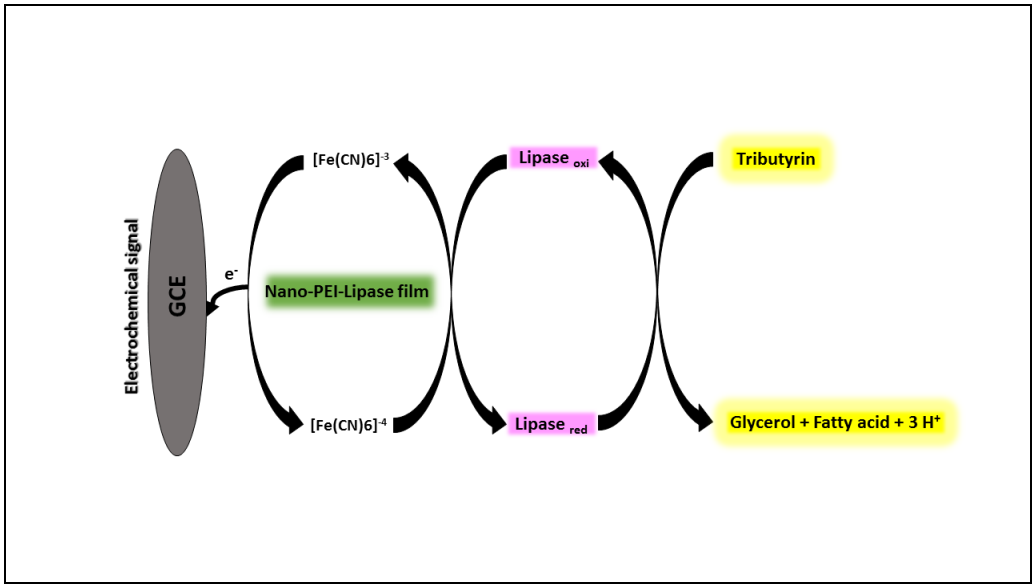
CHAPTER 3

DESIGN AND CHARACTERIZATION OF CONDUCTIVE NANO-PEI-LIPASE FILM BASED BIOSENSOR FOR EFFICIENT ELECTROCHEMICAL DETECTION OF TRIGLYCERIDES

Chapter 3. Design and Characterization of conductive Nano-PEI-lipase film for efficient electrochemical detection of triglycerides

Research Highlights:

- Novel Fabrication chemistry to coat the electrode and develop the electrochemical biosensor for detection of triglycerides.
- Covalent immobilization of Lipase enzyme onto the novel nano-polymer film.
- Linear range found for the triglyceride concertation is 100-500 mg/dL.



3.1. Introduction:

Triglycerides (TGAs) are natural fat esters having major role in metabolism, formed when a molecule of glycerol binds three molecules of fatty acids. TGAs serve as energy sources and transport the dietary fat in human body (Di Tocco et al., 2018). High concentration of triglycerides (TGA) is related with disease causing conditions such as lipid metabolism of various endocrine disorders, liver disfunction, diabetes, nephrosis and elevated risk of atherosclerosis. Thus, estimating triglyceride levels is considered important in routine medical check-up. Due to improved awareness for health and strict regulatory laws, triglyceride estimation in the food has become an important aspect in modern life (Dhand, Solanki, Sood, Datta, & Malhotra, 2009; Kartal, Kilinç, & Timur, 2007). Normal ranges of TGAs are 35-135 mg/dL and 40-160 mg/dL for women and men respectively. Standard methods for determination TGAs developed so far are enzyme based colorimetric, nuclear magnetic resonance, chromatographic, titrimetric, spectrophotometric and fluorometric methods (Di Tocco et al., 2018). The tests for the determination of TGAs are laborious, slow, complex, non-portable and costly. Moreover, most of these methods require sample pre-treatments to be carried out (Dhand et al., 2009). Thus, simpler, cheaper, portable, rapid and reliable methodologies should be developed to ease the testing procedures. Fabrication of electrochemical biosensors approaches to overcome all these limitations and make TGAs quantification cheaper, rapid and reliable (Di Tocco et al., 2018).

Electrochemical biosensors use lipase or multienzymes in combination with nanoparticles and polymer to detect TGAs. Lipases belong to hydrolases family and cause hydrolysis of esters producing fatty acids and glycerol. The mulienzyme reaction follows the steps given below:

$$\text{Triglyceride} + H_2O \xrightarrow{\text{Lipase}} \text{Glycerol} + 3\ \text{Fatty acids} + 3H^+ \quad \textbf{\textit{(Equation. 3.1)}}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol Kinase}} \text{Glycerol 3 - Phosphate} + \text{ADP} \quad \textbf{\textit{(Equation. 3.2)}}$$

$$\text{Glycerol 3 – Phosphate} + O_2 \xrightarrow{\text{Glycerol 3 – Phosphate oxidase}} H_2O_2 + \text{Dihydroxyacetone phosphate} \quad \textbf{\textit{(Equation. 3.3)}}$$

In multi enzyme TGAs biosensors, lipase in combination with glycerol kinase and glycerol-3-phosphate oxidase are used. Here, glycerol generated by lipase hydrolases is used by glycerol

kinase to convert it to glycerol-3-phosphate. Then, Glycerol-3-phosphate oxidase convert it to dihydroxyacetone phosphate and hydrogen peroxide. This hydrogen peroxide is oxidized to release oxygen and electrons which are measured by current change ***(Equation 3.1-3.3)*** (Dhand et al., 2009; Di Tocco et al., 2018; Kartal et al., 2007; Ono et al., 2019). Narang and Pundir in 2011 co-immobilized lipase, Glycerol 3- Phosphate oxidase and Glycerol kinase onto zinc oxide-chitosan nano-film. This whole film was coated onto platinum electrode and was characterized by cyclic voltammetry and electrochemical impedance spectroscopy against Ag/AgCl as reference electrode. The biosensors showed detection range and detection limit as 50-650 mg/dL and 20 mg/dL respectively at pH 7.5 (Narang & Pundir, 2011). Narwal and Pundir in 2017 coated commercially available nanoparticles of the enzymes lipase, glycerol kinase and glycerol 3-phosphate kinase onto pencil electrode for the estimation of triolein. As in the earlier study, the biosensor was characterized by cyclic voltammetry and impedance spectroscopy against Ag/AgCl as reference electrode at pH 7. The biosensor exhibited detection limit and linear range as 0.1 nM and 0.1-45 nM respectively. The sensor showed stability over 240 days having 80% of its original current (Narwal & Pundir, 2017). Release of fatty acid generated by lipase reaction causes changes in pH which can be detected by the 3 electrode system ***(Equation. 3.1)***. This kind of sensors uses only lipase enzyme rather than multi enzyme (Di Tocco et al., 2018). Solanki *et al*., in 2009 developed nanostructures cerium oxide film to develop lipase based biosensor. The researchers coated cerium oxide nano-film onto indium tin oxide glass plate. The nano-film was derived by sol-gel method. Lipase was immobilized on to the film for triglyceride detection assay. The fabricated electrode ITO/nano-CeO_2/lipase was characterized by scanning electrode microscope and cyclic voltammetry. The biosensor showed detection limit, linearity and shelf life as 32.8 mg/dL, 50-500 mg/dL and 84 days respectively (Solanki et al., 2009). Dhand *et al.*, in 2009 electrophoretically coated Polyaniline nanotube film onto indium tin oxide glass tube. Lipase was immobilized onto the film using glutaraldehyde by covalent bonding. Impedance was used to detect triglyceride into the samples. The sensors exhibited response time, sensitivity and linearity as 20 seconds, 2.59×10^{-3} $K\Omega^{-1}$ mg/dL, 25-300 mg/dL respectively (Dhand et al., 2009). Wu and his co-workers in 2014 modified a glassy carbon electrode (GCE) with conductive, biocompatible and nonporous gold composite (NPG) for the determination of tributyrin. For this purpose, the electrode was further followed by immobilization with lipase (GCE/NPG/lipase). The biosensor

showed shelf life with the detection limit and linear range as 2.68 mg/dL and 50-250 mg/dL respectively (Wu et al., 2014).

An alternative promising way of detecting TGAs is to measure glycerol produced by lipase activity. Glycerol has high oxidation potential and due to that its direct oxidation at bare working electrodes is not suitable for analytical applications. Development of metal nanoparticles such as copper, gold, rhodium, titanium oxide modified electrodes offer oxidation of alcohols and thus glycerol as well (Di Tocco et al., 2018). Tocco and his team immobilized lipase onto chitosan coated magnetic nanoparticles in combination with multiwalled carbon nanotubes, pectin, copper oxide nanoparticles on a glassy carbon electrode. The sensor thus developed hydrolyzed triglycerides from serum samples into fatty acids and glycerol. Glycerol oxidized in the reaction was measured by cyclic voltammetry and amperometry. The electrode showed detection limit from 3.2×10^{-3} g L^{-1} to 3.6×10^{-3} g L^{-1} (Di Tocco et al., 2018). Ono and his co-workers used nortropine-N-oxyl (NNO) to oxidize glycerol produced by lipase reaction with tributyrin on gold working electrode against Ag/AgCl as reference electrode. The proposed method could sense the concentration of tributyrin from 0.1 to 10 nM (Ono et al., 2019). Current TGAs biosensors still face problems like enzyme leaching, degradation, active site orientation as well as hindrance or less facilitation of electron transfer between the electrode surface and enzyme. These problems end up in affecting the performance of the sensor in terms of sensitivity, detection limits, shelf-life, specificity etc.

The performance of enzyme sensor and its stability are largely influenced by coating of the enzyme over the electrode surface. This can be achieved by either adsorption, covalent attachment or entrapment. Covalent attachment provides less chances of enzyme leaching and offers properly oriented catalytic sites for the reaction to take place (Ma, Cheong, Weng, Tan, & Shen, 2018). Use of nanotechnology in biosensor fabrication plays crucial role. Nanomaterials like carbon nanotubes, graphene oxides, metal nanoparticles in combination with polymers provide biocompatibility, higher sensitivity, more surface area, increased conductivity and mechanical strength, amenability for chemical modification and functionalization (Ma et al., 2018). This work reports novel fabrication chemistry to develop lipase-based triglyceride biosensor. Glassy carbon electrode was coated with reduced Graphene oxide (rGO), multi-walled carbon nanotubes (MWCNTs) and nanopolymer mixture of polyethylene imine (PEI)- titanium dioxide (TiO_2).

Glutaraldehyde(GA) was used to cross link both the polymer and lipase. Enzyme lipase hydrolyses the substrate tributyrin into glycerol and fatty acids and releases protons. The proton release causes changes in pH of electrolyte solution which then results in the increase of current. The magnitude of increase in the current was found to be proportional to triglyceride concentration. The sensor was exposed to various concentrations of triglyceride to measure its performance by cyclic voltammetry.

3.2. Experimental:

3.2.1. Materials and Methods:

Amano lipase PS, from *Burkholderia cepacia* (534641-10G) was purchased from Sigma Aldrich, India. Glycerol tributyrate was purchased from LOBA Chemie PVT. LTD., India. MWCNTs (size <100 nm and 5–8 μm) was purchased from Sigma Aldrich, India. Reduced graphene oxide or rGO (38818) and nano-TiO_2 suspension (SRL 94632-15 nm) were purchased from Sisco Research Laboratory, India. Polyethylenimine or PEI (P3143) and Glutaraldehyde (03965) were obtained from Sigma Aldrich and LOBA Chemie PVT. LTD., India respectively.

Glycerol tributyrate stock was prepared by mixing it in absolute ethanol to form 1g/ml stock solution and was stored at 4˚C. Triton X-100 was added along with glycerol tributyrate to a final concentration of 0.2% (w/v) in phosphate buffer system just before use. Lipase stock solution (20 mg/ml) was prepared in 0.1 M phosphate buffer or PB (pH 7.2) and was stored at 4˚C. Autoclaved double distilled water was used throughout. Potential used for cycling the voltage was -0.8 to +0.8 with the sample interval of 0.001 V at the scan rate of 80 mV/s. The electrolyte composition used during the experiments was 50 mM Phosphate buffer (pH 8.0) (9% NaCl + 50 mM $K_3[Fe(CN)_6]$). Groundnut oil was purchased from local market of Vadodara (Gujarat), India.

3.2.2. Apparatus:

FT-IR spectra were recorded using FT/IR-4700 type A (C019161788) (ATR PRO ONE-A062861809) spectrophotometer. All the electrochemical measurements were recorded by potentiostat CHI 660C (CH Instruments, Austin, TX, USA). The three electrodes configuration system containing a glassy carbon electrode (CHI 104), an Ag/AgCl reference electrode (CHI 111) and a platinum counter electrode was used. Ultrasonicator (Digital sonifier 450, Branson 50/60 HZ, USA) was used to disperse nanoparticles. Field emission gun scanning electron microscope (FE-SEM) JSM-7600F; Jeol, USA was used to study the surface of the coated electrode.

3.2.3. Fabrication of Lipase/Glutaraldehyde/PEI-TiO_2/MWCNTs/rGO/GCE electrode:

In present study, novel fabrication chemistry to coat the electrode combining the use of rGO, carboxylic acid modified MWCNTs and nano-TiO_2 dispersed into 0.03% (w/v) PEI was used to attach lipase enzyme for triglyceride detection. 0.03 % (w/v) of polyethyleneimine (PEI) stock solution was prepared to disperse titanium dioxide nanoparticles (TiO_2). 500 µL of readily purchased TiO_2 suspension was mixed with 500 µL of 0.03 % (w/v) of PEI and 500 µL of autoclaved double distilled water. The mixture was sonicated for 90 min. in ultrasonicator bath before use. 1 mg/mL stock of rGO was prepared by ultrasonicating it in autoclaved double distilled water for 1 h. 6 mg/mL stock of carboxylic acid modified MWCNTs was prepared by ultrasonicating in DMF (Dimethyl formamide) for 2 h. Amplitude used for ultrasonicating all the nanoparticles was kept same as 30% at 1 second intervals.

Before coating, the glassy carbon electrode (GCE) was polished with 0.3-0.05 µm alumina slurry followed by rinsing it in autoclaved double distilled water and ethanol. The polished electrode was then dried at room temperature before coating. 5 ul of rGO dispersion was drop casted on to GCE and was allowed to dry at room temperature for 15 min. It was then followed by 5 µL MWCNTs-COOH cast-off to the surface and dried at room temperature for 15 mins. Then, 5 µL TiO_2 nano-suspension prepared in polyethylenimine polymer was layered on to GO/MWCNT-COOH modified electrode surface. It was then allowed to dry at room temperature for 15 min. Then, 5 µL of lipase solution (0.1 M PBS, pH 7.2) (20 mg/mL) was layered on to it. The lipase was allowed to air dry at room temperature. Following it, 5 µL of 0.1% Glutaraldehyde as a cross linker was layered on to the surface. Change in the current at each step was also measured in the 10 mL system of the electrolyte prepared in 50 mM PB (pH 6.5) having 50mM $K_3[Fe(CN)_6]$ and 9% NaCl at 150 mV/s scan rate. Glutaraldehyde cross links both polyethylenimine polymer and lipase enzyme. The resulting electrode was washed with PBS (pH 6.5) and stored at 4°C in air. Fabrication of the electrode was confirmed initially by cyclic voltammetry (CV). The fabricated electrode with and without enzyme was also compared using CV. Other techniques such as FT-IR and FE-SEM were also used for confirming the fabrication chemistry.

3.3. Result and Discussion:

3.3.1. FT-IR studies:

FT-IR spectra of the nanopolymer films recorded with and without doping the lipase enzyme **(Figure 3.1)**. The strong vibrational peaks observed from 1000-1100 cm^{-1} correspond to C-C and C-N composite vibrations of the protein chains. The absorbance peak of the amide I region can be observed from 1600-1700 cm^{-1} due to the vibrational bending of C=O. The peak observed at 1670 cm^{-1} corresponds to β-turn of the enzyme. The less intense peak at 2060 cm^{-1} exhibits stretching of N=C between GA and lipase suggesting covalent binding between them. Strong peak observed at 1345 cm^{-1} corresponds to –OH bending of the lipase. C-H vibrations and imidazole ring stretching were observed at 2931, 2877 and 1462 cm^{-1} respectively. Thus, the FT-IR results confirm covalent attachment of the lipase onto the porous electrode surface (Cao et al., 2020; Ondul, Dizge, & Albayrak, 2012). Higher intensity of the peaks indicates high amount of enzyme loading onto the membrane surface.

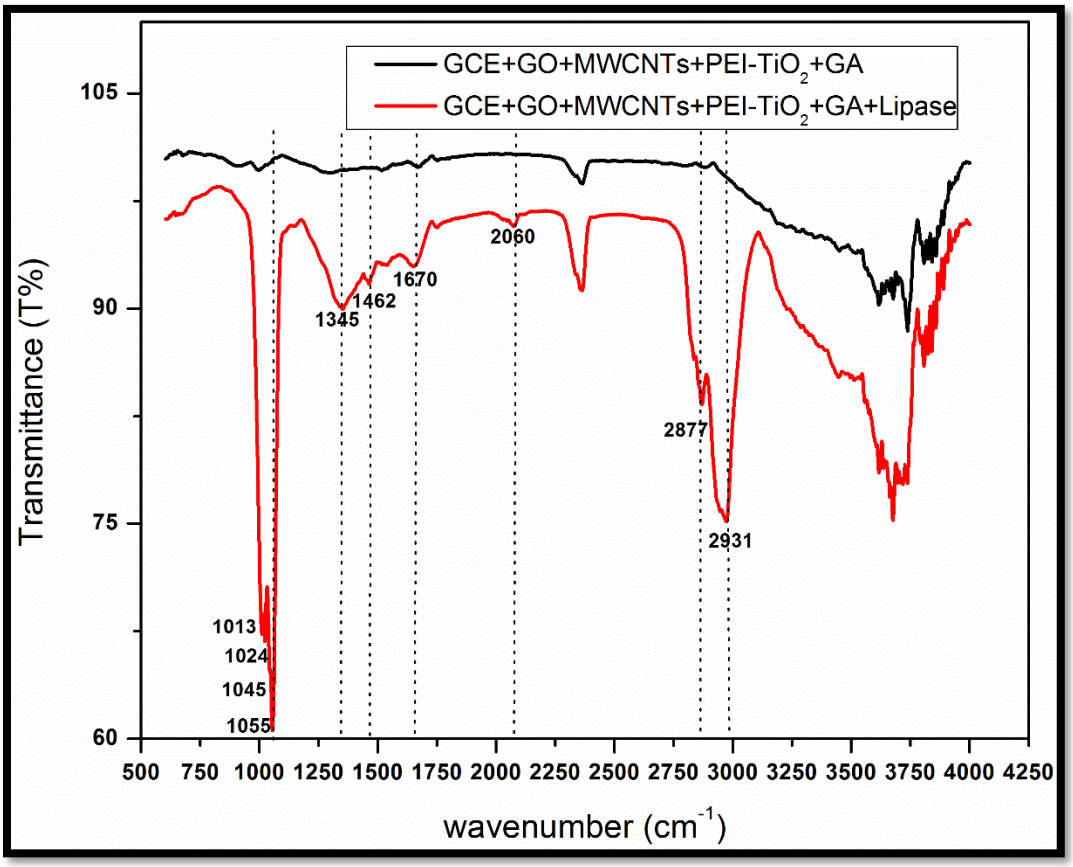


Figure 3.1. FT-IR Spectra of nano-polymer film with and without the enzyme lipase

3.2. Surface morphology studies:

Field emission gun scanning electron microscope was used to study the morphological features of the electrodes GCE/GO/MWCNTs, GCE/GO/MWCNTs/PEI-TiO_2/GA and GCE/GO/MWCNTs/PEI-TiO_2/GA/Lipase **(Figure 3.2 (a), (b) & (c))**. As evident from **Figure 3.2.**, at every step of electrode fabrication, the morphology of the surface undergoes change. In the first step of electrode modification, the electrode was first layered with GO, followed by layering with MWCNTs dispersion. Filamentous layer of MWCNTs can be seen in **Figure 3.2(a)**. Subsequently, porous structure of electrode surface was observed **(Figure 2(b))** when the electrode was layered with PEI-TiO_2 suspension. Then, **Figure 3.2(c)** shows the electrode with nano-polymer-enzyme film. It is evident from the **Figure 3.2(c)** that the enzyme was attached onto the surface of the electrode altering its previous surface morphology.

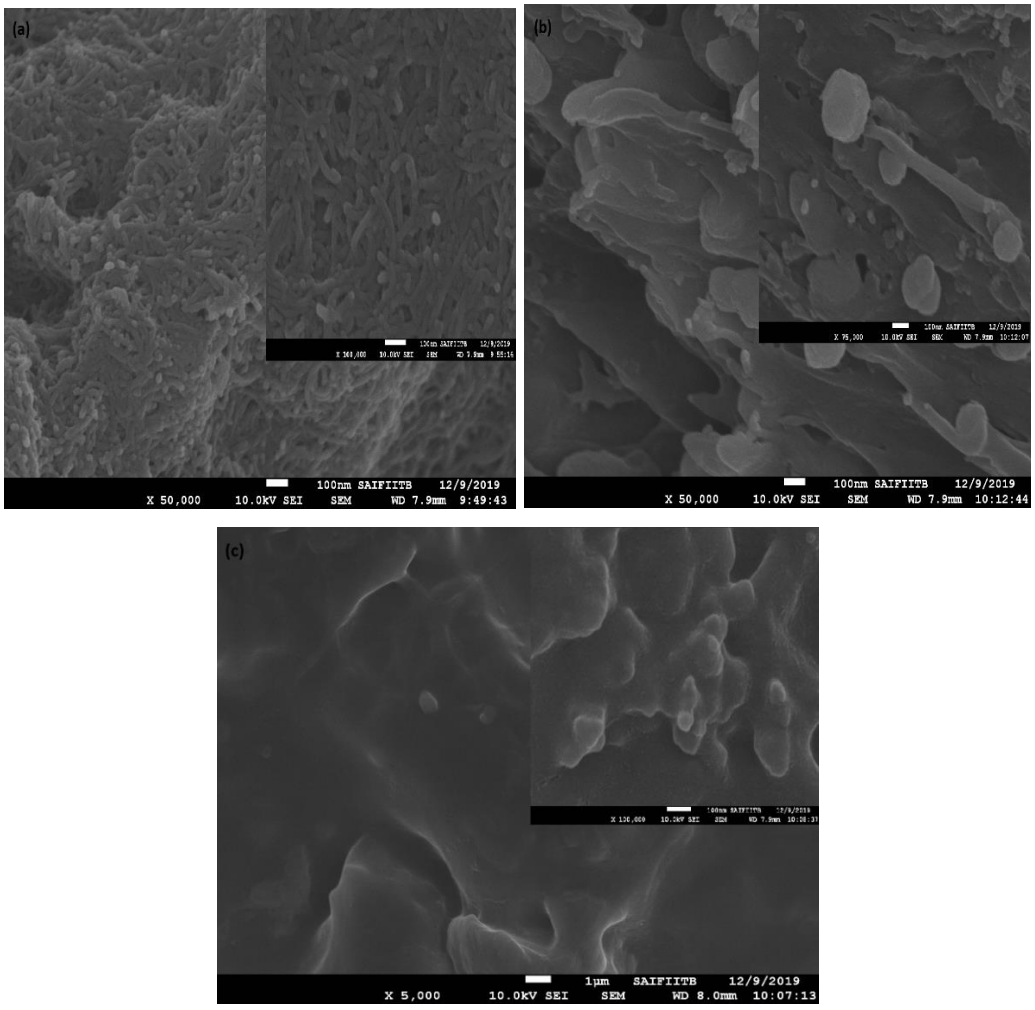


Figure 3.2. FE-SEM of GCE/GO/MWCNTs (a), GCE/GO/MWCNTs/PEI-TiO_2 (b), GCE/GO/MWCNTs/PEI-TiO_2/GA/Lipase (c)

3.3. Electrochemical studies of GCE/GO/MWCNTs/PEI-TiO_2/GA/Lipase:

Electrochemical properties of the bare GCE, GCE/GO/MWCNTs, GCE/GO/MWCNTs/PEI-TiO_2/GA and GCE/GO/MWCNTs/PEI-TiO_2/GA/Lipase were studied by running cyclic voltammetry in 50 mM PBS of pH 6.5 containing 50mM $K_3[Fe(CN)]_6$ and 9% NaCl at 150 mV/s scan rate. The magnitude of current increased after layering graphene oxide and MWCNTs. Higher electroactive surface offered by the nanoparticles on to the electrode resulted in enhanced electron transportation between the electrode surface and electrolyte. Following it, PEI-TiO_2 was layered on to the electrode and was cross-linked with glutaraldehyde. Current was found to decrease after coating with PEI-TiO_2/GA layer compared to GCE/GO/MWCNTs. The decrease in the magnitude of current shows PEI and GA causing insulating effect over the electrode surface. After that, when the electrode had lipase immobilized on to the surface through glutaraldehyde, it again showed further decrease current. Thus, PEI, GA and lipase here hinder the electron transfer involved in the redox process. The electrode GCE/GO/MWCNTs/PEI-TiO_2/GA/Lipase was fabricated in triplicates to check the sensor's replicability. Current response of each electrode was observed in 50 mM PBS (50mM $K_3[Fe(CN)_6]$, 9% NaCl, pH 6.5) at 150 mV/s scan rate. **Figure 3.4(a)** and **3.4(b)** explain the sensor can be replicated with the SD of 0.346 at its anodic peak.

The coated electrode GCE/GO/MWCNTs/PEI-TiO_2/GA/Lipase was then tested for different scan rates from 20-100 mV/s in 50 mM PBS (50mM $K_3[Fe(CN)_6]$, 9% NaCl, pH 5). It is evident from **Figure 3.5(a)** and **Figure 3.5(b)** that redox peak current magnitude depends on the scan rate. From **Figure 3.5(b)** showing the plot for square root of scan rate versus magnitude of redox peaks, the R^2 values for both the anodic and cathodic peaks were calculated and found to be 0.9932 and 0.9904 respectively. Thus, anodic peak showing more correlation will be considered for further experiments to estimate TGA concentration. Moreover, the ratio of cathodic and anodic peak current (Ipc/Ipa) approximates to 1. These results suggest that the electron transfer follows surface controlled process (Li et al., 2011; Liu, Lu, Li, Yao, & Li, 2007).

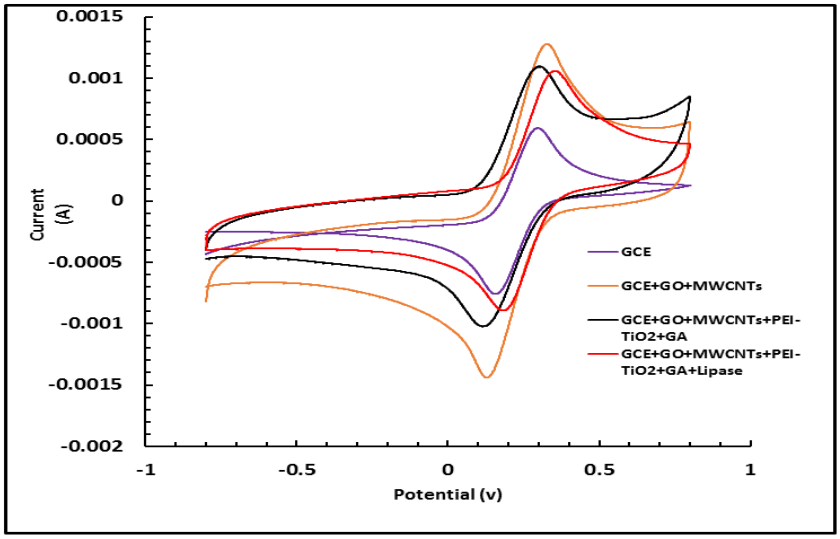


Figure 3.3. Cyclic voltammetry of bare GCE, GCE/GO/MWCNTs, GCE/GO/MWCNTs/PEI-TiO_2/GA and GCE/GO/MWCNTs/PEI-TiO_2/GA/Lipase in 10 ml system of the electrolyte prepared in 50 mM PB, pH 6.5, containing 50mM $K_3[Fe(CN)_6]$ and 9% NaCl at 150 mV/s scan rate.

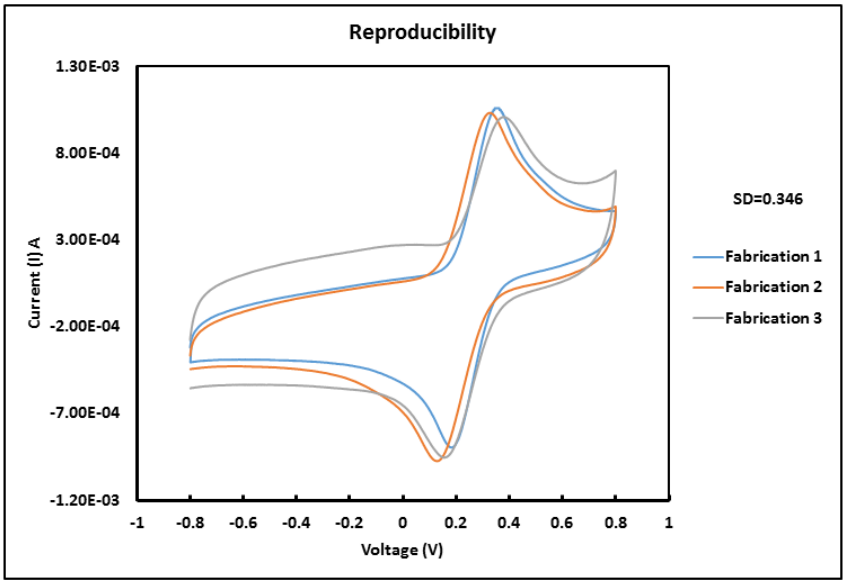


Figure 3.4(a) Cyclic voltammetric measurements of GCE/GO/MWCNTs/PEI-TiO_2/GA/Lipase electrode fabricated in triplicates in 50 mM PB, pH 6.5, containing 50mM $K_3[Fe(CN)_6]$ and 9% NaCl at 150 mV/s scan rate

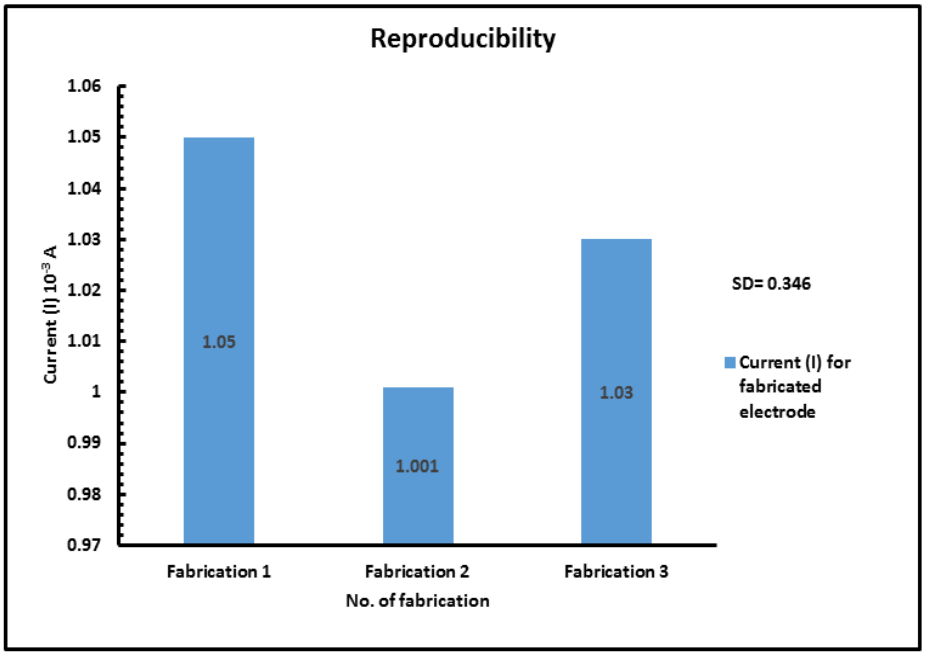


Figure 3.4(b) Anodic peak currents of GCE/GO/MWCNTs/PEI-TiO_2/GA/Lipase electrode in triplicates in 50 mM PB, pH 6.5, containing 50mM $K_3[Fe(CN)_6]$ and 9% NaCl at 150 mV/s scan rate

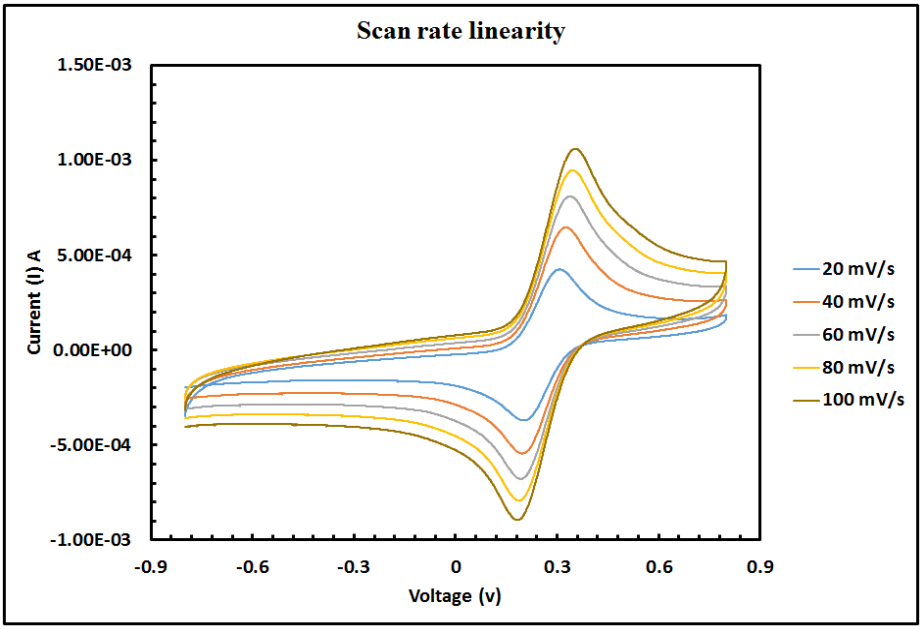


Figure 3.5(a): Cyclic voltammetry of GCE/GO/MWCNTs/PEI-TiO_2/GA/Lipase electrode at different scan rates from 20 mV/s to 100 mV/s in 50mM PB (50 mM $K_3[Fe(CN)_6]$, 9% NaCl, pH 5)

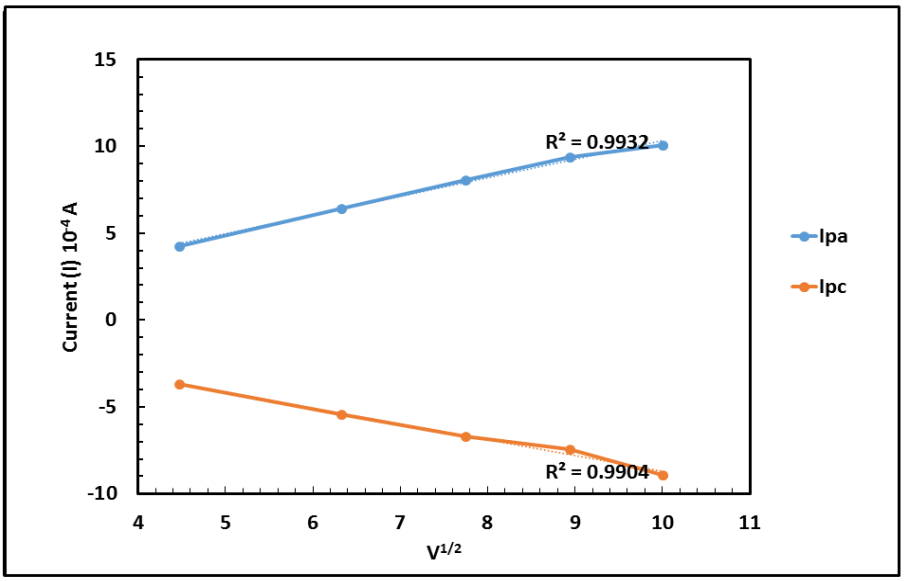


Figure 3.5(b) Square root of scan rate (20 mV/s to 100 mV/s) Vs current magnitude of GCE/GO/MWCNTs/PEI-TiO_2/GA/Lipase electrode in 50 mM PB, pH 6.5, containing 50mM $K_3[Fe(CN)_6]$ and 9% NaCl

3.4. Effect of pH on GCE/GO/MWCNTs/PEI-TiO_2/GA/Lipase:

The effect of pH on GCE/GO/MWCNTs/PEI-TiO_2/GA/Lipase was investigated by running cyclic voltammetry at a scan rate and sample interval of 80 mV/S and 0.001 V respectively. The electrolyte used was 50mM $K_3[Fe(CN)_6]$ and 9% NaCl in 50 mM PB (pH 5 to 8). It is clear from **Figure 3.6(a) and 3.6(b)** that the current peak of both anode and cathode decrease with increasing pH. This may be due to decrease of positive charge present on the electrode surface, leading to increased hindrance in electron transportation between the electrolytic medium and electrode. Thus, decreased value of electrochemical signal at elevated pH was observed (Dhand et al., 2009). This result confirmed that the electrode is sensitive to pH changes. During biochemical reaction, lipase hydrolyses triglyceride into fatty acid, glycerol and protons *(Equation i)*. These released protons would lower the pH of the medium and as a result the electrochemical signals of the biosensor would also change. **Figure 3.6(a) and 3.6(b)** show that the current was lowest at pH 8 for both the peaks. Thus, to have a positive correlation between the TGA concentration and current peak density, pH 8 was chosen as the optimized pH for the bioelectrode. All further measurements were taken at pH 8 and at anodic sweep segment of cyclic voltammetry (Dhand et al., 2009; Wu et al., 2014).

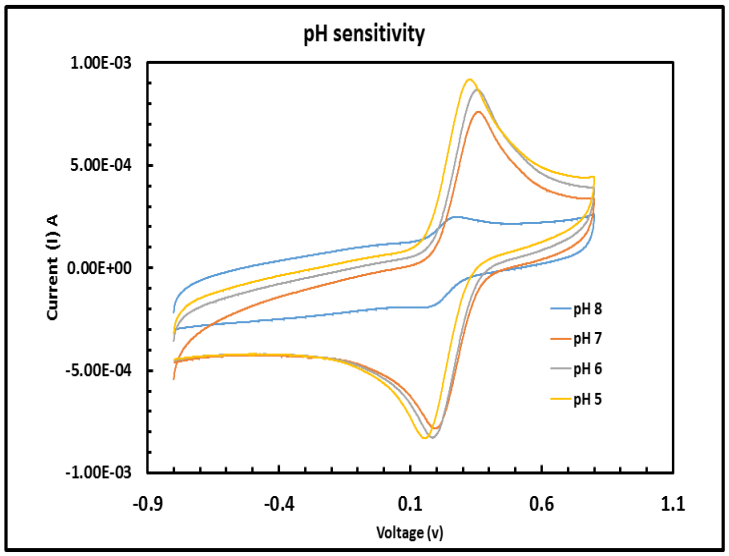


Figure 3.6(a) Cyclic voltammetry of GCE/GO/MWCNTs/PEI-TiO_2/GA/Lipase electrode as a function of pH in 50mM $K_3[Fe(CN)]_6$ and 9% NaCl in 50 mM PB (pH 5 to 8) at the scan rate of 80 mV/s.

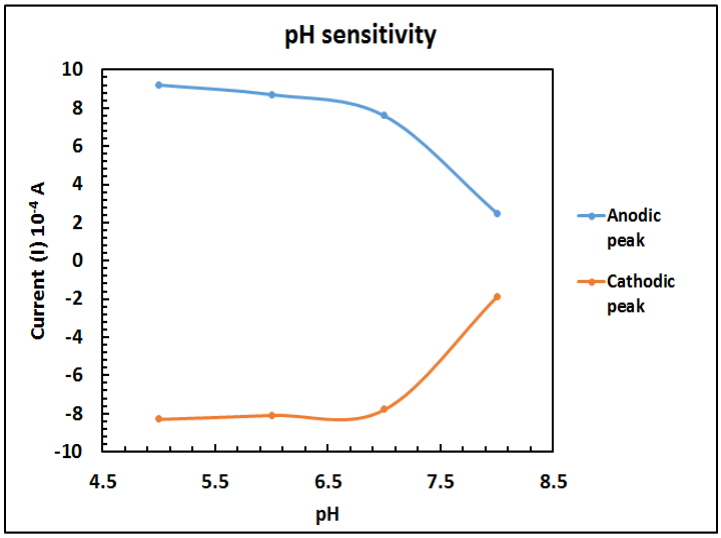


Figure 3.6(b) Effect of pH on the current density of both the peaks of GCE/GO/MWCNTs/PEI-TiO_2/GA/Lipase electrode

3.5. Cyclic voltammetric detection of triglyceride:

Cyclic voltammetric response at anodic sweep segment of the GCE/GO/MWCNTs/PEI-TiO_2/GA/Lipase bioelectrode as a function of triglyceride (TGA) concentration was recorded in 50mM $K_3[Fe(CN)_6]$ and 9% NaCl in 50 mM PB (pH 8) at the scan rate of 80 mV/s. It is evident from the **Figure 3.7(a)** that as the concertation of the TGA increases, anodic peak current

increases. This increase in magnitude of the current can be explained by the pH sensitive behavior of the bioelectrode. Protons are released during the enzymatic reaction by lipase which can alter the pH of the reaction system. This change in pH ultimately leads to change in magnitude of the current (Dhand et al., 2009). According to the **Figure 3.7(b)**, change in the anodic peak current magnitude with respect to the TGA concentrations follows linear regression model with the R^2 of 0.9485. The lower and higher detection limits found for TGA are 100 and 500 mg/dL respectively.

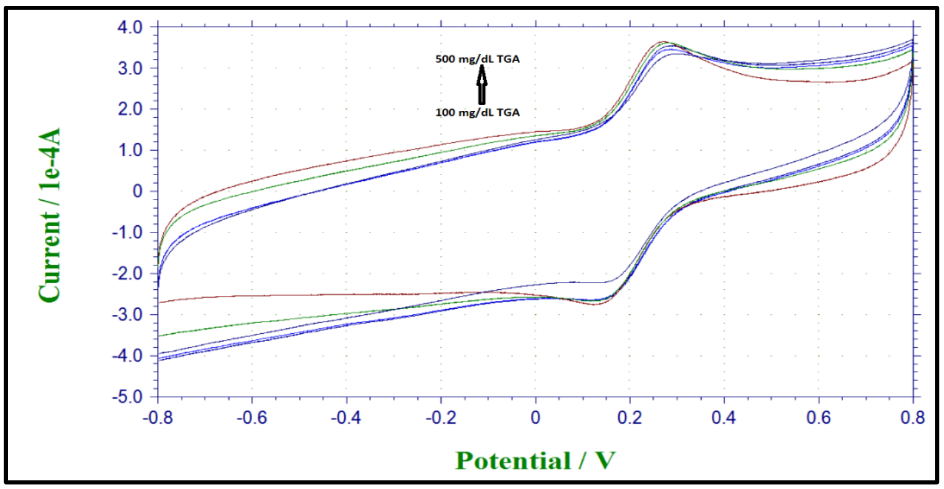


Figure 3.7(a) Anodic sweep segments of cyclic voltammetry of the electrode GCE/GO/MWCNTs/PEI-TiO_2/GA/Lipase as a function of TGA concentration at the scan rate 80 mV/s in 50mM PB ($K_3[Fe(CN)_6]$, 9% NaCl, pH 8) (100, 200, 300, 400, 500 mg/dL)

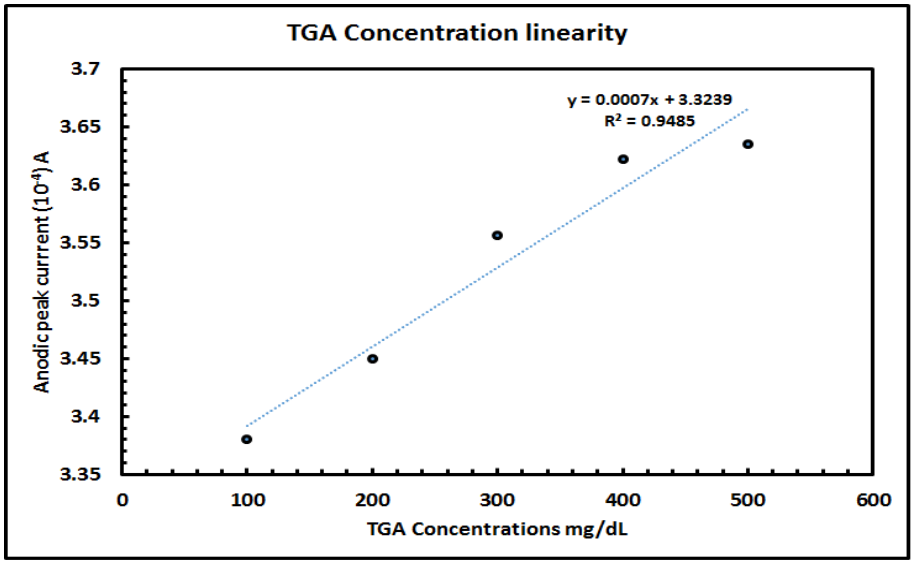


Figure 3.7(b) Linear range of the biosensor GCE/GO/MWCNTs/PEI-TiO_2/GA/Lipase for TGA detection (100-500 mg/dL)

3.6. Interference study:

Specificity of the biosensor is extremely important when it comes to practical use. Property of the enzyme decides the performance of the sensor. Though lipase is known to be highly specific for its substrate, its specificity in the presence of various interfering species was studied. 2 mM urea, 5 mM glucose, 0.05 mM cholesterol and 0.4 mM uric acid were added separately to 500 mg/dL TGA in 50mM PB (50 mM $K_3[Fe(CN)_6]$, 9% NaCl, pH 8). Interference in the anodic peak current due to each interfering species was checked by running anodic sweep segment of cyclic voltammetry. It is evident from **Figure 3.8(a) & 3.8(b)** that the magnitude of the anodic peak current varies from 100% to 99.03%, 101%, 97% and 98% when added glucose, cholesterol, uric acid and urea respectively. Thus, the performance of the biosensor seems to be negligibly affected in the presence of interfering species. This result confirms that the bioelectrode GCE/GO/MWCNTs/PEI-TiO_2/GA/Lipase has strong anti-interference characteristics.

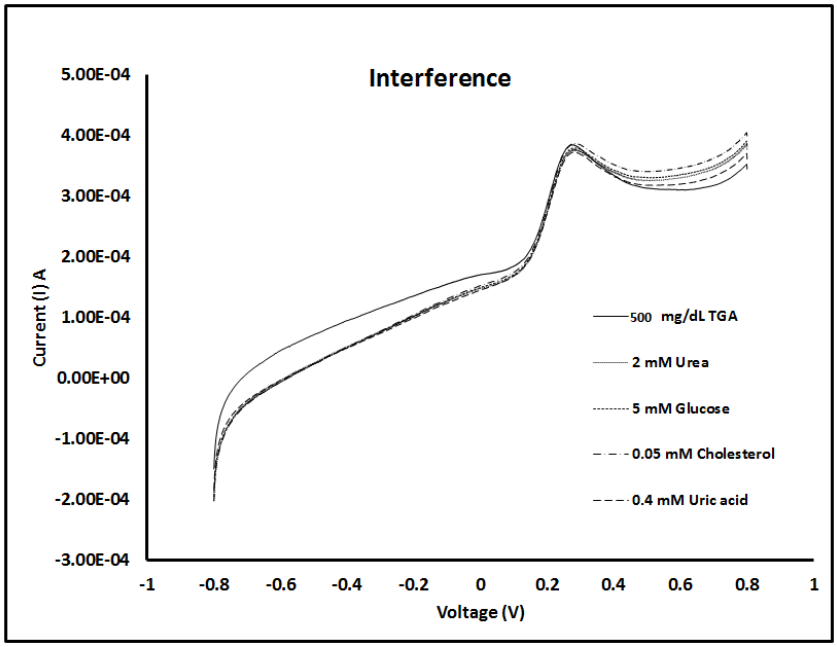


Figure 3.8(a) Anodic current changes at anodic sweep segments after adding interfering species in 500 mg/dL TGA at GCE/GO/MWCNTs/PEI-TiO_2/GA/Lipase bioelectrode

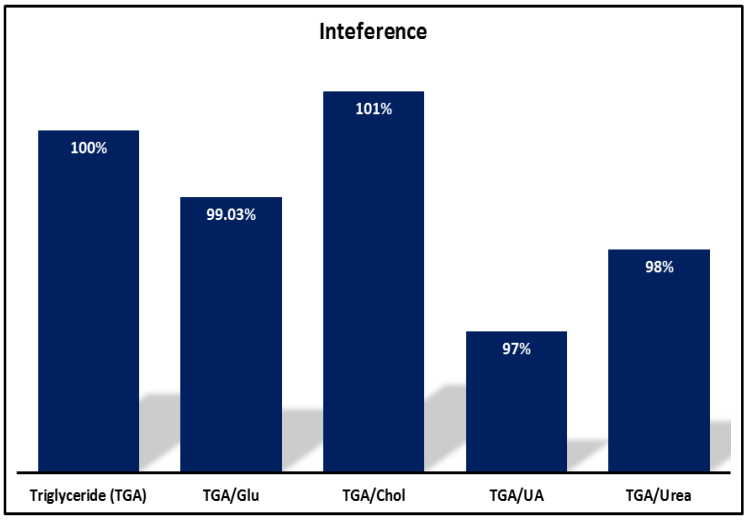


Figure 3.8(b) Effect on anodic current due to interfering species on GCE/GO/MWCNTs/PEI-TiO_2/GA/Lipase bioelectrode

3.7. Real sample detection:

To check the performance of the bioelectrode GCE/GO/MWCNTs/PEI-TiO_2/GA/Lipase for its practical use, the electrode was tested against locally manufactured ground nut oil (Gulab double filtered ground nut oil) mixed in 50mM PB ($K_3[Fe(CN)_6]$, 9% NaCl, pH 8). The samples were scanned at 80 mV/s scan rate. Stock solutions of the ground nut oil were made in ethanol and were dissolved into the reaction system just before use with the help of Triton X-100 (0.2 % W/V). The result shows relatable current change at anodic sweep segment of cyclic voltammetry **(Figure 3.9)**. Recovery of the real sample was found to be 91.94% for the ground nut oil 110 mg/dL **(Table 3.1)**.

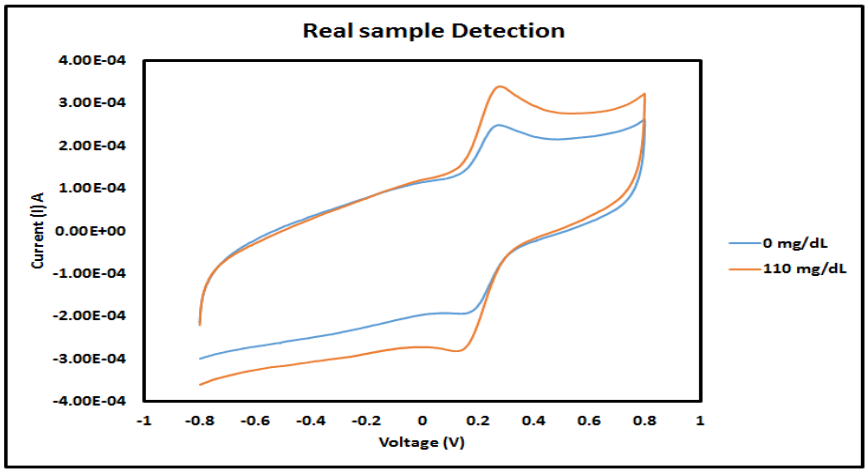


Figure 3.9. Real sample detection – ground nut oil

Triglycerides added (mg/dl) (ground nut oil)	**Triglycerides found (mg/dl) (ground nut oil)**	**Recovery**
110	**101.1428 ± 8.8572**	**91.94%**

Table 3.1. Real sample detection-Triglyceride determination

3.8. Conclusion:

In summary, nanoporous GCE/GO/MWCNTs/PEI-TiO_2/GA/Lipase bioelectrode was successfully fabricated using a novel chemistry for fabrication. This structure formed a good interface between active site of the enzyme and electrode surface. Enzyme lipase was immobilized on the PEI with glutaraldehyde covalent crosslinking. The electrode showed the linearity for triglyceride (TGA) from 100 mg/dL to 500 mg/dL with higher detection limit of 500 mg/dL. The electrode was checked for its specificity in the presence of various interfering species. The bioelectrode showed relatively good specificity for the triglycerides. The bioelectrode was checked against the real sample as ground nut oil for its practical application. The bioelectrode can be replicated with the SD of 0.346. The biosensor is expected to serve as promising tool to detect triglycerides. It will be interesting to immobilize other enzymes to develop biosensors for different metabolites and compounds with the same fabrication chemistry.