Journal of Applied Pharmaceutical Research 2017, 5 (1): 30 – 39

Journal of Applied Pharmaceutical Research (JAPTRonline)

ISSN No. 2348 – 0335 **www.japtronline.com**

BIOELECTRODE BASED CHITOSAN-NANO COPPER OXIDE FOR APPLICATION TO LIPASE BIOSENSOR

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Article Information

Received: 14th December 2016

Revised: 22nd December 2016

Accepted: 28th December 2016

Keywords

Chitosan (Chit), nano-CuO, bioelectrode, Lipase [LIP], cyclic voltammetry (CV), triglyceride [TG] biosensor

ABSTRACT

Chitosan (Chit)-nanocrystalline CuO composite prepared from Chitosan and CuO nanoparticles by a spin coating method. CuO nanoparticles (45 nm) synthesized by Sol-gel citrate method and characterized by X-Ray diffraction (XRD), Raman spectroscopy, UV-visible spectroscopy, Fourier transform spectroscopy (FTIR) and Scanning electron microscopy (SEM). The electrochemical studies revels that these Chit-nano CuO electrode provide favorable condition for immobilization of enzyme lipase [LIP] specific enzyme for triglyceride detection, resulting in enhanced electron transfer at the interface. The prepared bioelctrode (LIP/Chit-nano CuO/Au bioelectrode) is utilized for triglyceride [TG] sensing using cyclic voltammetry (CV) with hexacyanoferrate as mediator. The electrochemical response studies shows on improved sensing performance of bioelectrode exhibit high sensitivity, low detection limit and good linearity of tributyrin concentration with fast response time. The low value of Michallis-Menten constant indicates high affinity of LIP towards the analyte (tributyrin). The Redox behavior of nano CuO makes an efficient matrix with chitosan for triglyceride [TG] biosensor.

INTRODUCTION

Triglyceride (TG) can be generated by an esterification process of three hydroxyl (-OH) group of glycerol with three molecule of fatty acid that produce an ester as product[1].Lipase triglyceride ester hydrolase has many industrial applications, because it catalyses the hydrolysis of triacylglycerol into glycerol and fatty acid [2]. Triglyceride acts is important role in metabolism as an energy source and also as a dietary fat transporter. Cardiovascular diseases are major cause of global death [3], Triglyceride (TG) determination is crucial since its concentration could lead to hyperlipidemia [4, 5].Abnormal triglyceride concentration in blood leds to chronic obstructive pulmonary diseases which includes bronchitis, bronchopneumia, Sinusitis larystic etc. [6], Therefore, ensuring the level of triglyceride in our body in normal range women (35-135 mg/ dL) and men (40-160 mg/ dL) is a need[7, 8]. In the recent past, electrochemical nanobiosensors have been used for rapid determination of tributyrin in blood due to its high sensitivity and specificity. Gold, glassy carbon electrode and indium tin oxide are some commonly used working electrode in these biosensors. In order to detect tributyrin concentration with enhanced sensitivity and specificity, surface patterning of electrode with nanoparticles [9] is required. Recently use of lipase as catalyst to produce biodiesel by transporting triglyceride into fatty acid, alkyl acid have been reported [10]. However, free lipase is not favored in industrial development because it is difficult to recover for reuse and it has low stability .These drawback can be overcome by immobilization on various supports for instant Chitosan is polysaccharide carrying amino group useful. Chitosan supports have been used for lipase immobilization that permits to retain initial activity. Nanostructured metal oxide have been widely used in biosensor application as they provide a large surface to volume ratio, high catalytic activity, high electron conductivity due to small band gap and the strong adsorption ability[11]. Among the various methods available for determination of tributyrin (TG) [12], biosensors are comparatively more simple, sensitive, rapid and possible at beside the patient. Three types of TG biosensor

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have been reported: (i) DO metric TG biosensor which measured Dissolved oxygen consumed in enzyme reaction [13] (ii) electrochemical biosensor which measured electron under high potential [14] and (iii) potentiometric TG biosensor which measure the voltage [15], However electrochemical biosensor are more common in use as these are unaffected by environment factors and easy to operate. The most common device used for TG determination is an enzymatic amperometric triglyceride biosensor. Most triglyceride biosensor reported till date are based on multienzyme [16] however, there are works that used single enzyme (lipase) for triglyceride [17, 18].this methods is more preferable than multienzyme (lipase, glycerol, kinase-α-glycerophosphate oxidase and peroxidase) because single enzymatic method are less time consuming and inexpensive.

EXPERIEMENTAL

Materials:

Lipase (Aspergillus Niger, Himedia), Chitosan (Chit, Sigma Aldrich), Copper nitrate [Cu $(NO_3)_2.3H_2O$], Citric acid, ethanol, potassium chloride [KCl], Potassium fericynide $[K_3[Fe(CN)_6]^{3-}$, Sodium dihydrogen phosphate $[NaH_2PO_4]$, Sodium monohydrogen phosphate $[Na_2HPO_4]$ and all other chemicals were analytical grade. Aqueous solution and buffer were prepared in Mili Q.Water.

Synthesis of Copper oxide Nanoparticles

Copper oxide (CuO) nanoparticles (45nm) were synthesized by sol-gel citrate method [19] is shown in figure.1 by precursor using a copper nitrate (Cu $(NO_3)_2.3H_2O$), Citric acid ($C_6H_8O_7$), and ethanol (C_2H_5OH,>99% pure).Copper nitrate and citric acid were dissolved in ethanol with 1:1:2 proportion and stirred at 80°C for 3 hrs to get homogeneous solution was further heated at about 120°C for 12hrs in heating vessel to form the gel precursor. solution, then the The prepared product was subjected to 3hrs heat treatment (calcinated) at 350°C in a muffle furnace The dried powder then calcinated at 650°C in order to improve the crystallinity and sensitivity of the material.

Preparation of Chit-nano CuO/ Au electrode

Chitosan (Chit), a natural copolymer having β-1, 4, glucosamine and N-acetyl glucosamine units is a biodegradable polysaccharide exhibiting bioactive amino group [20]. Chitosan (200 mg) was dissolved in 100 ml of 0.2M acetic acid by stirring at room temperature for 3hrs, and then sol gel (figure 1) synthesized CuO nanoparticles were dissolved in 20 ml of chitosan solution and stirring for 30 minutes at room temperature. Finally, viscous solution of Chit-nano CuO was obtained. Then Chit-nano CuO solution was pour onto the surface of Au plate by spin coating technique and then, the Chit-nano CuO/Au electrode was kept too dry, finally dried Chit-nanoCuO/Au electrode was rinsed repeatedly with 50 mM phosphate buffer.

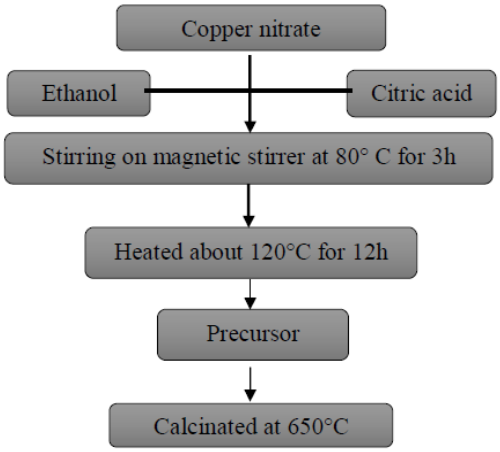

Fig.1. Sol-gel citrate method for synthesis of Copper oxide Nanoparticles

Fabrication of LIP/Chit-nano CuO/Au Bioelectrode

For preparation of bio electrode, enzyme Lipase (LIP) solutions were freshly prepared in phosphate buffer [50Mm], (pH.-7.0): solution prepared by adjusting the proportion of monobasic sodium phosphate and dibasic sodium phosphate solution of 0.05M Concentration by dissolving 1 mg of LIP [21]. Then, lipase was immobilized onto surface of Chit-nano CuO/Au electrode by physical adsorption method and kept in humid chamber for about 4hrs.The fabricated bio electrode (LIP/Chit-nano CuO/Au electrode) was then washed with phosphate buffer to remove any unbound enzyme and stored at 4°C when not in use.

RESULT AND DISCUSSION

The structural analysis of synthesized nano CuO has been done using X-Ray diffractometer; Fourier transform infrared (FTIR) studies have been performed to confirm the bonding in nano CuO. The morphological analyses have been conducted using scanning electron microscopy (SEM).The electrochemical analysis was done by using Electrochemical impedance spectroscopy (EIS) and Cyclic Voltametry (CV).

X-Ray Diffraction

Crystalline natures of prepared CuO nanoparticles were identified from their corresponding powder XRD pattern. The XRD pattern (Figure.2.) of nano CuO Particles exhibit reflection plane (2θ= 32.20, 35.10, 38, 48, 52.72.12, 58, 62, 66, 66.10, 72, and 75), 110, 002, 111, 202, 020, 202, 113, 311, 113, 311, 004 which have been assigned to the monoclinic hexagonal crystalline system of CuO (Std JCPDS File No: 048-1548 and 800-1917). The broad and well resolved diffraction peaks reveal the nano scale dimension of the plane along [111] plane. No characteristics peaks of any impurities were detected suggesting that high quality of CuO nanoparticles was prepared. The crystalline Size was calculated using Debye Scherer's formula

$$D = K\lambda / \beta \text{Cos}\,\theta$$

Where, D is crystalline size, K is usually taken as 0.94, known as Brags constant usually taken as 0.89, λ is the wavelength of X-Ray radiation (0.15418nm) for Cu-Kα, β is a full width half maximum of a diffraction peak measured at 2Θ.

The nanocrystalline size of CuO Nanoparticles was found to be 45 nm. The peaks with high intensities and narrow full width half maximum shows that crystallinity of the prepared material is good.

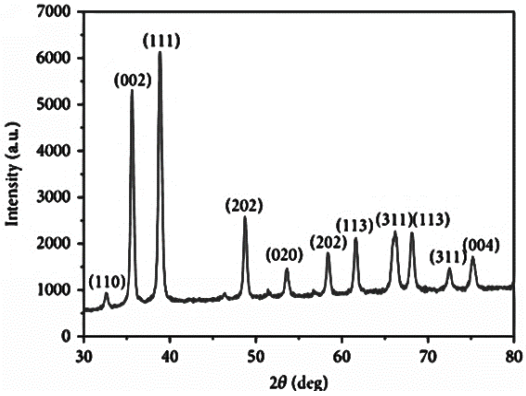

Fig.2 X-Ray diffraction pattern of synthesized CuO nanoparticles

Raman spectra

Raman spectra of CuO nanoparticles are shown in figure.3. It can be seen at three Raman peaks at 282, 330 & 616cm^{-1}. Synthesized CuO has monoclinic structure with space group C2h6. In Raman spectra the peak at 282cm^{-1} to the Ag modes and peak at 330 and 616cm^{-1} to the Bg modes which is consistent with previous work [22]. Raman active modes (Au+2Bu) occurred in raman observed spectra. This result shows obviously that pure single phase CuO nanoparticles are formed in the above experimental condition.

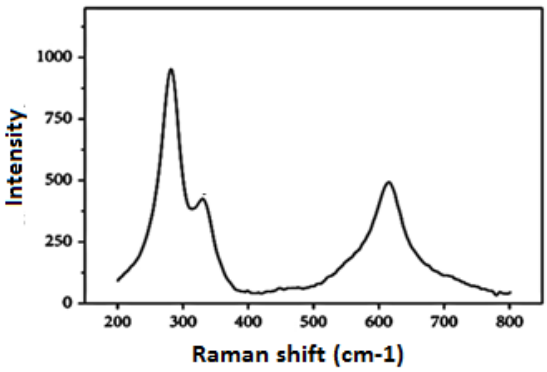

Fig.3 Raman spectra of synthesized CuO nanoparticles

UV Spectroscopy

Optical characterization of the sample was recorded on UV Visible absorption spectrophotometer shown in fig.4.In order to determine the band gap energy of CuO.Eg value of CuO according to the following equation,

$$Eg = h\nu_{freq}$$

Where, Eg is low gap energy is Planck's constant. ν_{freq} is frequency of emitted radiation .The band gap of nano CuO is calculated to be 3.42 eV, which is higher than the reported value of CuO [23]. The increasing in band gap may be due to the quantum size effect of sample [24].

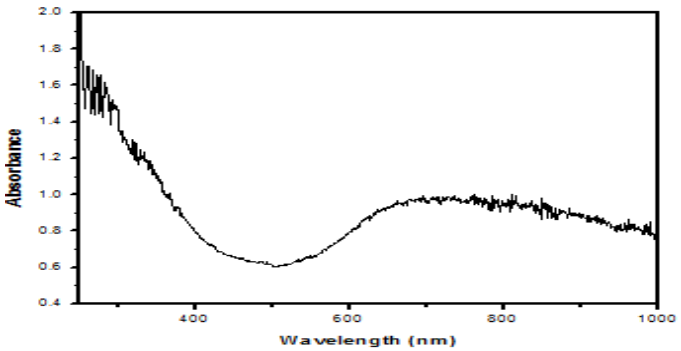

Fig. 4 UV Spectroscopy of synthesized CuO nanoparticle

FTIR Study

Fourier transform infrared Spectroscopy [FTIR] is a technique used to measure vibrational frequencies of bond in the molecule. FTIR spectroscopy analysis of CuO nanoparticles was scanned at room temperature in the range 4000-400 cm^{-1}. Figure.5 Shows FTIR spectra of CuO nanoparticles. The observed peaks at 453,494,609 Cm^{-1} corresponds to the characteristics stretching vibration of Cu-O bond in the CuO. There is a Sharp peak observed at 609 cm^{-1} in the spectrum on

CuO nanoparticles, which is a characteristic of Cu-O bond formation. Moreover, no other IR active mode was observed in the range of 605 to 660 cm^{-1}, which totally rules out the existence of another phase, i.e., Cu_2O [25]. The broad absorption peak at around 3466 cm^{-1} is caused by the adsorbed water molecule since the nanocrystalline materials exhibit a high surface to volume ratio absorb moisture. Thus, the pure phase CuO with monoclinic structure is also confirmed from the Fourier transform infrared Spectroscopy analysis.

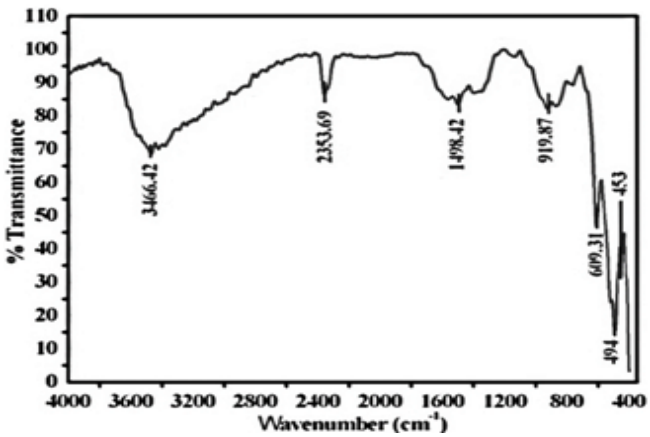

Fig.5 FTIR spectra of nanocrystalline CuO nanoparticles

Scanning electron microscopy

Scanning electron microscopy (SEM) have been used to investigate surface morphology of prepared material. Figure.6. [A], [B] shows SEM of CuO nanoparticles at different magnification [A], [B], which shows globular morphology.

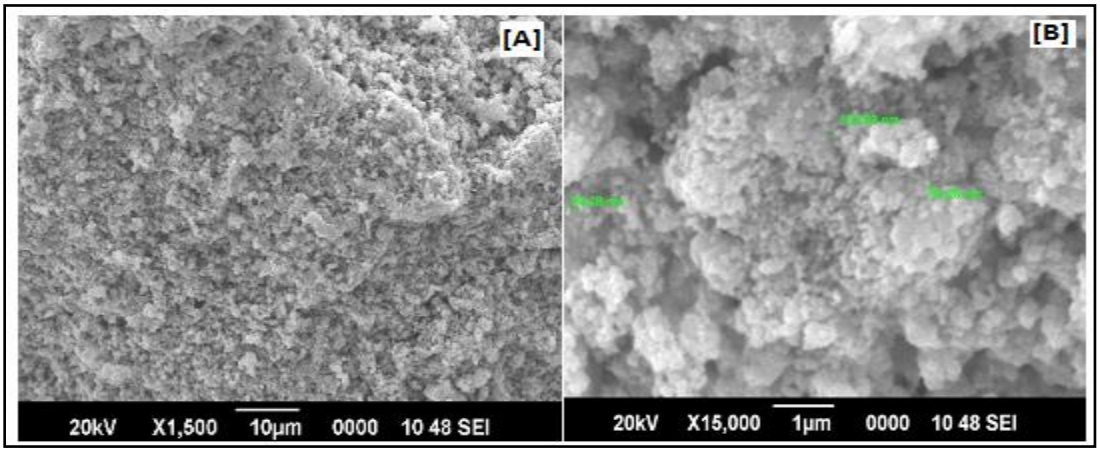

Fig.6. Scanning electron microscopy of Synthesized CuO nanoparticles

Figure.7 [A-D] shows Scanning electron microscopy (SEM) morphology of [A] Chit-CuO/Au electrode [B] Lipase/Chit-CuO/Au bio electrode respectively at different magnification from [A] to [D]. The SEM images of Chit-CuO/ Au electrode exhibit cotton globes like structure and also shows polymer is embedded in CuO nanoparticles. The revealed feature confirms the uniform pores are open and extend through the surface into the bulk which is believed to play important role in enzyme immobilization. Chit-CuO/Au bio electrode at higher magnification images [B] surface shows nanosized distribution of CuO nanoparticles provide high surface area of nanostructured and polymer matrix which can lead greater amount of an immobilized enzyme on the surface. After immobilization of enzyme lipase image [C] and [D] which shows morphology and perfect immobilization, as shape changes from cotton globes like structure to a crystal like structure. As the structure has varied, the corresponding surface area has also reduced and maximum absorption of enzyme occur. This shows perfect immobilization of enzyme.

ELECTROCHEMICAL STUDY:

The electrochemical studies have been carried out by dipping the electrodes in KCl (0.1M) containing 5mM $[Fe(CN)_6]^{3-}$ using electrochemical impedance spectroscopy (EIS) and Cyclic voltammetry (CV) at scan rate 10 mV/s.

Electrochemical impedance spectroscopy (EIS)

Electrochemical impedance spectroscopy (EIS) technique measure impedance of electrode surface as a function of frequency due to variation in the interfacial properties of the interface of the electrode. EIS is an effective and non destructive tool to investigate the process of modification with respective to enzyme on the surface of electrode.Nyquist plot includes a semicircle region observed at higher frequency

corresponding to electron transfer limited process on Z' axes is followed by straight line at 45°C two real axes at lower frequency revealing the diffusion limited process.

The complex impedance may be represented as a sum of real (Z') and imaginary (Z") components that originate from the resistance and capacitance of the cell including the ohmic resistance of the solution (Rs), Warburg impedance (Zw), capacity of electric double layer (Cdl) and surface electron resistance (R_{CT}) [26] and it appears when the current flows through LCR circuit (equivalent to randle circuit).

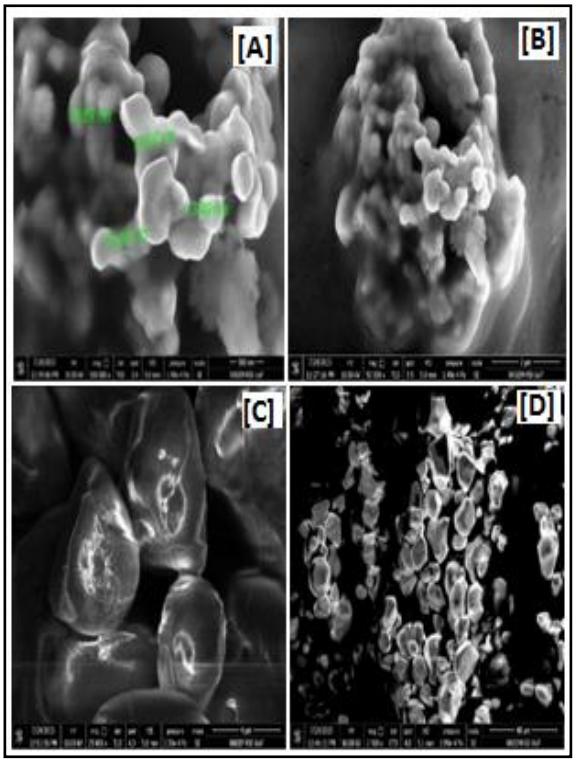

Fig. 7 Scanning electron microscopy of [A] Chit-CuO/ Au electrode [B] Lipase/Chit-CuO/Au bioelectrode

From figure. 8[A], R_{CT} value of electrode depends upon dielectric characteristics of the electrode/electrolyte interface. The Figure shows Nyquist plot ,after spin coating of Chit-nano-CuO onto the Au electrode, Rct value decreases to 32.13 KΩ,this decrease in Rct may be due to nanosized structure of nano CuO Particles that provide increased electro active surface leading to higher rate of electron transfer moreover, after immobilization of lipase onto Chit-nano CuO/Au electrode ,electron transfer by negatively charged redox marker $[Fe(CN)6]^{3-}$ may perhaps be hindrance resulting in increased Rct value 46.42 KΩ.

Electron transfer kinetics parameter (Ke) of chit-nano CuO/Au electrode and LIP/Chit-nano CuO/Au bioelectrode can be calculated from following equation,

$$ke = \frac{RT}{n^2F^2A\ Rct\ C} \quad \ldots\ldots\ldots\ldots [1]$$

Where, R is the gas constant ($8.314 Jmol^{-1}k^{-1}$), T is temperature (300 K), n is the number of electron transfer (1), F is faraday constant ($96485\ J\ mol^{-1}K^{-1}$), A is area of electrode ($1cm^2$) and C is the concentration of redox species in the electrolyte solution (5Mm), Heterogeneous electron transfer (Ke) values for chit-nano CuO/Au electrode and LIP/Chit-nano CuO/Au bioelectrode found to be 0.00166 $Cm.s^{-1}$ and 0.00115 $Cm.s^{-1.}$ High value for bioelectrode shows indicating a faster electron exchange between the redox species.

Cyclic voltammetry

In cyclic voltammetry, Current at the working electrode is plotted versus the applied voltage (i.e., the working electrode's potential) to give the cyclic voltammogram trace. Cyclic voltammetry is generally used to study the electrochemical properties of an analyte in solution. A standard CV experiment employs a cell fitted with three electrodes: reference electrode, working electrode, and the counter electrode. This combination is sometimes referred to as a three-electrode setup, Ag/AgCl as reference electrode, platinum as counter electrode, and LIP/Chit-nano CuO/Au bioelectrode as working electrode. The Electrolyte is usually added to the sample solution to ensure sufficient conductivity. The solvent, electrolyte, and material composition of the working electrode will determine the potential range that can be accessed during the experiment. Cyclic voltammogram of Chit-nano CuO/Au electrode and LIP/Chit-nano CuO/Au bioelectrode have been recorded at scan rate of 10mV/s using three electrode cell in a KCl solution containing $K_3[Fe(CN)_6]^{3-}$ as a mediator in potential range -0.2 to 0.6 V. Well defined Oxidation Reduction peaks are observed.

The Figure.8. [B] Shows cathodic peak current at 0.00166 mA, 0.00132mA for Chit-nano CuO/Au electrode and LIP/Chit-nano CuO/Au bioelectrode. The enhancement of cathodic peak current of Chit-nano CuO/Au electrode due to conducting nature of CuO nanoparticles and formation of a network between the positively charged CuO nanoparticles and the hydroxylamine group of CH. After immobilization of enzyme on nanoparticles the magnitude of current is found to be

decreased because of the inherent non conducting nature of enzyme which acts as barriers for electron transport due to ferro/ferri molecules in PBS electrolyte.

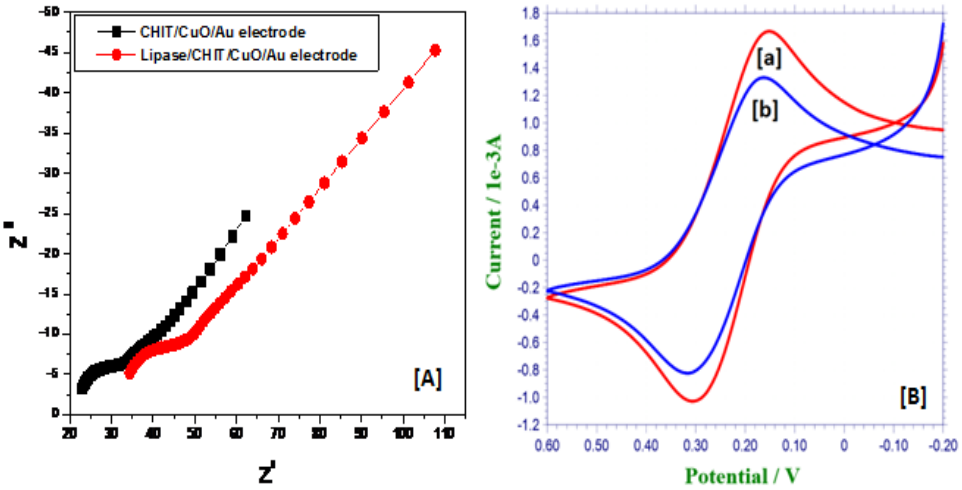

Fig.8. [A] Electrochemical impedance (EIS) and [B] Cyclic voltammetry (CV) study of [a] CHIT-CuO/ Au electrode [b] Lipase/CHIT-CuO/Au Bio electrode.

The CV investigation at various scan rate (10-70mV/S) have been performed for Lipase/Chit-CuO/Au bio electrode as shown in Figure 9.(A), indicating as we move towards higher scan rate peak current increases. The cathodic and anodic peak current found to be proportional to the square root of scan rate over the range (10-70mV/S) suggesting that it has controlled diffusion process (Figure 9.[B]), inset the figure (Figure 7.A), Linear sweep voltammetry of cathodic peak was carried out it perfectly indicate the current is proportional to current with increases in scan rate Figure 9. [C]), Peak current of bioelectrode proportional to scan rate according to equation (2) and (3).

Where, R is the gas constant (8.314Jmol^{-1}k^{-1}), T is temperature (300 K), n is the number of electron transfer (1), F is faraday constant (96485 J mol^{-1}K^{-1}), A is area of electrode (1cm^2) and C is the concentration of redox species in the electrolyte solution (5Mm), Heterogeneous electron transfer (Ke) values for chit-nano CuO/Au electrode and LIP/Chit-nano CuO/Au bioelectrode found to be 0.00166 Cm.s^{-1} and 0.00115 Cm.s$^{-1.}$ High value for bioelectrode shows indicating a faster electron exchange between the redox species.

Ic= 0.00166mA (mV/s) * Scan rate…….. (2)

Ia = 0.00132mA (mV/s) * Scan rate……. (3)

Redox peak current show linear behaviour with square root of scan rate ((Figure 9.B) revelling diffusion controlled process and follow equation (4) and (5)

I_c (A) =0.003(mA.V^{-1}.S^{-1}) +0.004(mA)* scan rate (m V /s), R^2=0.99……(4)

I_a (A) = -0.264 (mA.V^{-1}.S^{-1}) – 0.027(mA)* scan rate (mV /s), R^2=0.99…… (5)

The diffusion coefficient value (D) of redox probe [Fe(CN)6]$^{3-/4-}$ From electrolytic solution to corresponding to electrode surface has been calculated using Randles Sevcik equation [27] which is given as,

$$I_P = (2.69 * 10^5)n^{3/2}.A.D^{1/2}.C.V \quad \ldots\ldots\ldots\ldots (6)$$

Where, Ip is peak current of corresponding electrode, n is number of electron involved or electron stiochiometry (1), A is surface area of electrode (1cm^2), C is concentration (5Mm),v is scan rate (10 mV/s). The D value of Chit-nano CuO/Au electrode and LIP/Chit-nano CuO/Au bioelectrode was found to be 1.536*10^{-12} and 0.980*10^{-12} cm^{2}s^{-1} respectively. The higher value of D for Chit-nano CuO/Au electrode may have been due to better conductive nature of electrode compared to that of LIP/Chit-nano CuO/Au bioelectrode. The surface concentration of Lipase/Chit-CuO/Au bio electrode (1.55×10^{-3}mol/cm^2) estimated from plot of Ip vs. Scan rate (ŭ1/2)

$$I_{PC} = \frac{n^2F^2I^*AV}{4RT} \quad \ldots\ldots\ldots\ldots (7)$$

The reversibility of electron transfer kinetics did not depend on V; it also depends on standard heterogeneous electron transfer rate constant (ks) with Laviron model as given in equation.1.

$$Ks = mnFv/RT \quad \ldots\ldots\ldots\ldots (8)$$

Where, m is peak to peak separation of potential (V). The estimated value of Ks for Chit-nano CuO/Au electrode and LIP/Chit-nano CuO/Au bioelectrode indicated a fast electron transfer between the electrode surface and redox species. The value of Ks found to be 5.327 and 5.227 S^{-1}

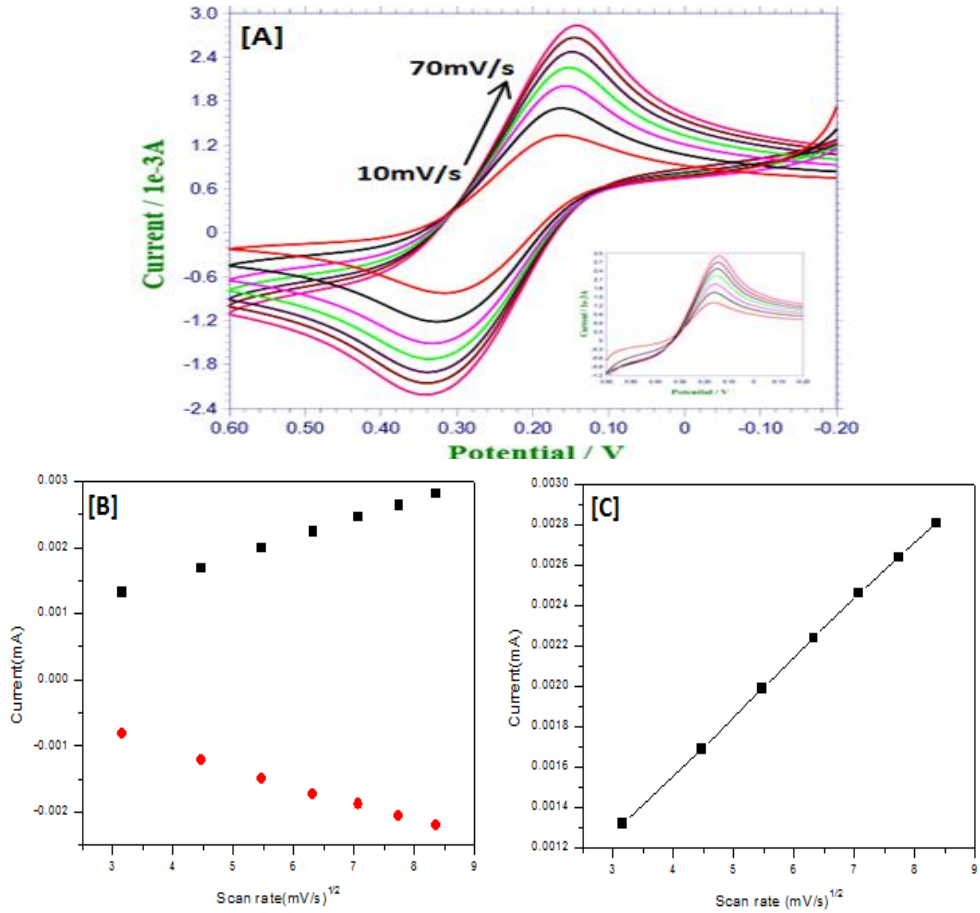

Fig.9.[A] Cyclic voltamogram of Lipase/Chit-CuO/Au Bio electrode at different scan rate (10-70 mV/S) and Linear sweep voltammetry inside [B]Redox current with respect to square root of scan rate of (10-70mV/s) [C] Linear sweep voltammetry of Cathodic peak current of Lipase/Chit-CuO/Au Bio-electrode(inset figure. [A] at scan rate (10-70mV/s)

Biosensing studies

Figure.10. [A] represent the CV studies of Lipase/CHIT-CuO/Au bio electrode carried out at different concentration of tributyrin (0.25-1.50mg/Ml) immersing the electrolyte in KCl (0.1M) containing [Fe $(CN)_6]^{3-/4-}$.it has been observed that there is a linear increase in the peak current with increase in analyte concentration indicating an increased in the charge transfer to the working electrode figure 10[B]. The mechanism involved in the detection of triglyceride using of Lipase/CHIT-CuO/Au bio electrode is shown in the figure.10[C]. LIP helps in the hydrolysis of tributyrin (triglyceride) which results in the production of fatty acid along with glycerol molecule. Sensitivity of prepared. Lipase/CHIT-CuO/Au bio electrode towards tributyrin is found to be 0.0006 mA (mg/mL) which is calculated from the slope of the plot of tributyrin concentration verses Current. The obtained sensitivity is much higher compared to other [28], with regression Coefficient (R^2=0.99) response time was found to be 2s revels the enhanced catalytic activity of enzyme on chit-nano CuO/Au electrode due to high charge transfer property of matrix. Detection limit (LOD) calculated for linear region using expression 3σ/sensitivity [29] is found to be 0.13 mg/mL where standard deviation for blank. The shelf life of bioelctrode has been monitored by measuring the electrochemical current response with regular interval of one week; it is observed that current response decreases about 4weeks. The reveal an affinity of enzyme (Lipase) for the substrate (tributyrin), enzyme substrate kinetics parameters has been observed. The value of apparent Michaelis-Menten constant (Km) for Lipase/CHIT-CuO/Au bio electrode calculated by using Line weaver–Burke plot (1/*I* versus 1/[C])

and K_m value has been found to be 1.76 mg/mL.This low value shows that a strong affinity between enzyme and substrate and the low value of km indicates enhanced affinity of lipase from Aspergillus Niger towards tributyrin.

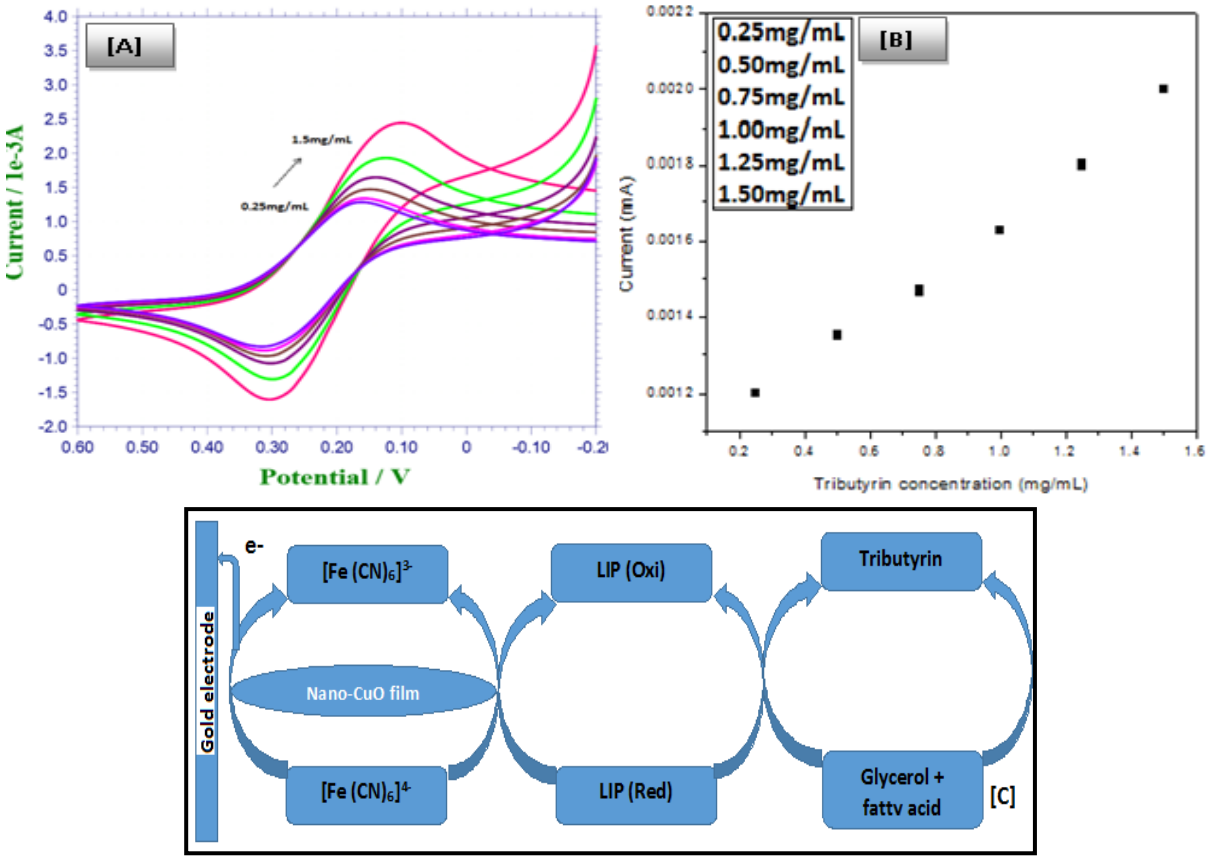

Fig.10 [A] Biosensing study of Lipase/CHIT-CuO/Au Bio electrode as a function of tributyrin concentration[0.25-1.50mg/mL [B] Calibration curve between current response and different concentration of tributyrin in KCl containing 5Mm $[Fe(CN)_6]^{3-}$[C] mechanism involved in the detection of triglyceride using of Lipase/CHIT-CuO/Au bio electrode

CONCLUSION

CuO nanoparticles (45 nm) successfully synthesized by Sol-gel citrate method and characterized by, X-Ray diffraction (XRD), Raman spectra, Fourier transform spectroscopy (FTIR), UV spectra, and Scanning electron microscopy (SEM).A successful immobilization of enzyme [Lipase] on the functionalized surface of Chitosan-CuO /Au electrode via physical adsorption was accomplished. Scanning electron microscopy of Chit-CuO/Au electrode and Lipase/Chit-CuO/Au bio electrode shows perfect immobilization of enzyme (Lipase).The electrochemical studies revealed that well defined Oxidation Reduction peaks are observed and also electrochemical impedance spectra shows good heterogeneous electron (Ke) behaviour. Lipase/Chit-CuO/Au bioelectrode bioelectrode shows improved biosensing characteristics like good linearity as 0.25-3mg/mL, low detection limit of 0.27 mg/dL, good response time, shelf life of 4 months, sensitivity of 0.0006 mA/mg mL with linear regression coefficient as 0.94 and standard deviation as 0.0004 mA/mg mL^{-1}. The low Km value obtained as 1.76 mg/mL indicates high affinity of Lipase/Chit-CuO/Au bio electrode bioelectrode for tributyrin

ACKNOWLEDGEMENT

We gratefully acknowledge for financial support from the Major Research project (MRP) [No.43-224/2014(SR)], University Grant Commission (UGC), New Delhi, and also Principal, Shri Shivaji Science College Amravati his kind cooperation during this research work

REFERENCES

1. Narang J, Chavhan N and Pundir, Construction of triglyceride biosensor based on nickel oxide-Chitosan Zinc oxide/Zinc hexacyano ferrate film. *International journal of Biological macromolecule* 2013; 60: 45-51.

2. Fernandez lafuente. R, Lipase from thermomyces lanuginose: uses and prospects as an industrial biocatalyst. *J. of molecular catalysis B: Enzymatic* 2010; 62 (3-4): 197-212.
3. Clellan M.C, Kessler for investigators D, A global analysis of technological change in health care: the case of heart attack. *Health aff, 1999*; *18* (3): 25-255.
4. Vijayalakshmi A, Tarunashree Y, Baruwati B, Manoram S, Narayana B, Johnson R and Rao N, Enzyme field effect transistor for estimation of triglyceride using magnetic nanoparticles. *Biosensor and Bioelectron* 2008; 23 (11):1708-1714.
5. Fossati. P, Triglyceride determined calorimetrically with an enzyme that produces hydrogen peroxide. *clin chemistry*, 1982; 28: 2077-80.
6. Nathell.L, Nathell.L, Almberg.P.M and Larsson.K, COPD diagnosis related to different guidelines and spirometry techniques. *Respir.Res*. 2007; 8: 89.
7. Caplan.L.A, Pesce.A.J, *Clinical chemistry theory, analysis and correlation Moshy C.V St. Louis*, 1989; 481
8. Fischbach Francis, *A manual of laboratory and diagnostic tests six Ed, Lippincott Williams and Wilkins, Philadelphia. 2000.*
9. Solanki P. R, Dhand C, Kaushik A, Anasari A. A, Sood K.N, Malhotra, Nanostructured cerium oxide film for triglyceride sensor. *Sens and Actuators B Chem 2009*; 141: 551-556.
10. Li. S. F, Fan. Y. H, Hu. R. F and Wu. W. T, pseudomonas cepacia lipase immobilized onto the electro spun PAN nanofibrous membrane for biodiesel production from soyabean oil. *J of molecular catalysis B: Enzymatic* 2011;72 (1-2): 40-45
11. Zhi.M, Xiang.C, Li.J, Wu.N, Nanostructured carbon metal oxide composite electrode for super capacitor: a review. *noscale* 2015;5(1): 72-88.
12. Okazaki.M, Komoriya.N, Tomoike.H, Inowe.N, Ttons, Hoosakis,J Chromatogram B. *Biomedical sci. Appl.*: 1998; 709: 179-87
13. Bhambim.M, Minakshi, Pundir.C.S, preparation of oxygen metre based biosensor for the determination of triglyceride in serum. *Sens.Tras*, 2006; 67: 561-7.
14. Kelly T.A, Kristan.G.D, Amperometric determination of glycerol and TG using an oxygen electrode. *Analyst*, 1984; 109: 453-6.
15. Reddy.R.R, Chadha.A, Bhattacharya.E, silicon based potentiometric triglyceride biosensor. Biosens Bioelectron.*Biosens.Bioelectron*, 2001; 16: 313-17.
16. Gowan Mc, M.W, Artiss .J.D, Strandbergh, Zak.B, A peroxidase coupled method for the colorimetric determination of serum triglyceride. *Clin.chem* 1983;29: 538-542.
17. Solanki P.R, Dhand.C, Kaushik.A, Ansari. A, Sood.K.N, Malhotra.B.D, Nanostructured Cerium oxide film for triglyceride sensor. Sensor *and actuators B: Chemical*, 2009; 141(2): 551-556.
18. PauliukaitP, Poherty.A.P, Murnghan.K.D, Brett. E.M, Application of room temperature ionic liquid to the development of electrochemical lipases bio sensing system for water insoluble analysis of analytes. *Electrochemical chemistry*, 2011; 656 (1-2): 96.
19. Kayani.zohara,Ali Yasmin,Kiran Faisa,Batool Iffat,Riaz Saira,Naseem Shahzad ,Fabrication of copper oxide nanoparticles by sol gel route.Material today proceding. 2(2015), 5446-5449.
20. Krajewska Barbara Membrane based processes performed with use of chitin/chitosan materials. *seperation and purification technology*. 2005; 41: 305-312.
21. Solanki .S, Pandey.C.M, Soni.A, Sumana.G, Biradar.A.M, An amperometric bienzymatic biosensor for thetriglyceridetributyrin using an indiumtin oxide electrode coated with electrophoretically deposited chitosan-wrapped nanozirconia. *MicrochimActa*, 2016; 183: 167-176.
22. J. F. Xu, W. Ji, Z. X. Shen et al., Raman spectra of CuO nanocrystals, "*Raman Spectroscopy*, 1999; 30, 5: 413–415.
23. Wang.N, He.H and Han.L, Room temperature preparation of cuprous oxide hollow microspheres by a facile wet-chemical approach. *Applied surface science*, 2010; 256: 7335-7338.
24. Swarnkar.R.K, Singh.S.C and Gopal.R, Optical characterization of copper oxide nanomaterial. *International conference on optics and photonics* 2009.
25. Zheng.L and Liu.X, Solution phase synthesis of CuO hierarchical nanosheets at near neutral PH and near room temperature. *Mater Lett*. 2007; 61: 2222-2226.
26. Kaushik.A, Khan.R, Solanki.P.R, Pandey.P, Alam.J, Ahmad.S and Malhotra.B.D,), Iron oxide nanoparticles – chitosan composite based glucose biosensor. *Biosens.Bioelectronics* 2008; 24(4):676-683.

27. Reichman.B, Bard.A.J and Laser.D, A digital simulation model for electrochemical processes Wo_3 electrode *of Electrochemical society*. 1980; 127: 647-.654.
28. Solanki.P.R, Dhand.C, Kaushik.A, Ansari.A, Sood.K.N, Malhotra.B.D, Nanostructured cerium oxide film for triglyceride sensor. *Sensors and actuators: Chemical*, 2009; 8, 141: 551-55.
29. Vidal JC, Garcia-Ruiz E, Castillo JR, Design of a multilayered cholesterol amperometric biosensor for preparation and use in flow system. *Electroanalysis*. 2001; 13(3): 229-235

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