

## Electrocatalytic Oxidation and Voltammetric Determination of Vitamin B<sub>6</sub> by a ssDNA-modified Electrode

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The human metabolic process is a series of reactions between biomolecules in nature. Vitamin B<sub>6</sub> helps protein metabolism and supports DNA repair against free radical damage. Here we reported on studying the electron transfer between DNA and vitamin B<sub>6</sub> by observing the electro-catalytic behaviors of a ssDNA-modified electrode towards vitamin B<sub>6</sub> and determine the quantitative content of vitamin B<sub>6</sub> using the ssDNA-modified electrode. The electron transfer coefficient  $\beta$  was evaluated to be  $0.36(\pm 0.02)$ , the standard rate constant  $k_0$  was estimated to be  $3.4(\pm 0.1) \times 10^{-4} \text{ cm s}^{-1}$  and the electron transfer number for the rate-determining step is equal to 1. The diffusion coefficient for vitamin B<sub>6</sub> was found to be  $4.2(\pm 0.1) \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ . The peak currents depend linearly on the concentration of vitamin B<sub>6</sub> from 0.10 to 6.00 mM with  $R = 0.99952$  ( $n=8$ ) and  $SD=0.07008$ . The linear regression equation was  $C \text{ (mM)} = 0.03245 \times I_p \text{ (\mu A)} - 0.04049$  with the detection limit of  $0.040(\pm 0.001) \text{ mM}$ . The determination for the real tablets yielded a good mean recovery of 99.46% with a relative standard deviation of 5.36%. Thus, the method is expected to find its applications in monitoring the content of vitamin B<sub>6</sub> in medical industry.

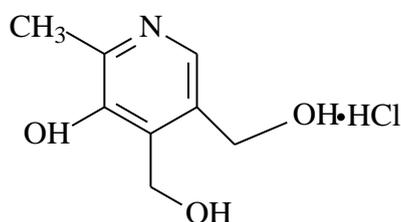
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**Keywords:** DNA-modified electrode; Vitamin B<sub>6</sub>; Electrocatalysis; Determination

### 1. INTRODUCTION

Vitamin B<sub>6</sub> is a very vital cofactor involving the metabolism of amino acids and lipids, as well as the synthesis of neurotransmitters, histamine and hemoglobin, and other key biologic processes [1]. Its biosynthesis is restricted to bacteria, protozoa, fungi, and plants, but, animals and humans require a constant supply of the vitamin in their diet or drugs [2]. Because vitamin B<sub>6</sub> helps protein metabolism and supports DNA repair against free radical damage, it is helpful to understand the role of vitamin B<sub>6</sub> at the view of molecular biology by studying the electron transfer between DNA and vitamin B<sub>6</sub>.

Vitamin B<sub>6</sub> is the derivatives of 3-hydroxy-2-methylpyridine, it includes three chemically, metabolically, and functionally related forms, pyridoxol, pyridoxal and pyridoxamine, in which alcohol, aldehyde, and amine groups, are located accordingly at the fourth position of the pyridine ring [3]. The structure of pyridoxol can be denoted in scheme 1, for instance. One can also obtain the structure of pyridoxal (pyridoxine) or pyridoxamine if the group at the fourth position is substituted with aldehyde or amine group.



**Scheme 1.** Structure of pyridoxol.

**Table 1.** Data of voltammetric detection for vitamin B<sub>6</sub>

Modified Electrodes	Oxidation potential (V)	Linear range (mol/L)	Detection limits (mol/L)	Reference
Vanady(IV)-salen complex	0.66	$4.5 \times 10^{-4}$ - $3.3 \times 10^{-4}$	$3.7 \times 10^{-5}$	15
Carbon nanotube	0.80	$5.0 \times 10^{-7}$ - $1.0 \times 10^{-4}$	$2.0 \times 10^{-7}$	16
Ru(bpy) <sub>3</sub> <sup>3+</sup> -modified boron-doped diamond	0.168	$0.28 \times 10^{-6}$ - $0.37 \times 10^{-3}$	$6.3 \times 10^{-8}$	17
Prussian blue	0.9	$1.0 \times 10^{-6}$ - $8.0 \times 10^{-5}$	$8.7 \times 10^{-7}$	18
Poly-methylene blue	0.57	0.010 - 1.03 mg·mL <sup>-1</sup>	1.34 mg mL <sup>-1</sup>	19
Activated glassy carbon	0.81	$2.5 \times 10^{-6}$ - $7.5 \times 10^{-3}$	$8.0 \times 10^{-7}$	20
Carbon disk	0.99*	$2.5 \times 10^{-6}$ - $1.0 \times 10^{-3}$	$1.0 \times 10^{-6}$	21
Carbon disk	0.85*	$5.0 \times 10^{-6}$ - $1.0 \times 10^{-3}$	$2.7 \times 10^{-6}$	22
Bare glassy carbon	1.18*	$1.0 \times 10^{-3}$ - $3.5 \times 10^{-2}$	$1.0 \times 10^{-8}$	23
Bare glassy carbon	0.74	$3.0 \times 10^{-7}$ - $3.3 \times 10^{-4}$	$1.0 \times 10^{-7}$	24
Carbon fiber	1.20	25-500 ng	25 ng	25
Graphite carbon paste electrode	0.60	$1.0 \times 10^{-6}$ - $2.0 \times 10^{-4}$	-	26

\*potentials versus Ag/AgCl

The quantitative analysis for vitamin B<sub>6</sub> is very significant to maintaining the one's health. Thus, some of determination methods have been developed such as ion exchange chromatography [4], liquid chromatography [5], high-performance liquid chromatography [3, 6-8], flow injection-solid phase spectrophotometry [9], fluorescence spectrometry [10-12], gas-chromatography-mass-

spectrometry [13]. Some of electrochemical detection methods also have been developed successfully. A multi-task flow system was fabricated for potentiometric analysis of vitamin B<sub>6</sub> [14]. The amperometric determination using modified electrodes such as vanady(IV)-salen complex [15], carbon nanotube [16], Ru(bpy)<sub>3</sub><sup>3+</sup> [17], Prussian blue [18], poly-methylene blue [19] and carbon electrodes [20-25] also have been reported since the electrochemical determination of pyridoxine was performed first by Söderhjelm and Lindquist [26], the linear ranges, detection limits and oxidation potentials for those voltammetric approaches were listed in Table 1.

As seen in table 1, the modified electrodes have displayed an advantage of the relatively low determination potentials, which is favorable to limiting the possible interference signals from coexisting components. As a matter of fact, modified electrodes were often used to fabricate the electrochemical sensors due to their desired selectivity, high sensitivity and low over-potentials [27-32]. Wang et al. studied the recognition of a ternary copper complex to single-stranded DNA and double-stranded DNA [33], Naik's group investigated DNA binding on carbaldehyde oxime [34]. Labuda et al. prepared DNA-based biosensors for the detection of deep DNA damage [35]. Some of DNA-modified electrodes for the determination of aromatic amines and drugs also reported [36-40]. Further, electrochemical DNA hybridization sensors were developed for the detection of specific DNA target sequences [41]. We previously reported on the interaction of double stranded DNA (dsDNA) with vitamin B<sub>6</sub>, the estimation of the binding equilibrium constant and the binding ratio of nucleotide to vitamin B<sub>6</sub> [42]. But so far, the DNA-modified electrode has not been developed to determine quantitatively the concentration of vitamin B<sub>6</sub>. We herein continue the work to investigate the electrocatalytic properties of single stranded DNA (ssDNA) towards vitamin B<sub>6</sub> and to fabricate a quantitative determination method of pyridoxol using the ssDNA-modified electrode.

## 2. EXPERIMENTAL SECTION

### 2.1. Chemicals

Salmon deoxyribonucleic acid (DNA) was obtained from Sigma (USA) and was used without further purification. Deionized water was obtained by a Milli-Q system with a resistivity higher than 18.2 MΩ cm<sup>-1</sup>. Stock solutions of dsDNA or ssDNA were prepared by dissolving an appropriate amount of DNA in deionized water and stored at 4 °C. The concentration of the stock solution of DNA was determined by ultraviolet absorbance at 260 nm using the molar extinction coefficient = 6600 mol L<sup>-1</sup> cm<sup>-1</sup> [43], the concentration of 2.0 mg mL<sup>-1</sup> dsDNA solution is equal to 5.1 mM nucleotide on DNA chain.

The solution of ssDNA was obtained by heating a solution of dsDNA 100°C for 30 min and suddenly immersing it into ice water.

Generally, the stock solutions of DNA are fresh for the tests, the storage time is not longer than 96 h. All other chemicals were of analytical reagent grade without further purification. Vitamin B<sub>6</sub>, a medicine injection solution, was from Jinan Limin Pharmaceuticals CO., LTD, China, was used for the experiments.

## 2.2. Apparatus

The cyclic voltammetric (CV) experiments were carried out with a CHI660C electrochemical workstation (CH Instrument Company, Texas, USA) in a three-electrode system at room temperature ( $25 \pm 2^\circ\text{C}$ ). A DNA-modified electrode was used as the working electrode, and a platinum sheet as the counter electrode, a saturated calomel electrode (SCE) as the reference electrode. All potentials are reported *vs* SCE. Ultraviolet-visible absorbance spectra were conducted with a double beam T1901 spectrophotometer, which was from Purkinje General Instrument Co., Ltd of China.

## 2.3. Preparation of DNA-Modified Electrode

A glassy carbon (GC) disk electrode of 3.0 mm diameter was used as a matrix of working electrode. It was polished on sand paper, 0.3 to 0.05  $\mu\text{m}$  alumina, and wool cloth to a mirror. After the bare mirror GC electrode was rinsed with deionized water, it was cleaned thoroughly in an ultrasonic cleaner with deionized water for ready to prepare DNA-modified electrodes.

A dsDNA-modified electrode was prepared like procedures described by Oliveira Brett and our previous work [33,44,45]. A 10  $\mu\text{L}$  solution of 5.1 mM dsDNA was dropped on the surface of the bare GC electrode with a microsyringe, then it was dried in air at 4  $^\circ\text{C}$  for 10 h to result in a dsDNA-modified electrode. Similarly, a ssDNA-modified electrode can be obtained. Before using the electrodes, they were immersed in 0.10 M NaOH solution for 15 min, then rinsed lightly with deionized water for the removal of unadsorbed DNA. Finally, the ssDNA-modified or dsDNA-modified electrode was used in electrochemical experiments.

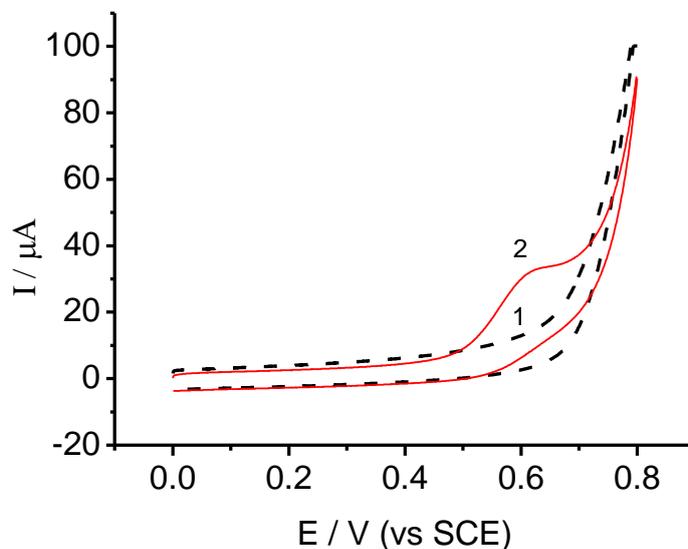
# 3. RESULTS AND DISCUSSION

## 3.1. Voltammetric Properties of DNA-Modified Electrode

The as-prepared dsDNA electrode based on the method mentioned was immersed in 0.10 M NaOH solution for the cyclic voltammetric experiments, the resulting voltammograms show a remarkable difference as compared with those yielded by the bare GC electrode in the same solution as shown in Fig. 1.

The dsDNA-modified electrode has a remarkable oxidative current peak at 0.58 V in the alkaline solution whereas the bare GC electrode displays no such oxidative current peak at the same potential in the same solution by comparison. The further studies showed that the peak current increases with increasing DNA concentration on the surface of the electrode, and the current decreases with decreasing DNA concentration on the GC surface. It revealed that the DNA was immobilized onto the surface of the glassy carbon electrode by the simple method [45,46]. The current of the DNA electrode is attributed to the electron transfer of guanine on DNA absorbed on the surface of the glassy electrode [47-49] because the dsDNA was easily denatured to ssDNA in the strong alkaline solution [50,51]. The backward scan did not lead to the corresponding reduced current, however, the second

subsequent cyclic scan showed a smaller oxidative peak and third scan appeared no any oxidative current peak at the same potential (not shown), suggesting the oxidation of the denatured ssDNA is an irreversible process [44-46]. The control tests showed that the dsDNA electrode had no current peak involving the redox of base groups in 0.10 M tris-HCl pH 7.00 buffer solution in the window potential of 0.00 to 0.80 V, suggesting the strong alkali took a pivotal role in transferring dsDNA to ssDNA.

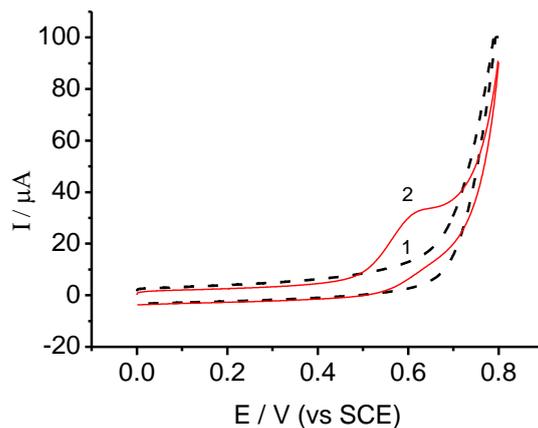


**Figure 1.** Cyclic voltammograms of electrodes in a 0.10 M NaOH solution in the potential range of 0.00 to 0.70 V at a scan rate of  $50 \text{ mV s}^{-1}$ . Curve 1, a bare GC electrode; curve 2, the DNA-modified electrode.

### 3.2. Electrocatalytic Oxidation of Vitamin B<sub>6</sub>

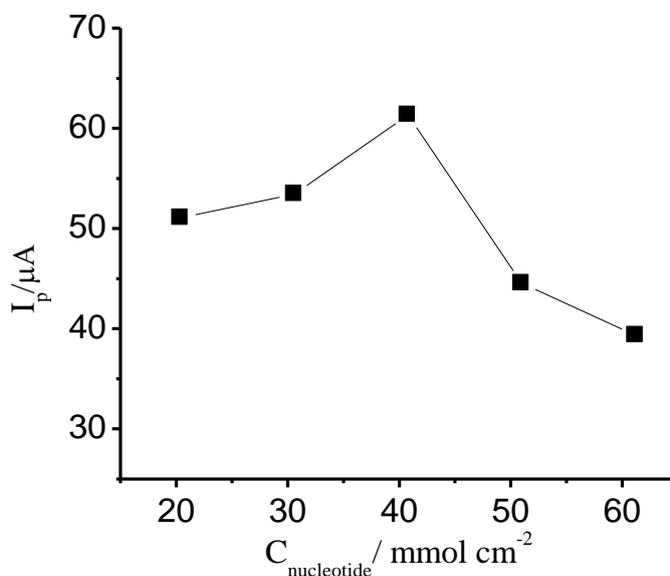
Because the metabolism and biologic processes of human's body involve the electron transfers between biomolecules, a ssDNA modified electrode was prepared for studying the electrocatalytic oxidation of vitamin B<sub>6</sub>. It was immersed 0.10 M NaOH supporting electrolyte and scanned at least for 3 cycles, resulting in a stable ssDNA modified electrode. Similarly, a dsDNA modified electrode and a bare GC electrode were also pre-treated in the alkaline solution for comparison. After the pre-treatment, the electrodes were taken in 3.2 mM vitamin B<sub>6</sub> solution including 0.10 M NaOH supporting electrolyte for the cyclic voltammetric tests. The resulting voltammograms are shown in Fig. 2.

Fig. 2 shows that electrochemical sweeps brought faradaic oxidative currents of  $38.6 \mu\text{A}$ ,  $21.6 \mu\text{A}$  and  $0.0 \mu\text{A}$  for the ssDNA electrode, the dsDNA electrode and the bare GC electrode, respectively, after they were subtracted by the background current. It means that, first, DNA can oxidize electrocatalytically vitamin B<sub>6</sub>, which is similar to the electrocatalytic oxidation of NADH by ssDNA [52,53](the electrocatalytic current is assigned to the guanine base because the electrocatalytic peak potential is in agreement with the oxidative peak potential of guanine base in our experiments); second, the catalytic activity of ssDNA is stronger than that of dsDNA.



**Figure 2.** Cyclic voltammograms of GC electrodes in 0.10 M NaOH solution containing 3.2 mM vitamin B<sub>6</sub> in a 0.10 M NaOH solution in the potential range of -0.20 to 0.80 V at a scan rate of 50 mV s<sup>-1</sup>. Curve (A), the bare GC electrode; (B), the dsDNA-modified GC electrode; (C), the ssDNA-modified electrode.

This stronger activity is attributed to their flexibility and better accessibility of nucleobases to the electrode surface. Heating made the base groups on DNA backbone thoroughly exposed outside, consequently increased the concentration of base groups on the surface of the GC electrode, and in turn, led to a larger catalytic current. This conclusion is also confirmed by four comparable cyclic voltammograms experiments: A series of various concentrations of the ssDNA were immobilized on the surface of the GC electrode, the resulting electrodes were rinsed in the same concentration of vitamin B<sub>6</sub> (1.2 mM) containing 0.1 M NaOH electrolyte for voltammetric sweeps, the obtained peak current trend was denoted in Fig. 3.



**Figure 3.** Dependence of the catalytic currents on the ssDNA concentration

Fig. 3 shows clearly the peak current raised with the increase of the ssDNA concentration added on the GC surface in the initial stage, and the catalytic current reached a maximum at 40 mmol cm<sup>-2</sup> nucleotide. Then, the peak current descended slightly when the ssDNA concentration added is 40 mmol cm<sup>-2</sup> nucleotide, which may be attributed to the mass transfer resistance in the thick film.

### 3.3. Kinetic studies

As seen in Fig. 3, vitamin B<sub>6</sub> can be oxidized electrocatalytically with ssDNA whereas it is not oxidized at the bare GC electrode in the same solution. To obtain information on the number of electron transfer (*n*) involved in the rate-determining step, a Tafel plot (Fig. 4) was drawn using background-corrected data (where there is no concentration polarization) from the onset of the current-voltage curves at a scan rate of 10 mV s<sup>-1</sup>. According to the current-overpotential equation [54], the net current can be expressed as

$$i = i_0 \left[ \frac{C_0(0,t)}{C_0^*} e^{-\alpha n F \eta / RT} - \frac{C_R(0,t)}{C_R^*} e^{(1-\alpha) n F \eta / RT} \right] \quad (1)$$

where *i*<sub>0</sub> is the exchange current density, *i* the net current or external current density, *α* the transfer coefficient (the scope of its value is from 0.3 to 0.7), *η* the overpotential, *C*<sub>0</sub>(0,t) the surface concentration of reducible species, *C*<sub>R</sub>(0,t) the surface concentration of oxidative species, *C*<sub>0</sub><sup>\*</sup> the bulk concentration of reducible species, *C*<sub>R</sub><sup>\*</sup> the concentration of oxidative species, *R*, *F* and *T* have their usual meanings. In the case that the difference between the surface and bulk of species involved may be negligible, the current-overpotential equation can be written as

$$i = i_0 \left[ e^{-\alpha n F \eta / RT} - e^{(1-\alpha) n F \eta / RT} \right] \quad (2)$$

If the working electrode is given a large positive overpotential, the exp[(1-*α*)*nFη*/RT] >> the exp(-*αnFη*/RT), equation 2 is become to equation 3

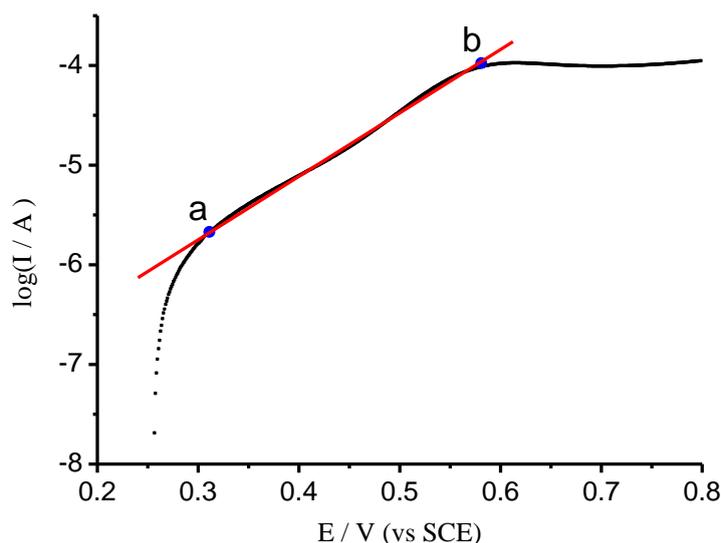
$$i = -i_0 e^{(1-\alpha) n F \eta / RT} \quad (3)$$

The logarithm of equation 3 leads to equation 4 (*β* = 1-*α*), if both the overpotential and the net current are positive values. Actually, this is another expression of the famous Tafel law.

$$\eta(V) = -(RT/n\beta F) \ln(i_0/A) + (RT/n\beta F) \ln|i/A| \quad (4)$$

At 25 °C it may be transformed to a more common form:

$$\eta(V) = -(0.059V/n\beta) \log(i_0/A) + (0.059V/n\beta) \log|i/A| \quad (5)$$



**Figure 4.** Tafel plot of the ssDNA modified electrode for the electrocatalytic oxidation of 0.40 mM vitamin B<sub>6</sub> in 0.1 M NaOH medium

Therefore, the Tafel slope can be obtained ( $b = 0.05917/n\beta$ ), its normal value is from 30 to 120 mV/decade.

In this system studied, the value of  $n\beta$  is 0.38 according to the Tafel slope in Fig. 4 for 0.40 mM vitamin B<sub>6</sub>. Similarly, the value of  $n\beta$  is 0.34 for 0.60 mM vitamin B<sub>6</sub>. Thus, an average value of  $n\beta$  is equal to 0.36. It could be deduced that the electron transfer number involved in the rate-determining step is  $n = 1$ . It will result in an unreasonable value of  $\beta = 0.18$ , if the value of  $n$  is equal to 2. Further, the electron transfer number was confirmed by calculating the difference between  $E_p$  and  $E_{p/2}$  in Fig. 2 by equation 6

$$|E_p - E_{p/2}| = 2.20(RT/nF) = 56.5\text{mV}/n \quad \text{at } 25^\circ\text{C} \quad (6)$$

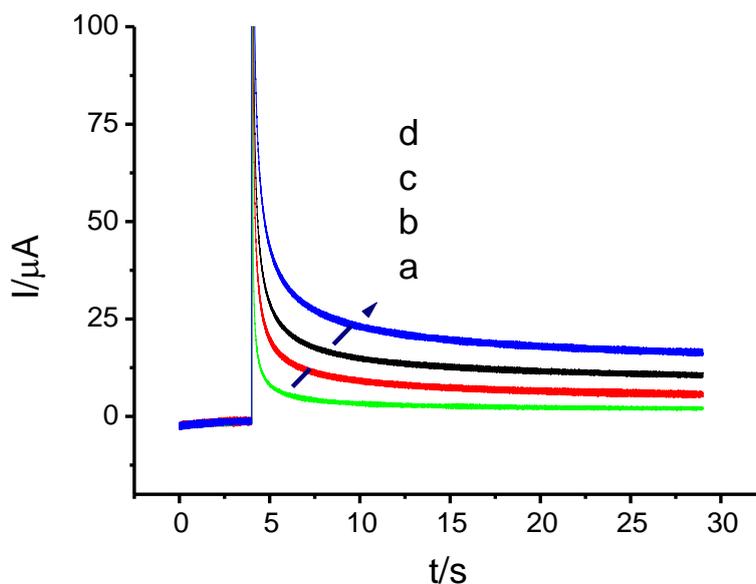
where the symbol  $E_p$  is the peak potential,  $E_{p/2}$  the half-peak potential. The potential difference between  $E_p$  and  $E_{p/2}$  was found to be 57 mV, confirming the electron transfer number for rate-determining step is equal to 1 ( $n = 1$ ), which is consistent with that reported by Razmi [18]. The Tafel slope of  $b = 164$  mV/decade was observed in this system, being greater than the normal slope 30–120 mV/decade, which showed the substrate–catalyst interaction [55-57], which may be attributed to a hydrogen bonding interaction between DNA and vitamin B<sub>6</sub>.

It is assumed that the value of  $\eta$  is taken as 0.0 V at the zero-current point [58], the exchange current density  $i_0$  may be obtained by taking the point of intersection of line a-b and the potential axis at  $\eta = 0.0$  V in Fig. 4. Thus, the average value of the standard rate constant  $k_0$  was calculated to be  $3.4(\pm 0.1) \times 10^{-4} \text{ cm s}^{-1}$ .

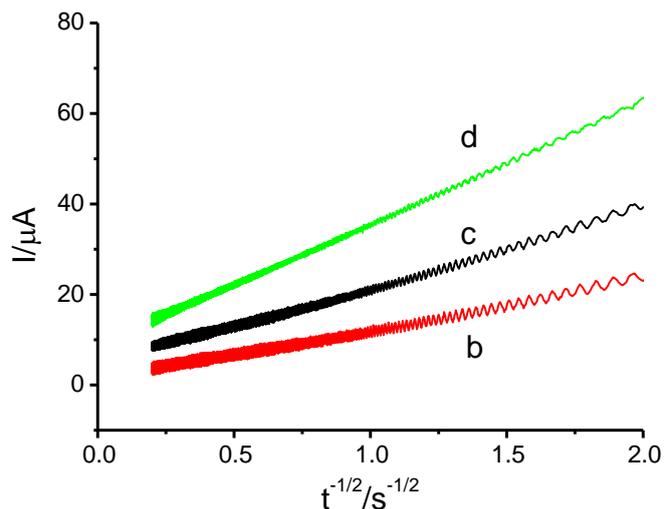
### 3.4. Determination of Diffusion Coefficient

The influence of potential scan rate on the peak current for the oxidation of vitamin B<sub>6</sub> has been investigated with cyclic voltammetry in 0.60 mM vitamin B<sub>6</sub> solution containing 0.1 M NaOH supporting electrolyte in the scan range of 0 - 400 mV s<sup>-1</sup>, the peak current (the background current was subtracted) is directly proportional to the square root of scan rate with a linear equation of  $I_p(\mu\text{A})=I_p(\text{ref}) + bv^{1/2}$  with  $R=0.99649$  ( $N=5$ ) and  $SD=2.14053$ , the reference current at 0.05 V/s is  $I_p(\text{ref})=I_p[\text{at } v^{1/2}=0.2236 \text{ (V/s)}^{1/2}]=27.98 \mu\text{A}$ , and the slope is defined as  $b=dI_p(\mu\text{A})/dv^{1/2}$ , its value is equal to  $132.67 \mu\text{A V}^{1/2}\text{s}^{-1/2}$ . The linear relationship indicates that the electrode process is a diffusion-controlled process. A shift of peak potentials appeared and the peak current only varied slightly while the scan rate was more than 400 mV s<sup>-1</sup>, which showed an electrochemical rate limit.

In order to obtain the information on the diffusion coefficient of vitamin B<sub>6</sub>, chronoamperometry was adopted in various concentrations of vitamin B<sub>6</sub>, the working potential was set at 0.65 V in the diffusion-controlled potential window. It can be seen from Tafel curve in Fig. 4 that the overall reaction rate in the potential window between a and b is controlled by the electron transition whereas the rate is controlled by diffusion only when the potential is more positive than that at point b (0.58 V). Therefore, the potential was stepped from 0.00 to 0.65 V in 0.00, 0.20, 0.40, 0.60 mM vitamin B<sub>6</sub> solutions, respectively. The bare test without vitamin B<sub>6</sub> was performed in the supporting electrolyte to obtain the background current for subtracting it from curves b, c, d in Fig. 5. The resulting curves of the corresponding faradaic currents against the inverse of the square root of the escaped time ( $t^{-1/2}$ ) were shown in Fig. 6.



**Figure 5.** Chronoamperometric curves of the ssDNA modified electrode in (a) 0.00, (b) 0.24, (c) 0.60, (d) 3.0 mM vitamin B<sub>6</sub> solutions containing 0.1 M NaOH supporting electrolyte, respectively. The working potential was stepped from 0.00 to 0.65 V vs SCE.

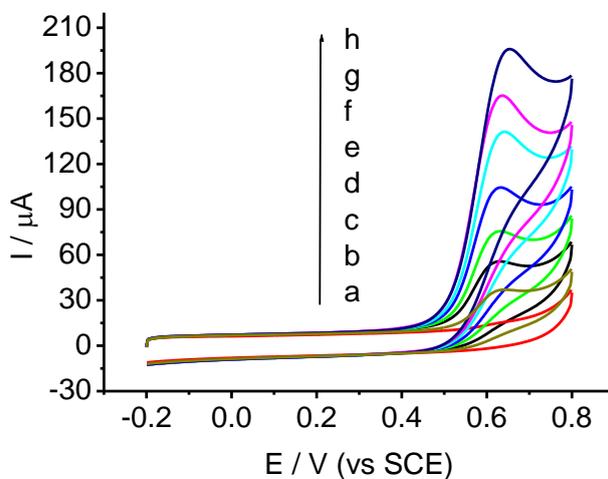


**Figure 6.**  $I_p-t^{-1/2}$  curves corresponding to those in Fig. 5.

It can be seen from Fig. 6 that the faradaic current against  $t^{-1/2}$  is a linear relationship, which follows Cottrell equation  $I_{(t)} = nFAD^{1/2}C/\pi^{1/2}t^{1/2}$ , where  $D$  is the diffusion coefficient ( $\text{cm}^2 \text{s}^{-1}$ ) and  $C$  the bulk concentration ( $\text{mol cm}^{-3}$ ) of vitamin  $B_6$ ,  $t$  the escaped time. Thus, we can obtain the diffusion coefficient ( $D$ ) of vitamin  $B_6$ , the average value of  $D$  was found to be  $4.2 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ .

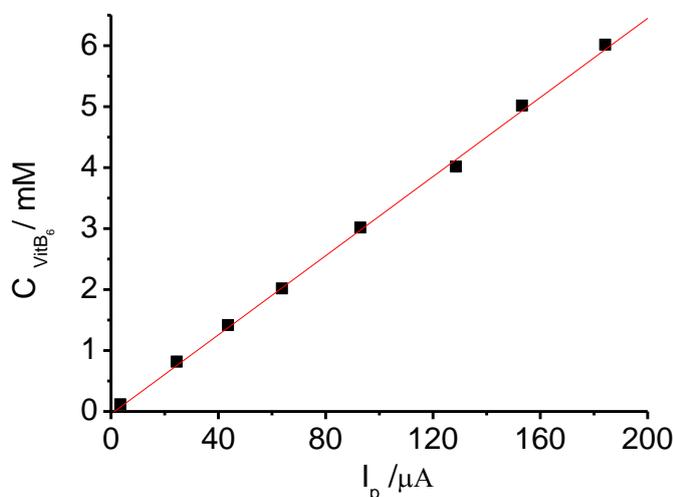
### 3.5. Analytical application

#### 3.5.1. Calibration curves



**Figure 7.** Cyclic voltammetric curves of the ssDNA-GC electrode in different concentrations of vitamin  $B_6$  solutions containing 0.1 M NaOH electrolyte: a, 0.10 mM; b, 0.80 mM; c, 1.40 mM; d, 2.00 mM; e, 3.00 mM; f, 4.00 mM; g, 5.00 mM; h, 6.0 mM. Potential range: -0.20 to 0.80 V; Scan rate:  $50 \text{ mV s}^{-1}$ .

The analytical performance of the ssDNA-modified electrode developed for vitamin B<sub>6</sub> determination was evaluated. It is observed that the peak current increases with the analyte concentration as shown in Fig. 7, and the anodic peak current appeared a linear relationship (Fig. 8) against the concentration of vitamin B<sub>6</sub> from 0.10 to 6.00 mM with R= 0.99952 (n=8) and SD=0.07008. The linear regression equation was  $C \text{ (mM)} = 0.03245 \times I_p \text{ (}\mu\text{A)} - 0.04049$  with the detection limit of 0.040(±0.001) mM.



**Figure 8.** Linear relationship between the concentrations of vitamin B<sub>6</sub> and the faradaic oxidative currents.

### 3.5.2. Interferences

To investigate the electro-catalytic selectivity of the ssDNA-modified electrode toward vitamin B<sub>6</sub>, several potential biomolecule interferences were tested. They are fructose, maltose, glucose, urea, vitamin C, vitamin B<sub>1</sub> and vitamin B<sub>12</sub>, respectively. The tolerable limit was defined as the concentrations of foreign substances that gave an error less than ± 5.0% in the determination of vitamin B<sub>6</sub>. The results exhibited that, although 1.00 mM vitamin C, 0.10 mM vitamin B<sub>1</sub> and 0.01mM vitamin B<sub>12</sub> have inference signals, 100 mM of fructose, maltose, glucose and urea have no inference signals for the determination of 1.00 mM vitamin B<sub>6</sub>, which means these substances can be allowed in higher concentration in the real sample. The selectivity of the method is thus acceptable and it may be applied to determine the content of vitamin B<sub>6</sub> in medicine samples.

### 3.5.3. Determination of Vitamin B<sub>6</sub> in Real Tablets

In order to evaluate the accuracy of the ssDNA modified electrode for the determination of vitamin B<sub>6</sub>, recovery experiments were carried out using real tablets containing vitamin B<sub>6</sub>. The determination for 4 real samples yielded a 99.46% mean recovery with a relative standard deviation only as 5.36% (Table 2). The detection average value is equal to 9.95 mg/tablet, which is very close to

10 mg/tablet (marked value). It is also very close to 10.04 mg/tablet, which is measured with ultraviolet spectroscopy. Therefore, the method is acceptable.

**Table 2.** Analytical results of vitamin B<sub>6</sub> in real tablets.

Marked value (mg/tablet)	Detection value (mg/tablet)	Recovery (%)	Average value (mg/tablet)	Mean recovery (%)	RSD (%)
10.00	9.83	98.31	9.95	99.46	5.36
	9.87	98.68			
	10.68	106.81			
	9.41	94.05			

#### 4. CONCLUSION

A ssDNA-modified electrode was prepared by immobilizing ssDNA on a bare glassy carbon electrode. The ssDNA-modified electrode has a role in electrocatalytic oxidation of vitamin B<sub>6</sub> in 0.1M NaOH solution. The peak currents depend linearly on the concentration of vitamin B<sub>6</sub> from 0.10 to 6.00 mM with R= 0.99952 (n=8) and SD=0.07008. The linear regression equation was C (mM) = 0.03245 × I<sub>p</sub> (μA) - 0.04049 with the detection limit of 0.040(±0.001) mM. The studies showed that glucose, fructose, maltose, urea at 100 mM concentration level, have no interference for the determination of vitamin B<sub>6</sub>. The determination for the real tablets at the ssDNA-modified electrode exhibited a mean recovery of 99.46%. It is expected to find its application in monitoring the content of vitamin B<sub>6</sub> in medical industry.

#### ACKNOWLEDGEMENTS

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