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Electrochemical determination of vitamin B_6 in water and juice samples using an electrochemical sensor amplified with NiO/CNTs and Ionic liquid

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Measurement of food additives helps a lot to check the food quality. Also, Vitamin B6 is one of the most important vitamins in food samples. In this study, we fabricated an electroanalytical food sensor based on the amplification of a carbon paste electrode (CPE) as well as NiO-CNTs nanocomposite and 1-methyl-3-octylimidazolium hexafluorophosphate (MOHFPE). The NiO-CNTs/MOHFPE/CPE showed the catalytic activity for the determination of vitamin B6 and also improved the oxidation current of vitamin B6 up to about ~ 2.5 times. Also, in comparison to CPE, the oxidation potential of vitamin B6 was shifted to a negative value of about 100 mV, which confirmed the successful modification of CPE with NiO-CNTs and MOHFPE. The results related to differential pulse voltammetric (DPV) investigations showed a linear between the current and vitamin B6 concentrations in the range of 4.0 nM - 500 μ M with a detection limit of 0.9 nM. The NiO-CNTs/MOHFPE/CPE, as an analytical tool, was successfully used to determine vitamin B6 in drinking and juices samples using the standard addition method.

Keywords: Vitamin B6, Food analytical sensor, NiO/CNTs, Modified electrode

1. INTRODUCTION

Vitamin B_6 (pyridoxine) is a water-soluble type of B vitamins [1]. This vitamin is not stored in the body, so people need to get it through nutrition [2]. Pyridoxine plays an important role in the body, which is involved in the body's response to more than 100 enzymes. Moreover, vitamin B_6 plays an important role in reducing the severity of nausea at early pregnancy stage [3-5]. Food is considered as an important source of Vitamin B_6 for the body [6-8]. Therefore, controlling foods to study their quality

and the presence of essential vitamins like B vitamins, by analytical methods is a good solution for researchers in the food industry [9, 10]. In this regard, many analytical methods are used for food sample's analysis since many years ago [11-15]. Recently, electroanalytical strategies showed more advantages compared to other analytical sensors, due to having many benefits such as low cost, simple operation, and portable ability [16-30]. In addition, electroanalytical sensors have more diversity due to different ways for modification and having an ability for using different types of modifiers [31-40].

In recent years, nanotechnology has become an integral part of many research topics [41-50]. Because many aspects and characteristics of materials are not discovered in nanoscale sizes yet, so many researchers are investigating this issue [51-60]. However, many research groups in different studies [61-65] have confirmed the high electrical conductivity of nanomaterials such as carbon nanotubes and metal-based nanoparticles. Therefore, CNTs and metal-based nanoparticle were suggested as powerful mediators in the fabrication of electrochemical sensors [66-69].

Ionic liquids with green properties and a low toxicity were introduced for large filed application since many years ago [70-72]. Accordingly, the high electrical conductivity of ionic liquids has enabled them to be used in the manufacture of electrochemical sensors, and especially in carbon paste modification [73-75]. In this regard, they have been introduced as suitable alternatives to paraffin in the manufacture of the modified carbon paste electrodes [76-80].

Based on the topics discussed in the previous paragraphs and considering the importance of vitamin B_6 analysis in food samples, in this research, we fabricated an amplified sensor (NiO-CNTs/MOHFPE/CPE in this case), as an analytical tool, to determine the amount of vitamin B_6 in food samples. The fabricated electroanalytical sensor improved the redox reaction of vitamin B_6 , which was also used successfully for the determination of this vitamin in food samples.

2. EXPERIMENTAL

2.1. Materials and instrument

Vitamin B₆ hydrochloride, graphite powder, and sodium hydroxide were purchased from Merck. 1-Methyl-3-octylimidazolium hexafluorophosphate, single wall carbon nanotubes, and paraffin oil was purchased from Sigma-Aldrich. Also, phosphoric acid, sodium dihydrogen phosphate, disodium hydrogen phosphate, trisodium phosphate, and nickel nitrate hexahydrate were purchased from Across Company.

pH meter Metrohm company was used to prepare the phosphate buffer solution (PBS). The Ivium-Vertex was used as an electrochemical workstation for performing electrochemical investigation. Linear sweep signals were recorded using scan rates 25, 50, 100, 200 and 300 mV/s and at pH = 7.0. Differential pulse voltammetric signals were recorded at pH= 7.0 and using E_{step} =0.02 V. Mira-3-XMU (Filed emission SEM) was used for the characterization of NiO/CNTs nanocomposite.

2.2. Synthesis of NiO/CNTs nanocomposite

1.5 g SWCNTs-COOH was dispersed in 100 mL distilled water solution, and then centrifuged for 15 min. 29.08 g nickel nitrate hexahydrate was dissolved in the previous solution under stirring. Subsequently, sodium hydroxide solution (2.0 M) was dropwise in a solution containing CNTs and nickel nitrate hexahydrate, and pH of this solution was checked. After stabilizing the conditions in neutral conditions (pH=7.0), the precipitated samples were filtered and then dried for 16 h at 100 °C. Afterward, the dried sample was calcinated for 4.0 h at 450 °C.

2.3. Preparation of NiO-CNTs/MOHFPE/CPE

The NiO-CNTs/MOHFPE/CPE was prepared by mixing 0.07 g NiO-CNTs + 0.93 g graphite powder in mortar and pestle in the presence of 10 cc diethyl ether. After evaporation of diethyl ether, the powder was converted to paste by adding 8 cc paraffin oil and 2 cc MOHFPE. The obtained paste was input at the end of the glass tube in the presence of copper wire.

2.4. Real sample preparation

Energy drinks, drinking water, and orange Juice were purchased from the local market. Notably, for drinking samples, real sample analysis was directly used. Orange Juices were filtered, centrifuged (3000 rpm for 30 min), and were then used for real sample analysis using the standard addition method.

3. RESULTS AND DISCUSSION

3.1. Characterization of NiO/CNTs

FE-SEM image of NiO/CNTs is presented in figure 1. As shown, the NiO nanoparticles with spherical shape and crystalline structure were decorated at surface single-wall carbon nanotubes

3.2. Electrochemical investigation

Redox behavior of vitamin B_6 is dependent on proton concentration due to the presence of phenolic structure [81-83]. Therefore, the oxidation signal of vitamin B_6 was recorded at the surface of NiO-CNTs/MOHFPE/CPE in the pH ranged between 5.0 and 9.0 (Figure 2 inset). The plot of the oxidation potential of vitamin B_6 in contrast with changing pH showed a linear relationship with equation E = 0.0604 pH + 0.9556 that confirmed the presence of equal values of proton and electron in redox behavior of vitamin B_6 (Figure 2) [84]. On the other hand, a comparison of the differential pulse voltammograms showed that the highest sensitivity to vitamin B_6 measurement occurred under neutral conditions. Accordingly, this pH was used as an optimum condition for future investigations.



Figure 1. FESEM image of synthesized NiO/CNTs nanocomposite

The role of NiO-CNTs nanocomposite and MOHFPE on redox behavior of vitamin B_6 was investigated in the modification process and at different electrodes. In addition, differential pulse voltammograms of vitamin B_6 at the surface of CPE, NiO-CNTs/CPE, MOHFPE/CPE, and NiO-CNTs/MOHFPE/CPE are presented in figure 3 curves a-d, respectively. As shown, by moving CPE to NiO-CNTs/MOHFPE/CPE oxidation current of vitamin B_6 has increased from 19.8 μ A to 4.94 μ A and the relative potential has also decreased from 626 mV to 526 mV. These results show that the presence of NiO-CNTs nanocomposite and MOHFPE can improve the electrical conductivity of CPE and create a good electrical condition for the fabrication of highly sensitive vitamin B_6 sensors.



Figure 2. E_p vs. pH plot for electro-oxidation of 50.0 μ M vitamin B₆ at the surface of NiO-CNTs/MOHFPE/CPE. Inset) DP voltammograms of 50.0 μ M vitamin B₆ at pH range 5.0-9.0



Figure 3. DP voltammograms of 50 μ M vitamin B₆ at the surface of CPE (a); NiO-CNTs/CPE (b), MOHFPE/CPE (c), and NiO-CNTs/MOHFPE/CPE (d) at pH=7.0.

Linear sweep voltammograms of vitamin B6 at the scan rate ranged from 25 to 300 mV/s were recorded at the surface of NiO-CNTs/MOHFPE/CPE (Figure 4 inset). The linear relation between current compared to $v^{1/2}$ with the equation I = 1.2352 $v^{1/2}$ + 1.2115 (R² = 0.9958) was detected for electro-oxidation of vitamin B₆ at the surface of NiO-CNTs/MOHFPE/CPE that confirmed the diffusion process [85-90] for redox behavior of vitamin B₆. Also, we detected a positive shifted in oxidation potential of vitamin B₆ with increasing in scan rates that suggest an irreversible behavior for electro-oxidation of vitamin B₆.



Figure 4. Plot of current *vs.* $v^{1/2}$ for electro-oxidation of vitamin B₆ at the surface of NiO-CNTs/MOHFPE/CPE at scan rates a) 25; b) 50; c) 100; d) 200 and e) 300 mV/s.

3.3. Effect of vitamin B₆ concentration

The differential pulse voltammograms of vitamin B_6 have been recorded in the concentration ranged from 4.0 nM to 500 μ M at the surface of NiO-CNTs/MOHFPE/CPE (Figure 5). Correspondingly, in this concentration range, oxidation current of vitamin B_6 showed a linear relation with its concentration with equation I = 0.0574 C + 1.2014 (R² = 0.9956). Also, in this study, the detection limit of 0.9 nM was determined for the measurement of vitamin B_6 using NiO-CNTs/MOHFPE/CPE, as an electro-analytical sensor.



Figure 5. The plot of current *vs.* vitamin B_6 concentration at the surface of NiO-CNTs/MOHFPE/CPE. Inset) DP voltammograms of vitamin B_6 at the surface of NiO-CNTs/MOHFPE/CPE in concentration ranges of 4.0 nM - 500 μ M.

3.4. Stability of NiO-CNTs/MOHFPE/CPE

The stability of NiO-CNTs/MOHFPE/CPE, as an electrochemical sensor for the determination of vitamin B₆, was investigated in a 40-day period. Accordingly, the result is presented in figure 6. The oxidation signal of vitamin B₆ has decreased from 4.94 μ A to 4.5 μ A in a 30-day period (91% initial signal) and then decreased to 3.0 μ A after 40 days. This point confirms the good stability of NiO-CNTs/MOHFPE/CPE for one month, as an electroanalytical sensor for the determination of vitamin B₆.



Figure 6. Current-days curve for electro-oxidation of 50.0 μ M vitamin B₆ at surface of NiO-CNTs/MOHFPE/CPE

3.5. Interference study and real sample analysis

In this study, the selectivity of NiO-CNTs/MOHFPE/CPE, as a new electroanalytical sensor for determination of 10.0 μ M vitamin B₆, was checked in this step. For this goal, an acceptable error of 5% in current and potential was selected. The results are presented in table 1, which confirmed the good selectivity of NiO-CNTs/MOHFPE/CPE, as a new electroanalytical sensor for the determination of vitamin B₆.

Table 1. The selectivity results relative to the determination of 10.0 μ M vitamin B₆ using NiO-CNTs/MOHFPE/CPE.

Species	Tolerant limits	
	(Winterference/Wgallic acid)	
F ⁻ , Li ⁺ , Ca ²⁺ , K ⁺	1000	
Glucose	550	
Valine, Methionine, glycine	300	

Also, the ability of NiO-CNTs/MOHFPE/CPE in determining vitamin B_6 in real samples was investigated using drinking and juices samples. The obtained results related to real sample analysis using standard addition method are presented in table 2. The recovery data showed the powerful ability of NiO-CNTs/MOHFPE/CPE, as a new electroanalytical sensor for the determination of vitamin B_6 in real samples.

Sample	vitamin B ₆ added	vitamin B ₆	vitamin B ₆	Recovery %
	(µM)	expected (µM)	founded (µM)	
Energy drink			8.78±0.68	
	10.00	18.78	18.28±0.76	97.33
Water drinking			<lod< td=""><td></td></lod<>	
	15.00	15.00	15.58±0.63	103.86
Orange Juices			<lod< td=""><td></td></lod<>	
	20.00	20.00	20.74±0.93	103.70

Table 2. Real sample analysis data relative to vitam	nin B ₆ using NiO-CNTs/MOHFPE/CPE.
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4. CONCLUSION

The present study described a new and highly sensitive electroanalytical sensor for the determination of vitamin B_6 . Accordingly, this sensor was constructed by the amplification of CPE by two conductive mediators (NiO-CNTs and MOHFPE). The NiO-CNTs/MOHFPE/CPE showed a catalytic effect on the determination of vitamin B_6 and also improved the oxidation current of vitamin B_6 up to about 2.5 times. In addition, the NiO-CNTs/MOHFPE/CPE showed recovery data of 97.33-103.86% for the determination of vitamin B_6 in food samples that confirm the powerful ability of the suggested sensor for determination of vitamin B_6 in real samples.

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