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Original scientific paper

Voltammetric determination of vitamin B6 in the presence of vitamin C based on zinc ferrite nano-particles modified screen-printed graphite electrode

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Abstract

The zinc ferrite nano-particles ($ZnFe_2O_4$) modified screen-printed graphite electrode ($ZnFe_2O_4$/SPGE) was used for the voltammetric determination of vitamin B_6 in real samples, using differential pulse voltammetry (DPV). It has been found that the oxidation of vitamin B_6 at the surface of such an electrode occurs at a potential about 150 mV less positive compared to an unmodified screen-printed graphite electrode. After optimization, a vitamin B6 sensor with a linear range from 0.8 to 585.0 µM and a detection limit of 0.17 µM. The $ZnFe_2O_4$/SPGE sensor exhibits good resolution between the voltammetric peaks of vitamin B_6 and vitamin C, making it suitable for detecting vitamin B_6 in the presence of vitamin C in real samples.

Keywords

Zinc ferrite nanoparticles; voltammetry; vitamin B6; vitamin C; modified electrode

Introduction

Vitamins are small organic molecules whose lack or excess may result in several diseases to the organisms that need them. They are classified into two groups by their solubilities, namely water-soluble vitamins (vitamin B and vitamin C) and fat-soluble vitamins (vitamin A, vitamin D, vitamin E, and vitamin K) [1 2].

Vitamin B_6 belongs to the water-soluble B complex vitamins group, commonly called pyridoxine. It is essential in the diet for the metabolism of amino acids and the maintenance of body cells. The nervous and immune systems need vitamin B_6 for efficient functioning; it also plays a major role in the conversion of tryptophan to niacin [3,4]. Also, It is found in different chemical forms (pyridoxamine, pyridoxine or 5-phosphate derivatives), but the most stable form is pyridoxine which is used in drug formulations such as multivitamin supplements or in enriched foods [5].

Vitamin C is one of the most important water-soluble vitamins and it refers to all compounds exhibiting equivalent biological activity to L-ascorbic acid (AA), including dehydroascorbic acid (DHAA), the oxidation product of AA, its isomers and esters. Vitamin C is an antioxidant necessary for the growth, development, and repair of all tissues [6,7]. Low levels of vitamin C can result in a condition called scurvy. Scurvy may cause symptoms such as rash, muscle weakness, joint pain, tiredness, or tooth loss. Vitamin C is an important antioxidant, along with vitamin E, beta-carotene, and many other plant-based nutrients. Antioxidants block some of the damage caused by free radicals, substances that damage DNA [8,9].

Studies show that vitamins B_6 and C are essential for the natural synthesis of dopamine in the human body. On the other hand, large doses of vitamins B_6 and C may reduce the risk of kidney stone formation in women. So, the simultaneous determination of these compounds is very important for pharmaceutical and biological investigation [10,11].

Several methods have been developed to determine vitamins B_6 and C, including high-performance liquid chromatography [12], spectrophotometry [13], and flow injection [14,15]. However, these methods require not only advanced technical expertise but also time-consuming, expensive and often need the pretreatment step. Electrochemical detection is an attractive alternative approach to these technologies because of the inherent advantages of simplicity, ease of miniaturization, cost-effectiveness, dependability, high sensitivity, and relatively low cost [16-21]. So far, electrochemical sensors have found widespread use in a variety of disciplines, including pharmaceutical, food, and clinical analyses [22-29].

Currently, the advancement of screen-printing technology for the fabrication of screen-printed electrodes (SPEs) is attracting enormous attention due to the advantageous properties of SPEs compared to conventional electrodes, such as cost-effectiveness, disposability, simplicity, versatility, availability of materials and patterns, elimination of electrode maintenance, the requirement for low volumes of solution, and appropriateness for outside laboratory measurement [30-32]. Chemically modified electrodes are best suited for the electrochemical determination of pharmaceutical, environmental, or biological samples. Chemically modified electrodes reduce the over-potential required for either the oxidation or reduction of the electro-active compounds [33-39]. Also, modification of electrodes is a powerful strategy for overcoming such limitations of un-modified electrodes as low selectivity, poor sensitivity, low stability, and the blockage of the electron transfer [40-45].

Nanostructured metal oxides crystallizing in the spinel structure type have been investigated intensively over the years and present a permanent interest due to their wide technological applications such as magnetic and optical materials, semiconductors, pigments, catalysts, or material for biomedical applications [46-51].

Ferrites are a well-known class of complex oxides of considerable technological importance. On the other side, nano $ZnFe_2O_4$ as spinel ferrites is found to be one of the most interesting spinel systems because of its unique properties, photochemical stability, good visible-light response and favourable magnetism [52,53]. A characteristic of $ZnFe_2O_4$ is that it has two different metal cations, Zn and Fe, with O as an anion. The cations occupy two different positions in a spinel structure: tetrahedral (Zn) and octahedral (Fe) sites along the face-centered cubic lattice formed by O_2^- cations. The use of bimetallic oxides as electrode materials could enhance both electrical conductivity by two orders of magnitude and electrochemical activity versus materials prepared with unitary metal oxides [54,55].

In the present work, the preparation and application of a screen-printed graphite electrode, modified with zinc ferrite nano-particles ($ZnFe_2O_4$), for the determination of vitamin B_6 in the presence of vitamin C is described. The electrochemical behavior of vitamin B_6 at $ZnFe_2O_4$/SPGE was investigated. The results showed the superiority of $ZnFe_2O_4$/SPGE to the bare electrode in terms of better sensitivity. We have also evaluated the analytical performance of the $ZnFe_2O_4$/SPGE for the quantification of vitamin B_6 in the presence of vitamin C in some real samples.

Experimental

Chemicals and instrumentation

All chemicals used were of analytical reagent grade purchased from Sigma-Aldrich and were used as received without any further purification. Double-distilled water was used throughout all experiments. Orthophosphoric acid was utilized to prepare the phosphate buffer solutions (PBSs), and sodium hydroxide was used to adjust the desired pH values (pH range between 2.0 and 9.0).

Cyclic voltammetry (CV), linear sweep voltammetry (LSV), chronoamperometry, and differential pulse voltammetry (DPV) investigations were performed in an electroanalytical system Autolab PGSTAT302N, potentiostat/galvanostat connected to an electrode cell, the SPGE (DropSens; DRP-110: Spain), containing graphite counter electrode, a graphite working electrode, and a silver pseudo-reference electrode. The system was run on a PC using General Purpose Electrochemical System (GPES) software. Solution pH values were determined using a 713 pH meter combined with a glass electrode (Metrohm, Switzerland).

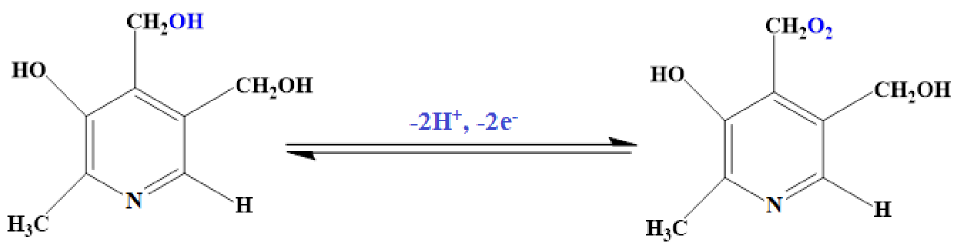
Preparation of modified electrode

$ZnFe_2O_4$ nano-particles were used to coat the bare screen printed graphite electrode. A stock solution of $ZnFe_2O_4$ nano-particles in 1 mL of the aqueous solution was prepared by distributing 1 mg of $ZnFe_2O_4$ nano-particles via ultra-sonication for 50 min, whereas 4 µL of aliquots of the $ZnFe_2O_4$ nano-particles suspension solution was cast on carbon working electrodes and evaporated at room temperature.

Results and discussion

Electrochemical behavior of vitamin B6 on the ZnFe2O4/SPGE

According to our knowledge, the electrooxidation of vitamin B_6 depends on the pH value of the solution (Scheme 1). So, the effect of pH was investigated using the DPV method. Results show that the oxidation peak current increased slowly from pH 2.0 to 7.0, and then the current conversely decreased when the pH value increased from 7.0 to 9.0. Consequently, pH 7.0 was chosen as the optimal experimental condition for other experiments.



Scheme 1. The proposed mechanism for the oxidation of vitamin B_6 at the $ZnFe_2O_4$/SPGE.

To investigate the vitamin B_6 behavior and the as-produced electrode response to vitamin B_6, the performance of $ZnFe_2O_4$/SPGE was compared to that of unmodified SPGE. Figure 1 shows the CV curve obtained for $ZnFe_2O_4$/SPGE (curve a) and unmodified SPGE (curve b) in the presence of 200.0 µM vitamin B_6-containing PBS at the scan rate of 50 mV/s. The results showed that the oxidation of vitamin B_6 is very weak on the surface of the bare SPGE, but the presence of $ZnFe_2O_4$ nano-particles could enhance the peak current and decrease the oxidation potential (decreasing the overpotential). A substantial negative shift of the currents starting from oxidation potential for vitamin B_6 and a dramatic increase of the current indicates the catalytic ability of $ZnFe_2O_4$/SPGE to vitamin B_6 oxidation. The results showed that the use of $ZnFe_2O_4$ nano-particle improved the characteristics of vitamin B_6 oxidation, which was partly due to excellent characteristics of $ZnFe_2O_4$ nano-particles such as excellent electrical conductivity and good chemical stability.

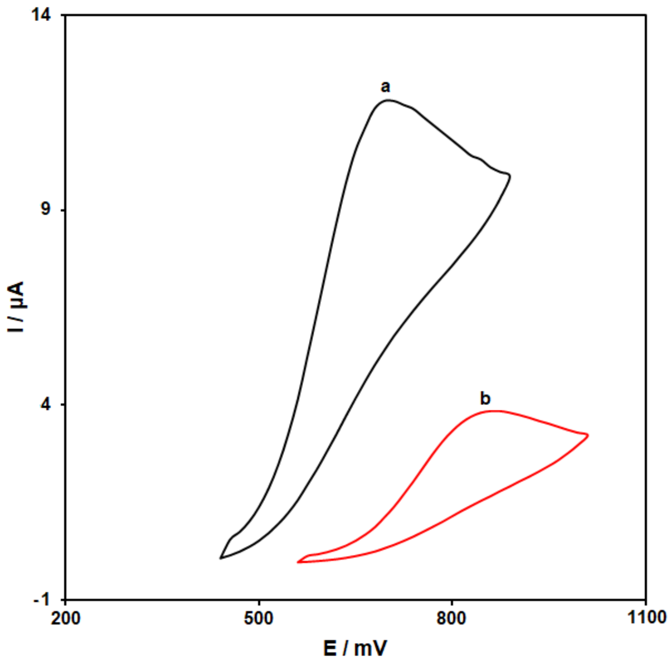


Figure 1. Cyclic voltammograms of a) $ZnFe_2O_4$/SPGE and b) SPGE in the presence of 200.0 µM vitamin B_6 at a pH 7.0 of *0.1 M PBS*, respectively.

Effect of scan rate

The linear sweep voltammograms measurements were carried out to evaluate the association of peak current with scan rate at varied scan rates (10-400 mV/s) in the 100.0 µM vitamin B_6-containing 0.1 M PBS (pH = 7.0) on the $ZnFe_2O_4$/SPGE (Figure 2). As shown in Figure 2, the peak currents of vitamin B_6 grow with increasing scan rates and there are good linear relationships between the peak currents (I_p) and the square root of the scan rate ($v^{1/2}$). The results also showed that the action is mass transfer of vitamin B_6 controlled at diffusion process.

To obtain further information on the rate-determining step, a Tafel plot was developed for the vitamin B_6 at the surface of $ZnFe_2O_4$/SPGE using the data derived from the rising part of the current–voltage curve (Figure 3). The slope of the Tafel plot is equal to $2.3RT/n(1 - \alpha)F$, which comes up to 0.1803 V decade^{-1}. We obtained the charge transfer coefficient (α) as 0.67**.**

Chronoamperometric measurements

The electrooxidation of vitamin B_6 by a $ZnFe_2O_4$/SPGE was also studied by chronoamperometry (Figure 4). Chronoamperometric measurements of different concentrations of vitamin B_6 at the $ZnFe_2O_4$/SPGE sensor were accomplished by setting the working electrode potential at 760 mV as the first step potential. Using chronoamperometric studies, we determined the diffusion coefficient, *D*, of vitamin B_6 in a buffer solution. The experimental plots of I_p versus $t^{-1/2}$ were employed with the best fits for different concentrations of vitamin B_6 (Figure 4A). The slopes of the resulting straight lines were then plotted versus vitamin B_6 concentrations (Figure 4B). Using these slopes and the Cottrell equation, we obtained $D = 9.1 \times 10^{-6}$ cm^2 s^{-1}.

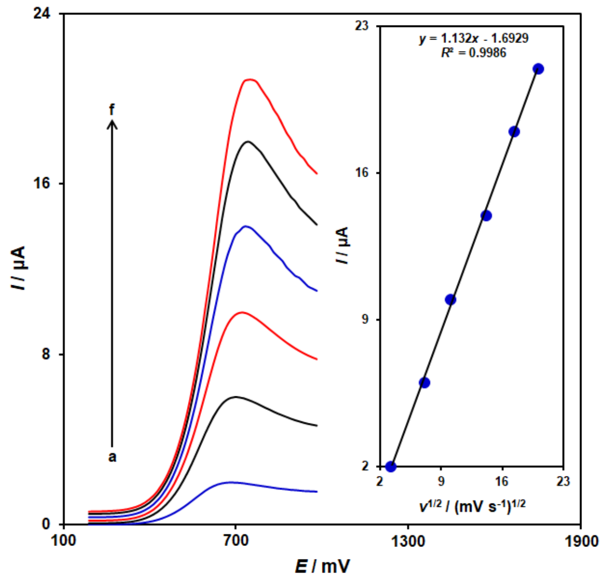


Figure 2. Linear sweep voltammograms of vitamin B_6 (100.0µM) at $ZnFe_2O_4$/SPGE at different scan rates of a) 10, b) 50, c) 100, d)200, e) 300, and f) 400 mV/s in 0.1 M PBS (pH 7.0). Insert: Plot of *I*p versus $v^{1/2}$ for the oxidation of vitamin B_6 at $ZnFe_2O_4$/SPGE.

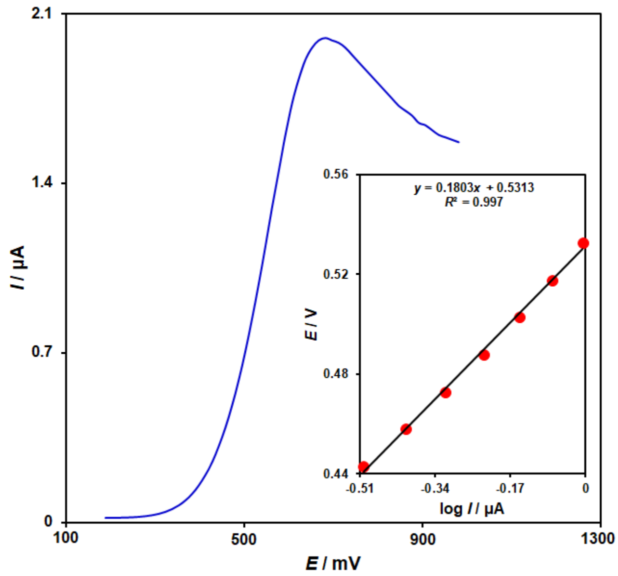


Figure 3. Linear sweep voltammogram for $ZnFe_2O_4$/SPGE in the presence of 0.1 M PBS (Ph 7.0) with 100.0 µM of vitamin B_6 at the scan rate of 10 mV/s; Points: outputs used in Tafel plot; Inset: Tafel plot of LSV.

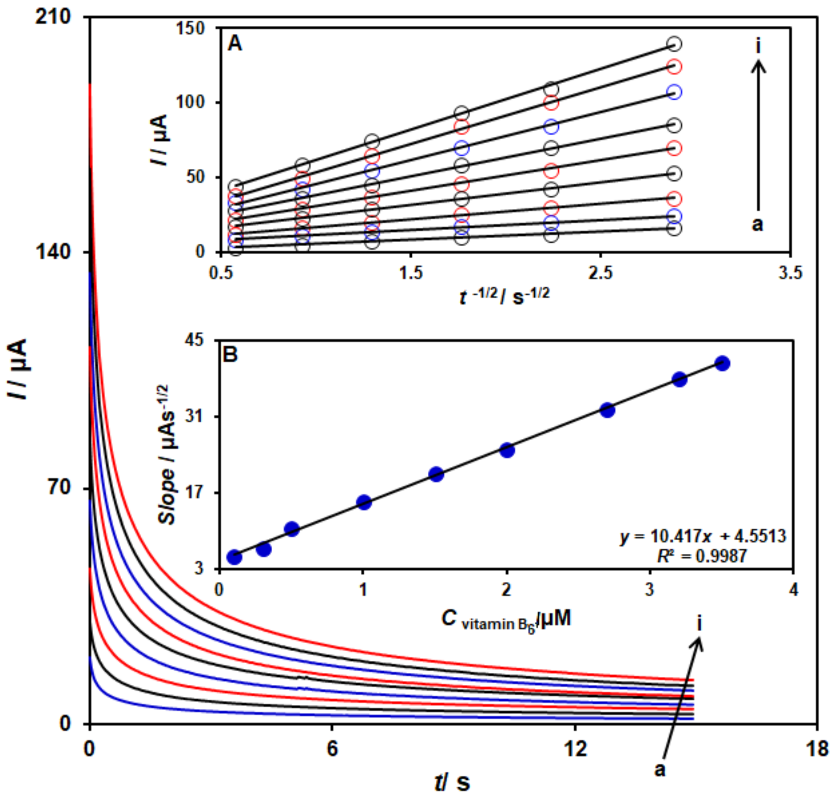


Figure 4. Chronoamperograms obtained at the $ZnFe_2O_4$/SPGE in the presence of a) 0.1, b) 0.3, c) 0.5, d) 1.0, e) 1.5, f) 2.0, g) 2.7, h) 3.2, and i) 3.5 µM vitamin B_6 in the0.1 M buffer solution (pH 7.0). A) Plot of *I* versus $t^{-1/2}$ for electrooxidation of vitamin B_6 obtained from chronoamperograms a–i. B**)** Plot of slope from straight lines versus vitamin B_6 level.

Calibration plot and limit of detection

Since DPV has a much higher current sensitivity and better resolution than CV and LSV, DPV was used for the determination of vitamin B_6. Figure 5 shows the DPV curves of $ZnFe_2O_4$/SPGE in the PBS buffer with variable vitamin B_6 levels (Step potential=0.01 V and pulse amplitude=0.025 V). It was found that the electrocatalytic peak currents of vitamin B_6 oxidation at $ZnFe_2O_4$/SPGE surface linearly depended on vitamin B_6 concentrations above the range of 0.8-585.0 μM (with a correlation coefficient of 0.9997), while determination limit was achieved to be 0.17 μM.

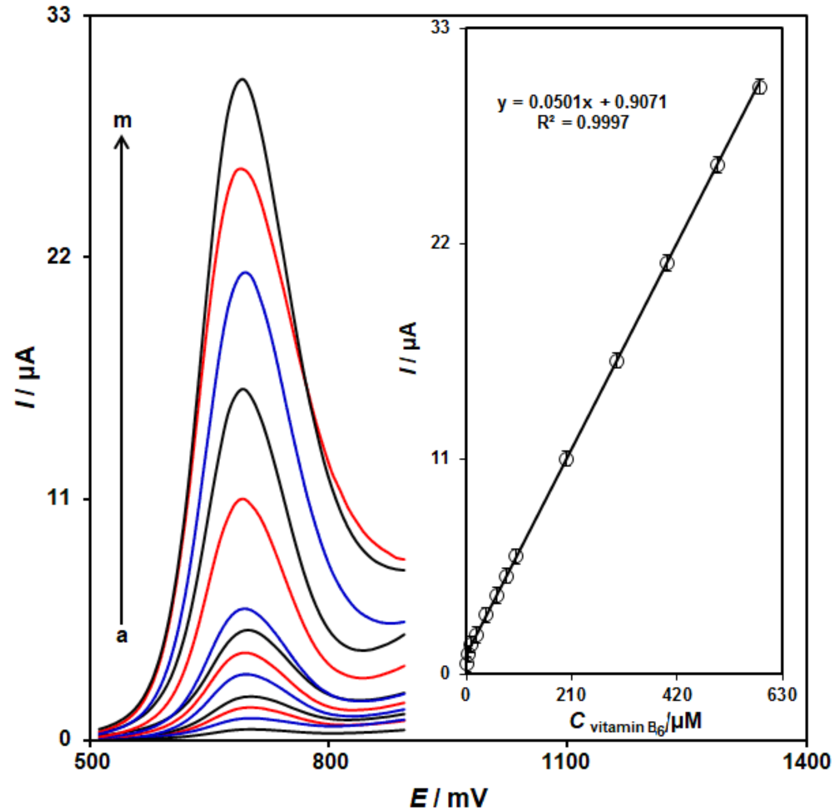


Figure 5. DPV curves of $ZnFe_2O_4$/SPGE in the 0.1 M buffer solution (pH 7.0) containing different concentrations of vitamin B_6. a-m corresponds to 0.8, 5.0, 10.0, 20.0, 40.0, 60.0, 80.0, 100.0, 200.0, 300.0, 400.0, 500.0, and 585.0 μM vitamin B_6. Inset: Plots of electrocatalytic peak current as a function of vitamin B_6 concentration.

Determination of vitamin B6 in the presence of vitamin C

The simultaneous determination of vitamin B_6 and vitamin C is one of the most important applications of the proposed modified electrode. This study investigated a simultaneous change in the concentrations of vitamins B6 and C by recording the DPV curves. The result showed two well-defined oxidation peaks with a 530 mV separation of the peaks (Figure 6). Insets A and B in Figure 6 show the dependence of DPV peak currents on the concentration of vitamin B_6 and vitamin C, respectively. The sensitivities towards vitamin B_6 in the absence and presence of vitamin C were found to be 0.0501 μA/μM (in the absence of vitamin C) and 0.0503 μA/μM (in the presence of vitamin C). These results demonstrated that the $ZnFe_2O_4$/SPGE successfully detected vitamin B6 and vitamin C simultaneously, both sensitively and selectively.

Stability of modified electrode

For checking $ZnFe_2O_4$/SPGE sensor stability, we kept the recommended sensor within the pH equal to 7.0 in the PBS for two weeks to test $ZnFe_2O_4$/SPGE stability and, consequently, we recorded the DPV of the solution consisting of 50.0 μM vitamin B_6 to be compared to the DPV observed prior to immersion. The oxidation peak of vitamin B_6 did not change and, in comparison to earlier responses to the current, showed a less than 4.5 % reduction in signal, reflecting acceptable stability of $ZnFe_2O_4$/SPGE.

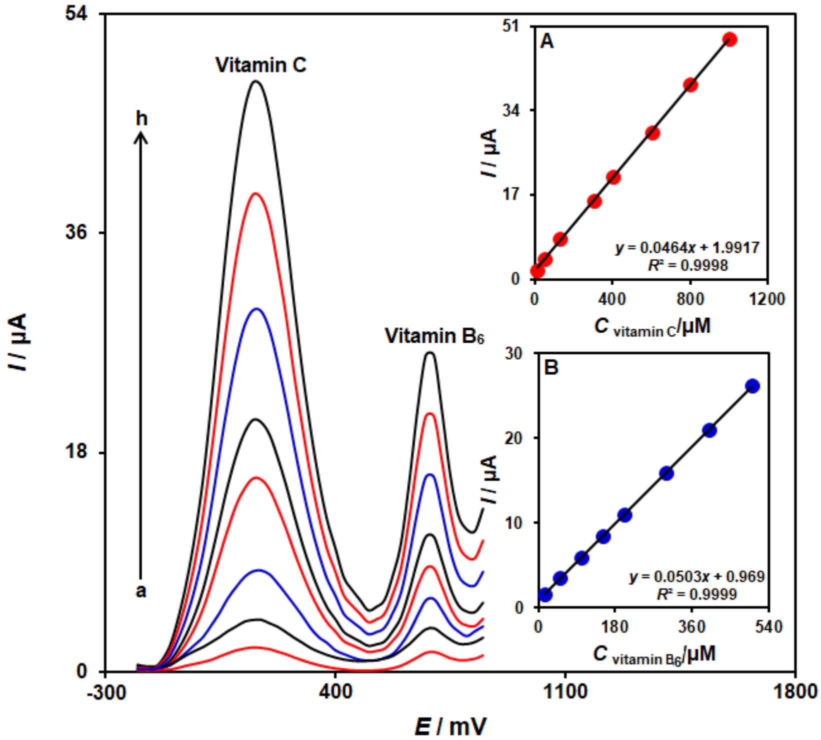


Figure 6. differential pulse voltammograms of $ZnFe_2O_4$/SPGE in 0.1 M PBS (pH 7.0) containing different concentrations of vitamin C and vitamin B_6 mixed solutions of: a)10.0+15.0 , b) 50.0+50.0, c) 125.0+100.0, d) 300.0+150.0, e) 400.0+200.0, f) 600.0+300.0, g) 800.0+400.0,and h) 1000.0+500.0 µM vitamin C and vitamin B_6, respectively. Insets: (A) plot of the peak currents as a function of vitamin C concentration and (B) plot of the peak currents as a function of vitamin B_6 concentration.

Conclusion

A sensor for voltammetric determination of traces of vitamin B_6 in real samples, based on the Zn-ferrite modified screen printed graphite electrode, was developed. The sensor exhibited a good linear response over the concentration range 0.8-585.0 µM with a detection limit of 0.17 µM for vitamin B_6. Also, the modified electrode successfully resolves the overlapped voltammetric peaks of vitamin B_6 and vitamin C by approximately 530 mV so that the modified electrode displays high selectivity in the DPV measurement of vitamin B_6 and vitamin C of in their mixture solutions. As well as, the proposed method could be applied to the determination of vitamin B_6 and vitamin C in real samples.

Conflict of interest*:* The authors declare no conflict of interest*.*

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