

Voltammetric Determination of Vitamin B₆ (Pyridoxine) Using Multi Wall Carbon Nanotube Modified Carbon-Ceramic Electrode

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A simple procedure was developed to prepare a carbon-ceramic electrode (CCE) modified with multi wall carbon nanotube (MWCNT). The electrochemical behavior of pyridoxine was investigated on the obtained electrode in phosphate buffer solution (PBS), pH 7.0. During oxidation of pyridoxine on the MWCNT/CCE, one irreversible anodic peak at $E_p = 716$ mV vs. SCE appeared. Cyclic voltammetric study indicated that the oxidation process is irreversible and diffusion controlled. The number of exchanged electrons in the electro-oxidation process was obtained, and the data indicated that pyridoxine is oxidized via two one-electron steps. The results revealed that MWCNT promotes the rate of oxidation by increasing the peak current, so that pyridoxine is oxidized at lower potentials, which thermodynamically is more favorable compared with on the bare CCE. A sensitive, simple and time-saving differential pulse voltammetric procedure was developed for the analysis of pyridoxine. Using the proposed method, pyridoxine can be determined with a detection limit of 95 nM. The applicability of the method to direct assays of some commercial pharmaceutical samples is described.

Keywords: Vitamin B₆, Pyridoxine, Voltammetric detection, Multi wall carbon nanotube, Carbon-ceramic electrode

INTRODUCTION

Vitamin B₆, also known as pyridoxine, is part of the B group vitamins and is required for both mental and physical health. The deficiency of pyridoxine has been suggested as the cause of many types of illness and disease [1]. The special attention has been paid to pyridoxine in clinical and pharmaceutical sciences because of its extensive and essential applications in bio-metabolisms. To date, various analytical methods have been developed for determination of pyridoxine, which include flow injections [2-4], high-performance thin-layer chromatography [5] and liquid chromatography with electrochemical detection [6]. Spectrophotometric

determination in presence of other vitamins has also been described by multi calibration techniques [7, 8]. Algar et al. [9] have determined pyridoxine in pharmaceutical preparations after sorption on Sephadex SP C-25 by native fluorescence method. A review on various methods for determination of pyridoxine in pharmaceutical formulations has been presented [10].

A few papers concerning the determination of pyridoxine by voltammetric techniques have been reported. Söderhjelm and Lindquist [11] were the first to study the voltammetric determination of pyridoxine using a carbon paste electrode. The determination of vitamin B₆ and its separation in pharmaceutical preparations and food with amperometric electrochemical detection, using a carbon disc electrode as an

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electrochemical detector in chromatography [12] and capillary electrophoresis [13], have also been reported. The literature also reports that pyridoxine was studied in pharmaceutical preparations by cyclic voltammetry using carbon paste electrodes modified with copper (II) hexacyanoferrate (III) [14] and with vanadyl (IV)-Salen complex [15]. Furthermore, there are reports where the glassy carbon electrodes have been modified using various modifiers such as a novel carbon nanotube [16] and polymethylene blue [17] and we have reported the electrocatalytic oxidation of pyridoxine on an aluminum electrode modified with metallic palladium particles/iron (III) hexacyanoferrate (II) film as a chemically modified electrode for the determination of pyridoxine [18]. Recently, electrochemical behaviour of pyridoxine hydrochloride at carbon paste electrode modified with crown ethers [19] has been reported.

Carbon nanotubes (CNTs) are molecular-scale wires with high electrical conductivity, extremely high mechanical strength and modulus. CNTs have attracted considerable attention due to their extraordinary structural, mechanical, electrical, and electrochemical properties as well as their promise in the field of material science. Now, CNTs have been widely used in electroanalytical chemistry. Recently, Pingarrón and co-workers [20] reviewed the role of carbon nanotubes in electroanalytical chemistry. Sol-gel technology involves the fabrication of material through the low temperature hydrolysis of a suitable monomeric precursor, followed by condensation and polycondensation to yield a polymeric oxo-bridged SiO₂ network. In 1994, Lev and co-workers [21, 22] introduced the sol-gel derived carbon ceramic electrodes (CCE) which comprised of dispersion of carbon powder into the starting sol-gel solution and this new kind of electrode had been largely utilized for the design of electrochemical devices whose surfaces could be renewed by a simple polishing step. Gavalas *et al.* [23] for the first time have reported the fabrication of carbon nanotubes composite electrode based on the sol-gel technique and described its favorable electrochemical characteristics, such as double-layer capacitance, electron transfer rates and selectivity with different silane precursors and different compositions. Thereafter, various procedures were employed for fabrication of CCEs modified with CNTs (multi and single wall) and have

been used in sensing of important organic and inorganic compounds.

In the current work, a simple and fast procedure was used for fabrication of carbon-ceramic electrode modified with MWCNT. The electrochemical properties and durability of the MWCNT modified CCE were investigated and the obtained electrode was described to electrochemical characterization of pyridoxine. After optimizing the experimental parameters, a voltammetric method was developed for the direct measurement of pyridoxine. The applicability of the method to direct assays of some commercial pharmaceutical samples is described.

EXPERIMENTAL

Chemicals

Multi wall carbon nanotube with a 95% purity (10-20 nm diameter) and 1 μm of length were obtained from Nanolab (Brighton, MA). Methyltrimethoxysilane (MTMOS), pyridoxine hydrochloride, vitamin B₁, vitamin B₂, vitamin B₁₂, uric acid, dopamine and other chemicals were purchased from Merck or Fulka and used without any further purification. Phosphate buffer solutions (PBS, 0.05 M) of different pH value were prepared from stock solutions of 0.05 M H₃PO₄, NaH₂PO₄, Na₂HPO₄ and NaOH. Doubly distilled water was used throughout the experiments.

Apparatus

The electrochemical experiments were carried out using an AUTOLAB PGSTAT-100 (potentiostat/galvanostat) equipped with a USB electrochemical interface and driven by a GPES 4.9 software package (Eco Chemie, The Netherlands) in conjunction with a three-electrode system and a personal computer for data storage and processing. A three-electrode cell system composed of a saturated calomel electrode (SCE) as the reference electrode, a platinum wire as the auxiliary electrode, and the MWCNT modified CCE (geometric surface area of 0.119 cm²) as the working electrode was employed for the electrochemical studies. A copper wire was inserted through the composite end of working electrode to establish electrical contact.

Procedures

Preparation of the Bare CCE

The blank carbon-ceramic electrode was prepared according to the procedure described by Lev and co-workers [21] by mixing 0.15 ml MTMOS, 0.3 ml methanol and 10 μ l hydrochloric acid (11 M). This mixture was magnetically stirred for 2 min, after which 0.3 g graphite powder was added and the resultant mixture shaken for additional 1 min. A 5-mm length of a 3-4 mm inner diameter Teflon tube was filled with the sol-gel carbon mixture and dried under ambient conditions (25 °C).

Preparation of the CCE Modified With MWCNT

The dried CC electrode was first carefully polished with polishing paper and subsequently rinsed with doubly distilled water and finally its surface dried with air drying. To obtain a uniform layer of MWCNT onto CCE surface, 15 μ l of DMF-MWCNT solution (0.4 mg/ml) was cast on the surface of CC electrode and dried in air to form a MWCNT film at electrode surface.

Preparation of Samples and Standard Solutions

Ampoules: a suitable volume of the ampoule was diluted to 100 ml in a volumetric flask with deionized water.
Tablets: Twenty tablets were weighed, finely powdered and portions equivalent to 50 mg pyridoxine were transferred into 100 ml volumetric flask; 50.0 ml distilled water was added, shaken thoroughly to dissolve and filtered through a Millipore filter (0.45 μ m). An aliquot of filtered solution was diluted with doubly distilled water in a 100 ml volumetric flask. Then 100 μ l of the resulting solution was added to a 100 ml volumetric flask and made up to volume with 0.05 M phosphoric acid buffer. All standard solutions were also prepared in 0.05 M phosphoric acid buffer solution.

RESULTS AND DISCUSSIONS

Electrochemical Properties of MWCNT/CCE

Fig. 1 shows the cyclic voltammogram of MWCNT modified CC electrode in phosphate buffer solution (pH 7.0). According to the cyclic voltammogram of MWCNT/CCE in buffer solution, we found no redox peak between -0.2 and 1.0

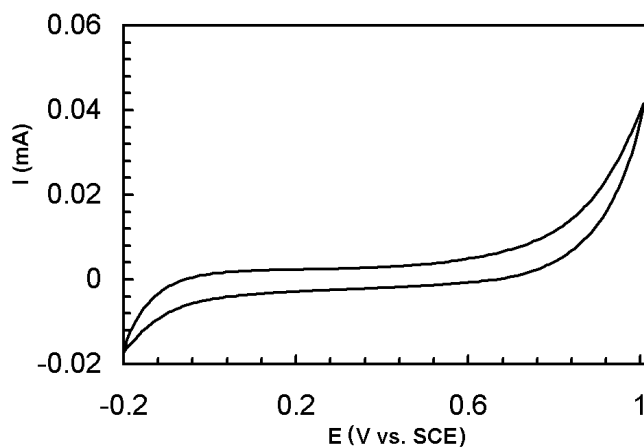


Fig. 1: Cyclic voltammogram of the MWCNT/CCE in 0.05 M PBS pH 7.0 at a scan rate 50 mVs⁻¹. Volume of MWCNT suspension: 15 μ l.

V and the background current was low; thus, this MWCNT/CCE provided a broad potential window to investigate the voltammetric behavior of catalyzed compounds. The effect of pH of buffer solution on the electrochemical behavior of modified electrode was investigated by recording cyclic voltammograms of MWCNT/CCE in various pH values from 3.0 to 12.0. The obtained results show that the cyclic voltammograms recorded in the studied pH range are similar and the electrode responses remain constant. The stability of the modified electrode and the reproducibility of the electrochemical behavior were also investigated by cyclic voltammetry. Under continuous potential sweeping of 50 mV s⁻¹ between -0.2 and 1.0 V in the same buffer solution (pH 7.0), an insignificant change in the electrochemical behavior of the electrode was observed (not shown).

Electrochemical Behaviors of Pyridoxine on the MWCNT/CCE

The cyclic voltammograms of a MWCNT/CCE in phosphate buffer at pH 7.0 in the absence and presence of pyridoxine were shown in Fig. 2. Within the potential range -0.2 to 1.0 V, there is no observable redox peaks (curve a), but upon addition of 1mM pyridoxine, a well-defined and sensitive oxidation peak appears at 716 mV (curve b). On the

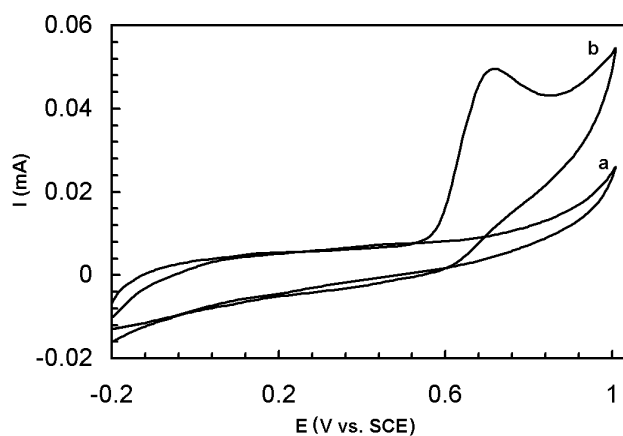


Fig. 2: Cyclic voltammograms of the MWCNT/CCE in the absence (a) and presence (b) of 1×10^{-3} M pyridoxine.

reverse potential scan from 1.0 to -0.2 V, there is no corresponding reduction peak observed for pyridoxine (even at high scan rates). This suggests that the electrode reaction of pyridoxine at the MWCNT/CCE is totally irreversible.

In order to illustrate the electrocatalytic effect of MWCNT toward pyridoxine, the electrochemical properties of pyridoxine at CCE without MWCNT was examined using cyclic voltammetry, and the result shown in Fig. 3. At bare CCE, 1 mM pyridoxine yields a very low oxidation peak at 0.80 V in phosphate buffer at pH 7.0 (curve b). However, the oxidation peak current of pyridoxine at the MWCNT/CCE increases significantly and the peak potential shifts towards low positive potential (Fig. 2 curve b) in comparison with that at bare CCE. The remarkable peak current enhancement and the fall of oxidation over potential undoubtedly testify the electrocatalysis of the MWCNT/CCE to the oxidation of pyridoxine. In conclusion, MWCNT/CCE greatly improves the determining sensitivity of pyridoxine on account of the unusual structure and properties of MWCNT (such as very large specific area, strong adsorptive ability, subtle electronic properties).

To characterize the MWCNT/CCE modified electrode, the number of electrons (n) and electron transfer coefficient (α) for pyridoxine on the electrode were evaluated. For an irreversible one-electron reaction, the electron transfer coefficient has been obtained using cyclic voltammetry by Tafel's plot [24] at a scan rate of 50 mV s^{-1} . The plot of $\log I$ vs E provides the value of slope:

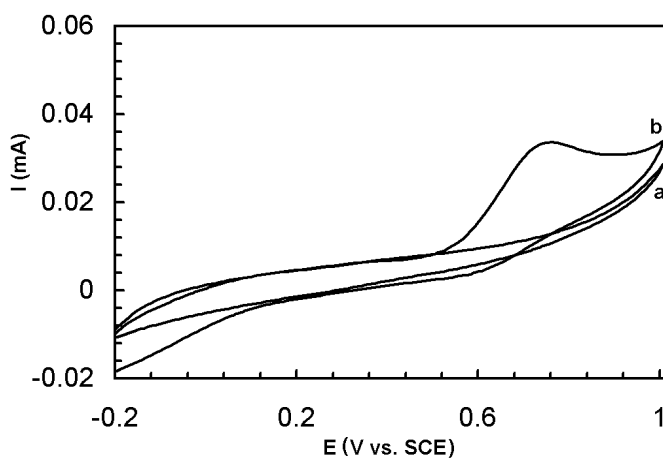


Fig. 3: Cyclic voltammograms of the bare CCE in the absence (a) and presence (b) of 1×10^{-3} M pyridoxine.

$$\text{Slope} = (1 - \alpha) F / 2.3 RT \quad (1)$$

The value of α from the slope of the Tafel plot obtained from the cyclic voltammogram for the modified electrode as given in Fig. 4A was found to be 0.57. This value of α has been utilized to calculate the value of n_α by cyclic voltammetry using the following equation [24],

$$|E_p - E_{p/2}| = 47.7 / \alpha n_\alpha \quad (2)$$

where, E_p is the anodic peak potential and $E_{p/2}$ is the potential where the current is half the peak value. The $E_p - E_{p/2}$ value was observed to be 89.5 mV at 50 mV s^{-1} scan rate, which is close to the theoretical value of 95 mV for a totally irreversible reaction. The value of n was calculated to be 0.935, which is close to one [19]. As E_p is a function of scan rate for a totally irreversible wave, the effect of scan rate on the oxidation of pyridoxine on the MWCNT/CCE has been studied. It was found that the anodic peak current increases with the increase in scan rate and the peak potential also shifts towards a more positive side with increasing scan rate as given in Fig. 4B. The plot of I_p vs $v^{1/2}$ indicates an initial linearity, which curves off at higher scan rates, suggesting that the reaction is initially diffusion controlled, but at faster scan rates ($v > 400 \text{ mV/s}$), the electron transfer becomes rate determining. Using the slope of I_p of cyclic voltammograms vs $C_{\text{pyridoxine}}$

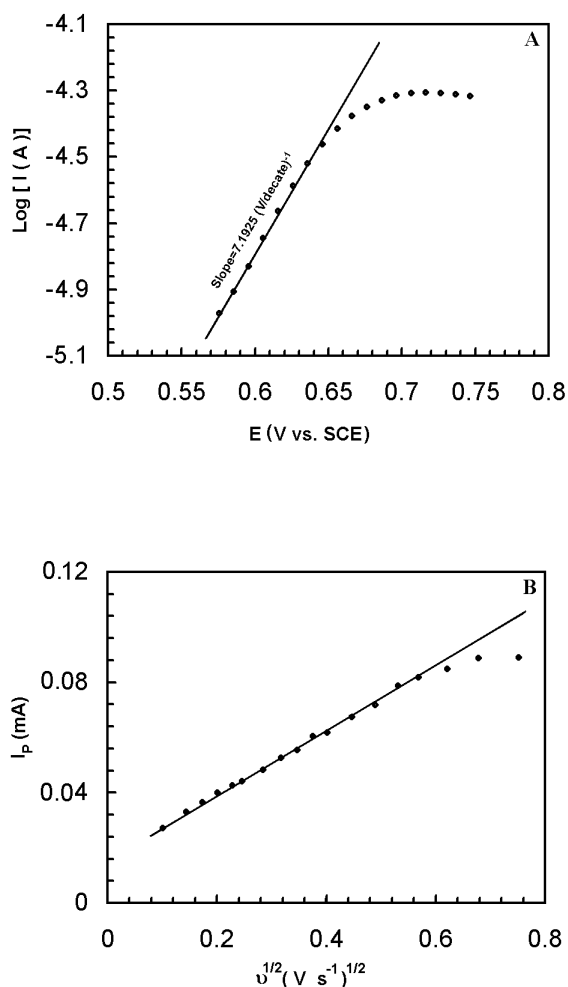


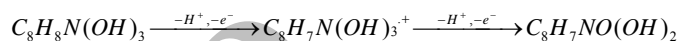
Fig. 4: Tafel plot (log I vs E) obtained from the cyclic voltammogram for the MWCNT/CCE in the presence of 1×10^{-3} M pyridoxine (A). Variation of anodic peak current with square root of scan rate (B).

($I_p = 2.99 \times 10^5 n [(an_a)^{1/2}] A C_{\text{pyridoxine}} D^{1/2} v^{1/2}$) and considering $an_a = 0.57$, $D_{\text{pyridoxine}} = 1.4 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ [18], $A = 0.119 \text{ cm}^2$, and $v = 50 \text{ mV s}^{-1}$, it is found that the total number of electrons involved in the anodic oxidation of B₆ is about 1.93, close to 2.

Based on the obtained results and according to the literature evidences [18, 19, 25-29], the mechanism of pyridoxine oxidation followed by:

firstly pyridoxine molecules undergoes one electron oxidation at the electrode surface resulting in a radical ion (pyridoxine^{•+}) formation, which produces the most stable of the resonating structure [19] and then the cation radical will produce a final stable product via another one electron oxidation process [18, 26, 27].

(3)



Optimizing of Some Parameters

Effect of Supporting Electrolytes and pH

The electrochemical oxidation behaviors of 1 mM pyridoxine on MWCNT/CCE in various medium such as pH 2.5-11.0 phosphate buffer, pH 1.0-5.0 sodium citrate-HCl buffer, pH 2.0-8.0 MacIlvaine buffer, pH 2.0-10.0 Britton-Robinson buffer (each 0.05 mol/L), were compared by cyclic voltammetry. The excellent oxidation response was obtained in phosphate buffer because the peak current is highest and the peak shape is well-defined. Thus, 0.05 mol/L phosphate buffer was chosen as the determining medium for pyridoxine.

Fig. 5 shows the changes of the peak potential (E_{pa}) and peak current (I_{pa}) of oxidation of pyridoxine with pH. Within the pH range of 2.5 to 11.0, the peak potential shifts negatively with rising pH, following the linear equation $E_{pa}(\text{V}) = 1.1682 - 0.064 \text{ pH}$ (correlation coefficient $r = 0.993$) (Fig. 5A). The slope of -64 mV pH^{-1} indicates that the transfer electron number is equal to the proton number [18] that has good agreement with above proposed mechanism. Meanwhile, the peak current gradually increases from pH 2.5 to pH 5.0 until reaches to the constant value in the pH range 5.3-9.0 (Fig. 5B) and then decreases at higher pHs. On the other hand, the anodic peak current of pyridoxine at MWCNT/CCE shows a hill shaped variation with pH in the range of 2.5-11.0 [14, 15, 28, 30]. A current decrease was observed when pH of the solution is lower than 5.0 ($\text{pH} < \text{pK}_{a1}$), due to the formation of the pyridoxine acidic form ($\text{pK}_{a1} = 5.0$ and $\text{pK}_{a2} = 8.96$) [31, 32]. Indeed the presence of high concentrations of the acidic form could influence the reaction kinetics of the pyridoxine with MWCNT on the electrode surface. Another current decrease was observed when the pH of the solution is higher than 9.0 ($\text{pH} > \text{pK}_{a2}$), which is related to the formation of anionic form of pyridoxine. It should be noted that, some part of current

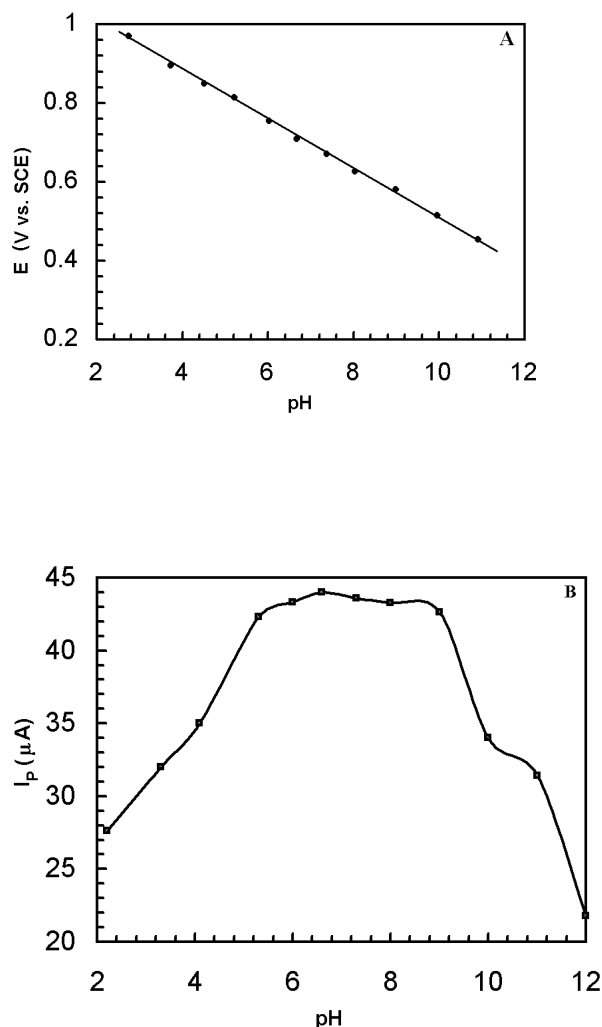


Fig. 5: Influence of pH on the anodic peak potential (A) and peak current (B) oxidation of 1×10^{-3} M pyridoxine.

decreasing in the high basic solution may be raised from the degradation of the pyridoxine as reported in literature [33]. Thus pyridoxine is expected to be in an anionic form only in strong basic solution ($\text{pH} > \text{pK}_{a2}$) due to the deprotonation of the -OH group bonded to the pyridinic ring and the cationic form in acidic solutions ($\text{pH} < \text{pK}_{a1}$) the pyridinic nitrogen is protonated [25]. On the other hand, decreases of anodic peak current in the high acidic and high basic pH may be also attributed to the change in electrostatic interaction between pyridoxine and MWCNT and the change in electrode reaction rates.

As can be seen, I_{pa} in the around of neutral is constant of nearly independent of pH, we will use phosphate buffer solution with $\text{pH} = 7.0$ (pH of biological medium) as supporting electrolyte in determination of pyridoxine.

Effect of the Amount of MWCNT Suspension

The amount of MWCNT suspension on the CCE surface directly determines the thickness of the MWCNT film. The relationship between the oxidation peak current of pyridoxine and the amount of MWCNT suspension is illustrated in Fig. 6. It is found that the oxidation peak current gradually increases, while gradually improving the volume of the MWCNT suspension from 5 to 15 μl . Further improving the amount of MWCNT suspension, the peak current almost remains stable. However, when the amount of MWCNT suspension exceeds 15 μl , the peak current conversely decreases. This is related to the variation of film thickness and solidity. Indeed if the film is thin, the pyridoxine amount adsorbed is small, thus the peak current is small. When it is too thick, the conductivity of the film drops, and the film no longer adheres tightly, since part of the MWCNT leaves the electrode surface. In addition, when the amount of MWCNT increases, the background current also increases, thus, the signal/noise (S/N) ratio changes. Taking into account the stability of the film and the S/N ratio, a 15 μl MWCNT suspension solution was used in this case.

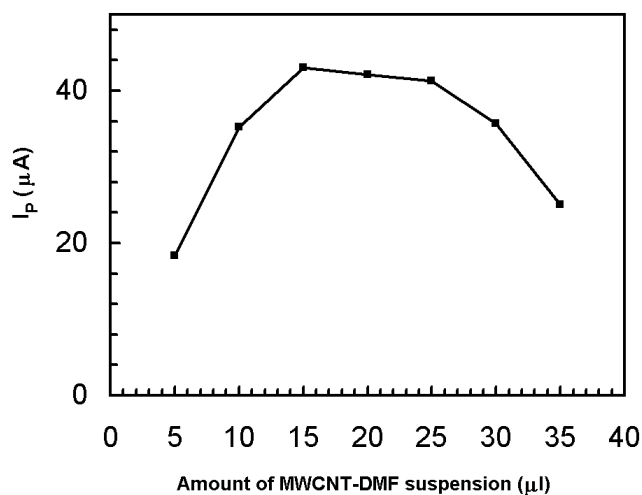


Fig. 6: Influence of MWCNT suspension (0.4 mg/ml) volume on the anodic peak current of oxidation of 1×10^{-3} M pyridoxine.

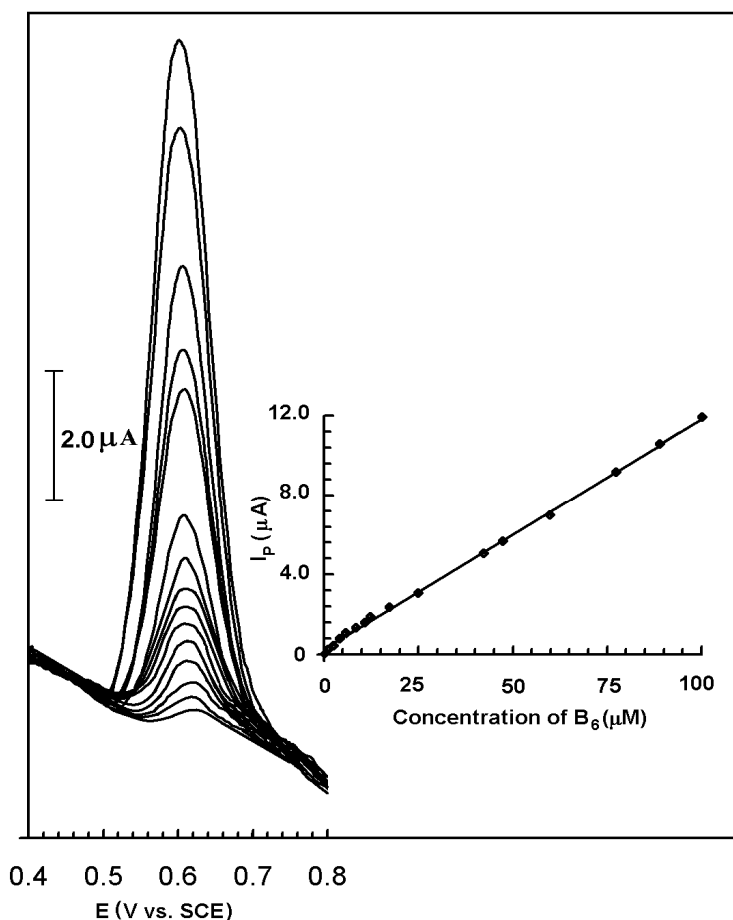


Fig. 7: Differential pulse voltammogram of pyridoxine at various concentrations: 0.25, 1.25, 2.75, 4.25, 5.875, 8.75, 11.0, 12.5, 17.5, 25.0, 42.5, 60, 77.5, 89 and 100 μM. Plot of anodic peak currents versus pyridoxine concentration (inset). Amplitude: 70mV, pulse width: 50ms.

Differential Pulse Voltammetric Determination of Pyridoxine

Since differential pulse voltammetry (DPV) has a higher current sensitivity and low background current via charging current than cyclic voltammetry, it was used in determination of pyridoxine at the MWCNT/CCE and estimating a lower limit of detection. The anodic peak was used for the determination of pyridoxine by DPV within the potential range scanned (+0.2 to +0.9 V vs SCE). The effect of pulse amplitude, at 50 and 100 mV, was also studied. As expected, an increase in peak current was observed at 100 mV; however, the better signal to background current characteristic could be obtained with pulse amplitude of 50 mV. The optimum

conditions for further studies were chosen as, pulse amplitude, 50 mV, and, scan rate, 10 mV s⁻¹. The oxidation peak currents were measured in 0.05 M pH 7.0 PBS (Fig. 7), and plotted against the bulk concentration of pyridoxine (inset of Fig. 7). The dependence of peak currents on the concentration of pyridoxine is a linear relationship in the range of 2.5×10^{-7} - 10×10^{-5} M (coefficient of variation = 0.9984). The detection limit (S/N = 3) is 95 nM. The relative standard deviation of 10 successive scans is 1.4% for 1×10^{-5} M pyridoxine indicating that the MWCNT modified electrode had an excellent reproducibility. The long-term stability of the MWCNT/CCE was evaluated by measuring the current responses at a fixed pyridoxine concentration of 1×10^{-5} M over a period of a week.

The MWCNT/CCE was used daily and stored in the air. The experimental results indicated that the current responses deviated only 2.2%, revealing that the MWCNT modified CCE fabricated by this way possesses moderate stability.

Interferences

To evaluate the interferences of foreign species (especially other B group vitamins) on the determination of pyridoxine at the level of 2×10^{-6} M, a systematic study was carried out. It is found that the MWCNT/CCE can tolerate interferences from other organic compounds. For example, 15-fold concentration of vitamin B₁, vitamin B₂, vitamin B₁₂ (i.e. 3×10^{-5} M), 30-fold concentration of uric acid, dopamine (i.e. 6×10^{-5} M) do not influence the current response of 2×10^{-6} M pyridoxine (signal change below 5%), suggesting that this proposed voltammetric method has excellent selectivity toward vitamin B₆.

Analysis of Pyridoxine in Pharmaceutical Samples

The modified electrode developed was applied to the voltammetric determination of pyridoxine in the commercial pharmaceutical samples with different compositions by the standard addition method. Voltammograms were recorded in samples solution and after addition of growing concentration of pure pyridoxine and the results are shown in Fig. 8 [as a typical for tablets] and Table 1. The results obtained with the MWCNT/CCE and are presented in Table 1 was realized in four replicate ($n=4$).

The results obtained by the MWCNT/CCE are in good agreement with the declared pyridoxine content. Further, in order to establish the suitability of the proposed method, known amounts of the standard pyridoxine were added into the analytical solution of the pyridoxine tablets and ampoules

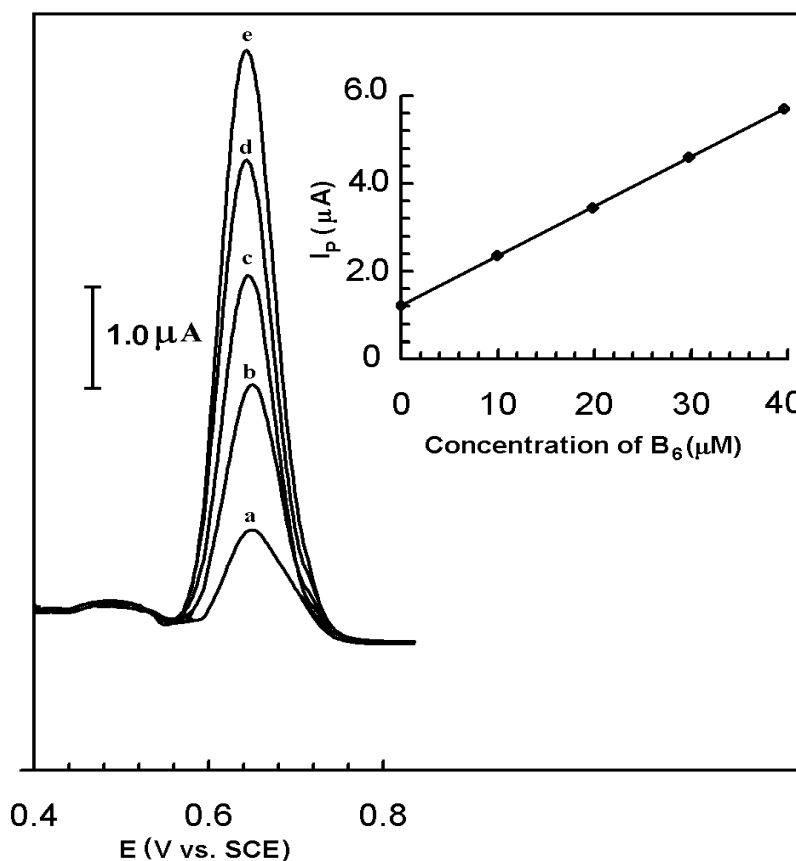


Fig. 8: Differential pulse voltammograms obtained for the oxidation of real tablet sample at MWCNT/CCE :a) unknown sample; b) sample + 10 μM; c) sample + 20 μM; d) sample + 30 μM; e) sample + 40 μM of pyridoxine.

Table 1: Determination of pyridoxine in commercial pharmaceutical samples by the proposed voltammetric method

Samples		Declared pyridoxine content	Declared found content	Recovery (%)	Recovery average %
Tablet	1	40.0 (mg/tablet)	38.97 (mg/tablet)	97.42%	104.76
	2	40.0 "	42.68 "	106.7%	
	3	40.0 "	43.56 "	108.9%	
	4	40.0 "	42.4 "	106.0%	
Ampoule	1	300 (mg/3ml)	303.12 (mg/3ml)	101.04	101.40
	2	300 "	304.76 "	101.59	
	3	300 "	306.35 "	102.12	
	4	300 "	302.48 "	100.83	

and the same procedure was applied. The recoveries indicate that the accuracy and repeatability of the proposed voltammetric method are very good. From above experimental results, it is obvious that this novel MWCNT-modified electrode has great potential for practical sample analysis.

CONCLUSIONS

A novel chemically-modified electrode, MWCNT/CCE, can be easily fabricated. The current of pyridoxine oxidation on the modified surface is enhanced and the oxidation process performed at thermodynamically favorable potentials, beyond the effect of real surface area enhancement. This is attributed to the nano-size, special electronics and structure of carbon nanotube. A differential pulse voltammetry procedure is successfully applicable for quantification of pyridoxine in bulk form and commercial pharmaceutical samples. The simplicity, sensitivity, selectivity and short time of analysis are the main advantages of the present method making it useful for routine analysis.

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