

ORDERED MESOPOROUS CARBON BASED SENSOR FOR SENSITIVE DETECTION OF VITAMIN B₆ IN PHARMACEUTICALS

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Abstract

A novel screen-printed sensor based on ordered mesoporous carbon was used for the electrochemically determination of pyridoxine (vitamin B₆) in pharmaceuticals. Various experimental parameters were studied and optimized such as the nature and the pH of the electrolyte solution, the scanning rate and the interference of different chemical species. Optimal electroanalytical conditions are represented by: phosphate buffer (pH 7.0) with the concentration of 0.01 mol/L and the potential scanning rate of 0.1 V/s. In these conditions, interferences of the main compounds found in pharmaceuticals were less than 1%. The linearity range of the sensor in order to determine the concentration of pyridoxine was from 1 to 200 µM with a detection limit of 0.42 µM. Voltammetry was successfully applied for the quantification of vitamin B₆ in pharmaceuticals. The results obtained for the ordered mesoporous carbon-based sensor were compared to those obtained by the standard spectrophotometric method resulting a very good correlation.

Rezumat

Un nou senzor serigrafiat pe bază de carbon mezoporos ordonat s-a utilizat pentru determinarea electrochimică a piridoxinei (vitamina B₆) în produse farmaceutice. Au fost studiați și optimizați diverși parametri experimentali, cum sunt: natura soluției de electrolit și pH-ul acesteia, viteza de scanare și interferențele diverselor specii chimice. Condițiile electroanalitice optime presupun folosirea tamponului fosfat (pH 7,0) de concentrație 0,01 mol/L, iar viteza de scanare a potențialului este de 0,1 V/s. În aceste condiții, interferențele principalilor compuși care se găsesc în preparatele farmaceutice sunt sub 1%. Domeniul de liniaritate al senzorului la determinarea concentrației de piridoxină a fost de la 1 până la 200 µM cu o limită de detecție de 0,42 µM. Metoda voltametrică a fost aplicată cu succes la cuantificarea vitaminei B₆ în produse farmaceutice. Rezultatele obținute cu senzorul pe bază de carbon mezoporos ordonat au fost comparate cu cele ale metodei spectrofotometrice standard, obținându-se o corelație foarte bună.

Keywords: pyridoxine, screen-printed sensor, ordered mesoporous carbon, voltametry

Introduction

Vitamin B₆ plays an essential role in the biochemical activities of various enzymes, being essential for proteins, carbohydrates and lipids metabolism [15]. Furthermore, it was reported that vitamin B₆ has a key influence in gene modulation, sexual behavior, and immune modulation in HIV-1 infection [13]. The biologically-active form of vitamin B₆ is pyridoxal phosphate, functioning as a coenzyme for the amino group transformation in the organism [3]. Vitamin B₆ deficiency may induce depression, anemia, weakness and skin disorders [14]. Due to its importance, developing a sensitive and simple analysis method for the detection and quantification of vitamin B₆ is very important in the pharmaceutical industry. For this purpose, some detection methods, such as spectrophotometry [11], high-performance liquid chromatography [9], ion fluorescence spectrometry [12], gas-chromatography

mass-spectrometry [8] and electrochemistry [6] were developed. The electrochemical determination methods were based on carbon disk electrode [4], glassy carbon electrode [16], pencil graphite electrode [5] etc. Until now, a small number of papers were related to the determination of vitamin B₆ using screen-printed electrodes [6]. Modification of screen-printed electrodes surface with nanostructured carbon materials (i.e. carbon nanofibers, carbon nanotubes, graphene, mesoporous carbon etc.) could improve the sensitivity of the sensors [1]. Among these nano-materials, ordered mesoporous carbon (OMC) is suitable for applications in sensing because it possesses a well-ordered pore structure with pore diameters in the mesopore range (2 - 50 nm) increasing the active surface area of the sensor. It can improve the sensitivity of the sensor by enhancing the velocity of electron exchange and the number of electro-active centers on the surface [1].

The aim of the present work was to assess the redox properties and analyze of vitamin B₆ with an ordered mesoporous carbon modified screen-printed carbon electrode using cyclic voltammetric method. After the optimization of experimental parameters, cyclic voltammetry was employed for the direct measurement of vitamin B₆ in pharmaceuticals. The method developed possessed numerous advantages, such as low detection limit, selectivity, simplicity, versatility and good reproducibility.

Materials and Methods

Chemicals and solutions

All solutions were prepared with ultrapure MilliQ-water (resistivity of 18.2 MΩ * cm). All chemicals were analytical reagent grade (from Sigma-Aldrich) and were used without further purification. Stock solution (1×10^{-3} mol/L) of pyridoxine was daily prepared in sodium phosphate buffer of pH 7.0. Supporting electrolytes used for electrochemical experiments were phosphate buffer solutions (PBS) of pH 7.0, 0.01 mol/L. For the buffer solutions with different pHs Na₂HPO₄ and NaH₂PO₄ stock solutions were mixed in appropriate ratios. The pH of the buffer solutions was measured with an Inolab pH 7310 pH-meter.

Electrochemical measurements

Ordered mesoporous carbon (OMC) modified screen-printed carbon electrodes, reference 110-OMC, were purchased from Dropsens Ltd (Spain). All voltammetric measurements were carried out by using a Biologic SP 150 potentiostat/galvanostat connected to a laptop for data acquisition and experiment control. All electrochemical measurements were carried out by using 30 mL thermostated glass cell with three-electrodes. The working electrode consists of carbon - ordered mesoporous carbon, the counter electrode consists of carbon and a pseudo-silver/silver chloride reference electrode. All electrodes were screen-printed on the same device. During the measurements, the solution in the cell was not stirred and the phosphate buffer solution (pH 7.0) was deaerated.

The spectrophotometric study was carried out by using a Rayleigh UV-1601 double beam spectrophotometer using a pair of 1 cm rectangular quartz cell of 10 mm path-length. The standard method, in agreement with the Romanian Pharmacopoeia Xth edition, consists in measuring the absorption in ultraviolet range at 290 nm in 0.1 mol/L hydrochloride solution [7]. A calibration curve in the range 1-100 μM was constructed and used for the quantification of vitamin B₆ in pharmaceuticals.

Preparation and analysis of pharmaceutical samples

Solid samples (Sicovit[®] B₆ (S.C. Sicomed S.A.), Anticârcel[®] (Zdrovit), Equilibra[®] (Antibiotice S.A.), Magne B₆[®] (Sanofi Winthrop Industrie), Neuromultivit[®]

(Lannacher Heilmittel GmbH)) containing pyridoxine hydrochloride were triturated in an agate mortar and a known amount of powder was dissolved in 0.01 mol/L phosphate buffer solution. Insoluble excipients were removed by filtering. The filtrate was transferred to a 50 mL volumetric flask and this volume was completed with phosphate buffer solution (PBS). The final concentration of PBS was 0.01 mol/L. The cyclic voltammograms were recorded in the potential range from -0.6 to 1.0 V at a scan rate of 0.1 V/s. The amount of pyridoxine in these samples was determined by the standard addition method and compared with spectrophotometric official method. Five replicates of all pharmaceutical samples were analyzed both in electrochemical and spectrophotometric methods.

Results and Discussion

Electrochemical behavior of vitamin B₆ at OMC-sensor

The cyclic voltammograms of an OMC-sensor in phosphate buffer at pH 7.0 with and without vitamin B₆ are illustrated in Figure 1.

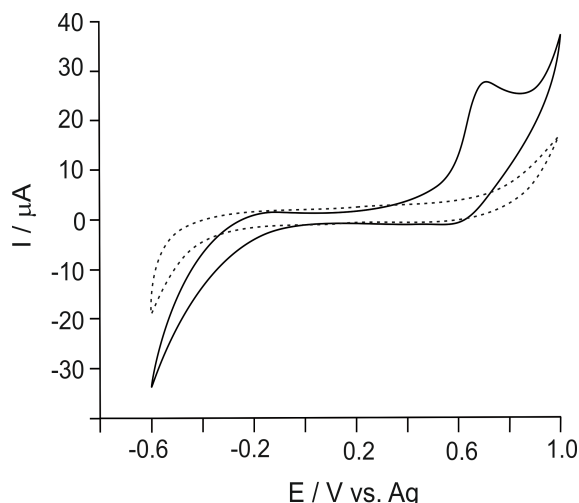
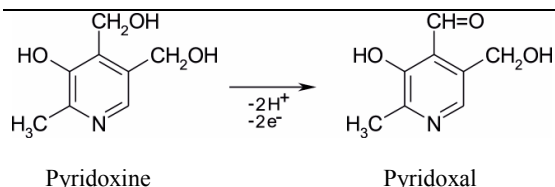


Figure 1.

Cyclic voltammograms of OMC-sensor in pH 7.0 phosphate buffer (dashed line) and in pH 7.0 phosphate buffer containing 50 μM vitamin B₆ (solid line)

In the potential range between -0.6 and 1.0 V, there are no observable redox peaks for the OMC-sensor (dashed line). However, upon addition of 50 mM vitamin B₆, a well-defined oxidation peak appeared and was observed at 0.70 V (solid line). On the reverse potential scan from 1.0 to -0.6 V, the reduction peak of vitamin B₆ was not observed. The oxidation process at the OMC-sensor surface is described by the following chemical reaction:



In the electrochemical process, two electrons and two protons are transferred.

Effect of the scan rate

The effect of potential scan rate on the peak currents of vitamin B₆ was evaluated by cyclic voltammetry (Figure 2 a).

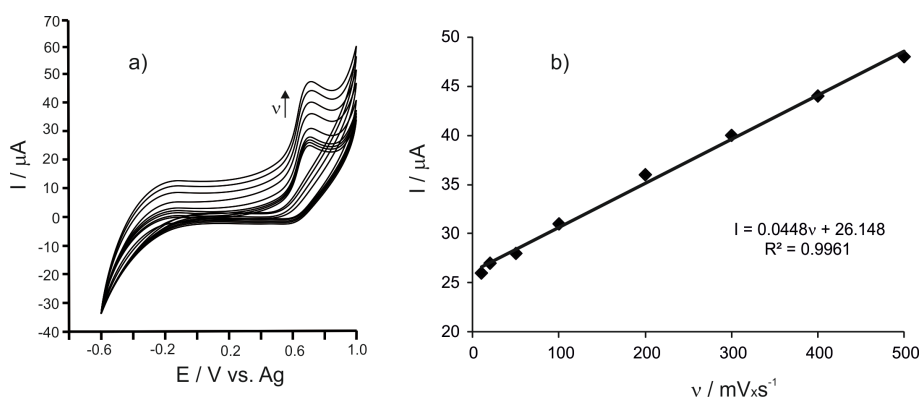


Figure 2.

a) Cyclic voltammograms of OMC-sensor immersed in 50 μM vitamin B₆ (electrolyte pH 7.0 phosphate buffer) solution. Scan rate range: 10 - 500 mV/s; b) Plot I vs. v

The optimal scan rate was 0.1 V/s because at this scan rate the peak is well defined, the background current is low and the time of analysis is short. The peak currents were proportional to the scan rate over 10 - 500 mV/s range (Figure 2 b), which demonstrates that an adsorption-controlled process was involved. The peak potential was slightly shifted to a more positive potential by increasing the scan rate. The value of $n\alpha$, the multiplication product of transfer coefficient (α) and number of electrons (n) transferred in the rate determining stage, was determined from Tafel dependence ($\log I$ vs. E) of the cyclic voltammograms [10]. The $n\alpha$ value was obtained as 0.249. In most systems, α can be approximated by 0.5 [10]. These results demonstrate that two electrons are involved in the oxidation of vitamin B₆.

Effect of pH

The effect of the pH of the electrolyte support solution on the electrochemical signal related to vitamin B₆ was investigated. The results obtained are shown in Figure 3. As it can be observed, the current increased with the electrolyte solution pH from 5.0 to 7.0, then decreased when the pH values were greater than 7.0. The maximum current increased when the pH of electrolyte solution was 7.0. The peak potential was linearly shifted to more negatively potentials with the increment of solution pH, indicating that protons are involved in the electrode oxidation process of vitamin B₆ [2]. Therefore, pH 7.0 was chosen for all electrochemical experiments.

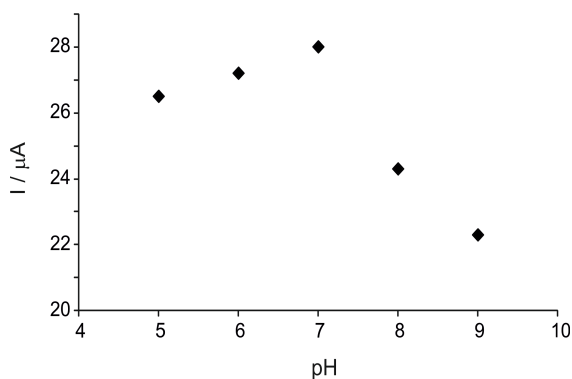


Figure 3.

The effect of pH on OMC-sensor peak currents immersed in 50 μM vitamin B₆ in phosphate buffer solution of pH 7.0

Study of interference

In this work, the interferences of several chemical species on the determination of vitamin B₆, such as lactose, starch, saccharose, magnesium lactate, potassium citrate, thiamine hydrochloride (vitamin B₁), cyan-cobalamin (vitamin B₁₂) were investigated. This compounds are found in the list of excipients from B₆ containing pharmaceuticals used in this research. The results are shown in Table I. From Table I it can be observed that no interferences existed for the determination of 50 μM vitamin B₆ in the presence of 100-fold lactose, starch and saccharose, 50-fold magnesium lactate, potassium citrate, 20-fold thiamine hydrochloride (vitamin B₁) and 10-fold cyan-cobalamin (vitamin B₁₂).

Table I

The interference of compounds included in excipients list

Interfering compound	Current ⁽¹⁾ /μA	Multiple amount added	Current ⁽²⁾ /μA	Relative standard deviation/%
Lactose	28.24	100	28.54	1.06
Starch		100	28.83	2.09
Saccharose		100	27.7	-1.91
Magnesium lactate		50	28.7	1.63
Potassium citrate		50	28.32	0.28
Thiamine hydrochloride (vitamin B ₁)		20	28.52	0.99
Cyancobalamin (vitamin B ₁₂)		10	28.35	0.39

⁽¹⁾the average peak current of OMC-sensor detecting vitamin B₆; ⁽²⁾the average peak current of OMC-sensor detecting vitamin B₆ in the presence of interfering compounds.

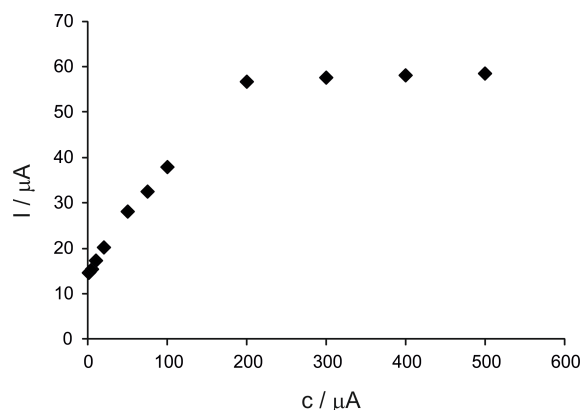
Calibration curve and repeatability

The calibration curve of vitamin B₆ detection was performed by cyclic voltammetry. Under the optimized experimental conditions, a series of different concentrations of vitamin B₆ were prepared and cyclic voltammograms were recorded. The current of the anodic peak at 0.70 V was used to obtain the calibration curve. The results showed that the anodic peak current was linear with the concentrations of vitamin B₆ in the range from 1 to 200 μM (Figure 4). The linear regression equation was $I (\mu A) = 0.2139c + 15.383$ (c in μM) ($R^2 = 0.9925$) with the detection limit of $0.42 \pm 0.26 \mu M$ ($3\sigma/m$ criteria, where: σ - standard deviation; m - calibration plot slope).

The reproducibility of the OMC-sensor was studied by successive quantifications of 50 μM vitamin B₆ by cyclic voltammetry ($n = 5$), the obtained peak current value was in the range of 27.42 to 28.86 μA with a relative standard deviation (RSD) of 1.49%.

Analysis of pharmaceuticals

The quantity of vitamin B₆ present in pharmaceuticals (tablet) is calculated from the calibration equation and the results are presented in Table II. The relative standard deviation of all quantifications was in the range 0.48 - 1.08 %, indicating the applicability of the proposed method for a great variety of pharmaceuticals.

**Figure 4**

Calibration curve

Supplementary, in order to establish the accuracy of the voltammetric method, the standard addition method was carried out. Well-known amounts of the standard vitamin B₆ were added into the analytical solution of the vitamin B₆ from tablets and the same procedure was performed. The recoveries obtained demonstrated the very good accuracy of the voltammetric method. As it can be seen, the excipients from tablets (lactose, starch, saccharose, magnesium lactate, potassium citrate, vitamin B₁, vitamin B₁₂) did not cause interferences on the voltammetric response of vitamin B₆.

Table IIQuantitative results for the determination of vitamin B₆ in tablets

Sample	Labeled mg per tablet	OMC-sensor			Compendial method	
		Found mg per tablet	RSD, n = 5 (%)	Recovery/%	Found mg per tablet	RSD, n = 5 (%)
Sicovit [®] B ₆	250	250.14	0.48	99.5	250.26	0.44
Anticârcel [®]	1	1.00	1.03	101.1	0.99	1.47
Equilibra [®]	5	5.06	1.08	99.3	5.04	1.63
Magne B ₆ [®]	5	4.97	1.06	100.5	4.95	1.86
Neuromultivit [®]	200	199.70	0.60	99.8	200.12	0.37

Conclusions

In this work, the electrochemical behavior of vitamin B₆ on ordered mesoporous carbon screen-printed electrode based sensor has been investigated by

cyclic voltammetry. The ordered mesoporous carbon based sensor provided a valuable tool for detecting and quantifying vitamin B₆, and it was successfully applied for quantifying vitamin B₆ in tablets with very good results. The developed procedure could be

an alternative or a complementary method for the analytical determination of vitamin B₆. It is a simple method which has low detection limit and low cost, and sufficient sensitivity, precision and accuracy.

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References

1. Apetrei C., Apetrei I.M., De Saja J.A., Rodriguez-Mendez M.L., Carbon paste electrodes made from different carbonaceous materials: application in the study of antioxidants. *Sensors*, 2011; 11: 1328-1344.
2. Bard A.J., Faulkner L.R., *Electrochemical Methods: Fundamentals and Applications*, 2nd Edition, John Wiley & Sons Inc., 2001.
3. Ciui B., Tertiş M., Peia D., Cristea C., Săndulescu R., Graphene oxide modified aptasensor for the mucin 1 detection. *Farmacia*, 2016; 64(1): 43-47.
4. Chen G., Ding X., Cao Z., Ye J., Determination of melatonin and pyridoxine in pharmaceutical preparations for health-caring purposes by capillary electrophoresis with electrochemical detection. *Analytica Chimica Acta*, 2000; 408: 249-256.
5. David I.G., Florea M.A., Cracea O.G., Popa D.E., Buleandra M., Iorgulescu E.E., David V., Badea I.A., Ciucu A.A., Voltammetric determination of B1 and B6 vitamins using a pencil graphite electrode. *Chemical Papers*, 2015; 69: 901-910.
6. Duţu G., Cristea C., Ede B., Hârceagă V., Saponar A., Popovici E.J., Săndulescu R., The electrochemical behavior of some local anaesthetics on screen printed electrodes modified with calixarenes. *Farmacia*, 2011; 59: 147-160.
7. *Farmacopeea Română*, Editura Medicală, Xth edition, Bucureşti, 1993.
8. Juhász Z., Dancs G., Marincs F., Vossen M., Allefs S., Bánfalvi Z., Vitamin C, B5, and B6 contents of segregating potato populations detected by GC-MS: a method facilitating breeding potatoes with improved vitamin content. *Plant Breeding*, 2014; 133: 515-520.
9. Lebedzińska A., Marszał M.L., Kuta J., Szefer P., Reversed-phase high-performance liquid chromatography method with coulometric electrochemical and ultraviolet detection for the quantification of vitamins B1 (thiamine), B6 (pyridoxamine, pyridoxal and pyridoxine) and B12 in animal and plant foods. *Journal of Chromatography A*, 2007; 1173: 71-80.
10. Matemadombo F., Apetrei C., Nyokong T., Rodríguez-Méndez M.L., de Saja J.A., Comparison of carbon screen-printed and disk electrodes in the detection of antioxidants using CoPc derivatives. *Sensors and Actuators B: Chemical*, 2012; 166-167: 457-466.
11. Muszalska I., Puchalska M., Sobczak A., Determination of vitamin B6 by means of differential spectrophotometry in pharmaceutical preparations in the presence of magnesium compounds. *Acta Poloniae Pharmaceutica*, 2011; 68: 845-851.
12. Ochiuz L., Luca M.C., Stoleriu I., Moscalu M., Timofte D., Tantar G., Stefanache A., Assessment of the in vitro release of alendronate sodium from mesoporous silica particles. *Farmacia*, 2016; 64(1): 131-134.
13. Senapati S., Patnaik A.K., Das S.P., Mohanty P., Electron Transfer Reaction of Pyridoxine (Vitamin B6) with Keggin Type 12 Tungstocobaltate (III) in Aqueous Perchlorate Medium. *American Chemical Science Journal*, 2014; 4: 242-254.
14. Spinneker A., Sola R., Lemmen V., Castillo M.J., Pietrzik K., González-Gross M., Vitamin B6 status, deficiency and its consequences-an overview. *Nutrición Hospitalaria*, 2007; 22: 7-24.
15. Tanaka T., Tateno Y., Gojobori T., Evolution of Vitamin B6 (Pyridoxine) Metabolism by Gain and Loss of Genes. *Molecular Biology and Evolution*, 2005; 22: 243-250.
16. Wu Y., Song F., Voltammetric Investigation of Vitamin B6 at a Glassy Carbon Electrode and Its Application in Determination. *Bulletin of the Korean Chemical Society*, 2008; 29: 38-42.