted date: 22 February 2016



Please cite this article as: Darko Kuzmanović, Muslim Khan, Eda Mehmeti, Ruqia Nazir, Naser Ramdan R. Amaizah, Dalibor M. Stanković, Determination of pyridoxine (vitamin B₆) in pharmaceuticals and urine samples using unmodified boron-doped diamond electrode, *Diamond & Related Materials* (2016), doi: [10.1016/j.diamond.2016.02.018](https://doi.org/10.1016/j.diamond.2016.02.018)

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Determination of pyridoxine (vitamin B₆) in pharmaceuticals and urine samples using unmodified boron-doped diamond electrode

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Abstract: Pyridoxine (vitamin B₆, VB₆) was detected in pharmaceutical preparation and human urine samples by employing differential pulse voltammetry (DPV) at unmodified boron doped electrode. In Britton-Robinson buffer solution (BRBS) at pH 6 VB₆ provides well defined oxidation peak at around +1.05 V vs. Ag/AgCl (3M KCl). The influence of most of the interferences present in real samples, supporting electrolyte and DPV parameters were investigated. A VB₆ sensor with linear range from 7 to 47 and a detection limit of 3.76 was obtained after optimization. Repeatability of the proposed procedure calculated after 7 measurements of 9 and 30 μM of VB₆ were 3.6 and 2.4 %, respectively. Proposed methodology was successfully applied for the determination of VB₆ in real samples, and from obtained results it can be concluded that proposed analytical procedure can be inexpensive alternative to the widely used separation methods.

Keywords: parameters; optimization; real samples; DP voltammetry;

Introduction

Pyridoxine (vitamin B₆) belongs to water soluble B complex vitamins group, commonly called pyridoxine. It is essential in the diet for metabolism of amino acids and for the maintenance of body cells [1]. It is reported that the deficiency of pyridoxine causes many of illnesses and diseases [2, 3]. Pyridoxine helps the body to create antibodies, transmit signals in the brain, maintain normal nerve function, make hemoglobin and keep blood sugar in normal range [4].

Due to its role in human body there is a need for fast, low cost and reliable analytical procedures for the selective and sensitive determination of pyridoxine. Nowadays, there are several analytical methodologies based on spectrophotometric determination including flow injection systems [5-9], high performance thin layer chromatography and liquid chromatography with electrochemical detection [10]. Also, there are several methods based on voltammetric determination of pyridoxine in pharmaceutical formulation already reported in the literature. These methods employ different electrodes such as carbon paste electrode [11], carbon paste electrode modified with vanadyl(IV)–Salen complex [12], chemically-modified glassy carbon electrodes [13], glassy carbon [14], ruthenium tris(2,2')bipyridyl-modified oxidized boron-doped diamond electrode [15].

Boron doped diamond (BDD) electrode present one of the best electrode material due to its wide potential window, low background current, long term stability, low adsorption and high sensitivity [16-19]. BDD electrode is powerful electrochemical sensor for the determination of biological active compounds [20-22], in the environment, food and drug analysis [23-27].

The aim of this work was to improve advantages of the BDD electrode and to develop sensitive, selective and low cost analytical procedure for the determination of pyridoxine. For this purpose differential pulse voltammetry was applied. After optimization of experimental conditions and interferences studies proposed method was successfully applied for the determination of pyridoxine in various samples.

Experimental

Apparatus and reagents

Pyridoxine, vitamins B₁, B₂ and B₁₂, boric acid, sodium hydroxide, ascorbic acid, uric acid, dopamine, acetic acid, phosphoric acid and sodium hydroxide were purchased from Sigma Aldrich and used as received without any further purification. Stock solution of 1×10^{-3} M of the tested compound was prepared by dissolving corresponding amounts in Millipore water. Calibration solutions were prepared from the stock solution by appropriate dilution with supporting electrolyte. The pH values of Britton-Robinson buffer were adjusted with sodium hydroxide (0.2 M).

The voltammetric measurements were performed using a potentiostat/galvanostat (Autolab PGSTAT 302 N, Metrohm Autolab B.V., The Netherlands) controlled by the corresponding electrochemical software (Nova 1.10). The cell (10 mL) consisted of a three-electrode system, a boron-doped diamond electrode (inner diameter of 3 mm; Windsor Scientific LTD, UK) embedded in a polyether ether ketone (PEEK) body with an inner diameter of 3 mm, a resistivity of $0.075 \Omega \text{ cm}$ and a boron doping level of 1000 ppm (as declared by the supplier) was used as the working electrode, an Ag/AgCl (3 M KCl) as a reference electrode and a Pt wire as a counter electrode. Prior to start the first measurement, the BDD electrode was rinsed with deionized water and gently rubbed with a piece of damp silk cloth until a mirror-like appearance of surface was obtained (with minimal probability of mechanical damage of surface). Subsequently, it was anodically pretreated by setting +2 V during 180 s in 1 M H₂SO₄ in order to clean the electrode surface (get rid of any impurities) followed by cathodic pretreatment at -2 V during 180 s to renew the hydrogen terminated surface of the working electrode. In order to confirm stability and advantages of the BDD electrode before starting measurements and at the end of working day, potential/current changes in the K₄[Fe(CN)₆] / K₃[Fe(CN)₆] couple was monitored. It was observed that during day these changes are lower than 5 %. All potentials reported in this paper are referred versus the Ag/AgCl (3 M KCl) reference electrode at an ambient temperature. All pH values were measured with a pH meter model Orion 1230 equipped with combined glass electrode model Orion 9165BNWP (USA).

The potential was swept over the range from 0 to 1.5 V (vs. Ag/AgCl) at 100 mV s⁻¹ (if not stated otherwise) for CV, and from 0 to 1.5 V vs. (Ag/AgCl) at optimized parameters for DPV (pulse amplitude 130 mV, and pulse time 50 ms).

Preparation of pharmaceutical tablets and urine samples

The analyzed multivitamin dissolving tablets (2 different suppliers, 4 and 4.5 g weight) were purchased from local shops in Graz, Austria. The tablets containing xylitol, magnesium stearate, potassium and dicalcium phosphate, riboflavin, starch, maltodextrins, folic acid, silica, vitamin C, orange flavor and pyridoxine. Each tablet was weighed, pestled and dissolved in the supporting electrolyte, sonicated for 30 min, and then filtered through filter paper to remove traces of undissolved species then transferred to a 100 ml flask and diluted with supporting electrolyte. The diluted solution was directly analyzed by proposed method and results were compared with those proposed by supplier.

The urine samples were collected from two different persons and for the measurements of pyridoxine concentrations, samples were spiked with aliquots of stock solution of pyridoxine. Each 1.0 mL of fresh urine sample was taken and diluted to 10 mL with supporting electrolyte and then directly analyzed.

Results and discussions

Electrochemical behavior of pyridoxine at BDD electrode, influence of pH and scan rate

Selection of appropriate supporting electrolyte and optimization of pH can play beneficial role for one voltammetric procedure. Britton-Robinson buffer solution can cover wide pH range it is inactive in wide potential window and due to which it is often used as supporting electrolyte [20-22]. In Figure 1. cyclic voltammograms in presence and absence of 0.1 mM of pyridoxine at BDD electrode in BRBS at pH 6 are presented, and inset figure shows dependence of peak current and peak potential from the pH of supporting electrolyte. In acidic media pyridoxine provides single oval-shaped and well-defined oxidation peak. The absence of corresponding reduction wave under these experimental conditions leads to the conclusion that this oxidation process of pyridoxine at BDD electrode belongs to the quasi-reversible or irreversible electrochemical processes. The peak disappeared when the pH is increased above 9. This

phenomenon was also observed from the other authors [4, 15, 28] and can be explained with pK_{a2} value of 8.9. Higher pH is followed with formation of hemiacetal form with negligible presence of electroactive free aldehyde. The highest peak current was obtained for pH 7, but at this pH peak was wide more than 400 mV which can cause big influence of interferences. At pH 6 of BRBS peak was oval-shaped with current value of 35 nA, and taking into account these values and peak resolution, this pH was selected as optimum. In the pH range from 4 to 7 peak potential of pyridoxine shifted linearly to less negative potential indicating that in electrochemical reaction in this pH range proton participating. The proposed electrochemical reaction at BDD electrode in BRBS at pH 6 is given in Scheme 1. This process can be observed as two steps where first step probably leading to the forming of aldehyde (from pyridoxine produced pyridoxal), which in further step as final product gives pyridoxic acid. These steps under proposed conditions are presented as only one overall oxidation process [4, 14, 15, 28].

Here Fig. 1.

At selected pH of BRBS effect of the scan rate on electrochemical behavior of pyridoxine was investigated and results are presented in Figure 2. Tested compound show good linearity of current from square root of scan rate (inset of Figure 2) indicating that process on the electrode surface is diffusion controlled rather than adsorption. The corresponding equation obtained from these measurements is expressed as: $I (\mu A) = -0.094 + 0.220 v^{1/2} (mV/s)^{1/2}$ with $R^2 = 0.9993$. With the increase of the scan rate negligible shift of peak potential is observed, which is characteristic for quasi-reversible and irreversible processes [21,22].

Here Fig. 2.

Here Scheme 1.

Optimization of detection performance for the determination of pyridoxine

Optimization of working parameters of electrochemical procedure plays important role for application in analysis of target analyte. Differential pulse voltammetry presents one of the most used electrochemical method which offers sensitive and selective quantification of a large number of different compounds [20,22,24]. For further improvement of this method optimization of its parameters such as pulse amplitude and pulse time was investigated. During these measurements the investigated parameter was changed while other was kept constant. Concentration of pyridoxine was 10 μM . With increasing pulse time from 10 to 100 ms, at fixed pulse amplitude of 50 mV, the most suitable peak, observing peak current, shape and width, was observed at the value of 50 ms. So, for optimization of pulse amplitude this value of pulse time was selected. It was found that with increasing peak amplitude from 10 to 130 mV peak current increases linearly. Further increase of pulse amplitude causes spreading of the peak and reducing in peak current. Pulse amplitude value of 130 mV and pulse time of 50 ms was selected as optimum for the quantification of pyridoxine.

Construction of calibration curve and analytical performance

Calibration curve for quantification of pyridoxine was constructed by plotting oxidation peak current versus concentration under previous optimized experimental conditions. Obtained voltammograms from these measurements and corresponding calibration curve are presented in Figure 3 A and B. The calibration curve was linear in the wide linear range from 7 to 47 μM with the corresponding equation $I (\mu\text{A}) = -0.043 + 0.008 c (\mu\text{M})$ ($R^2 = 0.9912$). The limit of detection, calculated as $3SD_{\text{intercept}}/\text{slope}$, was found as 3.76 μM . The repeatability of seven measurements of 9 and 30 μM of pyridoxine was 3.6 and 2.4 %, respectively. The obtained detection limit was at comparable value with those previously described in the literature for quantification of pyridoxine using electrochemical methods [4, 12-15, 28]. Benefits of this work lies in the wide working linear range and used solid, unmodified electrode, easily manipulation steps, without any sample pretreatment.

Here Fig. 3.

Interferences studies

Probably one of the most important parameter for analytical procedure is its selectivity. Influence of most of the common accompanies of tested compound was investigated in order to improve application of proposed procedure in different matrices. In Figure 4 A-E DPV voltammograms recorded for 10 μM of pyridoxine in presence and absence of tested interferences are presented. For multivitamin tablets, effect of vitamin B₁, B₁₂, riboflavin (vitamin B₂) and vitamin C was investigated. Also, vitamin C together with uric acid and dopamine can interfere for application of the proposed procedure for quantification of pyridoxine in urine samples. During these measurements concentration of pyridoxine was 10 μM and all tested interferences were in 5 time higher concentration. The content of selected interfering compounds in pharmaceutical preparations is expected to be lower than proposed for these measurements [14]. For every tested species it is considered to interfere if gives signal changes more than 10 %. In the case of pharmaceutical preparation from Figure 4 A, B and E it can be concluded that presence of vitamin B₁₂ under previously described conditions, disable determination of pyridoxine, as they provides oxidation peak at similar potential value. However, presence of vitamin B₁, B₂ and vitamin C does not induce changes in oxidation peak of pyridoxine, as vitamin B₁ does not provide electroactive behavior under these conditions and oxidation of vitamin C occurs at lower potential (Figure 4E). Riboflavin shows redox behavior at the potential lower than used in these experiments (data not shown). For interferences in urine samples from Figure 4 C, D and E it can be concluded that tested compound does not interfere with pyridoxine as oxidation of all of them occurs at lower potential.

From all these measurements it is noticeable that proposed methodology offer selective analytical approach for the determination of vitamin B₆ in various types of real samples.

Here Fig. 4.

Application of proposed analytical procedure in real samples analysis

The optimized proposed procedure was applied for the quantitative determination of pyridoxine in real samples analysis. Results obtained from these measurements and recovery studies are listed in Table 1. All samples were prepared as it is previously described. All results present average value of three replicate measurements and are in good agreement with those proposed by supplier. Also, results obtained from recovery studies for the both matrices are in good agreement with added amount of pyridoxine, which implies that our procedure can be successfully applied for the quantification of pyridoxine and satisfactory substitute to more expensive and time consuming separation methods.

Here Table 1.

Conclusions

Simple, fast, sensitive and selective electroanalytical procedure for the determination of pyridoxine in various real samples is described. Different parameters such as pH of supporting electrolyte, DPV experimental parameters, effect of possible interferent compounds were investigated. Wide linear range and limit of detection obtained by presented method were comparable with previously reported in the literature. The results indicated that presented method and boron doped diamond electrode are a potential alternative electroanalytical approach and satisfactory substitute to the separation methods.

Acknowledgments

This work has been supported by the Ministry of Education and Science of the Republic of Serbia (project No. OI 172030) and JoinEU-SEE-Penta Erasmus Mundus scholarship.

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Tables:

Table 1. Results^a obtained from the determination of pyridoxine in real samples and recovery^b experiments

Sample	Expected (μM)	Found (μM)	Added/found (μM)	Recovery %	Added/found (μM)	Recovery %
Phar. 1	10.00	10.83	5.00/16.98	107	5.00/22.34	102
Pharm. 2	10.00	10.62	5.00/16.32	104	5.00/22.31	105
Urine 1	/	0.00	10.00/10.20	102	5.00/15.33	101
Urine 2	/	0.00	10.00/10.09	101	5.00/15.08	100

^a n = 3

^b recovery was calculated as: $B_{\text{found}}/B_{\text{added}} \times 100\%$.

Figures and scheme captions:

Figure 1. Cyclic voltammograms in presence and absence (dash line) of pyridoxine (0.1 mM) at BDD electrode in BRBS at pH 6. Inset figure present dependence of peak current and peak potential (dotted line) from different pH.

Figure 2. CV voltammograms of 0.1 mM of pyridoxine at BDD electrode in BRBS at pH 6 at different scan rate, from 10-300 mV/s. Inset figure presents dependence of peak currents from the square root of scan rate.

Figure 3. A) DP voltammograms obtained for quantification of pyridoxine under optimized experimental parameters (7-47 μM). B) corresponding calibration curve constructed from these measurements.

Figure 4. DP voltammograms of A) vitamins B₁ and B₆; B) B₁₂ and B₆; C) dopamine and B₆; D) uric acid and B₆; E) vitamin C and B₆; as interferences. Concentration of pyridoxine is 10 μM and all tested interferences were in 5 time higher concentration. All experiments were done under optimized experimental parameters.

Scheme 1. Proposed oxidation mechanism for pyridoxine, in BRBS at pH 6, using BDD electrode

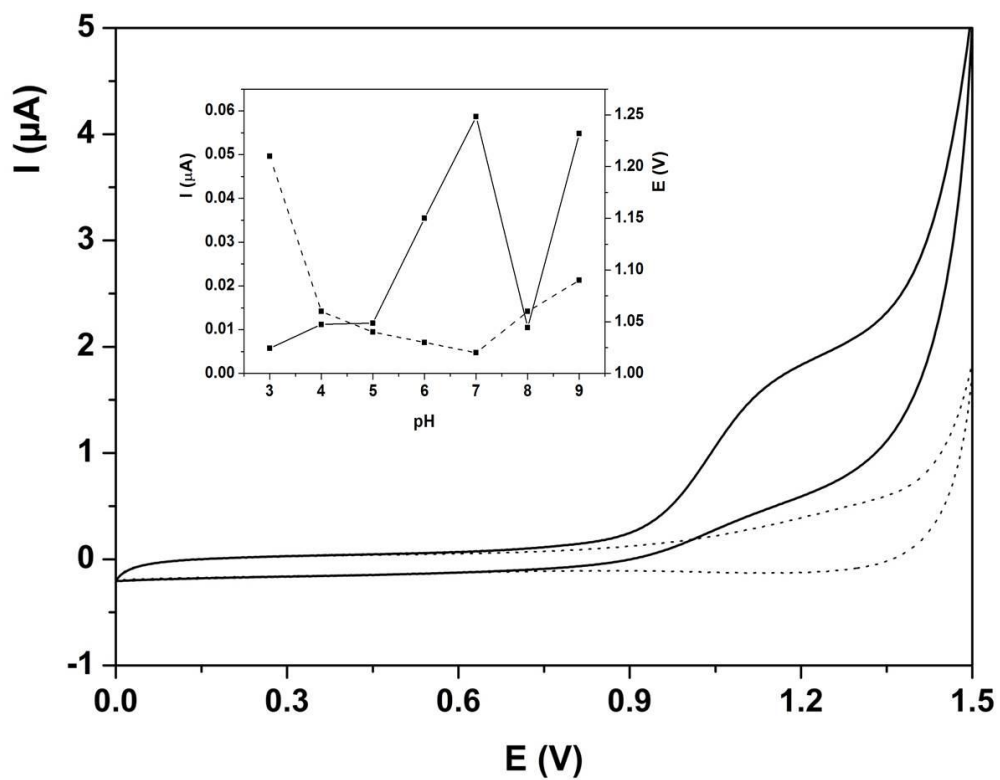


Figure 1

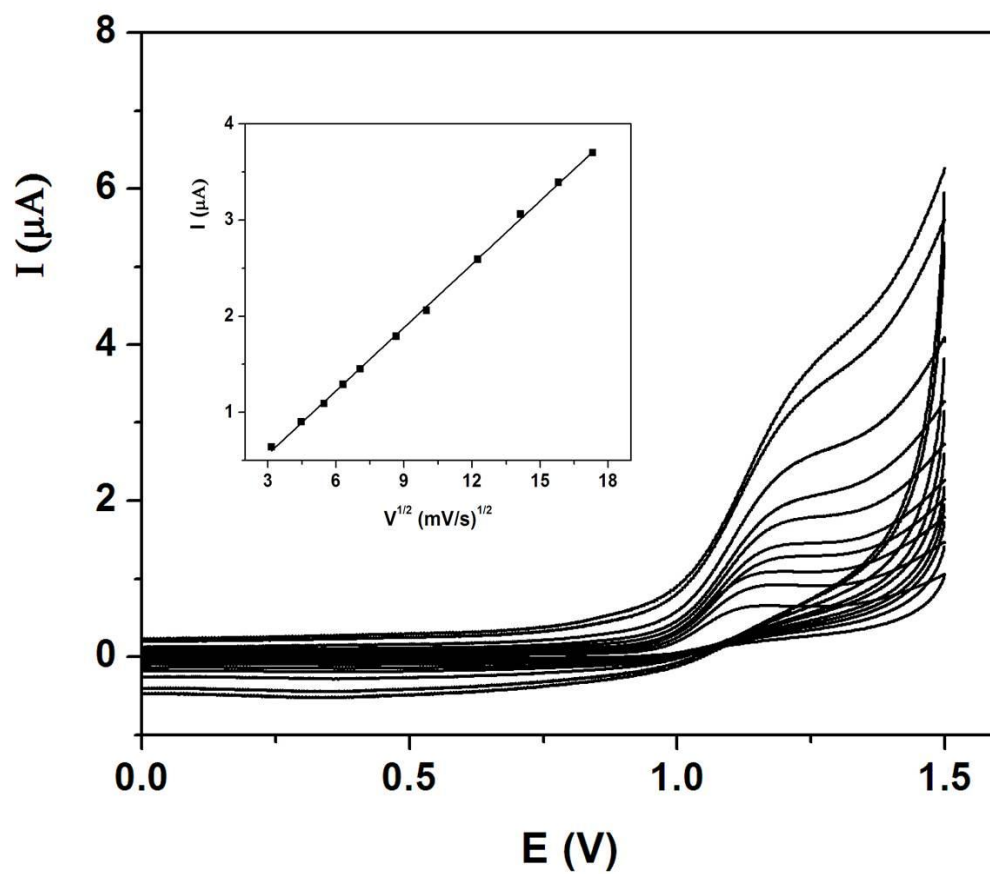


Figure 2

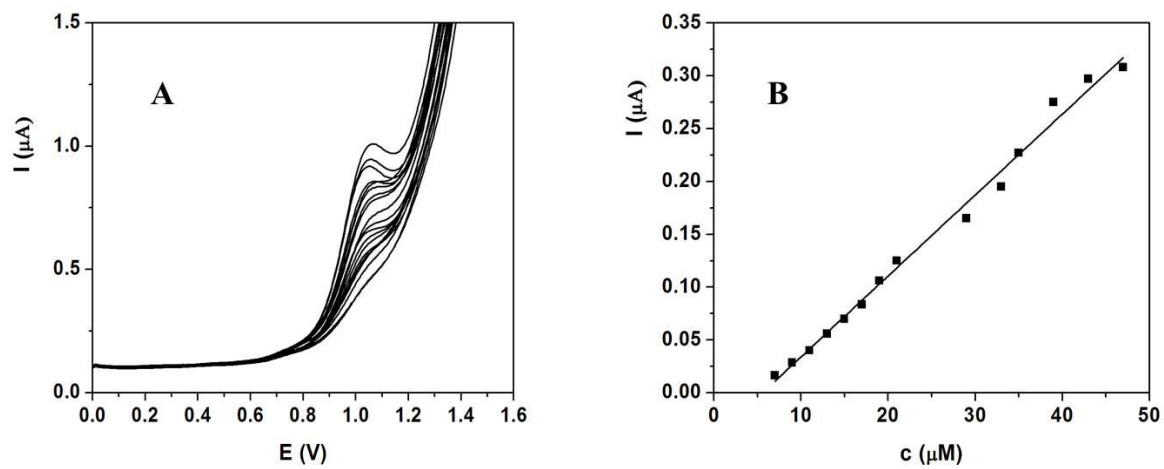
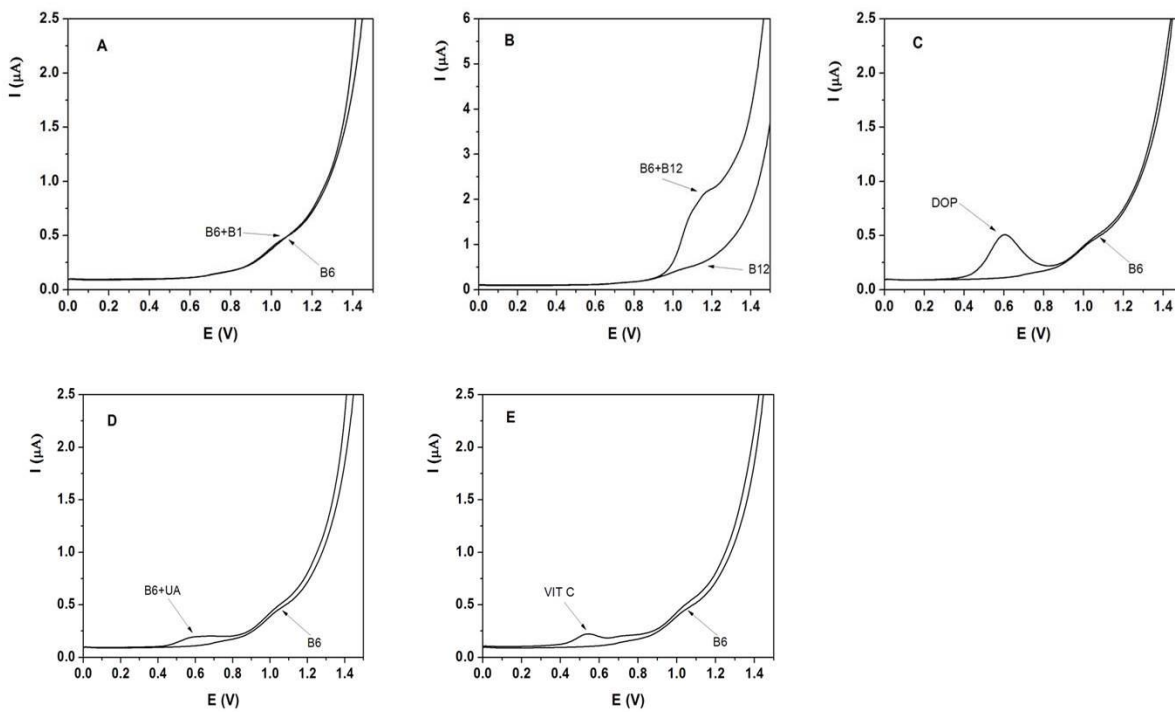
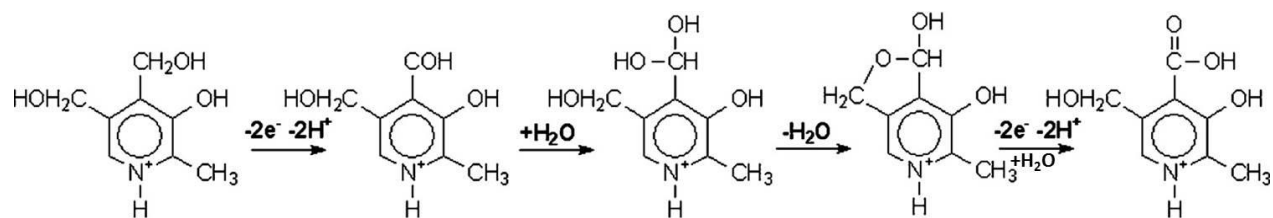


Figure 3

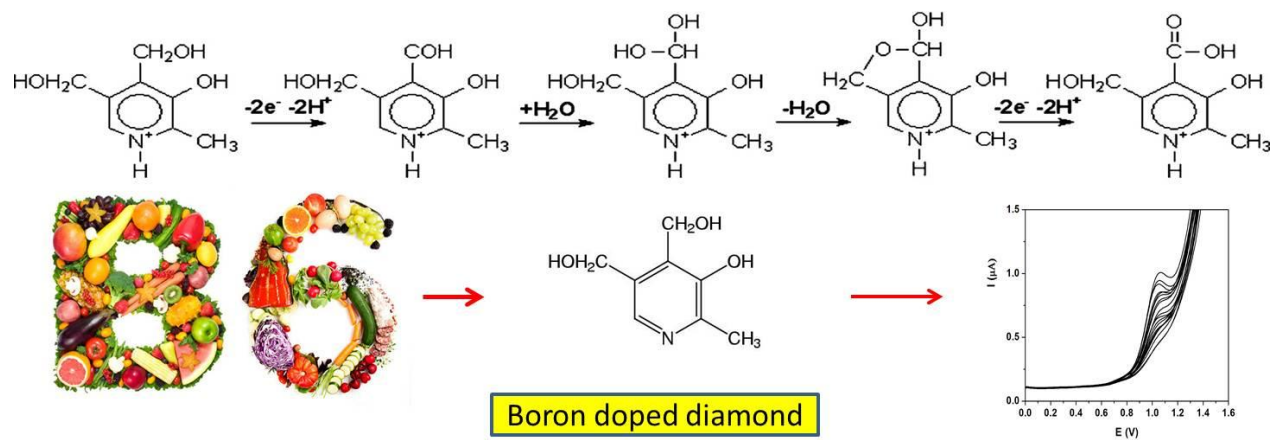
**Figure 4**



Scheme 1

Prime Novelty statement

This work presents the quantification of vitamin B₆, using differential pulse voltammetry, with boron-doped diamond electrode. BDD electrode is, up to date, one of the best electrode materials, with extremely low background current and negligible passivation. The aim of this study was to develop method for vitamin B₆ determination in various matrices, as can be seen from the results obtained procedure shows good recovery values in tested samples and could be satisfactory replacement to the separation methods and modified electrodes.



Graphical abstract

Highlights:

Vitamin B₆ was determined using unmodified boron-doped diamond electrode.

Differential pulse voltammetric detection was used.

The developed method was applied for determination of analyte in pharmaceuticals and urine samples.

Obtained results were in a good agreement with those proposed by supplier.