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Recent trends and perspectives in enzyme based biosensor development for the screening of triglycerides: a comprehensive review

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ABSTRACT

Clinical manifestations of the elevated plasma triacylglycerol (TG) include a greater prevalence of atherosclerotic heart disease, acute pancreatitis, diabetes mellitus, hypertension, and ischemic vascular disease. Hence, these significant health troubles have attracted scientific attention for the precise detection of TG in biological samples. Numerous techniques have been employed to quantify TG over many decades, but biosensors hold the leading position owing to their superior traits such as highly specific recognition for target molecules, accuracy, minituarization, small sample requirement and rapid response. Enzyme-based electrochemical biosensors represent an instantaneous resolution for the foremost bottlenecks constraining laboratory prototypes to reach real time bedside applications. We highlight the choice of transducers and constructive strategies to design high-performance biosensor for the quantification of triglycerides in sera and early diagnosis of health problems related to it. In the present review, a small effort has been made to emphasize the significant role of enzymes, nanostructured metal oxides, graphene, conducting polypyrrole, nanoparticles, porous silicon, EISCAP and ENFET in enabling TG biosensors more proficient and taking a revolutionary step forward.

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Biosensor; triglycerides; electrochemical; enzyme; nanocomposite

Introduction

Triglycerides (TGs, triacylglycerols), also acknowledged as natural fats, an ester composed of one glycerol molecule attached to three fatty acid molecules (Unsaturated/saturated or both). The ester bond is formed between the hydroxyl groups of glycerol and the carboxyl groups of the fatty acids. Triacylglycerols are the chief components of very-low-density lipoprotein (VLDL) and chylomicrons, which are a lead source of energy in metabolism besides transporting dietary fat throughout the bloodstream [1]. In the digestive tract, triglycerides are broken into monoacylglycerol and free unsaturated fats, in a procedure called lipolysis, with the discharge of bile and lipases, which in turn move to absorptive enterocytes, cells coating the digestive tract. Abnormally high rate of lipoprotein synthesis and low rate of catabolism results in accumulation of TGs. One more reason for triglyceride accumulation may be reduced activity of triacylglycerol lipase, which catalyzes the hydrolysis reaction of TG into free fatty acids and glycerol. Quantification of triglycerides (TGs) in human serum is crucial for health care diagnostics since its high levels in the bloodstream could result in hyperlipidemia. In human body, the level of TGs less than 150 mg/dl is considered to be normal. The condition is known as hyperlipoproteinemias when the level lies between 150–199 mg/dl and high risk of pancreatitis is associated with TGs level above 500 mg/dl. The reference range of triglycerides level in blood has been shown in Figure 1. The presence of excessive triglycerides (above 500 mg/dl) in the blood is one of the leading

causes of diabetes mellitus, liver obstruction, hypertension, atherosclerosis, Alzheimer disease [2] and various cardiovascular diseases [3]. In addition, the elevated amount of TGs causes reduction in nitric oxide level and raising the sum of many inflammatory compounds, which are partly responsible for vascular injury and endothelial dysfunction [4]. Consequently, the determination of total triglycerides level in serum has attracted clinical significance as a marker of deviation from the normal range, which could affect the lipid metabolism in our body and early diagnosis of a range of diseases associated with it.

Various methods including titrimetric method [5], fluorometric method [6], colorimetric method [7], micro method [8], spectrophotometric method [9], enzymic colourimetric [10], enzymatic centrifugal method [11], chromatographic methods [12], nuclear magnetic resonance method [13] and mass fragmentographic method [14] have been used for the triglyceride detection in serum or plasma. However, these conventional methods display some fine property but they are underprivileged in certain aspects such as low-sensitivity, non-specificity, instrument complexity, requirement of skilled personnel to operate, time consuming, etc. as shown in Table 1 [15–20]. In addition, there is requirement of pretreatment and derivatization step of sample as these methods are extremely complicated for small volume samples. Fortunately, biosensing methods overwhelms these drawbacks as they possess intrinsic advantages such as rapid sensing, broad detection range, easy operation, low-detection limit, ease of miniaturization and high sensitivity. This rare combination of

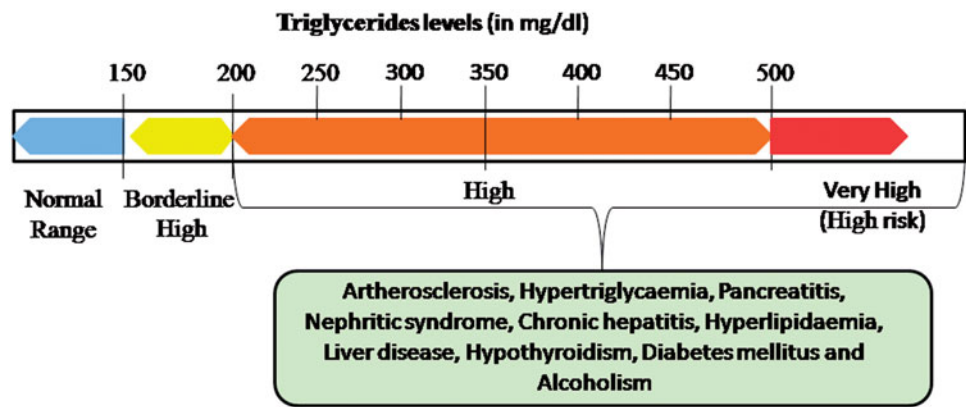


Figure 1 Reference range of triglycerides and various disorders associated with high level of TGs in human body.

Table 1. Various conventional methods for triglyceride determination with their merits and demerits.

Conventional Methods	Merits	Demerits	References
1. Chemical methods			
Titrimetric method	Reproducible results, easy to use	Non-specific, interference by other biological compounds	[5]
Micro method	Good linearity range	Expensive, requirement of many devices for analysis, less reliable	[8]
Autoanalyzer method	Speedy results, high reproducibility, low variation in results	Necessity of trained and competent personnel, poorly used in clinical laboratories	[15,16]
2. Chromatographic methods			
HPLC method	Faster, cost-effective, sensitive	Time consuming, costly equipments, requirement of expertise handling	[17]
Thin layer chromatographic method	Nanogram concentrations of triglyceride could be measured, high sensitivity	Difficulty in reproducing TLC results, faint and uneven spotting	[18]
Capillary ion-column gas chromatography method	Cost-effective, high signal to noise ratio, fast response time	Only compounds having high vapor pressures can be analyzed	[19]
3. Mass fragmentographic method	Sensitive	Requirement of expensive equipments	[20]

HPLC: High performance liquid chromatography.

properties makes biosensors suitable candidates for various promising applications in clinical diagnosis of diseases linked with high level of TGs at an early stage. Recently, the huge impact of nanomaterials as transducing element in the fabrication of improved/advanced biosensors has been reported with the progression of bionanoelectrochemistry. Many scientific articles have been published concerning the favorable alliance between the nanomaterials and biomolecules to enhance the electron transfer rate during the biochemical reaction. Nowadays, an imperative range of novel metal oxide nanostructures are being synthesized and gradually implicated in the construction of biosensors for monitoring specific biological molecules. The significance of using nanostructured modified electrodes is unquestionable as they display a unique property to augment the rate of electron transfer between the active site of enzyme and the electrode [21]. In this article, we describe a comprehensive review exploring the recent trends and principal strategies utilized for improvement in the field of TG biosensors.

Basic strategy of a biosensor

Technology has helped us to form a biosensor, a device in which an analyte is detected by combination of a biological component with a physico-chemical detector component (Figure 2). Basically, three parts constitute a biosensor – a biological element, detector element or the transducer and

allied electronic or signal processors [22]. Enzymes, microorganisms, tissues, organelles, antibodies, cell receptors, nucleic acids can be immobilized as a sensitive biological element for the detection of specific analyte. The transducer represents the second component of the biosensor body and works in a physico-chemical mode like electrochemical, optical, piezoelectrical or thermal that converts the resulting output signal from the interaction of the analyte with the biological element into another quantifiable signal. The continuous or discrete electric signal are generated, which are relative to the amount of single analyte or a group of analytes being sensed [23].

Working of TG biosensors

For the biosensor industry, the fabrication of an ideal biosensor for the monitoring of TGs in the human sera must be a major area of concern as TG is the biomarker of various diseases such as pancreatitis, cardiovascular disease, diabetes and Alzheimer's disease. Among various techniques and processes accessible for constructing TG biosensors, enzyme-based electrochemical biosensors have engrossed the utmost interest owing to their rapidity, simplicity, high-specificity, moderate-sensitivity and cost-effectiveness for the routine analysis [24]. The interaction of three enzymes (lipase (LIP), glycerol kinase (GK) and glycerol phosphate oxidase (GPO) is involved in the working of TG biosensors. Enzymes can be

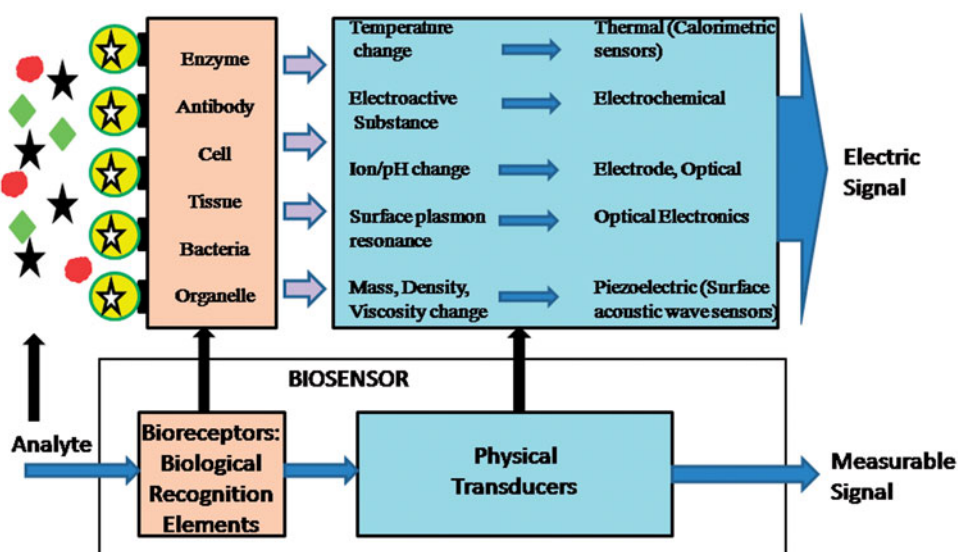


Figure 2 Schematic diagram showing basic principles of the biosensors.

immobilized on different surfaces in different modes: physical adsorption, covalent binding, entrapment and encapsulation. Mode of immobilization depends on the type of matrix or substrate and its site of action as well [25]. Tributyrin and triolein have been used as substrate for enzymes. It is essential to treat both tributyrin and triolein with surfactant (Triton X-100) to convert their hydrophobic nature into hydrophilic and then it is diluted with the distilled water as per requisite. The partial solubility of serum TGs poses another constraint and consequently hampering the analysis. To combat with this problem, it is obligatory to incubate the sera samples with 0.22 mM Triton X100 in phosphate buffer (pH-6.5). This step facilitates the preparation of efficient sample for biosensing techniques. Under optimum conditions, the standard curve between the concentrations of TG and response of the biosensor deduce the level of triglyceride present in the sample.

TG biosensors: categorization

The biosensors for the determination of TG as analyte can be categorized as electrochemical biosensors, metal oxide based biosensors, flow injection based biosensors, conducting polymer based biosensors, microgel based optical biosensors and enzyme nanoparticles based biosensors as described below:

TG biosensors based on electrochemical techniques

There are different measurements principles involved in the working of various electrochemical TG biosensors and thus classified accordingly: amperometric, conductometric and potentiometric biosensors.

Amperometric TG biosensors

In electrochemical techniques, amperometry is insightful in which current is the signal of concern linearly depending upon the concentration of the target analyte when a steady

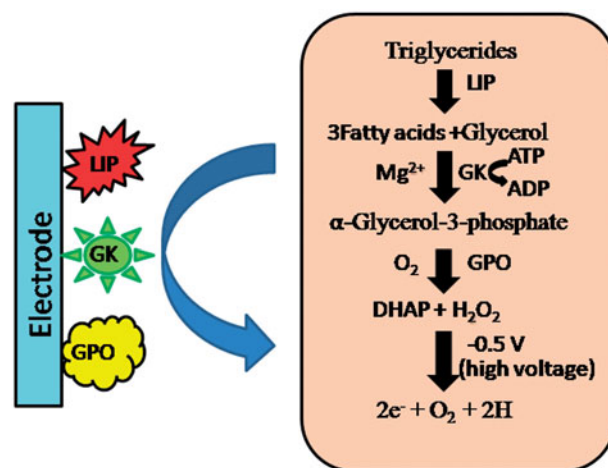


Figure 3 Schematic representation of electrochemical reactions involved in response measurement of enzyme based amperometric TG biosensor.

potential is applied [26]. In amperometric TG biosensors, the collective reaction of enzymes –lipase (LIP), glycerol kinase (GK) and glycerol-3-phosphate oxidase (GPO) generate H_2O_2 from TG, which is finally decomposed to release electrons at high voltages as shown in Figure 3. In an enzymatic reaction cascade, H_2O_2 produced is directly proportional to the flow of electrons, i.e. current which in turn, is directly proportional to the amount of analyte (TG) in the sample [27]. There are mainly two types of amperometric biosensors, which have been reported for determination of TG in past years: (1) Membrane based TG biosensors, (2) DO metric based TG biosensors

Membrane based TG biosensors

Amperometric TG biosensors employing different membranes such as polyvinyl acetate (PVA) [17], gelatin membrane [28], egg shell [29], cellulose acetate (CA) [16] and PVC membranes [15,30] have been fabricated in recent years. Various techniques such as physical adsorption, covalent binding, microencapsulation, entrapment, cross-linking were used to

Table 2. Membrane and DO metric based amperometric TG biosensors and comparison of their analytical properties.

Mode of Detection	Transducer	Immobilization Method	Linearity	Minimum detection limit	Storage stability	Response time	References
Amperometric	LIP/GK/GPO/HRP/PVC	Crosslinking	0.56–2.25 mM	0.11 mM	40 days	30 s	[15]
Amperometric	LIP/GK/GPO/GCE/ gel-atin membrane	Crosslinking	0.14–1.125 mM	0.14 mM	30 days	NR	[28]
Amperometric	LIP/GK/GPO/Egg shell membrane/Au	Physical adsorption	0.56–2.25 mM	0.28 mM	200 times used within 70 days	10 s	[29]
Amperometric	LIP/GK/GPO/HRP/PVC	Crosslinking	0.1–15 mM	0.1 mM	50% decay after 200 uses	NR	[30]
Amperometric	Diaphorase/GDH/ Collagen membrane	Physical adsorption	0.11–5.6 mM	0.1 mM	27 days	900 s	[31]
Amperometric	LIP/GK/GPO/POD/glass beads	Crosslinking	0.20–1.38 mM	0.20 mM	50% decay after 150 uses	1200 s	[16]
DO	LIP/GK/GPO/CA	Crosslinking	0.2–3.5 mM	0.2 mM	150 times used within 25 days	40 s	[16]
DO	LIP/GDH/Oxygen electrode	NA	0.05–0.3 mM	0.05 mM	NR	300 s	[32]
DO	LIP/GK/GPO/HRP/PVA	Crosslinking	0.56–2.25 mM	0.21 mM	50% decay in 50 days	2 s	[17]
DO	LIP/GK/GPO/PUM	Physical adsorption	0.005–1 mM	0.005 mM	NR	90 s	[33]
DO	LIP/GK/GPO/PVC	Crosslinking	5–20 mM	0.35 mM	NR	10 s	[34]

LIP: Lipase; GK: glycerol kinase; GPO: glycerol-3-phosphate oxidase; GDH: glycerol dehydrogenase; HRP: horse radish peroxidase; GCE: glassy carbon electrode; PVC: polyvinyl chloride; PUM: poly urethane membrane; PVA: polyvinyl alcohol.

immobilize enzymes on these membranes. In these biosensors, the membrane bound enzymes-lipase, GK and GPO were mounted onto a Pt or glass electrode (GC) or Au electrode (in case of egg shell membrane only) to assemble a working electrode. Then, the working electrode was connected to the reference electrode (Ag/AgCl) and auxiliary electrode (Pt wire) through the potentiostat. Under a potential of 0.4 V, the electrons generated from the H_2O_2 in the enzymatic reaction were measured by the fabricated biosensors. The measured current was directly proportional to the concentration of triolein/TG. In 1997, Schoemaker et al. constructed a sensitive three-electrode system for the determination of triglycerides [31]. Collagen membrane was used to immobilize diaphorase and GIDH enzymes. In the presence of phosphate buffer containing 2, 6 dichlorophenol-indophenol (DCPIP), incubation of serum samples was done with a microbial lipase. The ensuing glycerol was then oxidized by NAD^+ accompanying GIDH; the NADH generated was then oxidized by DCPIP. Serum triglycerides ranging from 10 to 500 mg/dL were simply assayed using small amount of sample in less than 15 min. Later, various TG biosensors based on membranes were fabricated, which displayed dynamic linear range along with low-detection limit in a short response time as shown in Table 2 with the comparison of their analytical properties. In the near future, it is anticipated that the use of biocompatible artificial membranes would be promising as the matrix for immobilization of enzymes, which would help in enhancing the selectivity and sensitivity of biosensors for the rapid, cheap and effective detection of TGs.

DO metric based TG biosensors

Dissolved oxygen (DO) which is consumed in the oxidation reaction of triglycerides by membrane bound with enzymes lipase, glycerol kinase (GK), glycerol-3-phosphate oxidase (GPO) is measured in these biosensors. This dissolved O_2 is directly proportional to the concentration of TG. These biosensors are very simple to handle and can be easily operated in the laboratories without employment of special trainee

and expertise. The disadvantage with DO based TG biosensors is that atmospheric oxygen can interfere in their working and thus resulting in low sensitivity. Earlier in 1984, Kelly et al. developed a TG measurement system, which involved indirect monitoring of NADH using its reaction with oxygen by horseradish peroxidase (HRP) [32]. The optimal response was shown at 33°C temperature and 7.5pH within 300 s by the proposed biosensor. The biosensor showed linearity and LOD as 0.05 to 0.3 mM and 0.05 mM, respectively. Table 2 provides a comparison of various DO metric based TG biosensors [33,34].

Conductometric/impedimetric TG biosensors

In a TG biosensor based on impedimetric technique, there is electrochemical deposition of polyaniline nanotubes (PANI-NT) onto the surface of indium-tin-oxide (ITO) coated glass plate. Then, covalent immobilization of enzyme- lipase is done by using glutaraldehyde. The change in the charge transfer resistance (R_{CT}) of PANI-NT film as a result of production of fatty acid molecules during TG hydrolysis, detects the varying concentration of TG. The fabricated Lipase/PANI-NT/ITO bioelectrode for the detection of TG in sera displayed sensitivity of $2.59 \times 10^{-3} K \Omega^{-1} \text{mg/dL}$ and linearity ranging from 25 mg/dL to 300 mg/dL with a short response time of 20 s [35]. These biosensors are easy to incorporate into micro-processor-controlled diagnostic tools and hence used as powerful method to estimate TG concentration. The demerits include the requirement of technological precision of the device along with the operating procedures [36].

Potentiometric TG biosensor

Catalytic hydrolysis of triglycerides into glycerol and free fatty acid by the lipase enzyme forms the basis of potentiometric TG biosensors. The production of fatty acid causes a change in the pH of the solution containing analyte, which is then deliberated by applying an open circuit potential configuration. In these biosensors, the potential difference between the

Table 3. Potentiometric /ion selective electrode based TG biosensors and comparison of their analytical properties.

Mode of detection	Transducer	Immobilization method	Linearity	Minimum detection limit	Storage stability	Response time	References
Potentiometric	Psi based electrode	Physical adsorption	5.9–21 mM	NR	180 – days	900 s	[38]
Potentiometric	Mesoporous Psi matrix	Physical adsorption	<5.6 mM	NR	17 hours working time within 60 days	NR	[39]
Potentiometric	EISCAP	NR	5–15 mM	5 mM	NR	NR	[40]
Potentiometric	Crystalline silica based miniaturized EISCAP	Covalent coupling	5 mM	NR	NR	30 s	[41]
Potentiometric	Microcantilever sensor	NR	0.024–0.48 mM	0.01 mM	NR	1200 s	[42]
Potentiometric	Silicon nitride layer of EISCAP	Covalent coupling	1–7 mM	5 mM	NR	2700 s	[42]
Potentiometric	EISCAP	NR	0.1–18 mM	0.1 mM	NR	1800 s	[43]
Potentiometric	Miniturized EISCAP	Covalent coupling	0.56–1.68 mM	0.56 mM	NR	300 s	[44]
Potentiometric	LIP/Pt wire (pH-FET)	Physical adsorption	0.6–3 mM	0.00009 mM	44 days	120 s	[45]
Potentiometric	Gate surface of a FET	Physical adsorption	5–30 mM	5 mM	45% decay after 14 days	<900 s	[46]

FET: Field effect transistors; Psi: porous silicon; EISCAP: electrolyte insulator semiconductor capacitors.

working electrode and the reference electrode is measured at zero current flow. The reference electrode generates a constant potential while a variable potential is produced by the working electrode, which rely on the analyte concentration [37].

Sub-categorization of potentiometric TG biosensors

On the basis of principle followed by different potentiometric biosensors, they can be additionally classified as porous silica, electrolyte-insulator-semiconductor capacitor (EISCAP), field effect transistor (ENFET) based TG biosensors. Various potentiometric/ion selective electrode based TG biosensors and comparison of their analytical properties have been described in Table 3.

TG biosensors based on porous silicon

The advantage of easy fabrication, greater adsorption property and controllable pore size makes porous silicon a leading candidate for various applications. In addition, it is attuned with standard silicon processing technology. Porous silicon (PSi) is prepared from the p-type crystalline silicon, which was oxidized thermally. It is utilized to immobilize lipase enzyme, which hydrolyses triglycerides to fatty acids. In 2003, Reddy et al. fabricated a potentiometric biosensor based on porous silicon for the quantitative estimation of triglycerides. The schematic representation of the biosensor has been shown in Figure 4. PSi was employed as an enzyme reactor. The enzyme was immobilized via physical adsorption on to the oxidized porous silicon, which was made-up from silicon. The employment of enzyme solution- oxidized porous silicon-crystalline silicon structure was done to sense the changes in pH of the solution during the hydrolysis of tributyrin as a result of shift in the capacitance-voltage (C-V) characteristics. The change in pH was proportional to the concentration of triglycerides present in the solution. The sensitivity of the sensor was high along with good reproducibility and reversibility. The LOD of the proposed biosensor for triglyceride was 1 μ M [38]. In 2016, Al-Hardan et al. developed a highly sensitive pH sensor, which utilized porous silicon (PSi) as an

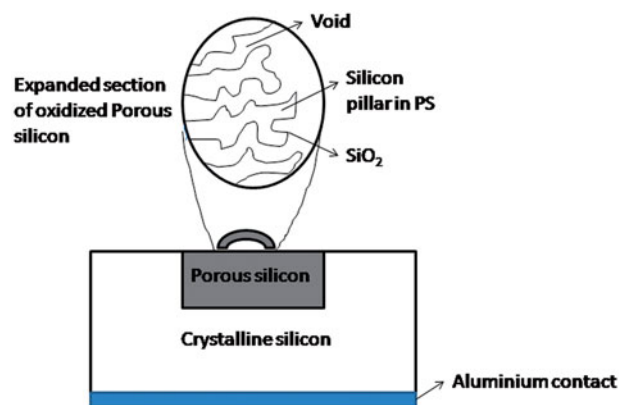


Figure 4 Diagrammatic representation of TG biosensor based on porous silicon.

extended gate field-effect transistor (EGFET). They prepared PSi with pore sizes in the range of 500 to 750 nm and a depth of approximately 42 μ m. Further when PSi was tested for sensing of hydrogen ion in different pH buffer solutions, it was revealed that the PSi had a sensitivity value of 66 mV/pH (a super Nernstian value). The sensor considers stability to be in the pH range of 2–12 was considered highly stable for the sensors based on PSi. The hysteresis values of the proposed PSi sensor were approximately 10.5 and 8.2 mV in the high and low pH loop, respectively [47]. Some of the drawbacks of these PSi based sensors include less specificity, high cost and complicated operation.

TG biosensors based on electrolyte-insulator-semiconductor capacitor (EISCAP)

Electrolyte–insulator–semiconductor capacitor (EISCAP) is mainly a pH sensor, which displays a shift in the measured capacitance-voltage (CV) characteristics with changes in the pH of the electrolyte (containing the bioanalyte) [39]. Owing to its easy preparation and possessing a planar surface, EISCAP is preferred over other transducers such as NH_4 -ISFET and pH-FET in the biosensors [40]. In EISCAP, enzyme molecules are immobilized via cross-linking, which ensures superior reproducibility. EISCAP turn into a particular biosensor because of the enzyme. When tributyrin, a short chain TG, is

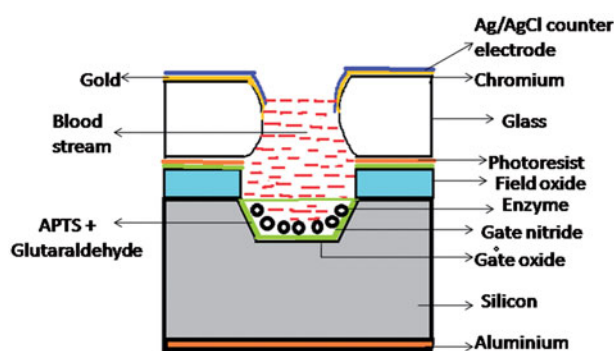


Figure 5 Schematic diagram of enzyme immobilized mini-EISCAP.

hydrolyzed in the presence of enzyme lipase, generates butyric acid and glycerol as a product resulting in more acidic electrolyte. The change in the pH of the solution is directly proportional to the amount of tributyrin produced. In 2008, Vemulachedu et al. developed a miniaturized TG biosensor based on the cubical pits of dimensions $1500\text{ }\mu\text{m} \times 1500\text{ }\mu\text{m} \times 100\text{ }\mu\text{m}$, which have a capacity to hold $0.1\text{ }\mu\text{L}$ of an electrolyte. During the course of the reaction, the flat band voltage shift in the capacitance voltage (C-V) characteristics was monitored and the changes in pH of the solution were sensed through the EISCAP. In comparison with the sensors of bigger dimensions, these miniature cells displayed high reaction rate. The drawbacks of the proposed sensor included complexity in preparation and requirement of 30 min post processing time [41]. In 2009, Fernandez et al. fabricated a biosensor for the estimation of triglycerides. For the stable measurements, the prepared biosensors imposed the existence of a buffer, which confines the sensitivity of the sensor at low amount of the analyte upto 1 mM [42]. In 2011, Preetha et al. developed a modified EISCAP sensor for the determination of triglycerides in blood. The EISCAP was optimized to operate at $25\text{ }^{\circ}\text{C}$ in 1 mg lipase in 1 mM Tris-HCl buffer (pH 7.4) and 0.25 mM phosphate buffer (pH 6) containing 1 M KCl. The sensor displayed sensitivity of $55 \pm 0.5\text{ mV}$ per unit change of pH and correlation coefficients of 0.979 along with higher storage stability of around 180 days [43]. In 2014, Veeramani et al. constructed a TG biosensor based on EISCAP as shown in Figure 5. It estimated the concentration of TG within the quantifiable range of 50–150 mg/dL in short time period of less than 5 min [44].

TG biosensors based on field effect transistor (ENFET)

Ion-selective field effect transistor (ISFET) is a strong platform to expand area of development in the field of biosensors. The FET devices have similar electronic properties as that of the conventional metal oxide semiconductor field-effect transistors (MOSFETs), therefore they respond quickly to the chemical environment changes, which facilitates a highly sensitive and steadfast pathway for the biomolecules detection. A variety of techniques have been utilized such as polymer entrapment or covalent bonding, to link enzymes on to the gate surface of a FET. In 1986, Nakako et al. developed an enzyme electrode based on hydrogen ion-sensitive field effect transistors (pH-FET's) for the analysis of neutral lipid.

The electrode was made up of two pH-FET's and a platinum wire. One of the FET's was immobilized with lipase membrane. The wide concentration ranges of triglycerides were determined by the proposed electrode within a short response time of 2 min [45]. The electrode suffered few technological and fundamental problems including instability in the functional groups of the sensing layer and impurities of the semiconductor. In 2008, Vijayalakshmi et al. fabricated a FET based novel sensor in which the thermostable lipase enzyme was immobilized on the magnetic nickel ferrite nanoparticles and a permanent magnet was applied below the gate of the FET. The TG concentrations within the range of 0.1–1.5% were estimated by the method [46]. The biosensor offered high-yield manufacturing, high speed and low cost, without compromising the sensitivity in diagnostics.

TG biosensors based on conducting polymer

Researchers have shown enormous interest in the electrochemical biosensors employing conducting polymer (CP) as they are cost-effective, stable, durable and easy to fabricate. In addition, these biosensors offer a direct electrical visualization of the analyte presence along with high selectivity and sensitivity when an appropriate enzyme is immobilized in the conducting polymer matrix. The utilization of conducting polymer simplifies the designs of biosensors as these materials behave both as transducers and sensing elements at the same time [48]. In 2007, Speiser constructed a TG biosensor using a nanocomposite film consisting of single-walled carbon nanotubes (SWCNT) and polyaniline (PANI). Using electrophoretic technique, the film was coupled onto the surface of ITO coated glass plate. The linear sweep voltammetry was used to carry out response studies [49]. In 2010, Dhand et al. fabricated a working bioelectrode represented as Lipase-GDH/PANI-SWCNT-TB/ITO, which could detect tributyrin in the concentration range of 50–400 mg/dL⁻¹ in a short response time of 12 s. The electrode displayed high sensitivity of 4.28×10^{-4} mA mg/dL and storage stability of 91 days [50]. In 2014, Jeong et al. developed a TG biosensor by immobilizing lipase, GK and GPO onto the polymer matrices via covalent binding. The detection range of the proposed biosensor was 15–20 mg/dL of micellar TG in serum of patients [51]. Poor processibility and chemical instability were critical challenges of these biosensors.

TG biosensors based on flow-injection analysis

FIA based biosensors are simple, cheap and flexible. Sample preparation and estimation is automated in these sensors along with quick start-up and shutdown times. In a flow-injection enzyme based electroanalytical system, enzymatic reactions are carried out in capillary taken after by electrochemical detection. In 2005, Wu and Cheng developed a biosensing system composed of lipase, GK and GPO, which exhibited a flow-injection analysis peak response of 2.5 min. For triglycerides, it displayed linearity range of 10^{-3} to 10^{-2} M. The storage stability was upto 2 months and 15 samples could be analyzed per hour [52].

TG biosensors based on nanostructured metal oxides (NMOs)

NMOs have moved toward becoming essential resources for biosensors, as they offer a powerful surface for immobilization of biomolecules with enhanced compliance, preferred orientation and high biological activity, which results in bringing about improved detecting attributes. These fascinating NMOs are relied upon to discover appliance in an innovative generation of smart and miniaturized biosensing strategies [53].

In 2007, Rejeb et al. developed an amperometric TG biosensor based on glycerol dehydrogenase/NADH oxidase, which employed a Prussian blue (PB) modified screen-printed electrode as a support for the two immobilized enzyme systems owing to their higher operating stability. The lipase activity against triglycerols was estimated using the proposed biosensor and storage stability, response time and effect of coenzymes were evaluated [54]. In 2008, Liao et al. fabricated a TG biosensor based on an iridium nano-particle modified carbon. This biosensor was evaluated using glyceryl tributyrates as a substrate. In human sera, a linear concentration range from 0 to 10 mM for glyceryl tributyrates and high sensitivity was observed. The inclusion of a selected surfactant and raising the incubation temperature resulted in better performance of the proposed sensor [55]. In 2009, Solanki et al. constructed a biosensor based on nanostructured cerium oxide for the determination of TG in sera. Lipase was immobilized onto the sol-gel derived Nano-CeO₂ film linked to the ITO coated glass plate. The biosensor showed minimum detection limit of 32.8 mg/dL and linearity range of 5–500 mg/dL. The shelf life of the biosensor was 12 weeks [56]. Later in 2011, Narang and Pundir fabricated an amperometric TG biosensor by co-immobilizing lipase, GK and GPO enzymes onto CHIT and ZnONPs composite film electrodeposited on the surface of a Pt electrode. The proposed biosensor displayed high sensitivity, wide linear range of 50–650 mg/dL and LOD of 20 mg/dL with fast response within 6 s. At 4 °C, the biosensor showed shelf life of 210 days [57]. In 2012, Narang et al. constructed TG biosensor based on the co-immobilization of the enzymes (lipase, GK and GPO) on to the AuPPy nanocomposite decked poly indole-5-carboxylic acid electrodeposited onto Au electrode. The biosensor exhibited LOD of 20 mg/dL and linear response range of 50–700 mg/dL. The response time was 4 s [58]. In 2013, Chauhan et al. developed a biosensor utilizing platinum nanoparticles (PtNPs) and polypyrrole (PPY) multilayered nanocomposite films. In this amperometric TG biosensor, lipase was covalently immobilized onto the nanocomposite films electrochemically deposited on the surface of ITO coated glass plate. LOD and linearity of the biosensor was 25 mg/dL and 50–500 mg/dL, respectively. The bioelectrode exhibited shelf life of 9 weeks [30]. In the same year, Narang et al. fabricated TG biosensor using nickel oxide-chitosan/zinc oxide/zinc hexacyanoferrate film. The enzymes (lipase, GK and GPO) were co-immobilized onto the hybrid film electrodeposited onto the Au electrode. The biosensor showed high sensitivity of 0.05 μ A/mg/dL and shelf life of 6 months when stored at 4 °C [59]. A disposable amperometric TG biosensor

with high sensitivity was developed by Phongphut et al. The proposed biosensor was prepared by co-immobilizing lipase, GK and GPO onto inkjet-printed Au/PEDOT-PSS nanocomposite deposited on the surface of screen-printed carbon electrodes (SPCEs). The biosensor displayed low LOD of 7.88 mg/dL, wide linear range of 0–531 mg/dL, good reproducibility and modest response time of 30 s [20]. In 2014, Gupta et al. constructed a TG biosensor utilizing sol-gel derived SiO₂-CeO₂ nanocomposite film decorated onto ITO coated glass substrate. Lipase was immobilized onto the surface of nano CeO₂-SiO₂/ITO electrode through physical adsorption. The biosensor displayed high sensitivity of 2.28 mA mg⁻¹ dL and linearity ranging from 50 to 400 mg/dL [60]. In the same year, Wu et al. [61] fabricated a biosensor using lipase-nanoporous gold (NPG) biocomposite modified glassy carbon electrode (GCE) for the estimation of triglycerides. The working electrode exhibited long shelf life, high conductivity as well as anti-interference ability against uric acid, glucose and urea. The LOD of the biosensor was 2.68 mg/dL [61]. Narang et al. developed TG biosensor by co-immobilizing enzymes onto a working electrode denoted as MNP-CHIT/ZnO-ZnHCF/GCE. The modified electrode showed wide dynamic range of 10–1000 mg/dL, low LOD of 10 mg/dL and excellent storage stability [62]. In 2015, Bhardwaj et al. prepared a working bioelectrode made up of reduced graphene oxide (ERGO), which exhibits excellent electrochemical properties, high electron mobility as well as high surface area. Using chronoamperometric method, a homogeneous film of reduced graphene oxide was electrodeposited on the surface of ITO electrode. The fabricated bioelectrode represented as LIP-G1DH/ERGO/ITO, displayed wide linear range of 50–300 mg/dL for estimating TG [63]. In 2016, Solanki et al. fabricated a TG biosensor by covalently immobilizing lipase and G1DH onto zirconium oxide nanoparticles (nano-ZrO₂)-CHIT nanocomposite electrophoretically deposited on the surface of ITO coated glass plate. The biosensor showed LOD of 155 μ g ml⁻¹ within a short response time of 45 s [64].

TG biosensors based on enzyme nanoparticles (ENPs)

In TG biosensors, direct immobilization of enzymes onto nanocomposite might be able to cause their denaturation, resulting in their instability and loss of activity. Enzymes nanoparticles can be used to triumph over this problem. As ENPs are the nanosize entities of enzyme molecules, they display exceptional chemical and physical properties [65]. Recently, Pundir and Aggarwal (2017) fabricated a sensitive TG biosensor by covalently immobilizing nanoparticles of enzymes (LIP, GK and GPO) onto the surface of gold electrode. The prepared biosensor displayed superior amperometric detection of triglycerides in blood sample. It exhibited dynamic working range of 10–500 mg/dL and LOD of 1.0 μ g/mL within a short period of time (5 s) [66]. Narwal and Pundir (2017) designed a TG biosensor by immobilizing nanoparticles of lipase, GK and GPO onto electrode made up of pencil graphite. This ENPs/PG working electrode showed a lower LOD of 0.1 nM within 2.5 s, long shelf life and high sensitivity.

Table 4. Conducting polymer, nanostructured metal oxides (NMOs) and ENPs based TG biosensors and comparison of their analytical properties.

Mode of detection	Transducer	Immobilization method	Linearity	Minimum detection limit	Storage stability	Response time	References
Amperometric	LIP/GDH/PANI/SWCNT/ITO	Covalent coupling	0.56–4.52 mM	0.56 mM	91 days	12 s	[50]
Amperometric	LIP/GK/GPO	Covalent coupling	0.17–2.26 mM	0.17 mM	NR	NR	[51]
Amperometric	LIP/GK/GDH/IrNPs/CPE	Covalent coupling	0–10 mM	0.1 mM	NR	NR	[55]
Amperometric	LIP/Nano-CeO ₂ /ITO	Covalent coupling	0.56–5.65 mM	0.37 mM	84 days	NR	[56]
Amperometric	LIP/GK/GPO/CHIT/ZnONPs	Covalent coupling	0.56–7.35 mM	0.23 mM	50% decay in 210 days	6 s	[57]
Amperometric	LIP/NPG/GCE	Covalent coupling	0.56–7.91 mM	0.23 mM	50% decay after 100 uses in 210 days	4 s	[58]
Amperometric	LIP/GK/GPO/PtNPs/PPY/ITO	Covalent coupling	0.56–5.65 mM	0.28 mM	270 days	NR	[30]
Amperometric	NiONPs-CHIT/ZnO-ZnHCF/LIP/GK/GPO/Au	Covalent coupling	0.56–7.91 mM	0.11 mM	50% decay in 180 days	4 s	[59]
Amperometric	LIP/GK/GPO/Inkjet-printed Au/PEDOT-PSS/SPCE	Covalent coupling	0–6 mM	0.09 mM	40% decay in 30 days	30 s	[20]
Amperometric	LIP/NanoCeO ₂ -SiO ₂ /ITO	Covalent coupling	0.565–4.52 mM	0.565 mM	NR	12 s	[60]
Amperometric	LIP/GK/GPO/MNP-CHIT/ZnO-ZnHCF/GCE	Covalent coupling	0.56–2.83 mM	0.03 mM	NR	NR	[61]
Amperometric	LIP/GK/GPO/MNP-CHIT/ZnO-ZnHCF/GCE	Covalent coupling	0.11–11.3 mM	0.11 mM	NR	NR	[62]
Amperometric	LIP/GDH/TB/ERGO/ITO	Covalent coupling	0.56–3.39 mM	0.56 mM	60 days	NR	[63]
Amperometric	LIP/GK/GPO/CHIT-nano-ZrO ₂ /ITO	Covalent coupling	0.0001–0.05 mM	0.000155 mM	85% decay in 77 days	45 s	[64]
Amperometric	LIPNPs/GKNPs/GPONPs/Au	Covalent coupling	0.11–5.65 mM	0.001 mM	90 days	5 s	[65]
Amperometric	LIPNPs/GKNPs/GPONPs/PGE	Physical adsorption	0.1–45	10 ⁻⁷ mM	20% decay in 240 days	2.5 s	[66,67]

LIP: lipase; GK: glycerol kinase; GPO: glycerol-3-phosphate oxidase; ITO: indium-tin-oxide; GDH: glycerol dehydrogenase; CHIT: chitosan; GCE: glassy carbon electrode; CeO₂: cerium oxide; ZnHCF: zinc hexacyanoferrate; TB: tributyrin; IrNPs: iridium nanoparticles; ERGO: electrochemically reduced graphene oxide; ZnO: zinc oxide; ZnONPs: zinc oxide nanoparticles; NPG: nanoporous gold; PPY: polypyrrole.

Various TG biosensors based on conducting polymers, nanostructured metal oxides and enzyme nanoparticles have been shown and analytically compared in Table 4.

Conclusion and future outlook

The potential of TG biosensors is apparent to healthcare industry for the monitoring of elevated level of triglycerides in human sera, which leads to several diseases. A brief outline of the recent progress in the design of enzyme based electrochemical biosensor platforms for the triglyceride estimation has been provided. Advancement in deployment of enzymes and nanoscale electrode materials for the evaluation of TG level in the sera has risen as a promising advent having enormous potential in terms of new functionality, high specificity, miniaturization, sensitivity and robustness in solving a wide range of challenges in field of biosensors. The utilization of conducting polymers, EISCAP, ENFET, porous silicon and nanostructured metal oxides to modify the electrodes could appreciably improve the capacity of electrochemical signal for selective determination of the target molecule and the major challenges pertaining to the fabrication of point-of-care biosensors could be resolved. In near future, biosensors must be unobtrusive, versatile, economic, non-invasive, sleek and wearable that would be able to accurately quantify triglyceride concentration in the sera in real time. The use of disposable, smart and user-friendly sensing devices will be preferred to diagnose the health status of patients by the healthcare professionals. Undoubtedly, the future prospects of enzyme based electrochemical biosensors are very promising and will

show an impending growth to reform the future personalized health care diagnostics. Biosensor expertise is still solicited by copious challenges to be tackled.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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