MINISTRY OF FOOD PROCESSING INDUSTRIES
GOVERNMENT OF INDIA

IIFPT

Indian Institute of Food Processing Technology

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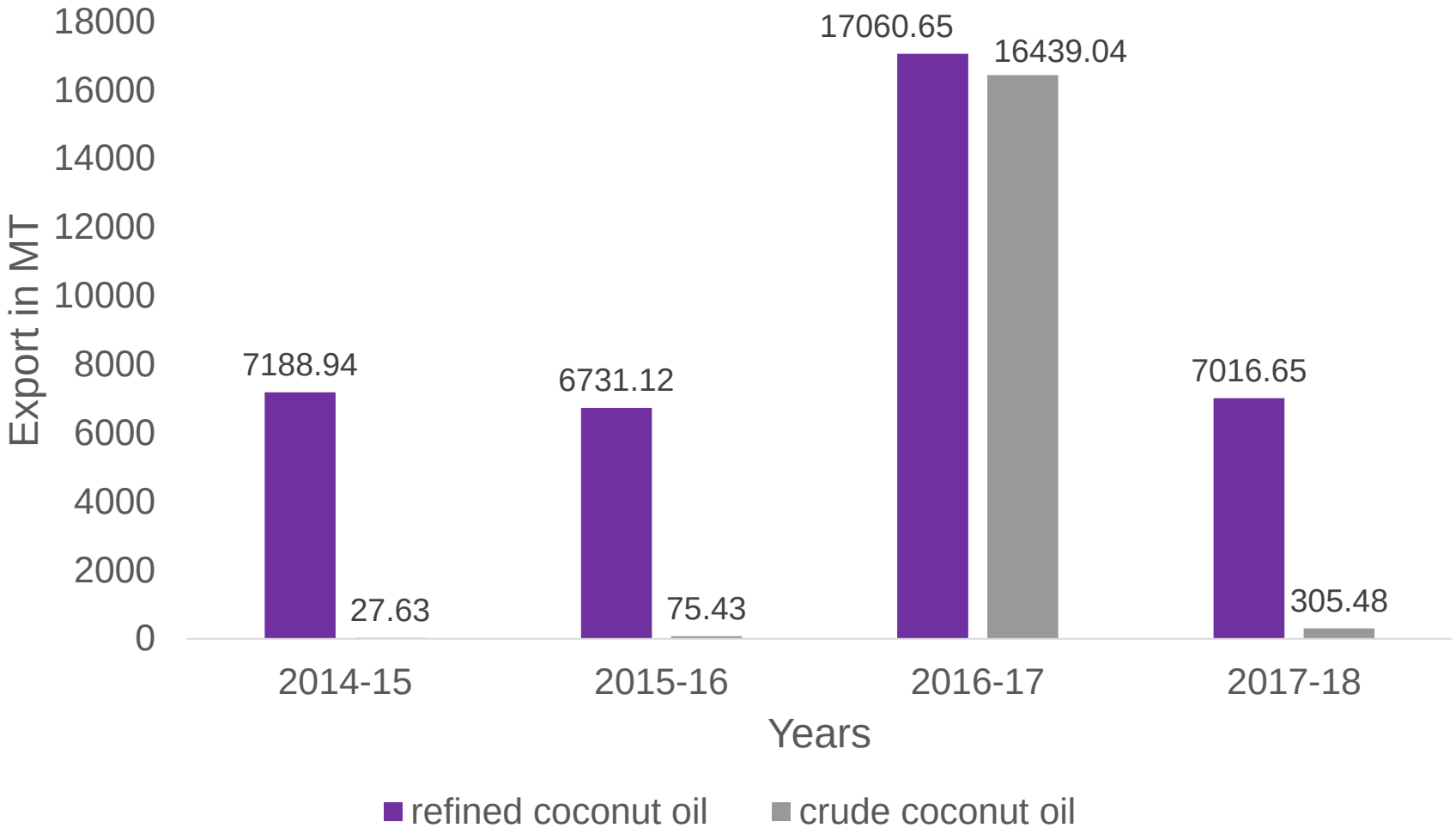
Development of amperometric multi-enzyme biosensor to evaluate the adulteration in Virgin coconut oil(VCO)

Coconut oil

- Coconut oil (CO) or copra oil is an edible oil extracted from the kernel or meat of the mature coconut (*Cocos nucifera*).
- It is extensively used for food and industrial purposes.

- Elevated cholestrol level
- High blood pressure
- Cardiovascular diseases

Global export scenario of coconut oil



Virgin Coconut Oil (VCO)

- VCO is quoted as **“healthiest oil in the world”** due to its medium chain fatty acids 48-53% of lauric acid. (Espino, 2006).
- VCO is growing in popularity as nutritional supplements and functional food (Villarino *et al.*, 2007).
- VCO is extracted from fresh coconut milk obtained from matured coconut of 12 months old by mechanical or natural means .

Why VCO is Adulterated?

- VCO has its own beneficial nature but its price is 10-20 times higher than coconut, corn, palm and sunflower oils (Rohman *et al.*, 2011).
- Some common adulterants of virgin coconut oil are palm kernel oil, palm oil, sunflower oil, corn oil, coconut oil, etc.
- In market coconut oil and virgin coconut oil is hard to differentiate.

Parameter	Virgin Coconut Oil	Coconut oil
Lauric acid(%)	48-53%	42-45%
Phenolic compounds	7.78 -29 mg	6.14 -28 mg
Diglycerides	**1.55 ppm**	**4.10ppm**

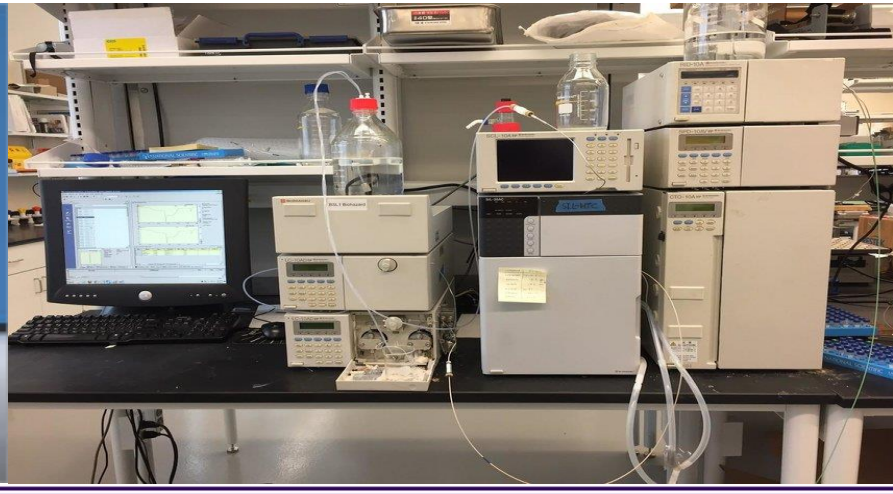
(Dayrit et al., 2011)

Existing methods of detection of diglyceride

- Existing techniques used are High Performance Liquid Chromatography (HPLC), Nuclear Magnetic Resonance (NMR), Fourier-transform infrared spectroscopy (FT-IR).

Disadvantages:

- Costly as well as laborious methods
- No simple/portable instrument



Fabrication and Assembly of Biosensor for VCO

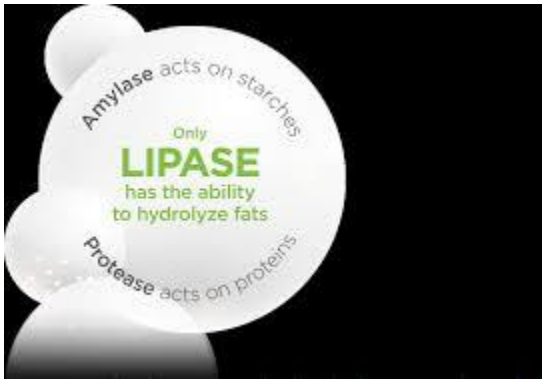
Mechanism

Diglyceride $+2H_2o$ —*lipase*→ Glycerol + fatty acid

Glycerol + ATP —*Glycerol kinase, $Mgcl_2$*→ Glycerol-3-phosphate +ADP

Glycerol3-phosphate $+O_2$ $+H_2O_2$ —*Glycerol-3-phosphate oxidase*→ dihydroxyacetone

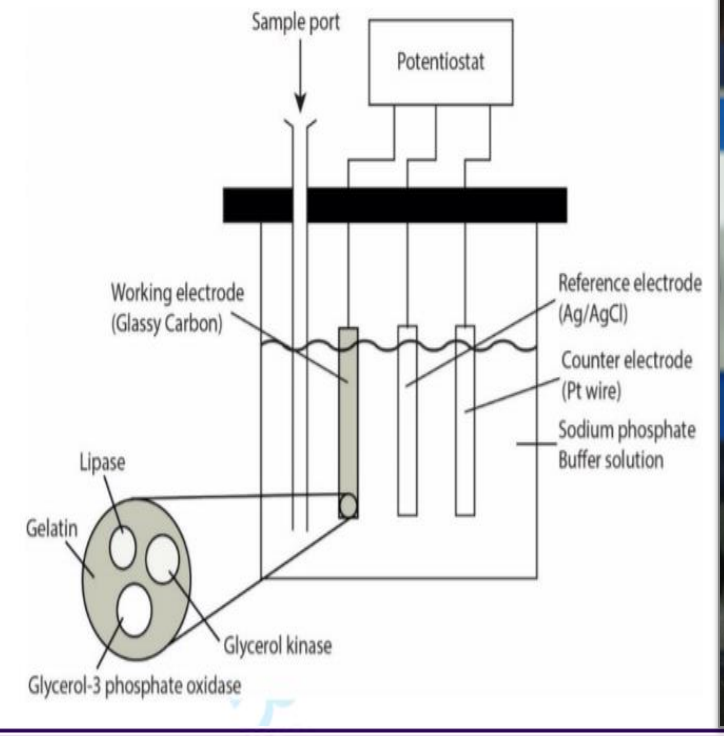
H_2O_2 → $2H^+ + O_2 + 2e^-$

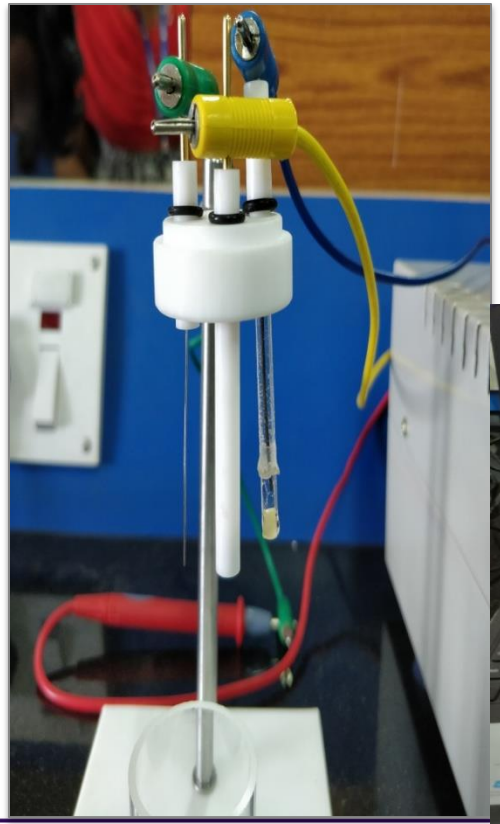


(Dayrit et al., 2011)

Electrode system and Setup

- Working electrode = Glassy carbon electrode
- Reference electrode= Ag/AgCl electrode
- Counter electrode= Pt wire electrode





Methodology

Preparation of 0.1M Sodium Phosphate Buffer

Preparation of gelatin membrane solution

Preparation of enzyme mixture(-20ºC)

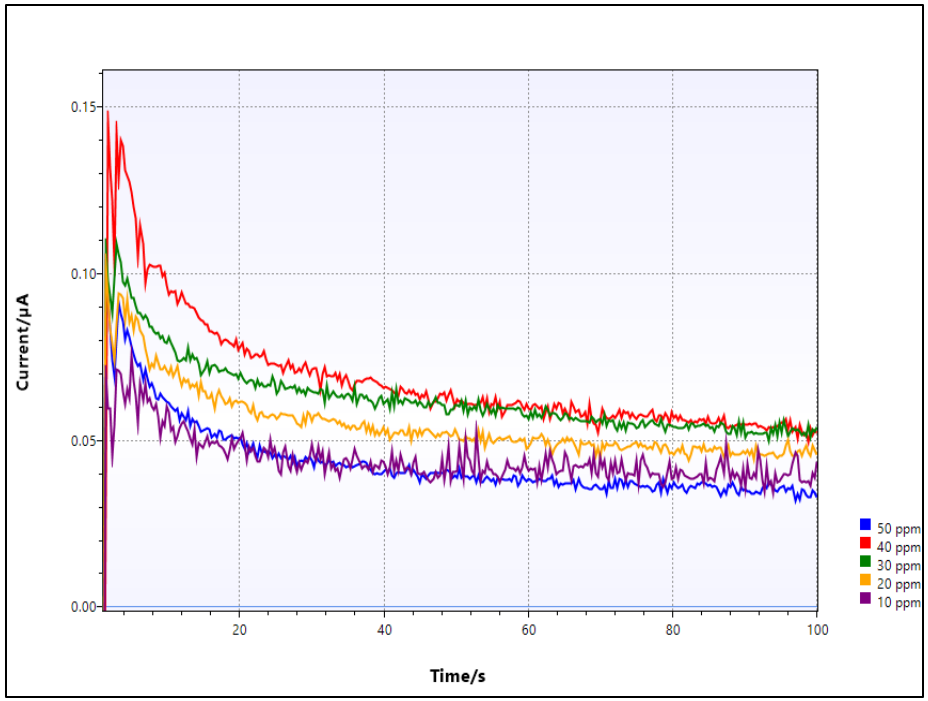
Dipping of working electrode in enzyme mixture containing membrane

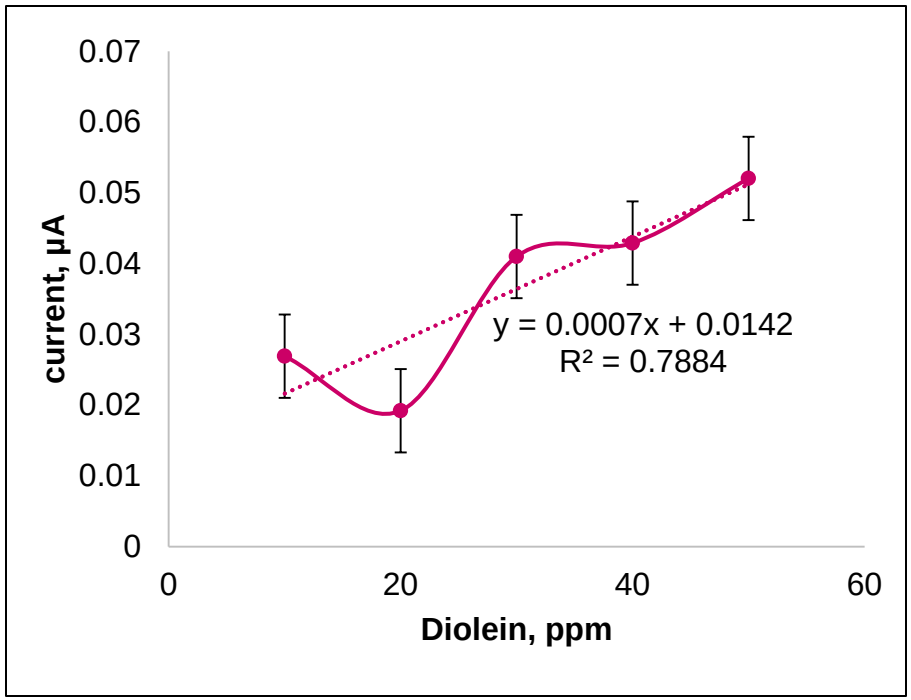
Dipping of working electrode in gluteraldehyde

Readings taken

Standardization of the immobilization technique

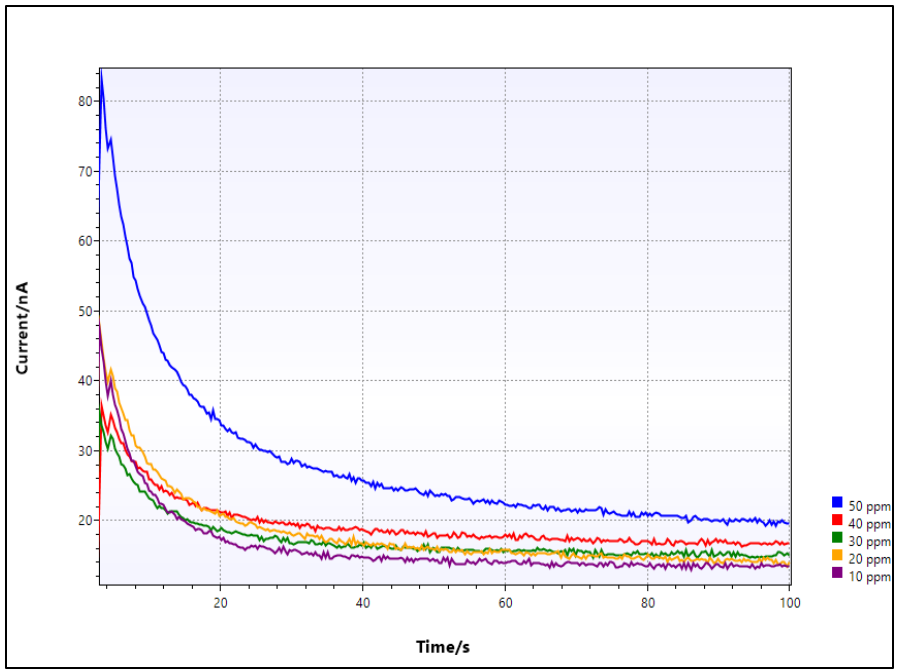
a. Physical adsorption

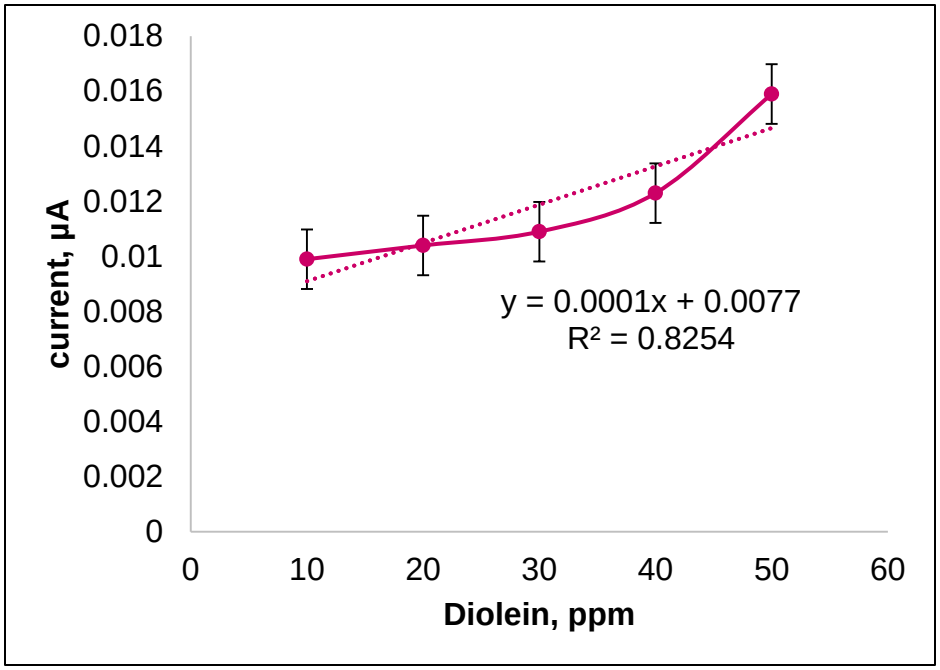




Standardization of the immobilization technique

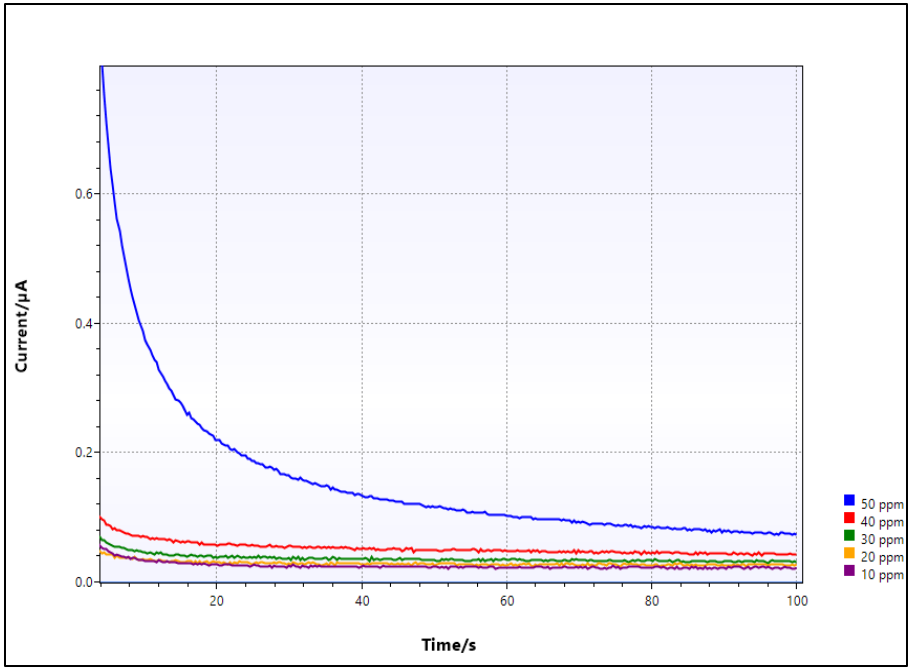
b. Layer by Layer Deposition

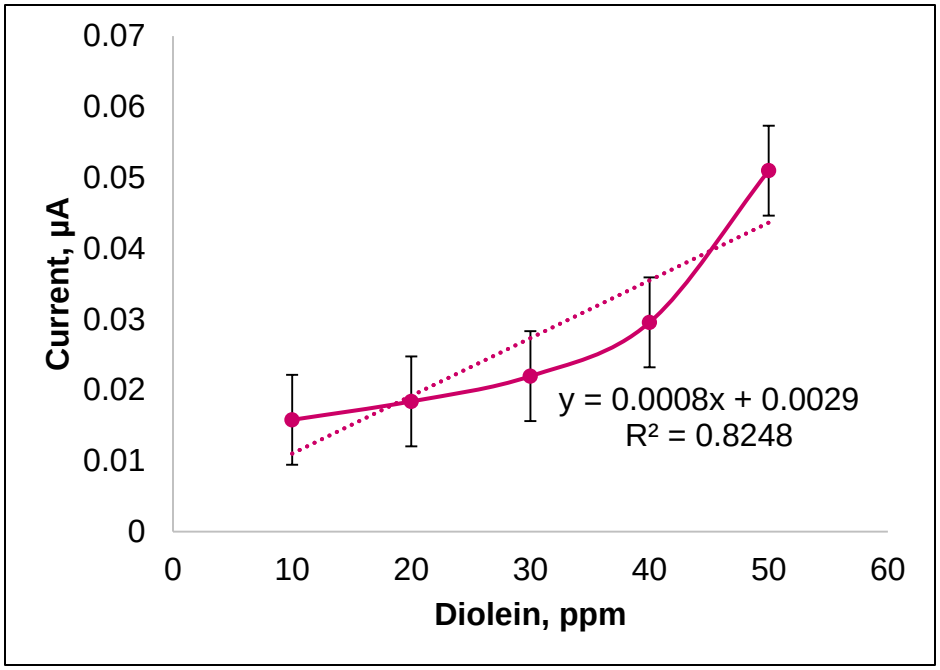




Standardization of the immobilization technique

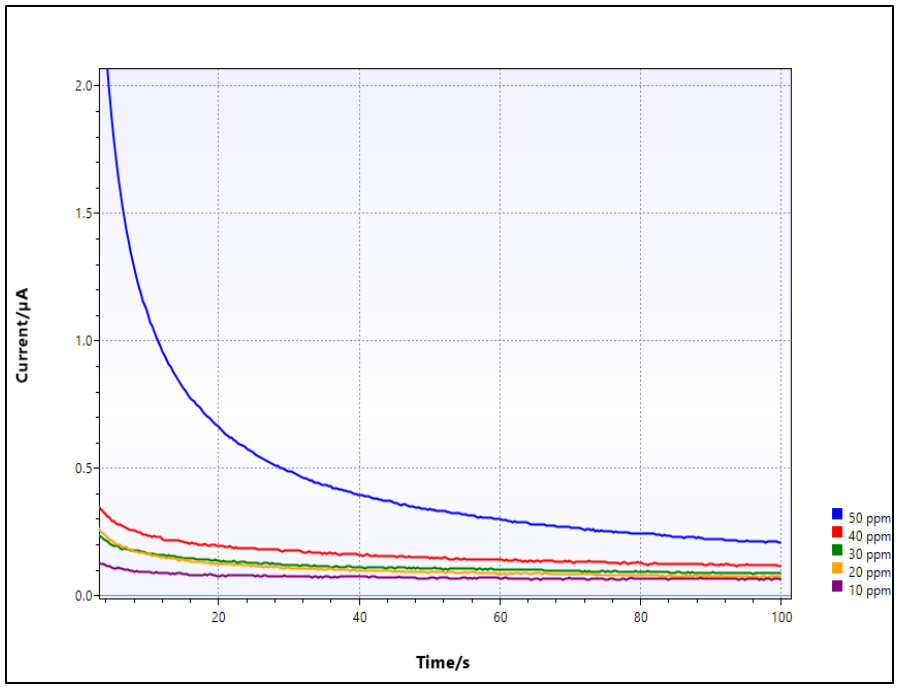
c. Covalent Bonding

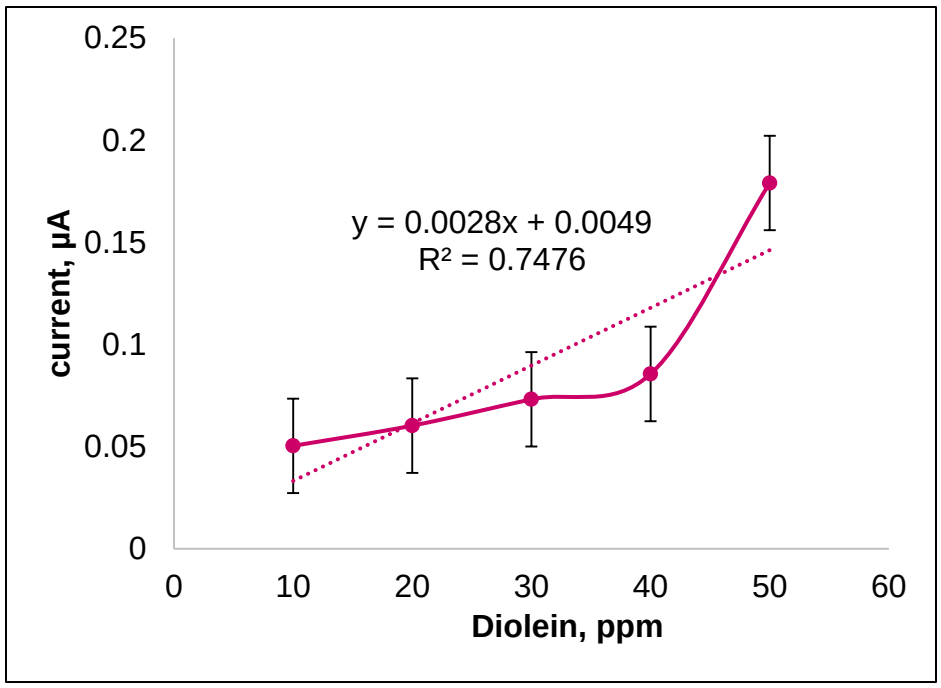




Standardization of the immobilization technique

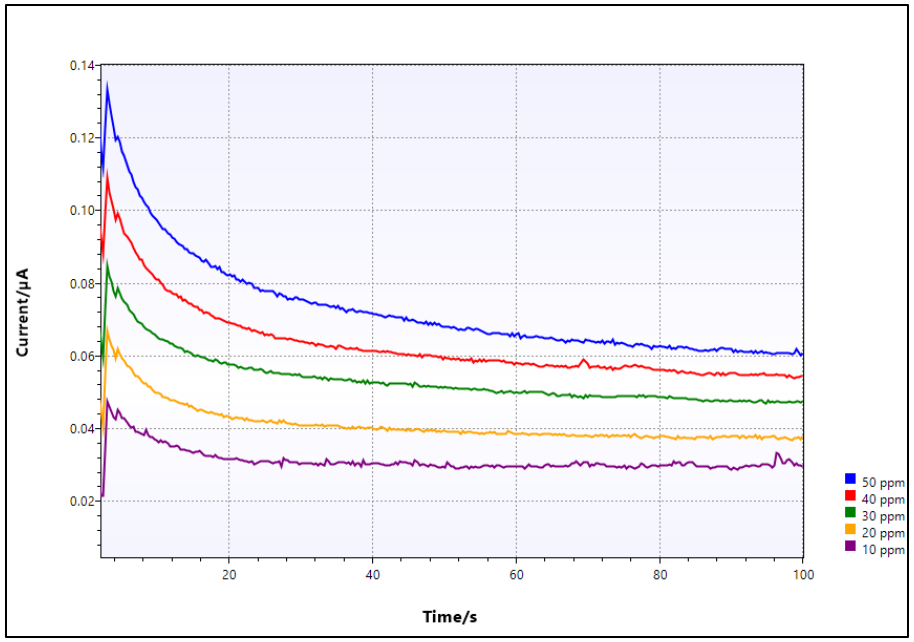
d. Sol gel entrapment

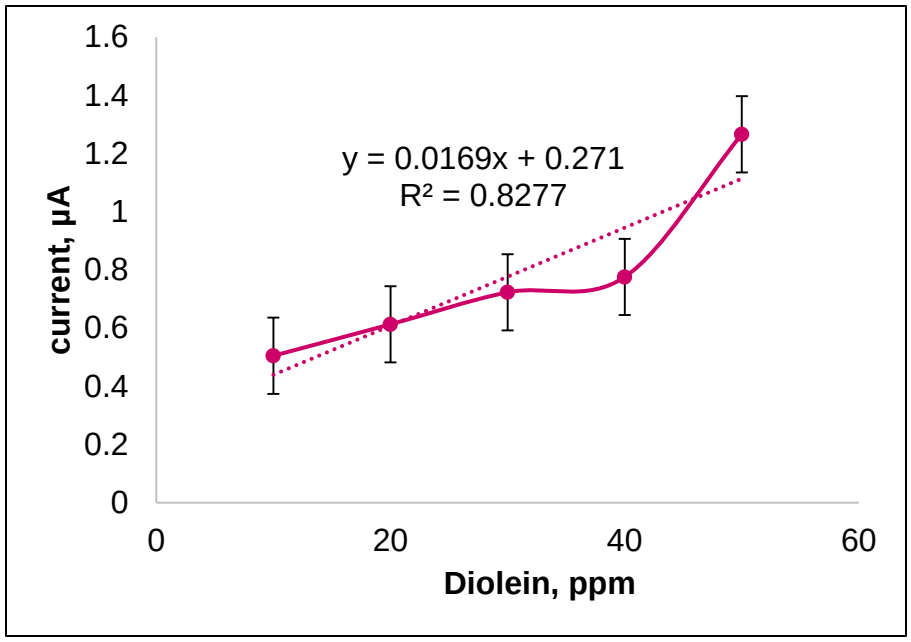




Standardization of the immobilization technique

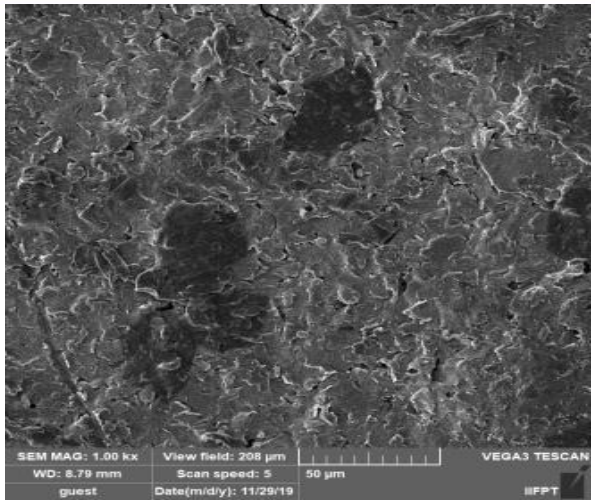
e. Cross Linkage

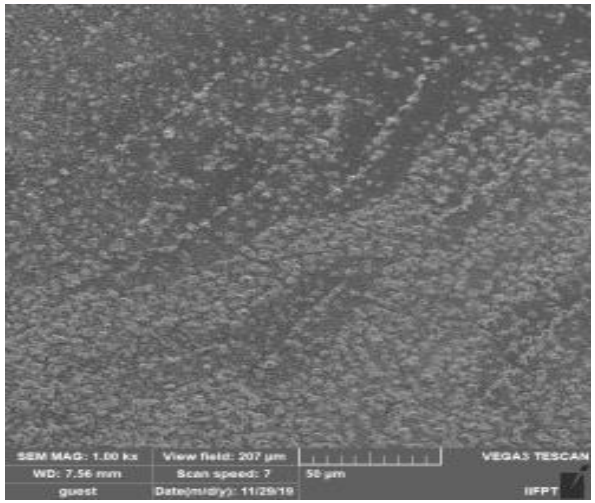


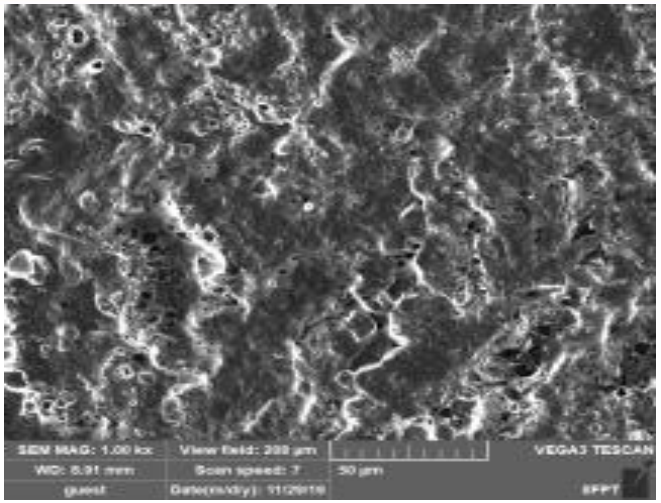


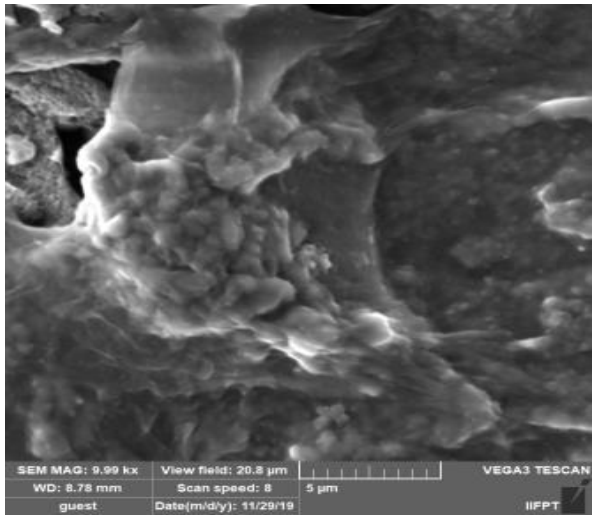
- In cross linkage, Coefficient of Determination(R^2 = 0.827) found to be greater than other techniques.
- Coefficient of Determination R^2 should be between 0.8 to 1.0.

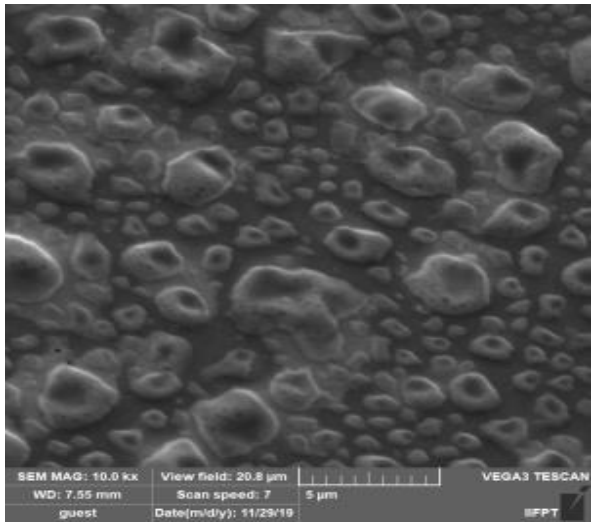
Surface Morphology of Working electrode: SEM Images

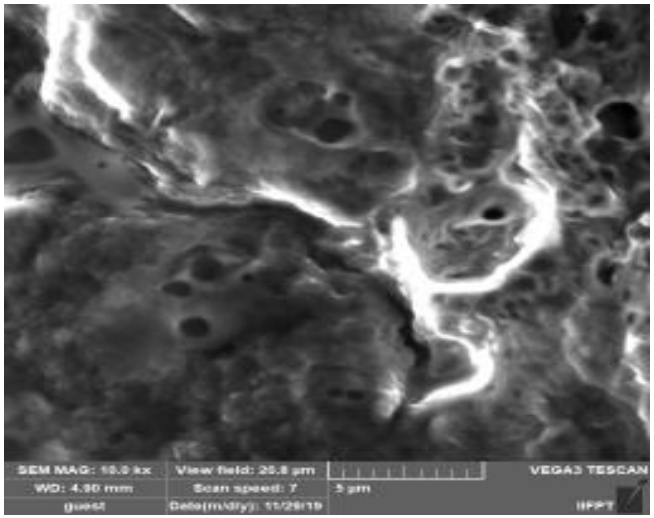






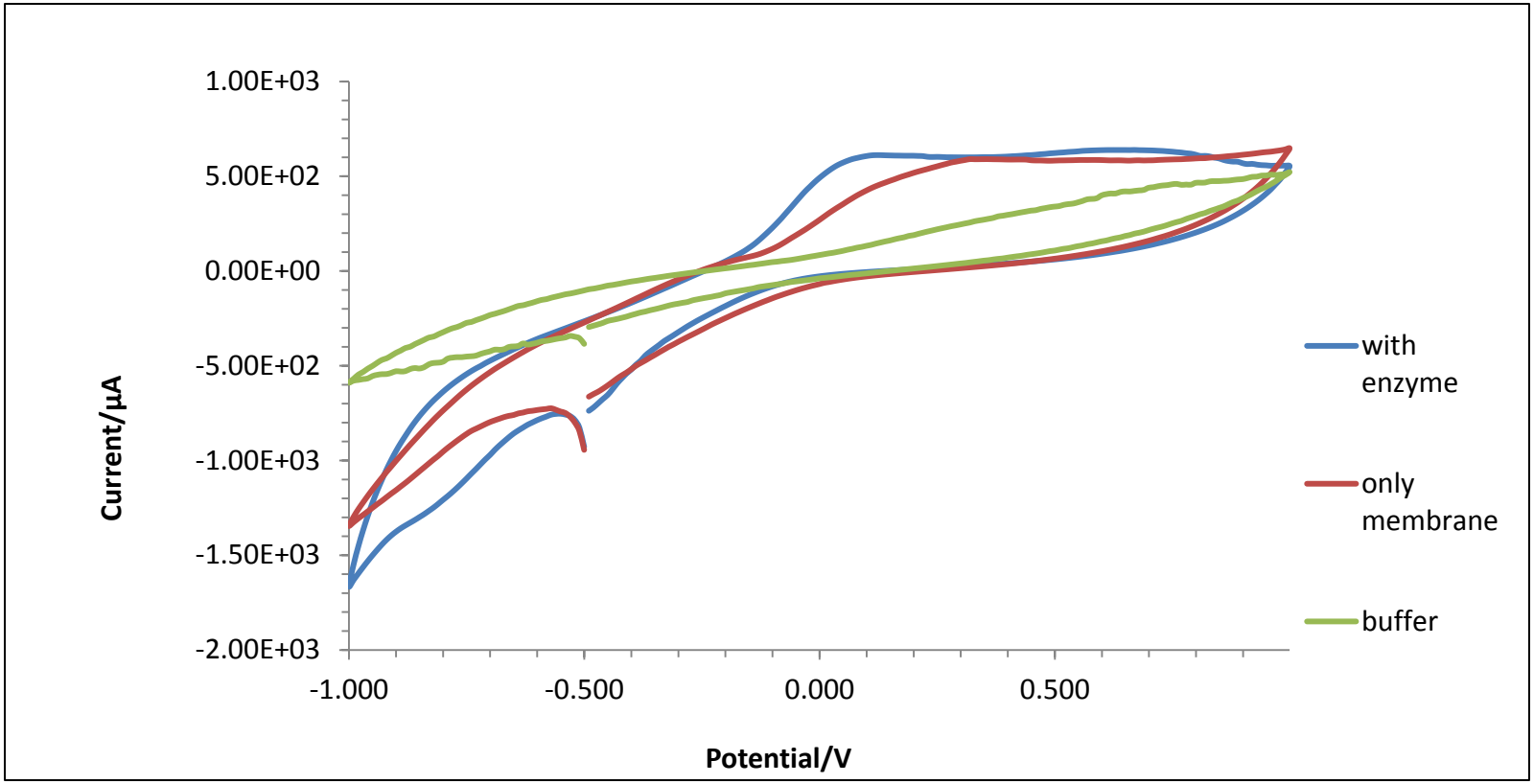






Bare Electrode | Gelatin | Immobilized surface

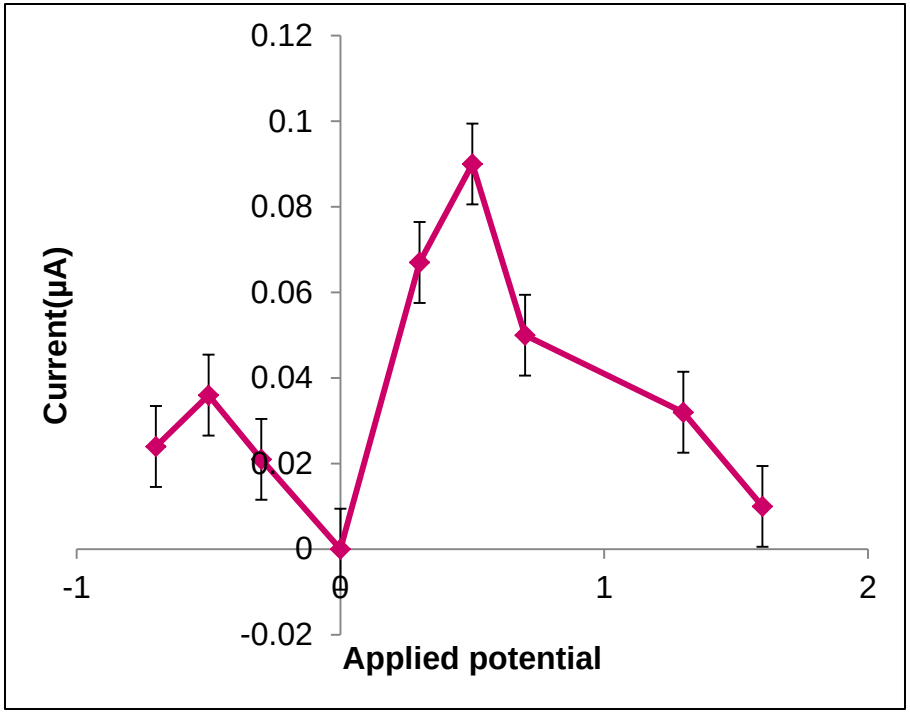
Characterization of electrode: Cyclic Voltammetry Study



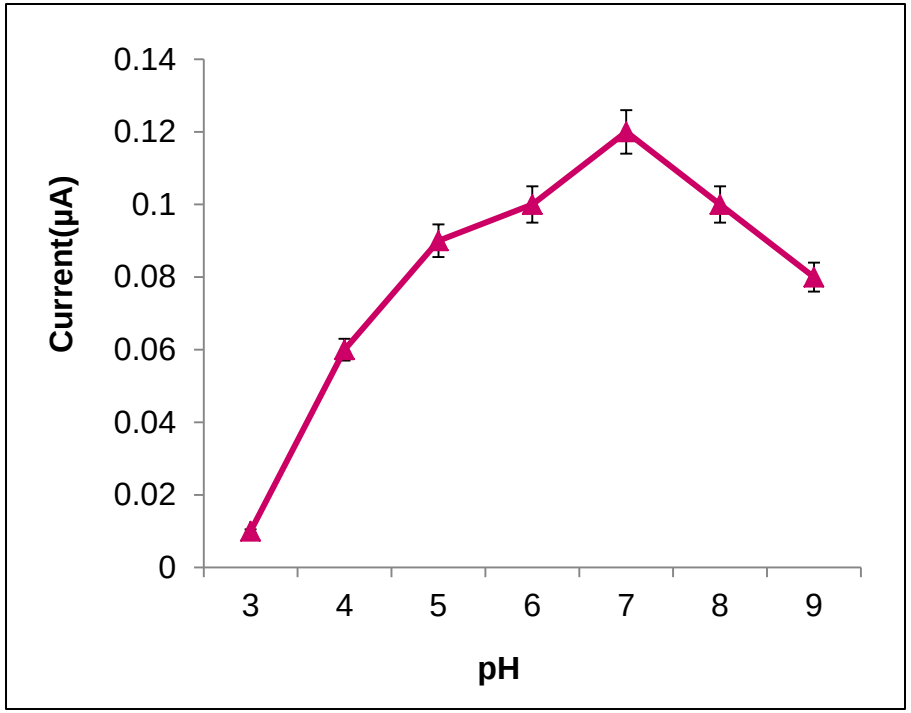
➢ The anodic potential shifted towards positive side with the increase of current and the cathodic peak potential shifted in the reverse direction in the presence of enzymes bound to that of the gelatin membrane.

Optimization of working electrode process parameter

a. Effect of potential



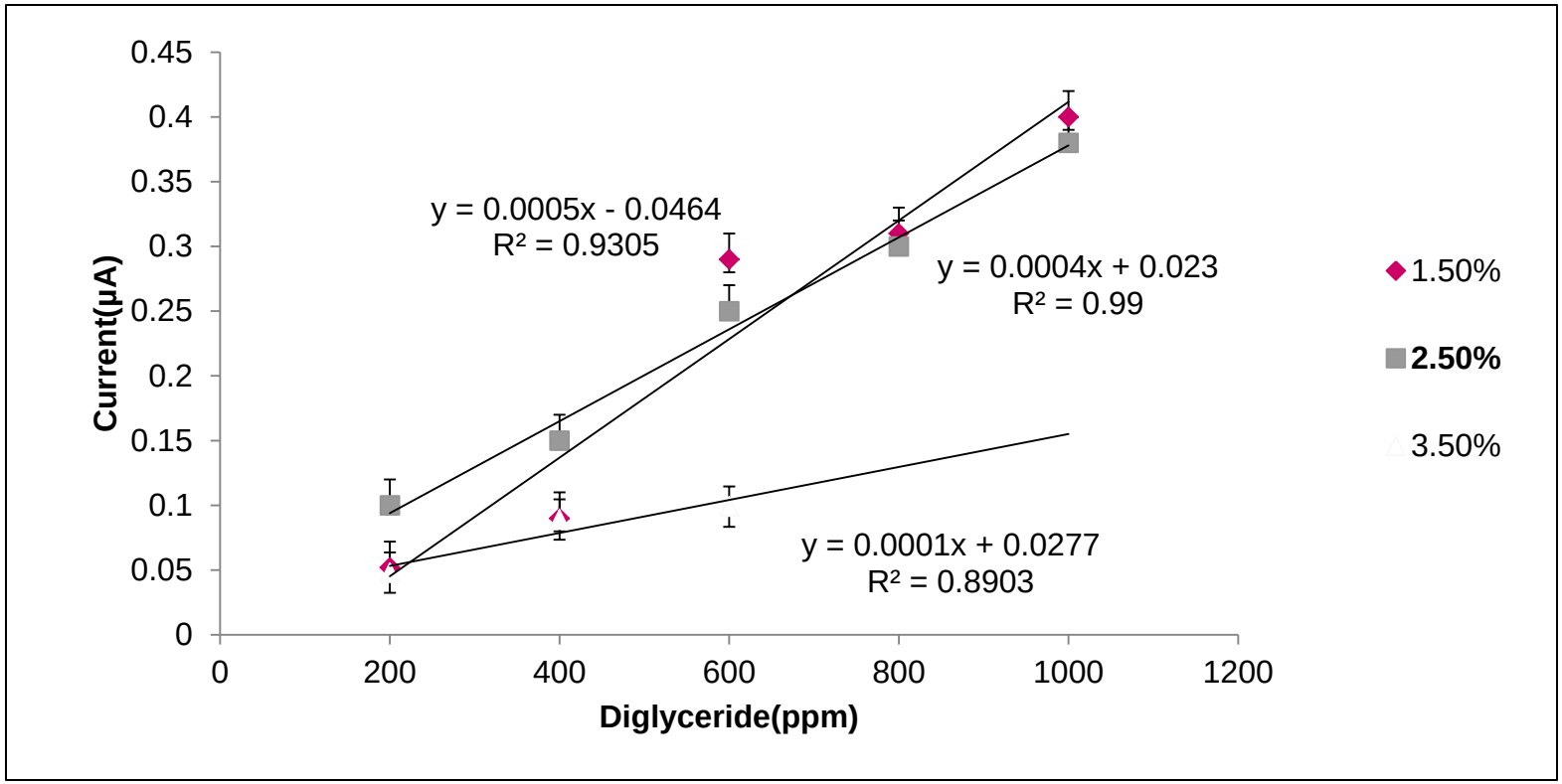
b. Effect of pH



- Optimized potential : +0.5V
- Optimized pH: 7.0

Optimization of working electrode process parameter

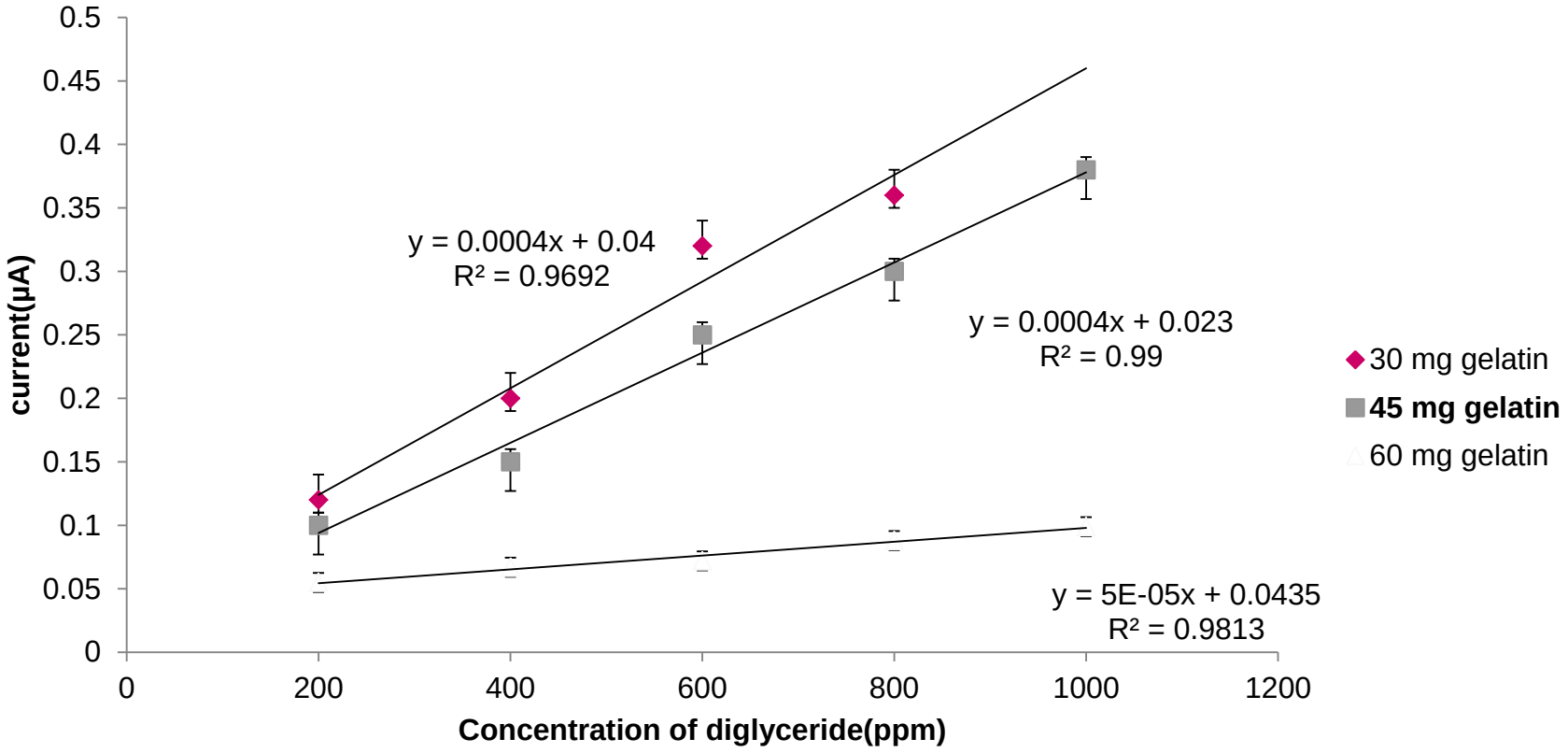
c. Effect of different % of gluteraldehyde



➢ In 2.5% concentration of gluteraldehyde Coefficient of Determination($R^2 = 0.99$) was found to be greater.

Optimization of working electrode process parameter

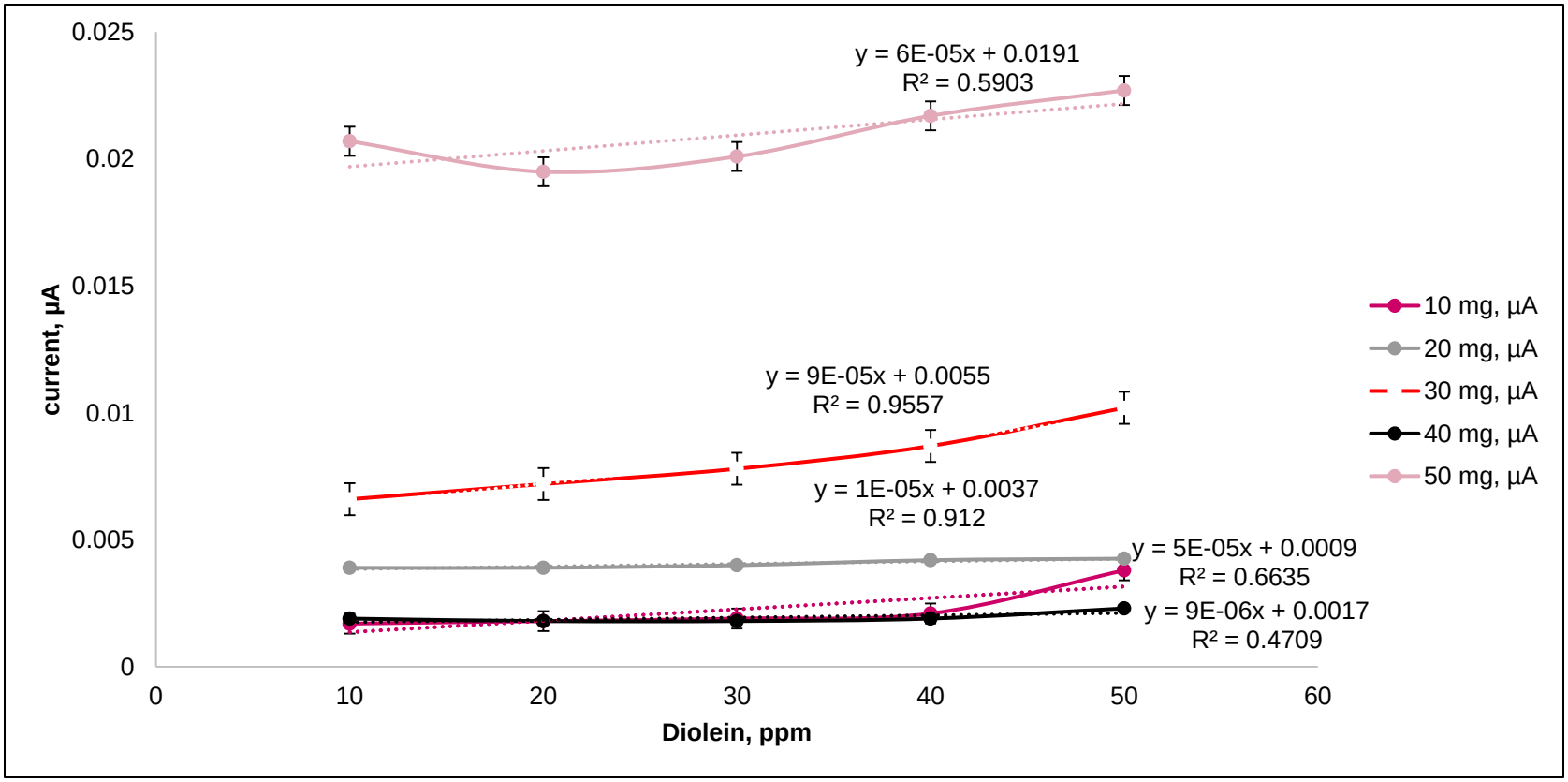
d. Effect of different mg of gelatin



➢ In 45 mg concentration of gelatin Coefficient of Determination($R^2 = 0.99$) was found to be greater.

Optimization of working electrode process parameter

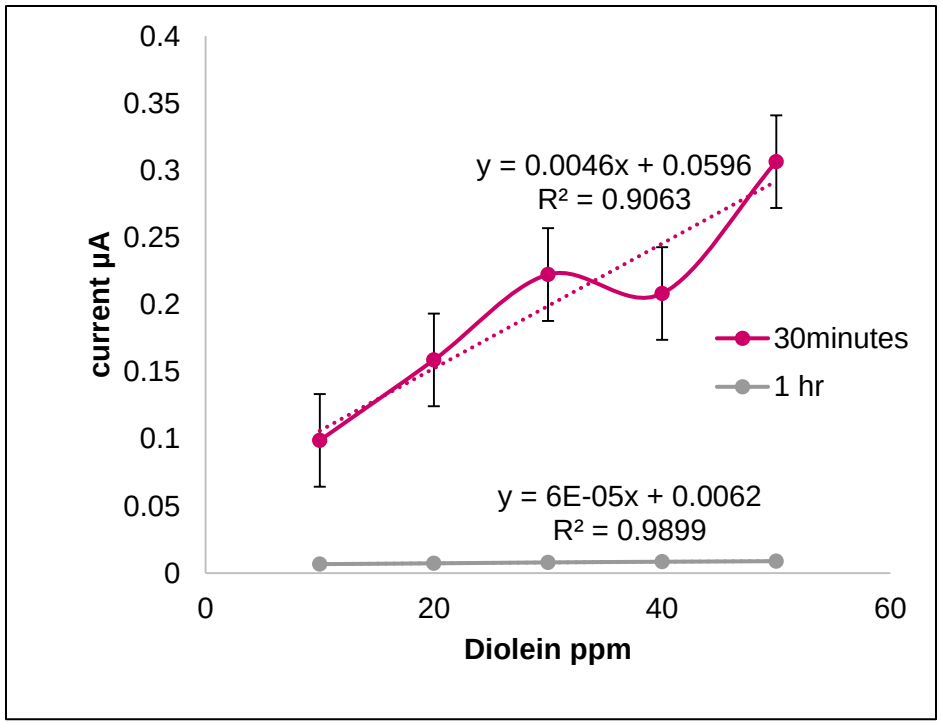
e. Effect of different concentration of BSA



➢ In 30 mg concentration of Bovin Serum Albumin (BSA) Coefficient of Determination($R^2 = 0.955$) was found to be greater.

Optimization of working electrode process parameter

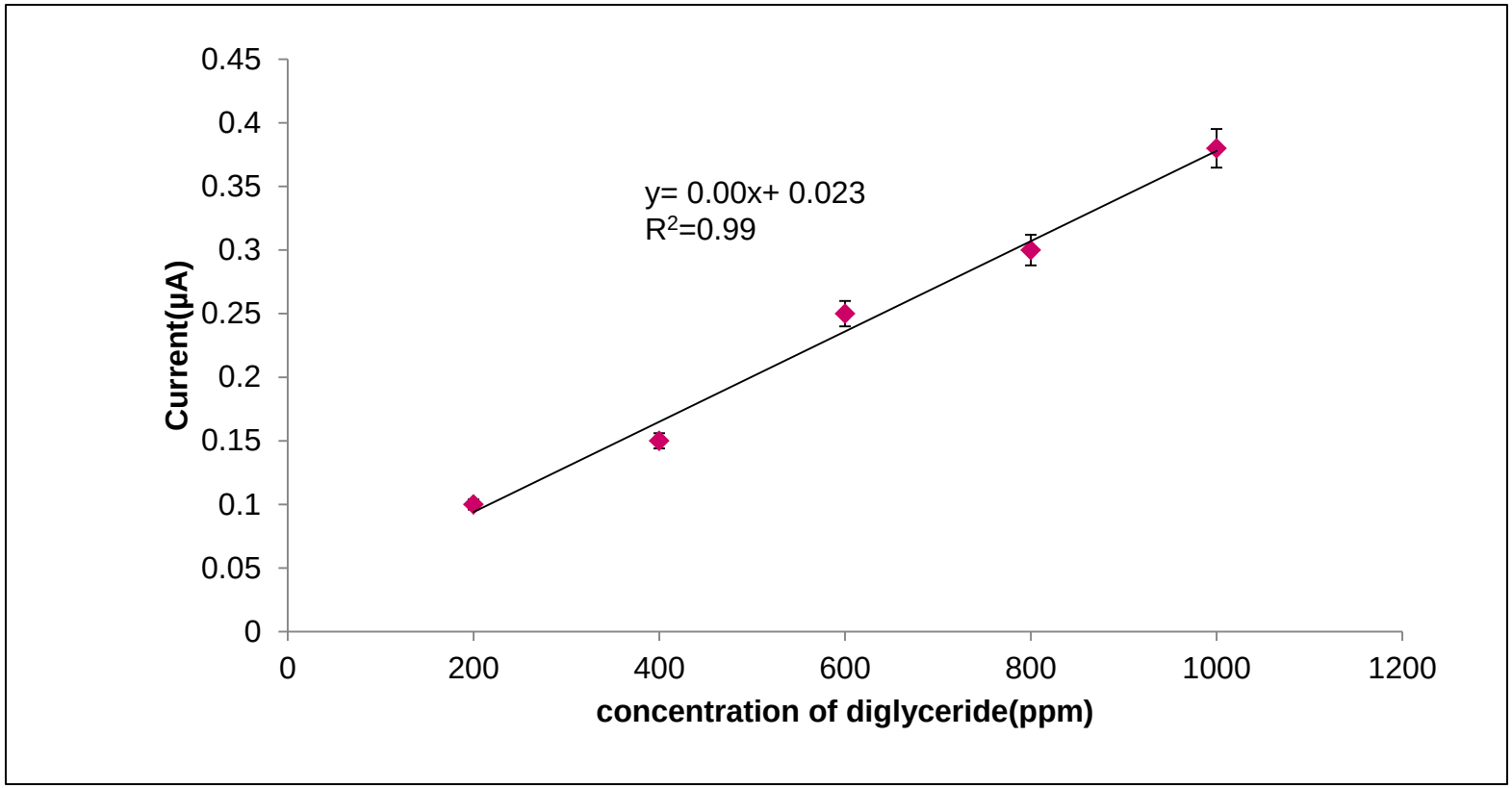
f. Effect of incubation time



Sl.No	Parameters	Optimized conditions
1.	pH	7
2.	Gluteraldehyde %	2.5%
3.	Applied potential	0.5 V
4.	Gelatin concentration	40mg
6.	BSA concentration	30 mg
7.	Immobilization time	1 hr
8.	Immobilization method	Cross Linkage method

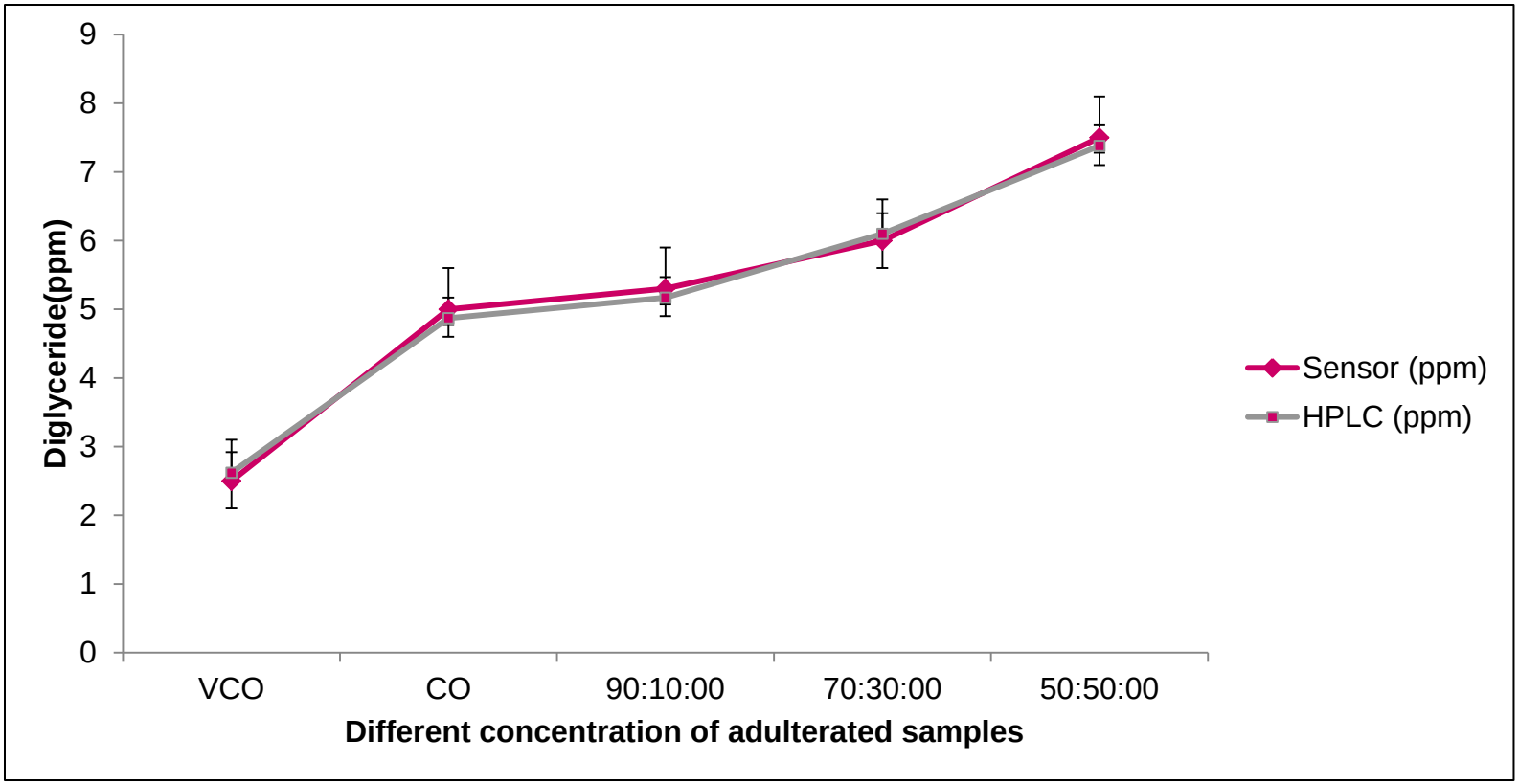
➢ The optimized conditions used for development of biosensor for virgin coconut oil

Empirical relation for the developed biosensor



- Response was recorded in terms of current obtained from the different concentration of diglyceride solution.
- The increase in concentration of diglyceride (ppm) there was increase in the current (µA) in a linear trend.

Validation of Biosensor



- Validation done by existing High Performance Liquid Chromatography(HPLC)
- Fresh VCO and CO was in the range between 2.25- 3 ppm and 4 -5 ppm respectively.
- The amount of diglyceride found in different proportions of adulterated samples of VCO:CO in ratio of 90:10,70:30,50:50 was increased in range from 5.3 ppm to 7.5 ppm

Performance of the Developed Biosensor

Precision

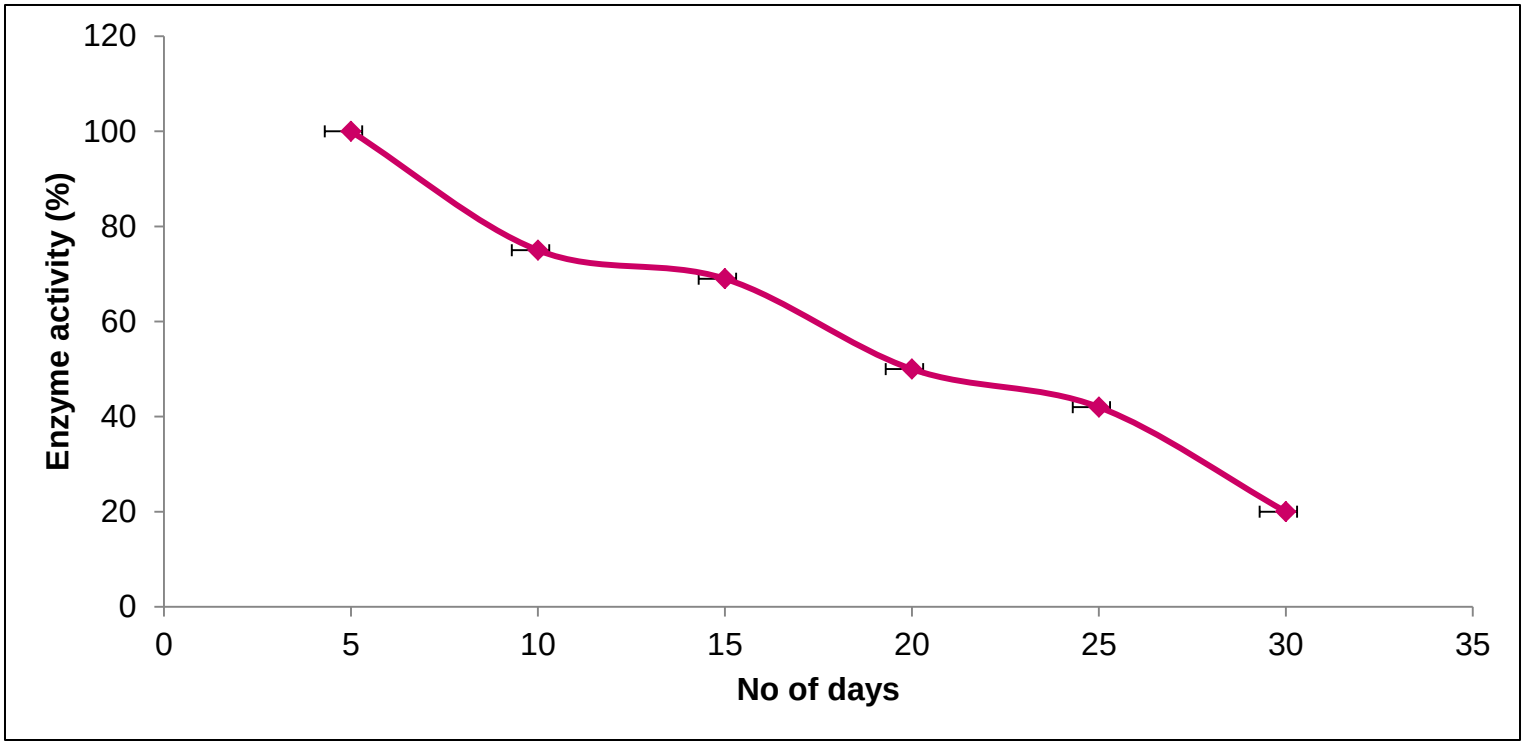
Days	Precision of the Biosensor		
1st day	Mean (ppm)	S.D	CV%
	2.2	0.34	0.15±0.56
7th day	2.5	0.25	0.1±0.32

$$\text{CV calculated} = \frac{Standard\ deviation(S.D)}{mean(x)}$$

✓ CV less than 1 (CV<1) means low variance i.e. precision is better.

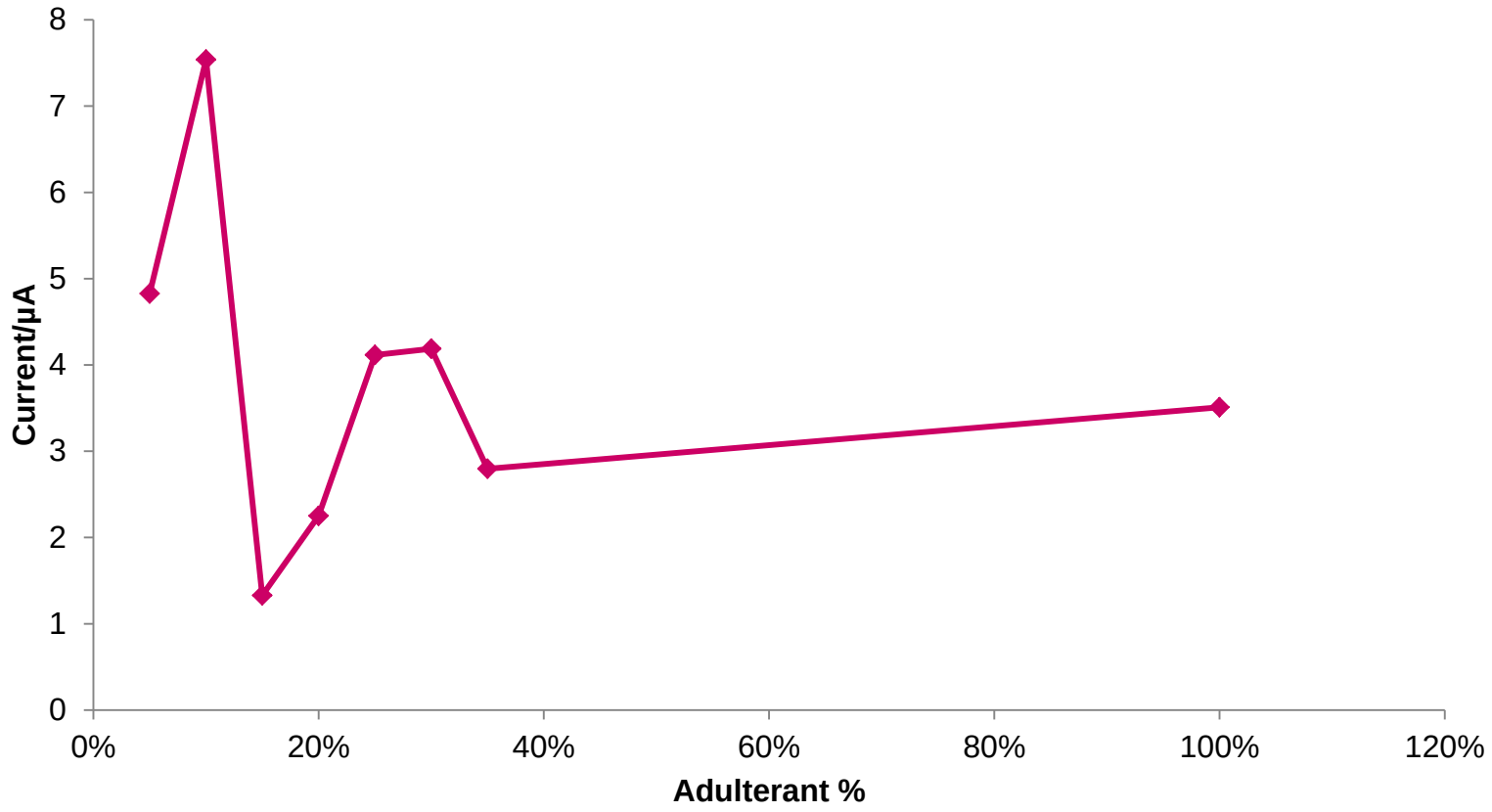
Performance of the Developed Biosensor

Stability of biosensor



- ✓ % of enzyme activity={(100/first day current reading) *(5th day current reading)}
- ✓ 50% activity was decreased within 15 days

Detection level in adulterated Virgin coconut oil (VCO)

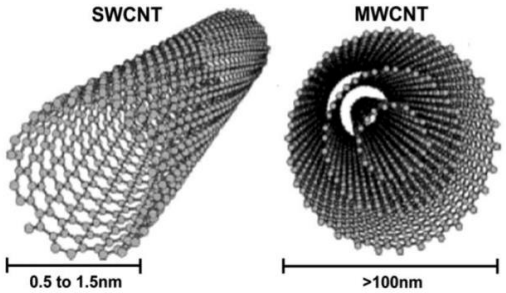


➢ The developed biosensor able to detect above 20 % adulteration of coconut oil in VCO

Conclusion

- Linear empirical relation developed was found to have coefficient of determination (R^2) =0.99.
- The validation study showed no significant difference at 95 % confidence level.
- The biosensor could detect above 20 % adulterated sample with detection time of 15 seconds per samples.
- The developed biosensor can be used to evaluate the adulteration in VCO and was observed to be reused for about 15 days.

Future Aspects

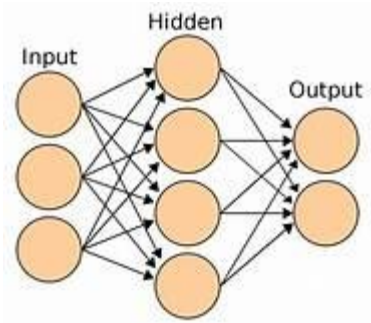


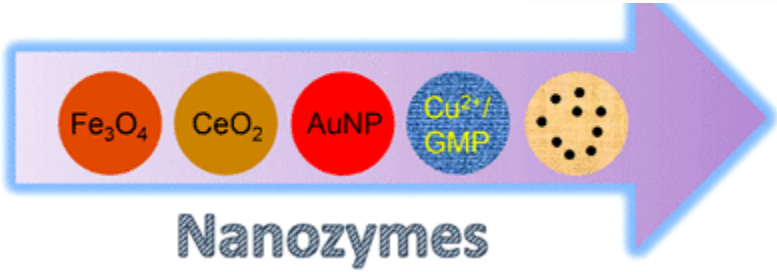
Modification of electrode

Nanoenzyme

Screen printed electrode

Artificial Intelligence





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THANK YOU

"Sensors are the integrated & smart approach to evolution"