Biosensors and Bioelectronics 100 (2018) 214–227

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Biosensing methods for determination of triglycerides: A review

C.S. Pundir*, Vinay Narwal

Department of Biochemistry, M.D.University, Rohtak 124001, India

ARTICLE INFO

Keywords:
Triglycerides (TGs)
Detection methods for TG
TG biosensors
Nano-materials
Biological materials
Hyperlipidemia

ABSTRACT

Triglycerides (TGs) are the major transporters of dietary fats throughout the bloodstream. Besides transporting fat, TGs also act as stored fat in adipose tissue, which is utilized during insufficient carbohydrates supply. TG level is below 150 mg/dL in healthy persons. Elevated TGs level in blood over 500 mg/dL is a biomarker for cardiovascular diseases, Alzheimer disease, pancreatitis and diabetes. Numerous methods are accessible for recognition of TGs, among them, most are cumbersome, time-consuming, require sample pre-treatment, high cost instrumental set-up and experienced personnel to operate. Biosensing approach overcomes these disadvantages, as these are highly specific, fast, easy, cost effective, and highly sensitive. This review article describes the classification, operating principles, merits and demerits of TG biosensors, specifically nanomaterials based biosensors. TG biosensors work ideally within 2.5–2700 s, in pH range, 6.0–11.0, temperature 25–39.5 °C and TG concentration range, 0.001–100 mM, the detection limits being in the range, 0.1 nM to 0.56 mM, with working potential – 0.02 to 1.2 V. These biosensors measured TG level in fruit juices, beverages, sera and urine samples and reused upto 200 times over a period of 7–240 days, while stored dry at 4 °C. Future perspective for further improvement and commercialization of TG biosensors are discussed.

1. Introduction

Triacylglycerols or triglyceride (TGs), known as natural fats, are made up of one glycerol molecule that is joined to three molecules of fatty acids (saturated/unsaturated or both) through ester bonds. TGs are the significant transporters of dietary fats throughout the circulation system. To a great extent,both very low density lipoproteins (VLDL) and chylomicrons are made up of TGs (Toth, 2011). Besides transporting fat, these can be used as fuel, when the body's energy demand is not fulfilled by carbohydrates (Dallongeville and Meirhaeghe, 2010). The normal level of TGs in blood is less than 150 mg/dL, while 150–199 mg/dL level is considered border line high and 200–499 mg/dL as high. The elevated TGs level over 500 mg/dL) in serum is used as a biomarker for cardiovascular disease (Sarwar et al., 2007), Alzheimer disease (Burgess et al., 2006), pancreatitis (Kota et al., 2012) and diabetes, due to abnormal lipoprotein metabolism. Accumulation of TGs may also be caused, by its high rate of synthesis as well as low rate of catabolism, in which the activity of triacylglycerol lipase and β-oxidase play an important role (Tirosh et al., 2008). The decreased activity of lipase catalyzing the hydrolysis of TG into free fatty acids and glycerol may result into accumulation of TGs (Ponec et al., 1995). The high TGs level affects the particle size of low density lipoprotein (LDL) negatively. Through a complex metabolic cooperation, TGs advance the development of small, dense LDL particles, which are especially atherosclerogenic (V et al., 1992). Indeed, even within the presence of normal LDL cholesterol, patients with high TGs level normally have endothelial dysfunction, due to which their blood vessels do not expand and contract properly (Lakatos and Hársági, 1988). Moreover, excess of TGs brings down nitric oxide levels and increment of numerous inflammatory compounds, which play a part in vascular injuries and endothelial dysfunctions (Yoshida et al., 2004). Therefore, the determination of TG in blood is very important in the clinical diagnosis and medical management of various diseases.

A number of methods are accessible for determination of TG such as titrimetric method (Klotzsch and McNamara, 1990), colorimetric method (Mamoru et al., 1997), enzymic colourimetric Fossati and Prencipe 1982; Kalia and Pundir, 2004) spectrophotometric method (Mocho´n and Leyva, 1984), chromatographic methods (Brunnekreeft and Leijnse, 1986), fluorometric method (Mendez et al., 1986), nuclear magnetic resonance method (Otvos, 1999), micro method (Kaplan and Lee, 1965), mass fragmentographic method (Björkhem et al., 1982) and enzymatic centrifugal method (Grossman et al., 1976; Hearne and Fraser, 1981). Titrimetric methods are non specific and affected by various interferents present in physiological samples (Greenfield and Clift, 1975). Colorimetric methods are unreliable, non-specific and insensitive, while enzymatic methods require expensive chemicals (enzymes) and applicable in limited concentration range. However, in

* Corresponding author.
E-mail address: chandraspundir@gmail.com (C.S. Pundir).

http://dx.doi.org/10.1016/j.bios.2017.09.008
Received 3 July 2017; Received in revised form 31 August 2017; Accepted 6 September 2017
Available online 09 September 2017

simple chromatographic methods, chiral separation is not possible (Parker, 1983). The advanced chromatographic methods are specific and highly sensitive, but require time consuming sample pretreatment, costly equipment and trained personnel to operate. Further, these methods are too inconvenient for small volume samples,as a sequence of steps are required for sample pretreatment and derivatization (Hou et al., 2016). The proton nuclear magnetic resonance method is slow, require skilled person and does not provide the absolute TG concentration (Braithwaite and Smith, 1999). In the micro method, the cost of equipment is very high. The visual determination of discrete LDL and high density lipoprotein (HDL) molecules by the EQ density gradient method is not as clear, as with native gradient centrifugation methods (Redfield, 1978). The fluorescent spectroscopic methods are highly sensitive and can even detect a single molecule, while fluorescence-based in vivo detection of TG is not practically applicable in clinical samples (Shireman and Durieux, 1993). In the centrifugal auto analyzer, a large number of analytical steps are required for TG determination in sera of hyper-triglyceridemia patients, which involve a high level of specialized ability and effectiveness (Kamata, 1985). Nevertheless, biosensing approach overwhelms these drawbacks, as these are specific, fast, easy, low-cost, and highly sensitive (Sadana, 2006). Recently, Nanomaterials have been used in fabrication of biosensors to enhance their analytic performance. Metal oxides nanostructures have unique capacity to advance quick electron transfer rate between electrode and the active site of desired enzyme (Hou et al., 2016). Now a days, with the progression of nanotechnology, a vital number of novel nanomaterials have been synthesized and their novel properties are being gradually discovered and applied to construct improved/advanced biosensors (Maeda, 2014; Pumera, 2014; Narwal and Singh, 2014). This article provides a comprehensive review of the state-of-the-art research activities which focus on several important metal-oxide nanostructures and nanocomposites in addition to the application of carbon nanomaterials for TG biosensing. The most commonly-used detection methods for the TG sensing are also discussed.

2. TG biosensors

A biosensor is an analytical device in which a biological element (enzyme, antibodies) is used to sense analyte(s) in a system (Turner, 2015; Narang and Pundir, 2017). The basic principle of TG biosensor is to generate an electric signal that is proportional to the quantity of an analyte or a series of analytes being detected (Evtugyn 2013). The development of an ideal TG sensor for detection of TGs (biomarker of cardiovascular disease, alzheimer disease, pancreatitis and diabetes) must be a hot issue for the biosensor industry (Pundir and Narang, 2013). Various processes and techniques have been used in construction of TG biosensors. Among them, the electrochemical TG biosensors have attracted the maximum attention, because of their simplicity, supreme sensitivity, specificity, rapidity and economic for routine analysis (Pundir et al., 2016; Palchetti et al., 2009).

3. Sample preparation for TG determination

In TG biosensors, triolein and tributyrin have been used as substrates for enzymes (Lipase, Glycerol Kinase and Glycerol phosphate oxidase). Both triolein and tributyrin are hydrophobic in nature, which require to be treated with the surfactant (Triton X-100) to be converted into hydrophilic and then diluted suitably in distilled water as per required concentrations. An additional constraint is the limited solubility of serum TGs in aqueous electrolytes, impeding the analysis. To solve this problem, TG sera samples are incubated with 0.22 mM Triton X100 in phosphate buffer (pH-6.5), enabling efficient sample preparation for biosensor signaling. In case of TG analysis in foods, samples (20–80 mg) are added into 8.0 mL Arabic gum solution in a polypropylene tube. The mixture is placed in a water bath at 65 °C for 5 min, stirred and filtered and then the filtrate is diluted to 10 mL with deionized water. An aliquot of 1.0 mL of this suspension containing the extracted lipid is added to the electrochemical cell containing 25.0 mL of PBS(Phosphate buffer saline) pH 6.5. TG level in the sample is intrapolated from the standard curve between TG concentrations and biosensor response prepared under the optimum conditions.

4. Classification of TG biosensors

The TG biosensors can be classified as electrochemical biosensors, conducting polymer based biosensors, metal oxide based biosensors, mid infra red fiber optic based biosensors, flow injection analysis based biosensors and microgel based optical biosensors as given below:

4.1. Electrochemical TG biosensors

The electrochemical TG biosensors can be classified according to their measurement principles: i.e., potentiometric, amperometric, impedemetric, conductometric sensors, metal oxide based biosensor, optical biosensor, flow injection analysis biosensor, microgel based biosensor and enzyme nanoparticles based biosensor.

4.1.1. Potentiometric TG biosensor

Potentiometric TG biosensors are based on lipase–catalyzed hydrolysis of the TG (tributyrin) to glycerol and free fatty acid (butyric acid). Fatty acid production induces changes in the pH of reaction buffer (analyte solution), which is measured using an open circuit potential configuration (Reddy et al., 2001).

4.1.1.1. Basic principle of potentiometric biosensor. Potentiometric biosensors are based on the principle of measure of the potential difference between the reference electrode and the working electrode at zero current flow. In these biosensors, a constant potential is generated by the reference electrode, while the working electrode conveys an erratic potential which depends on the concentration of analytes (Nakazato, 2013). The change in potential at the electrode-electrolyte interface from unbalanced activities of species i in the electrolyte phase (s) and in the electrode phase (β) is calculated by the following Nernst equation:

$$E = E_0 + \frac{RT}{Z_i F}\ln\frac{a_i^s}{a_i^\beta}$$

where E_0 represents the potential of standard electrode, R-gas constant, T-absolute temperature, F-faraday constant, a_i- species activity i, and Z_i - number of moles of electron involved (Yunus et al., 2013).

4.1.1.2. Sub-classification of potentiometric TG Biosensors. Potentiometric biosensors are classified further on the basis of principle, electrolyte–insulator–semiconductor capacitor (EISCAP), micromechanical and porous silica as follow

4.1.1.2.1. Electrolyte–insulator–semiconductor capacitor (EISCAP) based potentiometric TG biosensors. The biosensors based on an electrolyte–insulator-semiconductor capacitor (EISCAP) show a shift in the measured CV with changes in the pH of the electrolyte. Enzyme mediated biological reactions involved changes in the pH of the electrolyte (bioanalyte solution) and an EISCAP can be effectively used for detection of biological compounds (Setzu et al., 2011). When tributyrin, a short chain TG, is hydrolyzed by lipase, it results in the production of butyric acid as a product.

$$\text{Tributyrin} + \text{water} \xrightarrow{\text{Lipase}} \text{Glycerol} + \text{butyric acid}$$

The pH of the solution changes with the butyric acid produced,