INTERNATIONAL JOURNAL OF UNIVERSAL PHARMACY AND BIO SCIENCES

IMPACT FACTOR 1.89***
ICV 3.00***

| Pharmaceutical Sciences | RESEARCH   ARTICLE……!!! |

NOVEL DIAGNOSTIC TOOL TO DETECT VITAMIN B₆ USING Fe₃O₄ MODIFIED GLASSY CARBON ELECTRODE: A RAPID TECHNIQUE TO ASSAY BIOCLINICAL SAMPLES

Dr. Mrs. V. Violet Dhayabaran[1]*, T. Daniel Prakash[1], Mrs.A.Anita Margret[2], Valentine AdaikalaRaj[1]

[1] P.G and Research Department of Chemistry, Bishop Heber College, Tiruchirappalli- 620 017. Tamil Nadu, India.

[2] P.G and Research Department of Biotechnology and Bioinformatics, Bishop Heber College, Tiruchirappalli- 620 017. Tamil Nadu, India.

For Correspondence:
Dr. Mrs. V. Violet Dhayabaran *
Address: P.G and Research Department of Chemistry, Bishop Heber College, Tiruchirappalli- 620 017. Tamil Nadu, India.
Email:
violetdhaya@gmail.com

ABSTRACT

As Vitamin B_6 is water soluble, the human system cannot store them and the excess amount will be excreted through urine. Therefore a continuous supply of Vitamin B_6 is highly essential for healthy living. This leads to a greater demand for detecting the concentration of Vitamin B_6 in the body. The present study aims to explore detection of Vitamin B_6 even at very low concentration using Fe_3O_4 micro particle modified glassy carbon electrode as a new diagnostic tool. The micro particle of 1mg Fe_3O_4 with 3 mm diameter of GC electrode resulted an optimization of oxidation current and appears to be significant. The catalytic activity of vitamin B_6 was explored through various studies such as effect of pH, dependence of concentration, influence of scan rate, variation in temperature on the oxidation of vitamin B_6 in presence of modified electrode. The experimental results clearly indicates that modified electrode has an excellent sensitivity and selectivity.The mediated mechanism was derived by Michaelis Menten kinectics line and Weaver-Burk plot. The Eadie-Hofstee plot was drawn, kinetic parameter K_m was calculated and it was found to be 1×10^{-2} mol dm^{-3}. Experimental results show that the present study can be used for the development of chemical sensors. The modified electrode can also be used for the quantitative estimation of vitamin B_6 in different branded vitamin B_6 tablets. The detection limit of this modified electrode was found to be in the range of 10^{-3}M concentration of vitamin B_6.

1. INTRODUCTION:

In recent years, the fabrication of the chemically modified electrodes (CME) has been widely reported to improve sensitivity and selectivity in the determination of amino acids, vitamins, DNA and many more biomolecules. The mechanical attachment method was employed to modify the working electrode surface by attaching the multi walled carbon nanotubes (MWCNT) [1-2]. Fe_3O_4 nanoparticles, as one of the famous magnetic materials, have attractive properties, such as good biocompatibility, catalytic activity and low toxicity. They can interact with enzyme by some active groups such as ―OH, ―COOH and ―NH_2, providing a favourable microenvironment. Some application of Fe_3O_4 as sensors and biosensors has been researched, which mostly benefits from the ability to promote the electron-transfer reactions [3]. Vitamin B6 in coenzyme forms performs a wide variety of functions in the body and is extremely versatile, with involvement in more than 100 enzyme reactions, mostly concerned with protein metabolism [4]. Both PLP and PMP are involved in amino acid metabolism, and PLP is also involved in the metabolism of one-carbon units, carbohydrates, and lipids [5]. Vitamin B6 also plays a role in cognitive development through the biosynthesis of neurotransmitters and in maintaining normal levels of homocysteine, an amino acid in the blood [3]. Vitamin B6 is involved in gluconeogenesis and glycogenolysis, immune function (for example, it promotes lymphocyte and interleukin-2 production), and hemoglobin formation The analysis of Vitamin B_6 is very important because it involves a wide area of usage in the manufacture of drugs and gene modulation, and cancer research, immune modulation in HIV- infection. Vitamin B_6, also known as pyridoxine hydrochloride, is essential for the breakdown of food by the body, and turning carbohydrates, proteins and fat into energy. It is as well needed for the production of neurotransmitters (chemical messengers in the nervous system) and proper functioning of the nervous system and the immune system. As such it has been implicated in the treatment of depression and anxiety. It's also involved in the synthesis of hormones and red blood cells. Pyridoxine assists in the balancing of sodium and potassium as well as promoting red blood cell production. It is linked to cardiovascular health by decreasing the formation of homocysteine. Lack of pyridoxine may cause anemia, nerve damage, seizures, skin problems, and sores in the mouth [6-9].

In the present work, an attempt is made to describe the electrocatalytic oxidation of vitamin B_6 by Fe_3O_4 micro particle modified GC electrode using various voltammetric techniques.

2. MATERIALS AND METHODS

Acids, bases, ferrous sulphate, ferric chloride and KH_2PO_4 were purchased from Merck Chemical Co. All experimental solutions were prepared from double distilled water. The pH was adjusted using 0.1 M NaOH and dilute HCl. The micro Fe_3O_4 particle and Fe_3O_4 modified electrode were synthesized with reference to the literature reported earlier [8].

Electroanalytical Analysis

Cyclic voltammetry (CV) experiments were performed with a Princeton applied research with potentiostat driven by electroanalytical measuring software. The conventional electrochemical cell consisted of three electrodes with a glassy carbon (GC) electrode (3 mm diameter) as the working electrode, a Pt plate counter electrode and an Ag /AgCl/KCl$_{sat}$ reference electrode. The voltammetric experiments were carried out at 28 ± 2^0C using 0.1 M KH_2PO_4 as a supporting electrolyte. Solutions were degassed with nitrogen for 10 minutes prior to recording the voltammogram.

3. RESULTS AND DISCUSSIONS:

The electrocatalytic oxidation of vitamin B_6 was carried out on Fe_3O_4 / GC modified electrode. The catalytic activity of vitamin B_6 was explored through various studies in the presence of modified electrode.

Enhancement study

The cyclic voltammogram for the oxidation of vitamin B_6 (0.5 mM) with 0.1 M KH_2P0_4 (pH 5.0) as supporting electrolyte at the GC electrode and Fe_3O_4 as modified GC electrode (Fe_3O_4/GC) were recorded and represented in Fig.1. The figure depicts a significant electrocatalytic activity that is occurred during the oxidation of vitamin B_6 and exhibits an increase of oxidation current 15 µA compare to bare GC electrode. The oxidation of vitamin B_6 appears to be irreversible due to the absence of electro activity on the reverse scan during the cyclic voltammetry for both the electrodes.
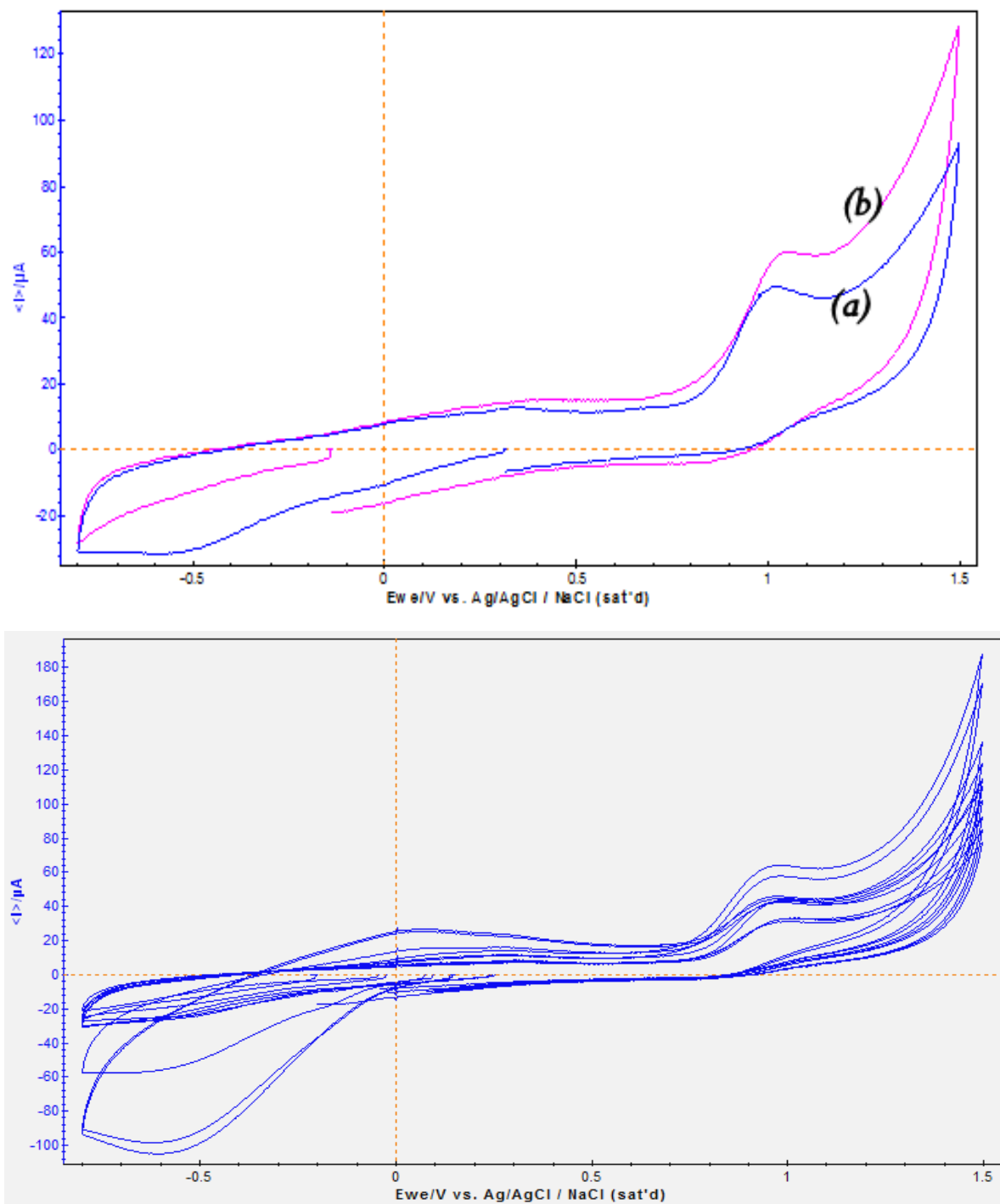
Fig 1.Cyclic voltammogram for oxidation of 0.5 mM vitamin B_6 obtained at (a) bare GC electrode and

(b)Fe_3O_4/GC in 0.1M KH_2PO_4, with potential scanning at a scan rate of 100mV/s at 28± 2°C and at pH 5.0

Scanning Electron Microscopy (SEM)

Scanning electron micrographs of Fe_3O_4 mechanically attached to a glassy carbon electrode (3 mm diameter) and immersed in 0.1M KH_2PO_4 electrolyte (a) before electrolysis (b) after electrolysis with an enlargement of 3000 times. The results shows in (Fig.2) that after electrolysis the microparticles of Fe_3O_4 appears not too different in sizes (2-10μm diameter), less lumpy with more well defined features of solid materials dominated by a 1-3 μm diameter microparticles indicating solid to solid conversion process. The stability of the coat is evident as the solids appear intact dominated by well defined edges of micro sizes solid particles even after 20 potential cycling. Scanning Electron Microscopy shows the effect of electrolysis (a-before electrolysis; b-after electrolysis) on the morphology of neutral Fe_3O_4 microparticles.

Influence of pH

The effect of pH on the oxidation of vitamin B_6 in 0.5mM concentration at the modified electrode (Fe_3O_4/GC) was studied over the range of 4.0 to 10.0. The experimental data obtained shows that the oxidation current decreases from pH 5 to 8.5 and slightly increases and remains constant until pH 10. From the fig.2 the optimum pH range for current enhancement is found and it lies at pH 5. The maximum enhancement is obtained at pH 5. Therefore pH 5 was employed for subsequent experiments.
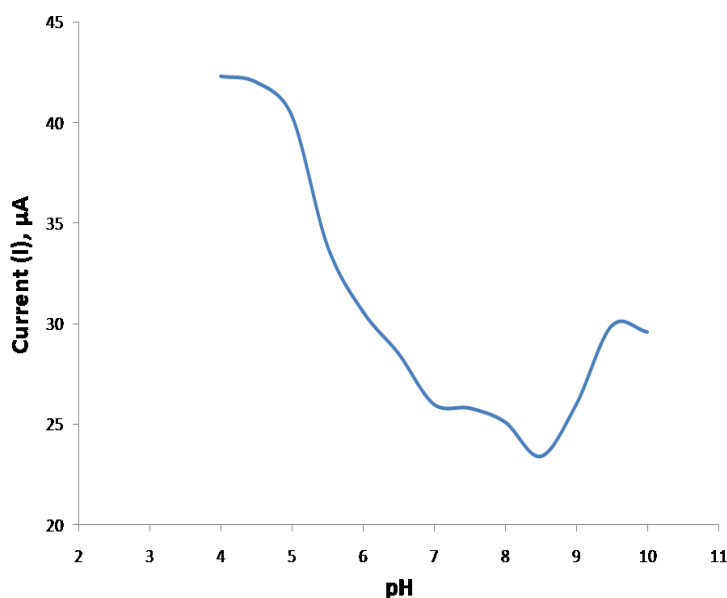

Fig.2 Influence of pH on oxidation current using Fe_3O_4/GC electrode in 0.5mM vitamin B_6, at 100m V/s at 28±2°c.

Influence of temperature:

The effect of temperature on the oxidation of vitamin B_6 was studied over the temperature range of $20°$ to $65°C$. The plot of log (oxidation current) of vitamin B_6 versus reciprocal of temperature (I/T) is shown in Fig.3. It was found that linearity is observed in agreement with thermodynamic expectation of equations (1) and (2) [1, 9]. The conductivity of the Fe_3O_4 microparticles with the increase in temperature also played a significant influence on the activation energy for diffusion of the substrate of interest, E_a as described by the Arrhenius equation (Eqs.1 and 2) given below.

$$\sigma = \sigma° \exp(-E_a/RT) \ldots\ldots \quad (1)$$

$$D = D° \exp(-E_a/RT)\ldots \quad (2)$$

Where σ/D is conductivity/diffusibility and $\sigma°/D°$ is standard conductivity and the initial diffusibility. The equations relate the influence on temperature on oxidation current. The activation energy (E_a) due to oxidation of vitamin B_6 at Fe_3O_4 modified GC electrode is found to be equal to 7.50 kJ.mol^{-1}
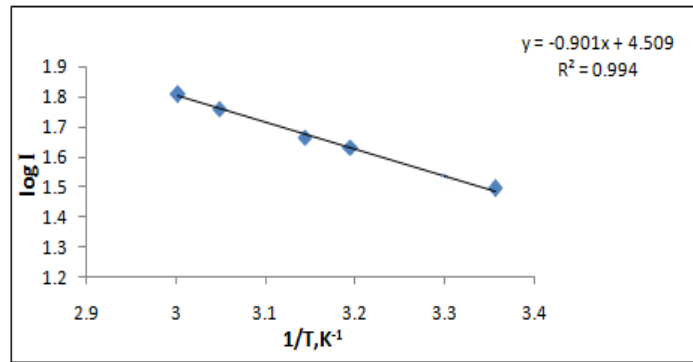

Fig.3 Arrhenius plot showing dependence of the current on temperature using 0.5mM vitamin B_6 in 0.1M KH_2PO_4 at pH 5 at Fe_3O_4/GC electrode.

Effect of concentration of vitamin B_6

The effect of concentration of vitamin B_6 in the oxidation process of the modified electrode was studied over the range of 0.5mM to 9.0 mM. The optimum pH 5.0 was fixed from the obtained experimental data. Fig.4 shows that peak current increases rapidly and linearly with increasing concentration of vitamin B_6. The plot of current versus concentration brings out the relationship between the concentrations of vitamin B_6 over the oxidation current. A good correlation is obtained ($R^2 = 0.9818$) with slope of 11.135μA M^{-1}
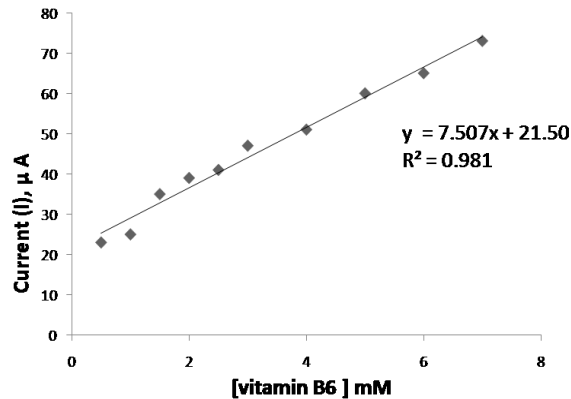
Fig. 4 A standard graph of vitamin B_6 over the concentration range of 0.5mM to 9.0 mM at Fe_3O_4/GC electrode immersed in 0.1M KH_2PO_4 at pH 5 with potential scanning at 100mV/s at 28 ± 2^oC.

Effect of metal oxide Dosages

The effect of dosage variation of iron oxide microparticles attached on the surface of glassy carbon electrode of 3mm over the range of 0.5mg to 0.1mg was studied and showed in Table1.The results show that there is a significant effect of micro metal oxide dosage on the oxidation of vitamin B_6.

As the dosage increases the oxidation current increase until 1.0mg however there is a decrease in oxidation current was observed after 1.0mg to 3.0mg. Therefore 0.7mg is employed because use of higher dosage would lead to higher error due to the small electrode surface area.

Table 1. Influence of Fe_3O_4 dosage onto GC electrode for oxidation of vitamin B_6

Weight.of Fe_3O_4, mg	Current I, μA
0.5	34.4
0.7	40.36
0.9	50.9
1	60.3
2	47.9
3	45.7

Effect of variation of scan rate

Effect of variation of scan rate (υ) on the voltammogram of 0.5mM vitamin B_6 using Fe_3O_4 modified GC working electrode in 0.1M KH_2PO_4 as supporting electrolyte was studied over the range of 5 to 1000 mV/s. The oxidation current of vitamin B_6 was observed to be increased with scan rate due to

heterogenous kinetics. Fig.5 shows the plot of log (peak current)Vs log (scan rate, υ) for the oxidation current of the first cycle and a straight line was obtained with slope 0.4869 with R^2 =0.9974 which is comparable with theoretical slope 0.5 for difffusion controlled process.
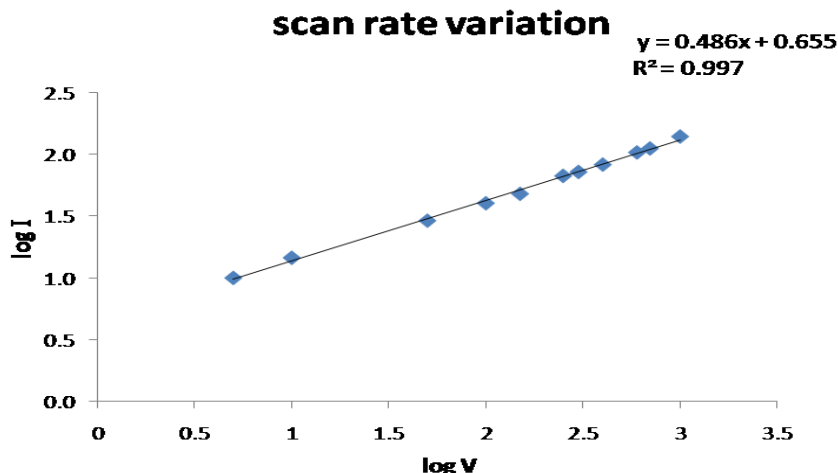

Fig.5. Plot of log I Vs log *v*. Effect of varying scan rates on oxidation of 0.5mM vitamin B_6 using Fe_3O_4/GC electrode in 0.1 M KH_2PO_4.

Mechanism of electrocatalytic activity of vitamin B_6

Fig. 1 shows atypical cyclic voltammogram response of vitamin B_6 in the absence and presence Fe_3O_4 in KH_2PO_4 buffer solution (pH 5) at scan rate of 100 mV/s.A clear catalytic anodic peak corresponding to oxidation of vitamin B_6 was noticed at approximately 1.0V (vs Ag/Agcl). Electro catalytic oxidation of vitamin B_6 is controlled by Fe_3O_4 microparticle. It is proposed that iron in iron oxide participate in the reaction mechanism. The most probable oxidation state of iron species is Fe (III)/Fe (II).

$$\text{Fe (II)} \longrightarrow \text{Fe (III)} + e^-$$
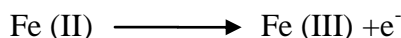
The fact that the anodic current increases by the addition of vitamin B_6 indicates that the oxidation is mediated by the surface confined Fe^{3+}, Fe^{2+} redox sites through surface catalytic path. The most probable mechanism of pyridoxine to pyridoxal can be represented as follows based on the mechanistic approach proposed by Jyh-MyngZen.
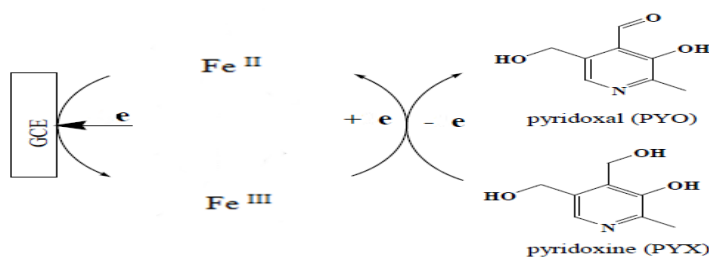

Fig.6 Mechanism of electrocatalytic activity of vitamin B_6

Recovery concetration of vitamin B$_6$ from tablets Neurobian and Becozinc.

Table 2.Recovery rate obtained for vitamin B$_6$ tablets using Fe$_3$O$_4$ for GC electrode in 0.1M KH$_2$PO$_4$ as supporting electrode.

| Sample	Recovery Concentration mM	Total Concentration mM	Error %
Neurobian	0.0143	0.07	80
	0.017	0.085	83
	0.0207	0.085	75
Becozinc	0.0737	0.1458	49
	0.444	0.1458	69
	0.6162	0.1458	57

An evalution of the efficiency of modified electrode (Fe$_3$O$_4$/GC) for the determination of vitamin B$_6$ in tablets (Neurobian) and (Becozinc) was carried out. 3mg of the tablet was diluted into 100 ml using 0.1 KH$_2$PO$_4$ buffer solution. Three replications were found to give recoveries of 20% , 17%, 25% from Neurobian, and similarly for Becozinc recovery concentrations of 51%, 31%, 43% were obtained and shown in the Table 2.

To assess the mechanistic aspects, electrochemical oxidation of vitamin B$_6$, the catalytic current (ΔI) is calculated as the difference between the baseline corrected limiting currents with and without vitamin B$_6$ in pH 5 solution.

Figure (7a) is the concentration (C_{bulk}) vs. anodic peak current (i_{pa}) plot for the vitamin B$_6$ oxidation reaction obtained by cyclic voltammetry at a slow scan rate of 10 mV s^{-1}. As can be seen, the catalytic current linearly increases with increase in concentration. It is indicating that in the concentration range studied, the reaction follows thefirst order kinetics. Michaelis- Menten equation in terms of current density (I) can be presented as,

$$I = nFAk_c\Gamma_tC_{\text{bulk}}/ (K_m + C_{\text{bulk}}) = I_mC_{\text{bulk}}/ (K_m + C_{\text{bulk}}) \qquad (1)$$

Where, $I_m = nFAk_c\Gamma_t$, $\Gamma_t$is the total surface concentration and other factors have their own significant values.
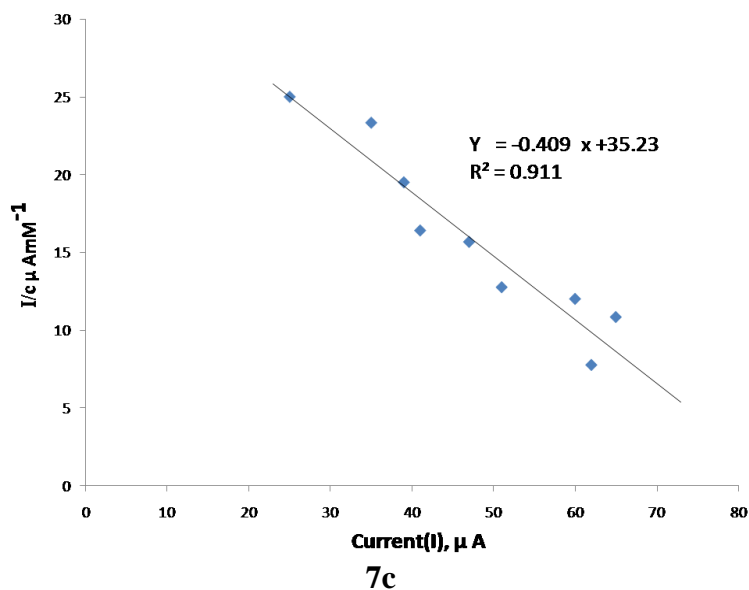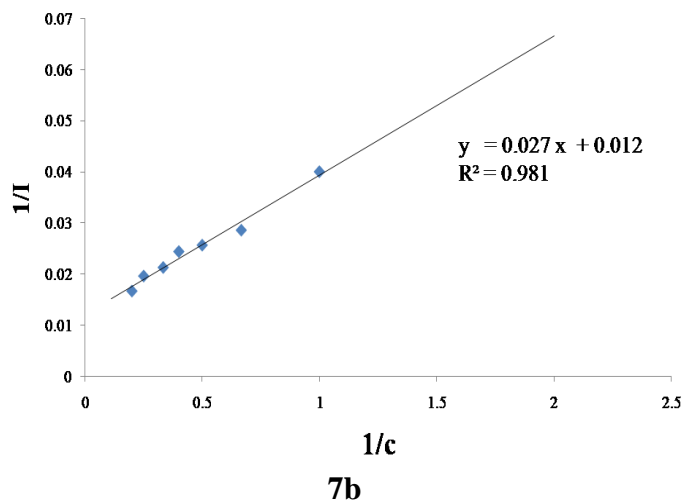

y = 6.6x – 17.8
R² = 0.988

7a

7b

7c
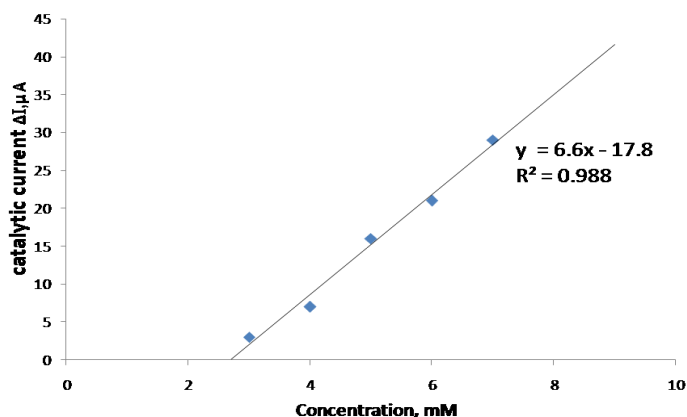
Fig.7a-7c Different methods for the estimation of Michaelis-Menten kinetic parameters (a) Michaelis-Menten plot; (b) Lineweaver-Burk plot and (c) Eadie-Hofstee plot

(c) Eadie-Hofstee plot: The other simplified expressions for calculating the kinetic parameters, namely, Lineweaver-Burk (LB) and Eadie-Hofstee (EH) methods are as follows.

Lineweaver-Burke (LB) Expression:

$$1/I = K_m/nFAk_c\Gamma_tC_{bulk} + 1/nFAk_c\Gamma_t \qquad (2)$$
$$1/I = S_{LB}C_{bulk} + I_{LB}$$
$$(S_{LB} = K_m/nFAk_c\Gamma_t \& I_{LB} = 1/nFAk_c\Gamma_t) \qquad (3)$$

Eadie-Hofstee (EH) Expression

$$i/C_{bulk} = nFAk_c\Gamma_t/K_m - i/K_m \qquad (4)$$
$$i/C_{bulk} = I_{EH} - S_{EH}i$$
$$(I_{EH} = nFAk_c\Gamma_t/K_m \& S_{EH} = -1/K_m) \qquad (5)$$

In eqns. (3) and (5), *S* and *I* denote for the slope and intercept of the linearized equations. (2) and (4), respectively. Figures (7b) and (7c) are typical plots of (i^{-1} vs. C_{bulk}^{-1}) and (i/C_{bulk} vs. I) for the LB and EH analysis, respectively. The calculated kinetics parameters, K_m based on these two methods was found to be 1×10^{-2} mol dm^{-3}.

Kinetic studies: The Fe_3O_4 modified electrode has a remarkable oxidative current peak at 1.0 V in 0.5mM vitamin B_6 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 Fe_3O_4 concentration on the surface of the electrode, and the current decreases with decreasing Fe_3O_4 concentration on the GC surface.

It revealed that the Fe_3O_4 was immobilized onto the surface of the glassy carbon electrode by the simple method. The current of the Fe_3O_4 electrode is attributed to the electron transfer of Fe^{2+} in Fe_3O_4 absorbed on the surface of the glassy electrode. 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 on any oxidative current peak at the same potential suggesting the oxidation of vitamin B_6 is an irreversible process.
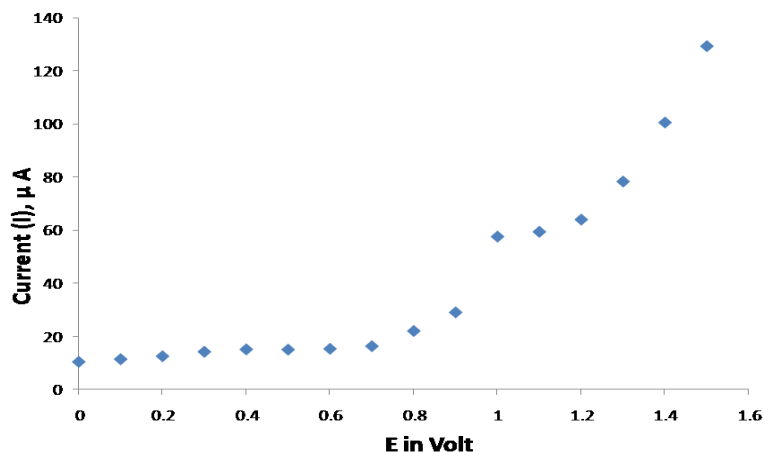

Fig.8 Cyclic voltammograms of 0.5mM vitamin B_6 solution in the potential range of 0.00 to 1.5 V at a scan rate of 100mV/s at 28± 2°C and pH 5.0 using Fe_3O_4/GC modified electrode.

As seen in Fig.8, vitamin B_6 can be oxidized electro catalytically with Fe_3O_4 whereas it is less 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.9) 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 100 mV s^{-1}.

According to the current-overpotential equation, the net current can be expressed as

$$i = i_o[(C_o(o, t)/C_o) \, e^{-\alpha nF\eta/RT} - (C_R(o, t)/C_R) \, e^{(1-\alpha)nF\eta/RT} \qquad (1)$$

where i_o is the exchange current density, i is the net current or external current density, α is the transfer coefficient, η is the over potential, $C_o(0, t)$ is the surface concentration of reducible species, $C_R(0, t)$ is the surface concentration of oxidative species, C_o is the bulk concentration of reducible species, C_R is 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

$$i = i_o \, e^{-\alpha nF\eta/RT} \, - \, e^{(1-\alpha)nF\eta/RT} \qquad (2)$$

If the working electrode is given a large positive over potential, the exp $[(1-\alpha)nF\eta/RT] \gg$ the exp $(-\alpha nF\eta/RT)$, equation 2 is become to equation 3

$$i = -i_o \, e^{(1-\alpha)nF\eta/RT} \qquad (3)$$

The logarithm of equation 3 leads to equation 4 ($\beta = 1-\alpha$) where α is the transfer coefficient, if both the overpotential and the net current are positive values. Actually, this is another expression of the famous Tafel law.

$$\eta(v) = -(RT/n\beta F) \ln(i_o/A) + (RT/n\beta F) \ln[(i/A)] \qquad (4)$$

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

$$\eta(v) = -(0.059V/n\beta) \log(i_o/A) + (0.059V/n\beta) \log[(i/A)] \qquad (5)$$

In this system studied, the Tafel slope obtained is 1.073 and $n\beta$ is found to be 59 mV, which confirms one electron transfer.
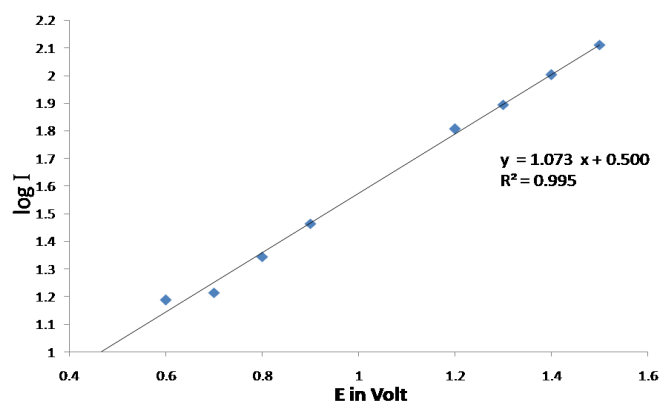

Fig.9. Tafel plot of the Fe_3O_4/GC modified electrode for the electrocatalytic oxidation of 0.5 mM vitamin B_6 at a scan rate of 100mV/s at $28 \pm 2^\circ$C at pH 5.0 using 0.7mg Fe_3O_4.

CONCLUSION:

Fe_3O_4/GC modified electrode was successfully fabricated and has exhibited excellent electrocatalytic effect on oxidation of vitamin B_6. The use of 1.0 mg dosage of Fe_3O_4 micro particle with 3.0mm diameter of GC electrode resulted in optimization of oxidation current and vitamin B_6 enhanced the current significantly. The exprimental results clearly indicates that modified electrode has an excellent sensitivity and selectivity. The present investigation pave a way to design a diagnostic tool to detect vitamin B_6 in body fluids as it plays a imperative role in human physiology.

Vitamin B_6 helps the body to: Synthesis antibodies, Maintain normal nerve function**,** Synthesize hemoglobin and a vitamin B6 deficiency can cause a form of anemia, Break down proteins, Keep blood sugar (glucose) in normal ranges.

The human body absorbs vitamin B_6 in the jejunum. Phosphorylated forms of the vitamin are dephosphorylated, and the pool of free vitamin B_6 is absorbed by passive diffusion Vitamin B_6 concentrations can be measured directly by assessing concentrations of (PLP) Pyridoxal 5' phosphate; other vitamers; or total vitamin B_6 in plasma, erythrocytes, or urine. Vitamin B_6 concentrations can also be measured indirectly by assessing either erythrocyte aminotransferase saturation by PLP or tryptophan metabolites. Plasma PLP is the most common measure of vitamin B6 status. PLP concentrations of more than 30 nmol/L have been traditional indicators of adequate vitamin B_6 status in adults. However, the Food and Nutrition Board (FNB) at the Institute of Medicine of the National Academies (formerly National Academy of Sciences) used a plasma PLP level of 20 nmol/L as the major indicator of adequacy to calculate the Recommended Dietary Allowances (RDAs) for adults.

Since there is a crucial need of assaying Vitamin B_6 this piece of work will find application in the medical field. As the size of the nanoparticle is more significant to enhance the function of the electrode.

There is a scope to improve the efficiency of the electrode by varied size, varied metal oxide and by introducing dopped metal oxide to detect vitamin B_6 even at very low concentration. Literature reveals the fact that excess of vitamin B_6 may also cause health problems like nerve damage due to deposition of vitamin B_6 in sub-epidermalvesicular dermatosis and sensory peripheral neuropathy. This can be conveniently applied to detect and quantify vitamin B_6 in different environment.

REFERENCES:

1. Mohammed Zidan, Tan Wee Tee, Abdul Halim Abdullah, Zulkarnain Zainal, GohJoo Kheng,(2011), *Int. J. Electrochem. Sci.,* 6, 289-300.

2. Koh Sing Ngai, Wee Tee Tan, Zulkarnain Zainal, Ruznizabinti Mohd Zawawi, Mohammed Zidan, (2012), *Int. J. Electrochem. Sci.,* 7, 4210 – 4222.

3. Zhang Jin-lei, Tan Xue-cai, Zhao Dan-dan, Tan Sheng-wei, Liu Li, Wang Lin and Huang Zeng-wei, (2011), *Chem. Res. Chinese Universities*., 27(4), 566-569.

4. Institute of Medicine. Food and Nutrition Board. Dietary Reference Intakes: Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. (1998) Washington, DC: National Academy Press.

5. McCormick D. (2006) Vitamin B6. In: Bowman B, Russell R, eds. Present Knowledge in Nutrition. 9th ed. Washington, DC: International Life Sciences Institute.

6. YunhuaWuandFajun Song, (2008), *Bull. Korean Chem. Soc*., 29(1), 38-42.

7. Yuzhong Zhang, Yuehong Wang, (2011), *American Journal of Analytical Chemistry*, 2, 194-99.

8. Marcos F. S. Teixeiraa, AlineSegninia, Fernando C. Moraesb, Luiz H. Marcolino-Júniorb, OrlandoFatibello-Filhob and Éder T. G. Cavalheiro, (2003), *J. Br. Che. Soc.,* 14 (2), 316-21.

9. Shou-Qing Liu, et al., (2012), *In. J. Elec. Sci.,* 7, 324-337.

10. Ki Do Kim, Sung Soo Kim, Yong-HoChoa, and HeeTaik Kim, (2007), *J. Ind. Eng. Chem.,* 13 (7), 1137-1141.

11. Weetee Tan, Mohammed Zidan, Zulkarnain Zainal, Abdul Halim Abdullah and JooKhengGoh, (2010), *Oriental Journal of Chemistry,* 26(1), 45-52.

12. Jyh-Myng Zen, Jyh-Cheng Chen and Annamalai Senthil Kumar Tamkang, (2002), *Journal of Science and Engineering,* 5 (4), 219-222.

13. J.Gonzalez-Rodriguez, J.M. Sevilla, T. Pineda, M. Blazquez, (2012), *Int. J. Elec. Sci.*7, 2221-29.

14. Solange M. Cottica, Jorge Nozaki, Helena S. Nakatani, Claudio C. Oliveira, Nilson E. de Souza, Jesui V. Visentainer,(2009), *J. Braz. Chem. Soc.,* 20 (3), 496-501.

15. Natori.Y, Oka.T,(1997), *Nutrition Research,* 17, 1199.

16. Tryfiates.G. P, Gannett.M.P, Peter.M, Arbogast. J.G, (1996), *Cancer Research,* 56, 3670.

17. Salhany, J. M.; Steven, M., (1996), *Aids Patient Careand Stds.*10, 353.

18. Voet.D, Voet.J.G, (1995), *Biochemistry*; Wiley: NY, U.S.A., 227.

19. Gorzalka.B.B, Moe.I.V,(1994), *Nutrition Research,* 14, 279.