Lipase Based Biosensors for Triglyceride Determination

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Abstract. A review of methods development in lipase based biosensor for triglyceride determination was briefly discussed. This review focuses on the basic principle of triglyceride biosensor that includes performances of triglyceride biosensor such as limit of detection, response time, and optimization.

1 Introduction

Triglycerides (TG) can be generated by esterification process of three hydroxyl (-OH) groups of glycerol with three molecules of fatty acids that produce an ester as product [1]. Triglyceride acts is important role in metabolisms as energy source and also as a dietary fat transporter. Fig. 1 shows the molecular structure of triglyceride:

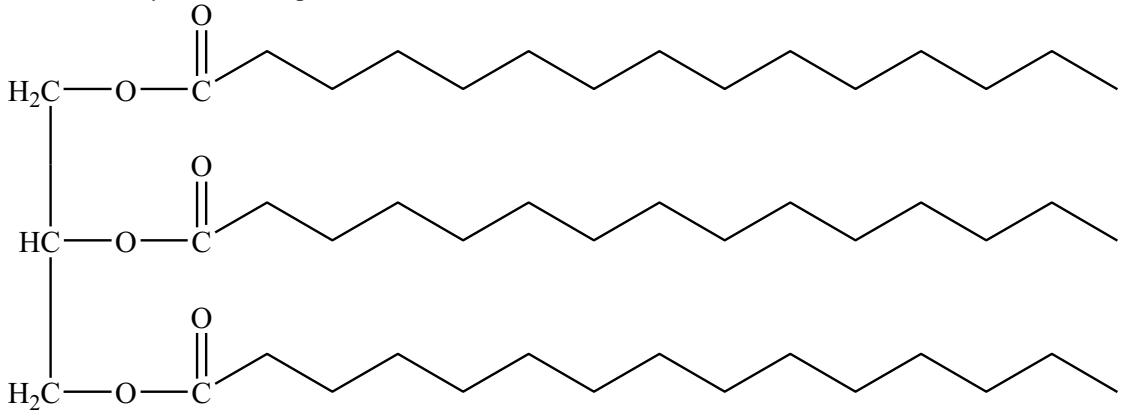

Figure 1. Molecular structure of Triglyceride (TG)

Determination of triglycerides (TG) is crucial since its high concentration could lead to hyperlipidemia. 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 [2]. Apart from coronary diseases such as heart attack and hyperlipidemia, are also associated to TG several disorders such as liver obstruction, diabetes mellitus and endocrine [3,4]. Hence, increased health awareness among community and stringent regulatory laws make the estimation of triglycerides content in food has become important nowadays. A biosensor is a device that enables the identification and quantification of an analyte of interest from a sample matrix such as food, water, blood and urine [5]. The most common device used for TG determination is enzymatic amperometric triglyceride biosensors. Normally, triglyceride biosensors are based on interactions of three enzymes, lipase, glycerol kinase (GK) and glycerol-3-phosphate oxidase (GPO). One of general strategies has been used for electrochemical sensing of Triglyceride is by measuring oxygen consumption or the amount of hydrogen peroxide produced by the enzymatic reactions [6]. The biosensor chemical reactions involved in the principle of this method re as equation (1) below; lipase hydrolyses triglycerides to glycerol and fatty acids [7]:

$$\mathrm{TG} + \mathrm{H_2O} \xrightarrow{\textbf{Lipase}} \text{Glycerol} + 3 \text{ free fatty acids} + \mathrm{H^+} \quad (1)$$

However, there are works that used single enzyme (lipase) for Triglyceride determinations (Solanki *et al.,* 2009) and (Pauliukaite *et al.,* 2011) [7,8]. This methods are more

preferable than multi-enzyme becaus MATEC Web of Conferences

e single enzyme methods are less time consuming and inexpensive.

2 Triglyceride (TG) biosensor

Triglyceride biosensor has been studied by other researchers using various properties and method. Most of the studies focus on surface of electrode, to determine triglyceride at optimum level. Table 1 shows recent studies on triglyceride biosensor.

There are several types of working electrode that have been used for triglyceride biosensor, such as Platinum (Pt), gold (Au), carbon (C), and indium tin oxide (ITO), most of this electrode were stable as base for working electrode. Screen printed carbon electrode (SPCE) has been used as working electrode in this study. Modification on surface, able the detection of hydrogen ions, (H^+) at optimum level. The H^+ ions produced during the enzymatic reaction, will be detected electrochemically on bioelectrode surface at low applied potential [9]. Furthermore, present researches has describes the improvement of TG biosensor by employing ionic liquid on the surface of SPCE which is relatively cheaper compared to other common working electrode such as glassy carbon electrode (GCE), Au and ITO [9].

Meanwhile, triglyceride can be detected by electrochemical or amperometric detection. Besides that, performance of triglyceride biosensor is measured by identifying its detection limit, linear range, response time, sensitivity and storage ability. Based on Table 1, Narang and Pundir (2011) shows a modest detection limit, nevertheless it shows wide linear range that are corresponding with triglyceride concentration that varies in the range between 30 and 550 mg/dL [14,15]. Furthermore, time taken for the electrode to response is faster and the life time is longest. Therefore, high performance of triglyceride biosensor must have lower detection limit. A linear working range that correspond with triglyceride concentration, fast response time (2 sec) and long life time (210 days) in which the biosensor can be used several time with constant performance.

3 Electrochemical characterization for Triglyceride biosensor

Phongphut *et al.,* (2013) state that cyclic voltammetry was used to optimize the electrochemical response of modified electrode, hence triglyceride detection was then performed by chronoamperometry at a constant applied potential [15]. Solanki *et al.,* (2009) shows cyclic voltammetric (CV) studies of ITO modified electrode and bioelectrode have been carried out in PBS containing $[Fe(CN)_6]^{3-/4-}$ at scan rate of 50 mV/s [8]. Cyclic voltammetric (CV) studies have been carried out in phosphate buffer solution (PBS) at potential range -1.0 to 1.0 V. Furthermore, calibration curve, response time and detection limit are then able to be estimated from the amperometric responses.

Pundir *et al.,* (2010) stated that, an amperometric triglyceride biosensor was applied in their research, as current produced from the cyclic voltammetry (CV) was optimum at potential 0.4 V, and at that potential has been selected for standardization of working conditions for triglyceride, determination in amperometry [13]. Therefore, cyclic voltammetry was used for triglyceride determination, in present work due to its rapid capability in measuring redox behavior over a wide potential range [16].

4 Parameters optimization for Triglyceride biosensor

There are several parameters that have been study to increase optimization level of triglyceride biosensor, such as effect of triglyceride biosensor towards applied potential, enzyme concentration, pH, and temperature. Phongphut *et al.,* (2013) stated that, the operational potential for triglyceride determination on Au electrode have been characterized by ranging the potential from 0.1 V to 0.6 V, and the optimum potential for biosensor was at 0.4 V, most of optimum potential was at lower potential since higher applied potential leads to the interferences [15]. Pundir., (2008) stated that, effect of pH towards biosensor have been studied, and optimum pH level for biosensor to response was at pH 6.5, while result that found by Narang and Pundir*.,* (2011), the biosensor was found optimum at pH 7.5, most of triglyceride biosensor shows optimum pH between 6.5 to 7.5, after that it start decreasing [11,14]. Meanwhile, for optimum temperature, Narang *et al.,* (2013) state that, the changing in temperature will give an effect on current response of the biosensor and from their result, it reached at approximately 35 ˚C [1]. In contrast with result from Pundir., (2008), the biosensor shows the temperature was optimum at 25˚C [11].

In our study, we incorporated ionic liquid (IL) that has a high ionic conductivity and well biocompatibility to enhance the electrochemical response. A single enzyme which is lipase with ionic liquid modified electrode as a base for tributyrin sensor was developed for determination of lipase activity. IL was used to ensure fast and easy electron transfer, since most of IL does not give harm towards enzyme [7]. Besides that, it presented as suitable compounds for biosensor because most of them seem to have the ability to solubilize proteins without denaturation [17]. The optimization parameters of SPCE based IL electrode was carried out, and pH 7, 30°C and 5 % of lipase enzyme loading resulted in optimum performance.

Table 1. Analytical properties of recent triglyceride (TG) biosensor

Authors	Type	Type of support for immobilization	Type of electrode	Detection Limit (mg/L)	Linear range (mg/L)	Response Time (s)	Storage stability
[7]	Electrochemical	MWCNT–RTIL ZnO(NPs)-CHIT	GCE	0.02	0-1603	NR	NR
[14]	Amperometric	NiO(NPs)-CHIT/ZnO-ZnHCF	Pt	200	500-6500	6	210 days
[1]	Amperometric	Au/PEDOT-PSS CeO_2/TCO	Au	100	500-7000	4	50% loss in 180 days
[15]	Amperometric	Cellulose acetate, CA	SPCE	7.9	0-531	30	40% loss in
[9]	Electrochemical	C/Iridium(NPs)	TCO	0.60	100-599	5	30 days
[11]	Amperometric	CeO_2	Pt	180	180-3100	40	42 days
[10]	Electrochemical	Egg shell membrane PVA	SPCE	NR	0-8850	10	25 days
[8]	Electrochemical		ITO	328	500-5000	20	NR
[12]	Amperometric		Pt	247	500-2000	10	84 days
[13]	Amperometric		Pt	190	500-2000	2	70 days
							50% loss in 50 days

5 Conclusion

In conclusion, cyclic voltammetry is the fastest and simplest way for TG determination. Most of studies shows optimize conditions at pH (6.5-7.5) and temperature (25-35°C). Modification of working electrode improves TG biosensor performance.

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