

## **CHAPTER 6**

---

---

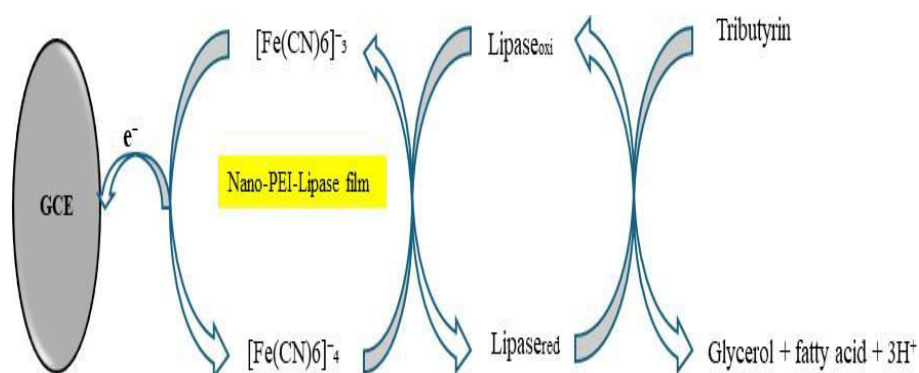
# **TESTING OF TRIGLYCERIDE REAL SAMPLES USING CONDUCTIVE NANO-PEI-LIPASE FILM BASED BIOSENSOR**

---

**Research highlights:**

- Real sample detection using previously made TGA biosensor.
- Successfully detected TGA in real samples, coconut oil, sunflower oil and groundnut oil by cyclic voltammetry.

**Graphical abstract:**



## 6.1 Introduction:

Triglycerides (TG) are the products of the chemical reaction between fatty acids and glycerol that serve a crucial role in energy metabolism and fat transportation within the human body. The typical range of TG in human blood and serum is between 150–190 mg/dl. Elevated TG levels in the blood can indicate various health conditions such as nephritic syndrome, chronic hepatitis, pancreatitis, liver disease, hypothyroidism, hyperlipidemia, diabetes mellitus, and alcoholism (Pundir et al., 2017).

The common techniques used for measuring glycerol are liquid chromatography and spectrophotometric analysis, which rely on chemical and enzymatic oxidase–peroxidase method respectively (Fossati et al., 1982). Both the methods have drawbacks, as the methods are hard, slow, complicated, not easy to move around, and cost a lot. Also, many times need extra steps before testing (Dhand et al., 2009). So, we need simpler, cheaper, amenable, quick, and trustworthy ways to do these tests. Making electrochemical biosensors seems like a good alternative to solve these problems and make measuring TGAs cheaper, quicker, and reliable (Tocco et al., 2018).

The main objective of the study was to test the application of the TGA biosensor developed earlier, for the analysis of real samples. This biosensor was designed for the electrochemical detection of triglycerides (TGA). To make it work better, the research used a combination of reduced graphene oxide nanoparticles, carbon nanotubes, titanium oxide nanoparticles, and polyethyleneimine.

The study previously done used a novel fabrication chemistry for designing the TGA sensing electrode. The materials used for the preparation of the electrode comprised of rGO, carboxylic acid modified MWCNTs, and nano-TiO<sub>2</sub> dispersed into 0.03 % (w/v) PEI. The nanoparticles were covalently attached to the modified MWCNTs for triglyceride detection. The glassy carbon electrode was polished, then coated with rGO dispersion, MWCNTs-COOH, TiO<sub>2</sub> nano- suspension, lipase solution, and 0.1 % glutaraldehyde was used as a crosslinker. The electrode was then washed with PBS and stored at 4°C in air. The electrode demonstrated a linear response for triglyceride (TGA) concentrations ranging from 100 mg/dL to 500 mg/dL, with a detection limit extending up to 500 mg/dL. To check its specificity, the electrode was tested in the presence of different interfering substances. The bioelectrode exhibited notable specificity for triglycerides. The fabrication process was optimized to increase the amount of enzyme on the

sensor, which in turn improved the efficiency and sensitivity for detecting triglycerides. The results obtained using cyclic voltammetry were tested and standardized. However, the analysis with real samples was not completed yet. In the present study, the study with the electrode was completed using sunflower oil, coconut oil, and groundnut oil as real samples (Jinal et al., 2023).

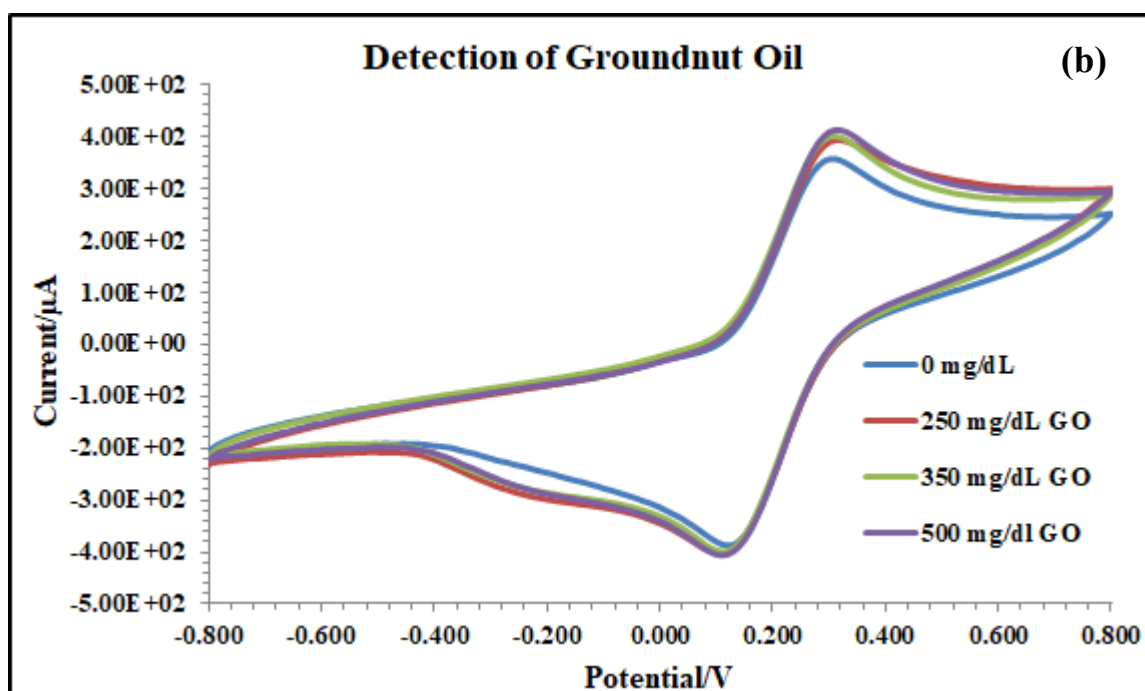
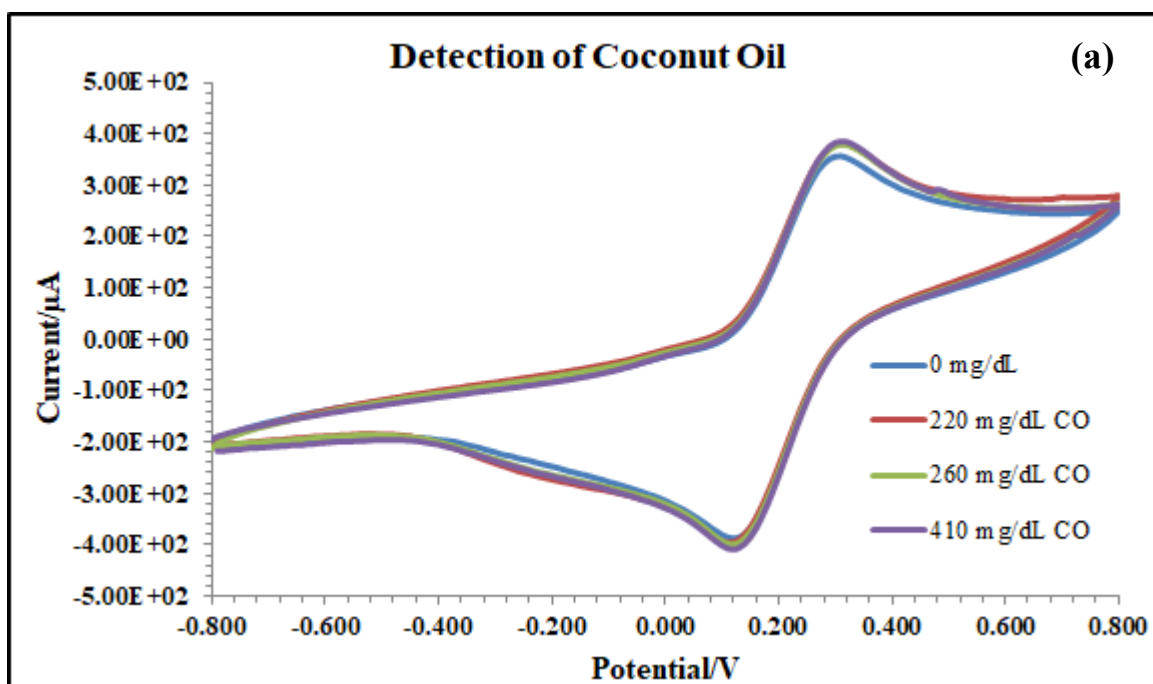
## 6.2 Materials and methods:

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  $\mu\text{m}$ ) was purchased from Sigma Aldrich, India. Reduced graphene oxide or rGO (38818) and nano-TiO<sub>2</sub> 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. Sunflower oil, coconut oil, and groundnut oil were purchased from a local market of Vadodara, Gujarat.

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<sub>3</sub>[Fe(CN)<sub>6</sub>]. Groundnut, coconut and sunflower oils were purchased from local market of Vadodara (Gujarat), India

## 6.3 Results and Discussion:

To check the performance of the bioelectrode GCE/GO/MWCNTs/ PEI-TiO<sub>2</sub>/GA/Lipase for its practical use, the electrode was tested against commercially available double-filtered ground nut oil, coconut oil and sunflower oil. The oils were separately mixed in 50 mM PB, pH 8.0 containing K<sub>3</sub>[Fe(CN)<sub>6</sub>], 9 % NaCl. The samples were scanned at an 80 mV/s scan rate. Stock solutions of the ground nut oil, coconut oil and sunflower oil were made in ethanol and were dissolved at three different concentrations as shown in Table 1, into the reaction system just before use with the help of Triton X-100 (0.2 % W/V). The results showed relatable current change at the anodic sweep segment of cyclic voltammetry. The average recovery of the real sample calculated using linear equation  $y = 0.0007X + 3.3239$  and current was found 101.05 %, 98.03 % and 95.47 % with coconut oil, groundnut oil and sunflower oil respectively (Table 1 and Fig. 1(a), (b) and (c)).



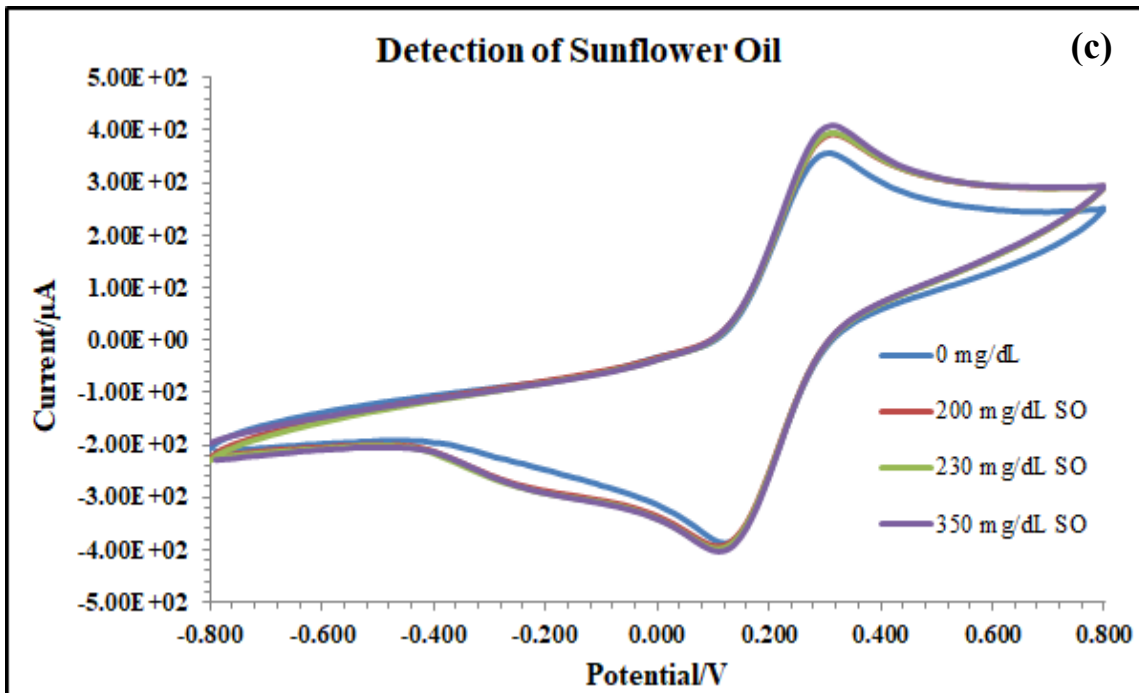


Figure 6.1 Real sample detection. Real samples coconut oil (a), groundnut oil (b) and sunflower oil (c) were used in the study at three different concentrations (n=3).

Sample	Triglycerides added (mg/dL)	Triglycerides found (mg/dL)	Recovery
Coconut Oil (CO)	220	222.857 ± 1.29	101.29%
	260	265.71 ± 2.2	102.2%
	410	408.57 ± 0.35	99.65%
Ground nut Oil (GO)	250	251.42 ± 0.56	100.56%
	350	351.4 ± 0.4	100.4%
	500	465.71 ± 6.86	93.14%
Sunflower Oil (SO)	200	180.0 ± 10.0	90%
	230	222.86 ± 3.98	96.02%
	350	351.428 ± 0.4	100.4%

Table 6.1: Detection of real samples - TGA (Triglycerides)

#### **6.4 Conclusion:**

In summary, GCE/GO/MWCNTs/PEI-TiO<sub>2</sub>/GA/Lipase bioelectrode was checked against the real sample of ground nut oil, coconut oil and sunflower oil for its practical application. This biosensor is expected to serve as an effective tool for the detection of triglycerides. Further, the fabrication chemistry is promising for developing biosensors of different metabolites and compounds using other enzymes.