Chapter 22

Lipase, Phospholipase, and Esterase Biosensors (Review)

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Abstract

A biosensor is a device composed by a biological recognition element and a transducer that delivers selective information about a specific analyte. Technological and scientific advances in the area of biology, bioengineering, catalysts, electrochemistry, nanomaterials, microelectronics, and microfluidics have improved the design and performance of better biosensors. Enzymatic biosensors based on lipases, esterases, and phospholipases are valuable analytical apparatus which have been applied in food industry, oleochemical industry, biodegradable polymers, environmental science, and overall the medical area as diagnostic tools to detect cholesterol and triglyceride levels in blood samples. This chapter reviews recent developments and applications of lipase-, esterase-, and phospholipase-based biosensors.

Key words Biosensor, Enzyme, Lipase, Esterase, Phospholipase, Application

1 Introduction

The first biosensor named "enzyme electrode" was developed in the 1960s by Clark and Lyons [1] and consisted of a glucose oxidase enzyme coupled to an oxygen electrode. Since then biosensor technology has improved and impressively increased their application in areas such as environmental pollution control, biodefense, clinical analysis, metabolic engineering, clinical analysis, agricultural and bioprocess monitoring, etc. [2, 3]. Biosensors have been used in fermentation process to monitor key variables such as substrates and product concentrations allowing to improve process yields and productivity through the design of control strategies [4, 5]. A biosensor coupled to a telemetry system was used to monitor glucose and ethanol at distance in craft breweries [6]. Environmental applications of biosensors include determining toxic contaminants in water [7–9], as well as pesticides, heavy metals, and pollutants like dioxins, phenols, and polycyclic aromatic hydrocarbons [10–12]. In food industry, biosensors have been used for contamination of primary materials and processed foods and for quality control [13–15]. Detection of pathogenic microorganisms is an

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active research area in food industry and bioterrorism [16–18]. The most important biosensor market is related with clinical and pharmaceutical applications. Biosensors have been used as a diagnostic tool for monitoring glucose [19], urea [20], and ethanol [21], among others [22]; however, current applications are focused to opportunely detect in humans microbial diseases [23] and other serious disorders such as cancer, ischemia, etc. [24, 25], among other important medical applications [26]. Within the clinical and industrial sector, enzymes have been extensively employed as a biological recognition element in biosensor design since they present some advantages as high selectivity and fast response and some of them are commercially available or can be isolated and purified from diverse sources [27].

Esterases, lipases, and phospholipases are enzymes that catalyze the hydrolysis of esters, fats and oils, and phospholipids, respectively. Lipases hydrolyze long-chain fatty acid triglycerides, while esterases hydrolyze shorter fatty acids [28, 29]. Lipase production is important since they had diverse applications such as detergents, agrochemicals, biodegradable polymers, textile industry, oleochemical industry, cosmetics and flavors, and resolution of racemic mixtures [30]. On the other hand, esterases are used to degrade natural materials and industrial pollutants, such as plastics and cereal wastes [31], while phospholipases are related to food and oil industries [32]. Lipases are also important drug targets or marker enzymes in the medical sector. They can be used as diagnostic tools, and their presence or increasing levels can indicate certain infection or disease [33]. In this sense, it is estimated that in the year 2022, the biosensor market for medical, environmental, food, and agriculture industries will reach to US $27.06 billion [34]. Thus, lipase, esterase, and phospholipase biosensor development is relevant and still is an open issue, particularly for clinical and pharmaceutical applications. Herein we describe recent developments and applications of lipase, esterase, and phospholipase enzymatic biosensors. The chapter is organized as follows: Subheading 2 describes the generalities in biosensor design; Subheading 3 is related on enzymatic biosensors; Subheading 4 describes lipase, esterase, and phospholipase biosensors; and finally Subheading 5 states new trends in biosensor design.

2 Biosensor Preliminaries

2.1 Biosensor Definition

An electrochemical biosensor has been defined as a "self-contained integrated device, which is capable of providing specific quantitative or semi-quantitative information using a biological recognition element retained in direct spatial contact with an electrochemical transduction element" [35]. In general a biosensor is a device contained in a small package, where the biological recognition

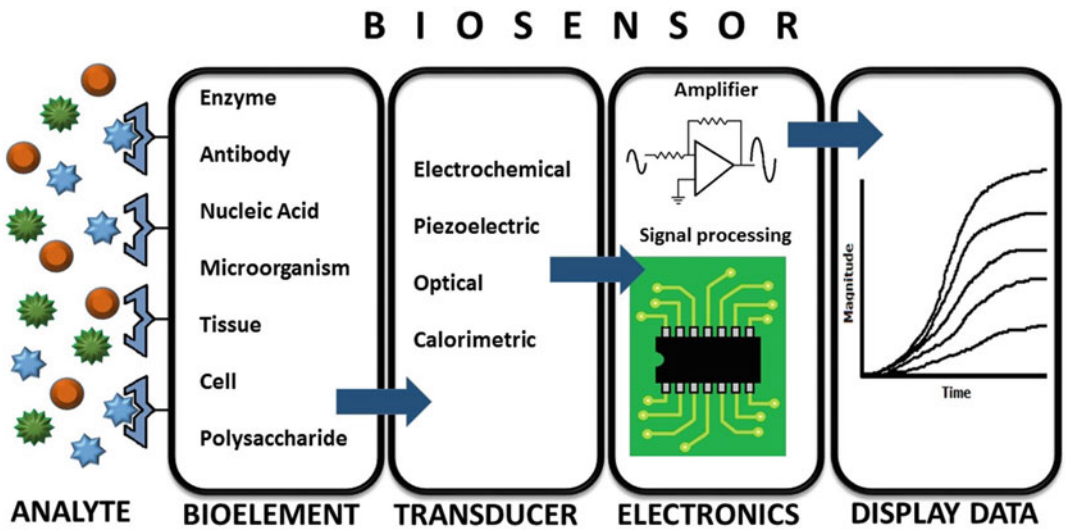

Fig. 1 Basic structure of a biosensor

element is in direct contact with the transducer. The biological recognition system translates biochemical information, usually an analyte sample, into a chemical or physical output signal with a defined sensitivity. The recognition system provides the sensor with high selectivity for the analyte to be measured and does not recognize other analytes. Biological recognition elements or bioelements include enzymes, antigens, antibodies, nucleic acids, receptors, cells, and cell organelles. The transduction element could be optical fibers, piezoelectric devices, crystals, and various types of electrochemical electrodes. The principle of detection in a biosensor is the specific binding of the analyte of interest to biorecognition element immobilized on a suitable support medium. The specific interaction results in a change of physical-chemical properties such as pH, electron transfer, mass, and heat transfer which are detected and measured by the transducer [36]. Figure 1 shows the basic structure of a biosensor.

2.2 Biosensor Classification

Biosensors may be classified based on their transducer type in electrochemical, optical, calorimetric, and piezoelectric, although there are other transducers as bioluminescence (Fig. 2). Thermal detection biosensors are based on the absorption or production of heat that changes the temperature of the medium when a reaction takes place. When the sample comes in contact with the enzyme, the heat reaction of the enzyme is measured; the heat produced or absorbed is proportional to the molar enthalpy and the total number of molecules in the reaction [2, 37]. In optical biosensors, the output signal is light, where energy of the electromagnetic radiation measured can provide information about changes in the local environment surrounding the analyte, its molecular vibrations, or

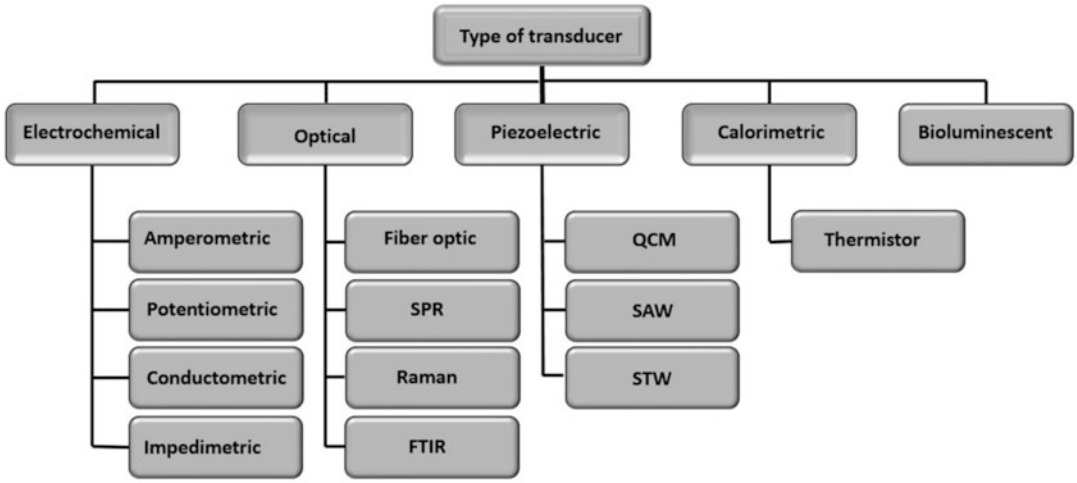

Fig. 2 Biosensor classification based on the type of transducer

the formation of new energy levels; this information may be determined with many types of instruments such as fiber optics [38], Raman spectroscopy [39], and surface plasmon resonance (SPR) [40], among others [41]. Piezoelectric biosensors are based on crystals designed to vibrate at a specific frequency when an electrical signal excites them. The frequency of oscillation is therefore dependent on the electrical frequency applied to the crystal as well as the crystal mass. Therefore, when the mass increases due to binding of chemicals, the oscillation frequency of the crystal changes and the resulting change can be measured electrically and be used to determine the additional mass of the crystal [42]. The surface acoustic wave (SAW) and the quartz crystal microbalances (QCM) are two approaches for piezoelectric biosensors. A SAW device generates and detects acoustic waves on the surface of a piezoelectric crystal [43], while the QCM biosensor is composed by a quartz crystal located between two metal electrodes; when an oscillating electric field is induced, the mass change at the crystal surface is proportional to the acoustic wave [44]; both devices feature low costs. Recently, bioluminescence biosensors have appeared to detect viability of bacteria, since only metabolically active cells can produce light [45]. The last classification is given by electrochemical biosensors which will be discussed in more detail in the next section.

2.2.1 Electrochemical Biosensors

If a biological system features electrochemical properties, then it is natural to think that a biosensor could take advantage of those characteristics, e.g., to catalyze the reaction of a biochemical compound transforming the response of the sensing material into electrical signals [46]. In an electrochemical biosensors, enzymes are commonly used as the biological recognition element in which analytes are detected by the physicochemical transducer providing

a measurable signal [47]. Electrochemical biosensors are divided in conductometric, potentiometric, amperometric, and impedimetric according to the way they generate a signal. The amperometric biosensor measures the current resulting from an electrochemical reaction at a constant applied potential in a Pt or Au electrode [35]. The measured current is directly correlated to the oxidation or reduction taking place in an electrode in an electrochemically active solution [48]. Potentiometric biosensors are based on the principle that a transducer senses the variation in protons in a biological sample such as an enzyme which is correlated with the analyte concentration [49]. Potentiometric biosensors are commonly designed from ion-selective electrodes (ISE) and ion-sensitive field-effect transistors (ISFETs). The transducer may be an ISE which is an electrochemical sensor based on thin films or selective membranes as recognition elements [50]. An ISFET is composed of an ion-selective membrane applied directly to the insulated gate of the field-effect transistor (FET). In a potentiometric biosensor, ISFETs are coupled with a biocatalytic layer and are usually called enzyme field-effect transistors (ENFETs) [51]. In a conductometric biosensor, it measured the biological and chemical changes in the conductance between a pair of metal electrodes in a bulk solution [52]. Resistance and reactance in a biological sample are measured with an impedimetric biosensor [53]. Electrochemical biosensor has shown advantages such as appropriate detection limits, easy miniaturization, and use of small analyte volumes and may be used in turbid biofluids and robustness [54].

2.2.2 Bioaffinity and Catalytic Sensors

Biosensors could also be classified as bioaffinity and catalytic sensors. Bioaffinity biosensor operation is based on interaction of the analyte with macromolecules that have been isolated from their original biological environment or engineered. There is no consumption of analyte by the immobilized biocomplexing agent since equilibrium is usually reached. Typical receptors used in bioaffinity biosensor include antibodies and nucleic acids. These biosensors can be used to detect genetic material of microorganisms, for the presence of pathogens or pesticides or any type of substances that may cause an immune response [55]. Catalytic biosensors are based on a reaction catalyzed by macromolecules where continuous consumption of substrate is achieved by the immobilized biocatalyst incorporated into the sensor. Typical receptors used in catalytic biosensor include enzymes, whole cells, and tissues [35].

2.3 Biosensor Requirements

Biosensor performance may be appraised on sensitivity, response times, dynamic ranges, robustness, and simplicity of fabrication. Recent advances in technology involved in biosensor design and construction have allowed to overcome the technical difficulties, inexpensive, sufficiently sensitive, accurate, and easily manufactured at high rates [56]. Some important characteristics that biosensor

may have include high selectivity and sensitivity, accuracy and repeatability, linearity, fast response, long lifetime with a low price, and miniaturizable [55]. The biosensor must be so selective to interact exclusively with the compound of interest and not to others with similar properties; this is achieved through highly specific recognition elements. The biosensor must have enough sensitivity to detect concentrations of certain analytes. Sensitivity and linear range are a function of the physical design and the molecular recognition element [57]. The biosensor has to be reliable so it cannot be altered by the sample and should be insensible to temperature, electrical noise, or other environmental interferences. Some industrial process will require fast response from the biosensor in order to get rid of contaminated or damaged products. Therefore, low cost, long lifetime, and reusability are other desired characteristics. The size of the device is another important factor; advances in electronics and nanotechnology have allowed miniaturizing increasing the application field of these devices.

3 Enzyme Biosensors

Enzymes are proteins that catalyze chemical reactions in living organisms. In a reaction catalyzed by an enzyme, substrate binding occurs in a specific region of the enzyme called the active site, comprising a binding site and a catalytic site. An enzyme biosensor is an analytical device that combines an enzyme with a transducer to produce a signal proportional to target analyte concentration. This signal can result from a change in proton concentration; release or uptake of gases, such as ammonia or oxygen; light emission; absorption or reflectance; heat emission; and so forth, brought about by the reaction catalyzed by the enzyme. The transducer converts this signal into a measurable response, such as current, potential, temperature change, or absorption of light through electrochemical, thermal, or optical means. This signal can be further amplified, processed, or stored for later analysis [58]. Advantages of enzymatic biosensors include high selectivity, fast response, possibility of regeneration, simplicity involved in constructing the devices, and low costs [59]. Drawback will include sensitivity to environmental conditions, limited lifetime, and inhibition by substances in the sample [55]. Enzyme clinical-based biosensors may be classified as offline, in vivo, and online detectors [26]. In offline detectors, biological samples are put in the measuring chamber of the biosensor where the concentration of the analyte of interest is measured; commercial glucose belongs to this type of detectors. The entire process lasts only for a few seconds, and these devices are disposable [60]. Online detectors are coupled with a flow line coming from a sampling device in contact with the biological sample. In vivo detectors are biosensors implanted in the patient's

body and continuously read the concentration of the analyte of interest. Diverse transducers have been used to design enzyme biosensors such as thermistor [61], amperometric [48], FET [62–64], and optical [65], just to cite some of them. Biosensors based on oxygen measurements belong to the first generation of enzyme electrodes, i.e., Clark's glucose biosensor [1]. Second generation comprises specialized molecules named electron transfer mediators (ETM) to generate the response between the enzyme and the electrode, while the third generation is related with the direct response of the enzyme with the electrode through electrochemistry, showing high selectivity and sensitivity [66].

3.1 Immobilization Methods

Immobilization is a key stage in biosensor design and is the basis to achieve the interface between the biological material and the transduction system. Biosensors are usually designed with high enzyme loading to insure sufficient biocatalyst activities, and the enzymes are provided with an appropriate environment to sustain their activities [67]. Additionally the stability of the enzyme in the biosensor is of paramount importance. In order to obtain stable activity for a long time, enzymes are fixed on suitable solid supports. The process is known as immobilization. Immobilization means associating the biocatalysts with an insoluble matrix, so that it can be retained in proper reactor geometry for its economic reuse under stabilized conditions [68]. The immobilization method will depend on the recognition biological element, the type of transducer, the physicochemical properties of the analyte, and the operating conditions. The improper strategy of selection for the development of immobilization technique can cause damage to the conformation of biomolecules leading to the deactivation of biomolecular activity [69].

Immobilization methods include adsorption, covalent binding, cross-linking, encapsulation, and entrapment (*see* Fig. 3). With the adsorption method, the biomaterial is attached to the surface of the sensor by van der Waals forces, hydrophobic forces, hydrogen bonds, and ionic forces. Organic substances like charcoal, silica gel, and clay and inorganic substances as cellulose, starch, and collagen are used to immobilize enzymes with this method on the biosensor. In covalent binding the sensor surface is treated as a reactive group to which the biological materials can bind [68]. It requires mild conditions under which reactions are performed, such as low temperature, low ionic strength, and pH, in the physiological range [67], and their cost is expensive [70]. Substances as agarose, cellulose, and resins are commonly used for immobilization in this method. In the cross-linking method, biological material is chemically bounded to solid supports or cross-linking agents as glutaraldehyde and hexamethylene diisocyanate. This process is known as coreticulation, since it creates complex matrices that make multienzyme immobilization possible [71]. In the

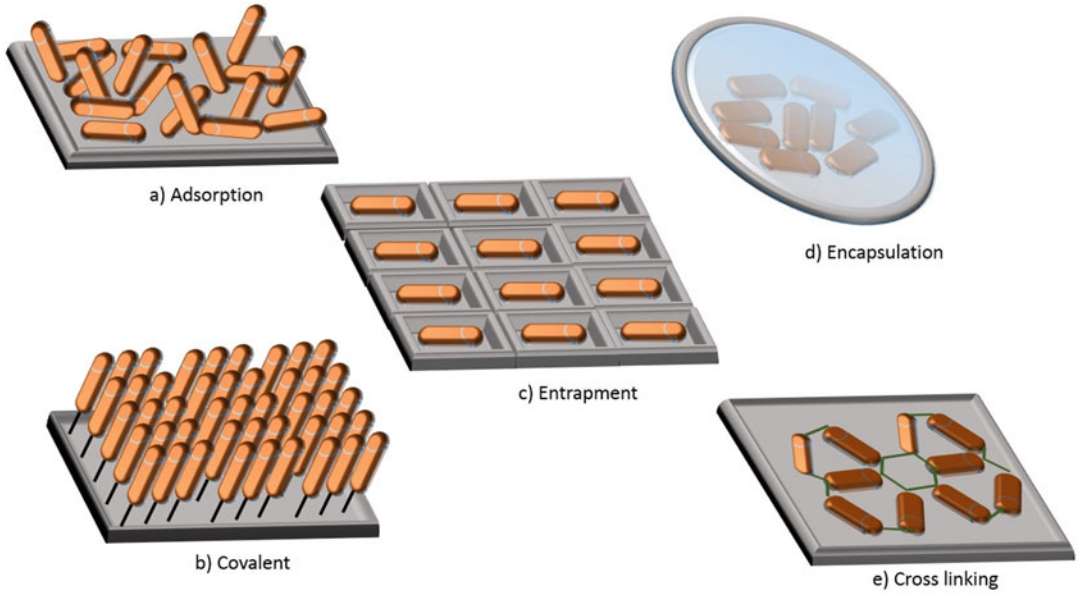

Fig. 3 Main immobilization methods for biosensors

entrapment method, a semipermeable membrane separates the analyte and the bioelement where the sensor is attached to the bioelement. The porous entrapment scheme is based on forming a porous encapsulation matrix around the biological material that helps in binding it to the sensor [68]. Substances as gelatin, collagen, alginate, and polyacrylamide have been commonly used for immobilization in the entrapment method. Other techniques such as encapsulation, sol-gel, photopolymerization, electropolymerization, etc. are described in [72].

3.2 Nanotechnology

Nanotechnology has an important role in biosensor. Nanotechnology involves the study, manipulation, creation, and use of materials, devices, and systems typically with dimensions smaller than 100 nm [73]. Electrochemical biosensors incorporating enzymes with nanomaterials are new materials with synergistic properties originating from the components of the hybrid composites. Biosensor based on nanotechnology has an excellent scenario as a new generation of bioelectronic devices with high sensitivity and stability [74]. Nanoscale materials have been used to achieve direct wiring of enzymes to electrode surface, to promote electrochemical reaction, to impose nanobarcode for biomaterials, and to amplify signal of biorecognition event [75]. Commonly used nanomaterials for enzyme biosensors are CNT and gold. Carbon nanotubes (CNTs) are graphite sheet rolled up into a nanoscale, which have diameters ranging between fractions of nanometers and tens of nanometers and lengths up to several centimeters with both their ends normally capped by fullerene-like structures [76]. Gold as a precious metal

shows high catalytic capability for many organic reactions. Metal nanoparticles have been used in biosensors to catalyze biochemical reactions, since they behave in the reaction medium as do conventional homogeneous catalysts, and can be easily recovered after the reaction [73]. Recent advances in nanomaterial to elaborate enzyme biosensors have been reported in [77], in which graphene is one of the most used nanocomponents [78, 79], with potential applications in environmental and medical areas [80, 81].

4 Lipase, Esterase, and Phospholipase Biosensors

4.1 Hydrolases

Hydrolases classified as EC 3 have shown wide substrate specificity and based on the bonds they act on are classified in diverse subclasses as amides, esters, etc. Esterases represent a special group of hydrolases (EC 3.1) that catalyze the cleavage and formation of ester bonds and are subdivided in carboxylic ester hydrolases (EC 3.1.1), thioester hydrolases (EC 3.1.2), phosphoric monoester hydrolases or phosphomonoesterases (EC 3.1.3), and phosphoric diester hydrolases or phosphodiesterases (EC 3.1.4). Carboxylic ester hydrolases (EC 3.1.1) are also classified as carboxylesterases (EC 3.1.1.1) and triacylglycerol (TAG) hydrolases or lipases (EC 3.1.1.3) based on their substrate specificity [29].

4.2 Lipase Biosensors

Lipases (triacylglycerol ester hydrolases, EC 3.1.1.3) are ubiquitous enzymes that catalyze the hydrolysis of fats and oils as long-chain mono-, di-, and triacylglycerols (TAGs), phospholipids, and cholesterol esters [82]; *see* Chap. 1 in this book. The main reaction occurs at the lipid-water interface. This characteristic distinguishes lipases from other hydrolytic enzymes and makes them unique target proteins with regard to selectivity and inhibition [83]. Diverse biochemical methods to detect lipase activity have been reported. Titrimetric, colorimetric, fluorimetric, turbidimetric, chromatographic, radiometric, enzymatic, physical, and immunological procedures may be used to detect lipase activity [84]. Existing methods to control the lipase activity are not yet suitable for non-purified samples and for large-scale analyses due to their cost and time requirements [85]. Therefore, specific devices like biosensors are an alternative for lipase activity detection [86]. A biosensor should be distinguished from analytical systems such as high-performance liquid chromatography (HPLC) or flow injection analysis (FIA) which incorporates additional separation steps or hardware. HPLC or FIA systems may incorporate a biosensor as a detecting device; however, a FIA system, containing a reagent reservoir, an enzymatic or immunological reactor, and, downstream, an electrochemical sensor, is not a biosensor [35].

Diverse biosensors have been reported to detect lipase activity. A surface acoustic wave was developed to detect pancreatic lipase

activity [87]. The sensor was based on the change in conductance of the solution caused by the release of a fatty acid, and triolein was used as substrate. A linear relationship between frequency response and enzyme concentration was obtained. A lithium niobate piezoelectric crystal-based surface acoustic wave (SAW) sensor connected to two parallel Pt electrodes in a detection cell was used to detect dimethoate, an organophosphate insecticide [88]. A lipase solution was put in the detection cell. The surface acoustic wave sensor was used to record the change in resonance frequency with time with a linearity of.167–1.34 μg/mL dimethoate and detection limit of 81 ng/mL dimethoate. An amperometric sensor for detecting lipase activity based on glycerol dehydrogenase/NADH oxidase was reported in [89]. A Prussian blue-modified screen-printed electrode was selected as substrate for the immobilized enzyme systems. Diverse parameters such as cofactor and coenzyme concentrations, pH effect, response time, and storage stability were evaluated and optimized. In spite of the importance in determining lipase activity, most lipase biosensors are related in using lipases for determining other compounds.

Lipases are important drug targets or marker enzymes in the medical sector. They can be used as diagnostic tools, and their presence or increasing levels can indicate certain infection or disease [33]. High concentration of cholesterol can cause heart disease, hypertension, arteriosclerosis, coronary artery disease, and cerebral thrombosis [69]. High concentration of triglycerides can cause hyperlipidemia which has been associated with several disorders including diabetes mellitus and liver obstruction [90]. Detection of triglycerides and cholesterol is of extreme importance in order to prevent unwanted disorders. Lipase biosensor has strongly focused on development of devices for detection of triglycerides and cholesterol, and the basic concept is to utilize a lipase to generate glycerol from triacylglycerol and quantify the released glycerol or alternatively the nonesterified fatty acid by chemical and enzymatic method, allowing a physician to diagnose patients with cardiovascular complaints [91].

The production of fatty acids by means of lipolysis reaction under specific reaction conditions may result in pH changes [92]. A pH ISFET sensor and a microreactor containing silica gel beads with surface-immobilized lipase were used to detect triglycerides [93]. Immobilization methods given by chemical bond to the surface of glass beads coated with keratin and adsorption onto nitrocellulose sheets had satisfactory behavior. The highest sensitivity was obtained for tributyrin (0.478 pH/mM for concentration < 4 mM), while the widest linear range was obtained for triacetin (up to 30 mM). Enzyme solution-oxidized porous silicon-crystalline silicon structure was used to detect changes in pH during the hydrolysis of tributyrin as a shift in the capacitance-voltage characteristics. Porous silicon, prepared from p-type (100)

crystalline silicon, was thermally oxidized and used to immobilize lipase, an enzyme, which hydrolyzes triglycerides resulting in the formation of fatty acids. This causes a change in the pH of the solution [94]. Glycerol and triglycerides were determined in an amperometric biosensor by means of glycerol dehydrogenase and lipase [95]. Glycerol dehydrogenase was adsorbed on the electrode, entrapped in gelatin, and immobilized in polylysine gel orb organic salts. Sensitivity of the electrodes varied from 2 to 9 nA/mM glycerol with steady state. NADH was produced by the catalytic oxidation of glycerol in the presence of glycerol dehydrogenase immobilized on the surface of an electrode. Glycerol and triacylglycerol were monitored with a flow injection enzymatic system. The best approach to measure glycerol and triacylglycerols was with the connection of a lipase column and a silica-fused capillary column co-immobilized with glycerokinase and glycerol-3-phosphate oxidase. Lipase helped the breakdown of triacylglycerol to yield free fatty acids and glycerol. The proposed biosensor exhibited a flow injection analysis peak response of 2.5 min and a detection limit of 5×10^{-5} M, with linear working ranges from 10^{-4} to 10^{-2} M for glycerol and from 10^{-3} to 10^{-2} M for triacylglycerol. The enzyme reactor was stable up to 2 months in continuous operation, and it was possible to analyze up to 15 samples per hour [96]. An amperometric triglyceride biosensor that was designed based on commercial lipase, glycerol kinase, and glycerol-3-phosphate oxidase was co-immobilized onto egg shell membrane through covalent coupling [97]. The biosensor showed optimum response within 10 s. The current was proportional to concentration of triglyceride in the range 0.56–2.25 mM. The minimum detection limit of the method was 0.28 mM. The biosensor could be used 200 times in a period of 70 days. Biosensor was still hampered by the awkward nature of the detection method requiring different enzymatic reactions; nevertheless, amperometric biosensors have the best perspectives out of all electrochemical biosensors [86].

With the advances in nanotechnology, it has been possible to design more sensitive and specific devices. A triglyceride potentiometric biosensor is based on immobilizing enzymes in an ISFET. The new approach of this biosensor consisted in using magnetic nanoparticles to immobilize the enzymes over the ISFET gate surface [62]. Enzymes immobilized could be conveniently retained on the ISFET gate surface by providing a laboratory magnet underneath the gate. Tributyrin, trioctanoate, and triolein were detected in the range of 5–30 mM, with fast response (<5 min). The biosensor could be used for several days without any loss of linearity. Additionally the measurements could also be made in small volumes (0.2 mL) reducing the requirements of large sample volume. A nanotube made of polyaniline was used to covalently immobilization of lipase via glutaraldehyde. This biosensor is based on a conductometric technique. Fatty acid molecules

produced during triglyceride hydrolysis result in change in charge transfer of the polyaniline nanotube film varying triglyceride concentration [98]. The biosensor featured linearity between 25 and 300 mg dL^{-1}, sensitivity of 2.59×10^{-3} $K\Omega^{-1}$ mg^{-1} dL, and response time as 20 s. Another version of this biosensor was reported and includes nanocomposite film comprising of polyaniline and single-walled carbon nanotubes (SWCNT) mounted onto indium tin oxide-coated glass plate using electrophoretic technique. In this case glycerol dehydrogenase and lipase were immobilized via *N*-ethyl-*N*′-(3-dimethylaminopropyl) carbodiimide and *N*-hydroxysuccinimide. Response of the biosensor states that tributyrin could be detected in the range 50–400 mg dL^{-1}, response time of 12 s, high sensitivity as 4.28×10^{-4} mA mg^{-1} dL, and storage stability of about 13 weeks [99]. Lipases for triglyceride detection were immobilized in sol-gel-derived Nano-CeO_2 film (35 nm). The lipase/nano-CeO_2/indium-tin-oxide biosensor shows linearity as 50–500 mg/dL, low detection limit of 32.8 mg/dL, response time of 20 s, shelf life of 3 months, and sensitivity of 0.195 mA/mg dL cm^2 [100]. Triglycerides were determined with a biosensor where the detection method was based on fast Fourier transform (FFT) in a flow injection system. Lipase and glycerol dehydrogenase enzymes were immobilized on to the CeO_2 nanoparticles and multiwall carbon nanotubes. Under optimal detection conditions, the linear response was in the range of 1–100 mg/L with detection limit of 0.5 mg/L [101]. A biosensor to detect diglycerides hydrogen peroxide, formed through the enzymatic breakdown of DG via lipase, glycerol kinase, and glycerol 3-phosphate oxidase, was electrochemically oxidized at an applied potential of +0.5 V in Ag/AgCl reference electrode. The current generated was proportional to diglyceride concentration in the range of 0–25 μM in phosphate buffer and bovine serum media [102]. The performance of two sensors to detect tributyrin was compared. The electrolyte-insulator-semiconductor capacitor (EISCAP) that had limited sensitivity of the EISCAP was limited to less than 500 μM whereas the microcantilever, a micromechanical device, was capable to detect 10 μM of tributyrin levels [103]. Enzymes can be monitored in real time through sequential injection analysis systems (SIA) that operates in programmed time interval, reducing wear-out and maintenance of the components and at the same time saving reagents. A lab-made SIA system was used to monitor offline lipase/esterase activity using a substrate p-nitrophenyl butyrate, determining hydrolytic activity in the linear range 0.05–1.60 U/mL, kinetic reaction of 5 min, five samples/h, and a relative standard deviation of 8.75% [104]. Table 1 displays a list and characteristics of diverse biosensors related to triglyceride detection.

Table 1
Description of diverse triglyceride lipase biosensors

Biosensor transducer	Linearity (L) Detection limit (D) Sensitivity (S)	Response time (R) Timelife (T) Repeatability (RSD)	Enzyme involved (E) Novelty (N)	Application	Reference
Piezoelectric Acoustic	L: 0.02–0.03 mol/L D: 13 U/L	–	E: lipase N: SAW sensor	Pancreatic lipase	[87]
Electrochemical Amperometric	L = up to 1.5 mM S: 0.5–0.7 μA/mM	R: 8–20 min	E: lipase and glycerol dehydrogenase (GDH) N: carbon electrodes were modified with adsorbed Meldola blue, Nile blue or toluidine blue	Glycerol and triglycerides	[95]
Piezoelectric Acoustic	L: 0.167–1.34 μg/mL D: 81 ng/mL	–	E: lipase N: SAW resonator	Lipase to detect dimethoate	[88]
Electrochemical Amperometric	L: 2.2×10^{-5} mol/L–2.0×10^{-3} D: 81 ng/mL	R: 5–10 min T: 30 days R.S.D.: 2.8%	E: lipase N: immobilized lipase on microemulsion-based gel MBG	Serum triglycerides	[105]
Electrochemical Amperometric	L: 10^{-4}–10^{-2} M glycerol 10^{-3}–10^{-2} M triacylglycerol D: 5×10^{-5} M	R: 2.5 min T: 2 months R.S.D.: 4.30%	E: lipase, glycerokinase (GK), and glycerol-3-phosphate oxidase (GPO). N: flow injection enzymatic analytical	Triglyceride detection	[96]
Electrochemical Amperometric	L: 1–50 μmol L^{-1} D: 0.7 μmol L^{-1} S: 350 mA mol^{-1} L cm^{-2}	R: 10 s T: 7 days R.S.D.: 25–10%	E: lipase, glycerol dehydrogenase, and NADH oxidase N: Prussian blue-modified screen-printed electrode	Triglyceride detection	[89]

(continued)

Table 1
(continued)

Biosensor transducer	Linearity (L) Detection limit (D) Sensitivity (S)	Response time (R) Timelife (T) Repeatability (RSD)	Enzyme involved (E) Novelty (N)	Application	Reference
Electrochemical Amperometric	L: 1–50 0.2 to 3.5 mM D: 0.20 mM	R: 40 s T: 25 days R.S.D.: 7.62%	E: lipase, glycerol kinase (GK), and glycerol-3-phosphate oxidase (GPO) N: Ag/AgCl CA membrane-bound	Triglyceride detection	[106]
Electrochemical Impedimetric	L: 25–300 mg dL^{-1} S: 2.59×10^{-3} $K\Omega^{-1}$ mg^{-1} dL	R: 20 s T: 10 weeks R.S.D.: 7%	E: lipase N: LIP/Glu/PANI-NT/ITO	Triglyceride detection	[107]
Electrochemical Potentiometric	L: 50–500 mg/dL D: 32.8 mg/dL S: 0.195 mA/mg dL cm^2	R: 20 s T: 3 months R.S.D.: 3%	E: Lipase N: Sol-gel CeO_2/ITO	Triglyceride detection	[108]
Electrochemical Potentiometric	L: 1 to 100 mg/L D: 0.5 mg/L	R: 25 s T: 55 days R.S.D.: 0.34–4.1%	E: Lipase and glycerol dehydrogenase N: GC electrode surface with MWCNTs/CeO2 NPs	Triglyceride detection	[100]
Electrochemical Amperometric	L: 50 to 400 mg dL^{-1} S: 4.28×10^{-4} mA mg^{-1}dL	R: 12 s T: 13 weeks	E: Lipase and glycerol dehydrogenase N: PANI-SWCNT/ITO	Triglyceride detection	[98]
Electrochemical Amperometric	L: 0.56–2.25 mM S: 0.28 mM	R: 10 s T: 70 days R.S.D.: 3.48%	E: lipase, glycerol kinase, and glycerol-3-phosphate oxidase N: immobilized covalently onto egg shell membrane	Triglyceride detection	[97]

Electrochemical Amperometric	L: 0.56–2.25 mM D: 0.21 mM	R: 2 s T: 50 days R.S.D.: 5.85%	E: lipase, glycerol kinase, glycerol-3-phosphate oxidase, and horseradish peroxidase N: covalently immobilized onto PVA membrane through glutaraldehyde	Triglyceride detection	[109]
Electrochemical Amperometric	L: 0–531 mg/dL D: 7.88 mg/dL S: 2.66 μA/mM	R: 30 s T: 30 days R.S.D.: 9.74%	E: lipase, glycerol kinase, and glycerol-3-phosphate oxidase N: Au/PEDOT-PSS	Triglyceride detection	[110]
Electrochemical Potentiometric	L: 15 to 200 mg/dL D: 15 mg/dL	T: 5 days R.S.D.: 6.7%	E: lipase, glycerol kinase, and glycerophosphate oxidase. N: enzymatic cascade reaction	Triglyceride detection	[111]
Electrochemical Amperometric	L: 0.14–1.125 mM D: 0.14 mM	T: 30 days R.S.D.: 2.21%	E: lipase and glycerol-3-phosphate oxidase (GPO) N: glassy carbon, Ag/AgCl reference electrode and Pt auxiliary electrode	Triglyceride detection	[112]
Electrochemical Amperometric	L: 0.25 to 4 mg mL^{-1} D: 155 μg mL^{-1} S: 0.155 mg mL^{-1}	R: 45 s T: 11 weeks	E: lipase N: LIPGDH/Chit-nano-ZrO_2/ITO	Triglyceride detection	[113]
Electrochemical Potentiometric	L: 10–100 mg/dL D: 0.1 mg/dL	R: 5 s T: 90 days R.S.D.: 1.84%	E: lipase N: NPs/Au electrode	Triglyceride detection	[114]
Electrochemical Amperometric	L: 0.1 mM–45 mM D: 0.1 nM S: 1241 ± 20 mA cm^{-2} mM^{-1}	R: 2.5 s T: 240 days R.S.D.: 0.06%	E: lipase N: lipase NPs/GKNPs/GPONPs	Triglyceride detection	[115]
Electrochemical Potentiometric	L: 0.25–3 mg/mL D: 0.27 mg/dL S: 0.0006 mA/mg mL	T: 4 months	E: lipase N: Lip/Chit-CuO/Au	Triglyceride detection	[116]

(continued)

Table 1
(continued)

Biosensor transducer	Linearity (L) Detection limit (D) Sensitivity (S)	Response time (R) Timelife (T) Repeatability (RSD)	Enzyme involved (E) Novelty (N)	Application	Reference
Electrochemical Amperometric	L: 6 to 25 mM D: 0.075 mM	R.S.D.: 7.35%	E: lipase N: polydopamine-gold nanocomposite PDA/AuNP enzyme	Triglyceride detection	[117]
Electrochemical Potentiometric	L: 0.1 mM–45 mM D: 10.6 mg/dL S: 1.232 μA mg/dL^{-1} cm^{-2}	R: 10 s T: 50 days R.S.D.: 1.52–1.67%	E: lipase N: electrospuncarbon nanofibers (CNFs)/Ag impregnated carbon nanofibers (AgCNFs)	Triglyceride detection	[118]
Electrochemical Amperometric	L: 50 mg dL^{-1}–300 mg dL^{-1} D: 4.67 mg dL^{-1} S: 0.40 μA mg^{-1} dL cm^{-2}	T: 1 month R.S.D.: <6%	E: lipase N: ε-polylysine-heparin microparticles	Triglyceride detection	[119]
Electrochemical Potentiometric	L: 1×10^{-12}–1×10^{-9} mol L^{-1} D: 0.33×10^{-12} mol L^{-1}	R: 30 min	E: pancreatic lipase N: Au electrode	Target DNA	[120]
Electrochemical Potentiometric	L: chlorfenvinphos 281.5 μM, malathion 843 mM D: chlorfenvinphos 100–900 μM, malathion 100–900 mM	R: 5 min R.S.D.: chlorfenvinphos 4.5%, malathion 6.3%	E: lipase N: p-Nitrophenol by p-Nitrophenyl acetate hydrolysis by free lipase enzyme	Organophosphate pesticides, methyl parathion, and malathion detection	[121]

4.3 Esterases Biosensors

Most of the esterase biosensors are related to cholesterol detection. The cholesterol esterase (ChEt) also known as bile salt-activated lipase catalyzes the hydrolysis of dietary cholesterol esters, triacylglycerols, phospholipids, and vitamin esters via a serine protease mechanism [69]. Several biosensors for cholesterol detection using a mixture of cholesterol esterase and cholesterol oxidase have been reported. A voltammetric- and chronoamperometric-modified carbon biosensor was used for the direct determination of cholesterol. Cholesterol ester hydrolase and cholesterol oxidase were used as sensing elements. The current generated was proportional in the range 1.0×10^{-6}–1.5×10^{-4} M, with a response time of 30 s [122]. Three enzymes, cholesterol esterase, cholesterol oxidase, and peroxidase, combined with flow injection potentiometry were used to detect cholesterol. The cholesterol esterase converts esterified cholesterol to free cholesterol, which is then oxidized by cholesterol oxidase with hydrogen peroxide as product. A fast response of 30 s was observed for this sensor, with a linear slope between 0.05 and 3.0 mM [123]. A patented device for cholesterol detection is described in [124]. The biosensor consisted of a feeder strip where the sample is put and easily transferred to the detection zone. An amperometric technique was used to detect the cholesterol/cholesterol oxidase reaction. Linear response in the range 2.81–13.0 mM was achieved for this device. Cholesterol esterase and cholesterol oxidase were immobilized individually and co-immobilized on to arylamine glass beads through diazotization [125]. Similar to the other reported methods, cholesterol ester is hydrolyzed by cholesterol esterase to free fatty acid and cholesterol, which is oxidized by cholesterol oxidase to cholestenone and H_2O_2. The lower detection limit of the method was 42.8 mg/L for individually immobilized enzymes and 21.4 mg/L for co-immobilized enzymes. The biosensor could be used 300 times without losing activity. A crystal sensor to measure in real time cholesterol concentration in buffer and serum using cholesterol esterase (ChEt), cholesterol oxidase (ChOx), and horseradish peroxidase (HRP) was developed in [126]. Linear range between 50 and 300 μM and 25 and 400 μM was found for free cholesterol and low-density lipoprotein cholesterol, with a response time of less than 25 min. Authors claim that the biosensor could be used for cholesterol determination in clinical samples. A method was developed for total serum cholesterol determination employing cholesterol esterase, cholesterol oxidase, and peroxidase co-immobilized onto alkylamine glass beads through glutaraldehyde coupling with a conjugation yield of 2.3 mg/g of support and 76% retention of initial activity. The minimum detection limit of the method was 50 mg/dL with linear response between A_{520} and cholesteryl acetate concentration ranging from 5 mg to 50 mg/dL

[127]. A biosensor based on an oxygen electrode with gold cathode and Ag/AgCl anode electrode was developed for cholesterol detection. Cholesterol esterase and cholesterol oxidase were immobilized by cross-linking with glutaraldehyde and bovine serum albumin. The sensor presented linearity in the range of 2–50 mg/dL and could be used over 30 times. The biosensor was used to determine cholesterol in food samples such as egg and meat [128]. A biosensor made of polyvinyl chloride beaker was chemically modified for covalent immobilization of cholesteryl esterase and cholesterol oxidase using glutaraldehyde as a coupling agent. A fabricate enzyme reaction beaker acts as an amperometric electrochemical cell for three electrode-based systems for measurement of serum total cholesterol. The sensor response was of 20 s. The reaction cell lasted over 100 days and lost the 50% of its initial activity during its regular use of 200 times [129]. A chronoamperometric biosensor for the determination concentrations of esterified and free cholesterol was developed using homemade potentiostat and disposable strips immobilized with Fe_3O_4, cholesterol oxidase, and cholesterol esterase. The sensor was based on the detection of reduction signal of hydrogen peroxide generated in two enzymatic reactions. The sensing device had a linear response over the range of 100–400 mg/dL for cholesteryl oleate and detection limit of 19.4 mg/dL [130].

A wireless biosensor system to continuously monitor the total cholesterol concentration in fish (Nile tilapia, *Oreochromis niloticus*) was reported in [131]. Cholesterol oxidase and cholesterol esterase were immobilized using glutaraldehyde on sensor built with Pt–Ir wire (φ0.178 mm) as the working electrode and Ag/AgCl paste as the reference electrode. The sensor had a linear output correlated with the cholesterol level in the range of 2.65–403 mg dL^{-1} and could be used over 40 h to monitor total cholesterol levels in free-swimming fish in an aquarium. An amperometric biosensor by entrapment immobilization of cholesterol esterase and cholesterol oxidase onto conducting polypyrrole was developed to detect cholesterol. The biosensor had linear response from 1 to 8 mM and shelf life of 4 weeks with about 15 estimations of cholesterol. The sensitivity of the electrode was of 0.15 μA/mM [132]. A recent amperometric biosensor based on a sensing electrode and a reference electrode in simultaneous contact with an integrated reagent layer was developed to detect total cholesterol. The integrated reagent was formed by cholesterol esterase, cholesterol dehydrogenase, coenzyme, redox mediator, surfactant, stabilizer, filler, and at least one aqueous thickening agent. The sensor had a linear response of 50–500 mg/dL cholesterol acetate and a minimum detection limit of 50 mg/dL [107]. Table 2 displays a list and characteristics of diverse biosensors related to cholesterol detection.

Table 2
Description of diverse cholesterol biosensors based on esterases and other enzymes

Biosensor transducer	Linearity (L) Detection limit (D) Sensitivity (S)	Response time (R) Timelife (T) Repeatability (R.S.D.)	Enzyme involved (E) Novelty (N)	Application	Reference
Electrochemical Amperometric	L: 0.05–3.0 mM D: 0.01 mM	R: 30 s R.S.D.: 3%	E: cholesterol esterase (CE), cholesterol oxidase (COD), and peroxidase (POD) N: FIP- ferrocyanide	Cholesterol detection	[122]
Electrochemical Amperometric	L: Free cholesterol 50–300 μM, and (LDL) 25–400 μM DL: S:	R: 25 min R.S.D.: 3%	E: cholesterol esterase (ChEt), cholesterol oxidase (ChOx), and horseradish peroxidase (HRP) N: AT-cut quartz crystal sensor	Cholesterol detection	[125]
Electrochemical Amperometric	L: 0.3–0.35 mmol L^{-1} D: 5.7–100 μ mol L^{-1} S: 0.96 to 43.99 nA $mmol^{-1}L$	R: 7–15 s R.S.D.: 0.7–12.9%	E: cholesterol oxidase and/or cholesterol esterase N: Pt/Pt/PPy-COx/*o*PPD	Cholesterol detection	[133]
Electrochemical Amperometric	L: 1 to 8 mM S: 0.15 μA/mM	T: 4 weeks	E: cholesterol esterase (ChEt) and cholesterol oxidase (ChOx) N: PPY/ChEt/ChOx	Cholesterol detection	[134]
Electrochemical Amperometric	L: 1.2 μM–1 mM DL: 11.9 nM	R: 2 min T: 25 days R.S.D.: 4%	E: cholesterol esterase (ChEt), cholesterol oxidase (ChOx), and peroxidase (HRP) N: FIA-Ag/AgCl/3 M NaCl	Cholesterol detection	[135]
Electrochemical Potentiometric	L: 50 mg dL^{-1}–500 mg dL^{-1} D: 25 mg dL^{-1} S: 0.042 μA mg dL^{-1}	R: 240 s T: 6 weeks	E: cholesterol oxidase, cholesterol esterase, and peroxidase N: PANI/ChEt/ChOx/POD	Cholesterol detection	[136]
Electrochemical Potentiometric	L: 50 to 500 mg/dL S: 7.5×10^{-4} nA/mg	R: 40 s T: 10 weeks	E: cholesterol esterase (ChEt) and cholesterol oxidase (ChOx) N: PANI/ChEt/ChOx	Cholesterol detection	[137]

(continued)

Table 2
(continued)

Biosensor transducer	Linearity (L) Detection limit (D) Sensitivity (S)	Response time (R) Timelife (T) Repeatability (R.S.D.)	Enzyme involved (E) Novelty (N)	Application	Reference
Electrochemical Amperometric	L: 2–50 mg/dL	T: 9 weeks	E: cholesterol esterase (ChEt) and cholesterol oxidase (ChOx) N: oxygen gold cathode and Ag/AgCl	Cholesterol detection	[127]
Electrochemical Potentiometric	L: 10–60 μM S: 0.85 nA/μM	T: 15 days	E: cholesterol oxidase (COX) and cholesterol esterase (CE) N: Au-NWs-based MEMS microfluidic platform	Cholesterol detection	[138]
Electrochemical Amperometric	L: up to 780 mg dL^{-1} D: 12 mg dL^{-1} S: 1.61 nA mM^{-1}	R: 180 s T: 1 month	E: cholesterol oxidase (ChOx) and cholesterol esterase (ChEt) N: Tetraethylorthosilicate sol-gel/ChEt/ChOx	Cholesterol detection	[139]
Electrochemical Amperometric	L: 2.65–403 mg dL^{-1}	T: 40 h R.S.D.: 4.58%	E: cholesterol oxidase and cholesterol esterase N: Pt–Ir wire Ag/AgCl	Cholesterol detection	[130]
Chronoamperometric	L: 100–400 mg/dL D: 19.4 mg/dL	R: 15 s T: 1 month R.S.D.: 5.06%	E: cholesterol oxidase (ChOx), and cholesterol esterase (ChEt) N: homemade potentiostat and disposable strips Fe_3O_4	Cholesterol detection	[129]
Electrochemical Amperometric	L: up to 12 mM D: 0.5 nM S: 2.07 ± 0.1 μA/μM/cm^2	R: 3 s T: 3 days	E: cholesterol oxidase and cholesterol esterase N: nanosized Pt particle decorated graphene-based	Cholesterol detection	[140]

Electrochemical Amperometric	L: 50–500 mg/dL D: 50 mg/dL	R: 1 min T: 100 days R.S.D.: 5%	E: cholesterol esterase and cholesterol dehydrogenase N: disposable screen-printing test strip	Cholesterol detection	[132]
Electrochemical Potentiometric	L: 0.97–7.8 mM D: 0.5 mg dL^{-1}. S: 29.33 μA mM^{-1} cm^{-2}	T: 60 days R.S.D.: 5%	E: cholesterol oxidase (ChOx), cholesterol esterase (ChEt), and horseradish peroxidase (HRP) N: Ti/NPAu/ChOx–HRP–ChEt	Cholesterol detection	[141]
Electrochemical Amperometric	L: 50–300 μM D: 15 μM S: 443.25 μA mM^{-1} cm^{-2}	R: 12 s R.S.D.: 5.4%	E: cholesterol oxidase (ChOx) and cholesterol esterase (ChEt) N: (ChEt/ChOx)-FG/Gr	Cholesterol detection	[142]
Electrochemical Impedimetric	L: 2.5×10^{-4}–6.5×10^{-3} M/l D: 2.5×10^{-4} M/l S: 196 Ω/mM/cm^{-2}	R: 25 s T: 7 weeks R.S.D.: 5%	E: involved enzyme: cholesterol esterase (ChEt) and cholesterol oxidase (ChOx) N: PtNP/PPY/ITO	Cholesterol detection	[143]
Electrochemical Amperometric	L: 50 μM to 15 mM D: 12 μM	R: 20 s T: 6 months	E: cholesterol oxidase and cholesterol esterase N: ferrocene-capped gold nanoparticle or ferrocenemethanol(Fc-OH)	Cholesterol detection	[144]
Electrochemical Potentiometric	L: 1.29–12.93 mM D: 0.47 mM S: 0.384 mA mg^{-1} dL cm^{-2}	R: 5 s	E: cholesterol esterase (ChEt) and cholesterol oxidase (ChOx) N: ChEt–ChOx/CHIT–CdS QDs	Cholesterol detection	[145]
Electrochemical Amperometric	L: 10–700 mg/dL D: 0.1 mg/dL	R: 5 s T: 3 months R.S.D.: 3%	E: cholesterol esterase (ChEt) and cholesterol oxidase (ChOx) N: ChOxNPs þ ChENPs/Au electrode	Cholesterol detection	[146]
Electrochemical Amperometric	L: 0.5–250 mg/dL D: 0.5 mg/dL	R: 20 s T: 2 months R.S.D.: 0.98%	E: cholesterol esterase (ChEt), cholesterol oxidase (ChOx), and horseradish peroxidase (HRP) N: c-MWCNT/AuNP	Cholesterol detection	[147]
Electrochemical Amperometric	L: 5–5000 μg/mL D: 3.0 μg/mL	R.S.D.: 5.5%	E: cholesterol oxidase (CHOD) and cholesterol esterase (CHER) N: Ag/CHER and CHOD/AuNPs/SPE	Cholesterol detection	[148]

(continued)

Table 2
(continued)

Biosensor transducer	Linearity (L) Detection limit (D) Sensitivity (S)	Response time (R) Timelife (T) Repeatability (R.S.D.)	Enzyme involved (E) Novelty (N)	Application	Reference
Electrochemical Amperometric	Linearity: 1×10^{-6}–1.5×10^{-4} M S: 9500 μA M^{-1}	R: 30 s T: 1.5 years	E: horseradish peroxidase N: carbon paste modified by the peroxidase HMF	Cholesterol detection	[103]
Electrochemical Amperometric	L: 0.05–6.2, 6.2–11.2 mM D: 10 μM S: 90.7 μA mM^{-1} cm^{-2}	R: 7 s T: 30 days R.S.D.: 4.2%	E: cholesterol oxidase (ChOx) N: ChOx/AuPt–Ch–IL/GCE	Cholesterol detection	[131]
Electrochemical Differential pulse voltammetric	L: 25–125 μM D: 6.3 μM S: 0.18 μA/cm^2/μM	T: 1 month R.S.D.: 0.52%	E: cholesterol oxidase (ChOx) and horseradish peroxidase (HRP) N: GCE/PTH/ChOx/HRP	Cholesterol detection	[149]
Piezoelectric Acoustic	Detection limit: 0.8 mM	T: 10 days	E: cholesterol oxidase (ChOx) and horseradish peroxidase (HRP) N: TEM-PAA-ChOx-HRP	Cholesterol detection	[150]
Chemiluminescence	L: 0.05–10 mM D: 1.5 μM	T: 2 weeks R.S.D.: 2.4%	E: cholesterol oxidase (ChOx), and peroxidase-like activity N: Peroxidase-like activity of copper nanoclusters	Cholesterol detection milk	[151]
Electrochemical Amperometric	L: 0.5–300 mg dL^{-1} D: 0.416 mg dL^{-1} S: 13.28 μA mg^{-1} dL cm^{-2}	–	E: cholesterol oxidase (ChOx) and horseradish peroxidase (HRP) N: ChOx/HRP/K3[Fe(CN)6]/GCE	Cholesterol detection	[152]
Electrochemiluminescent	L: 8.33×10^{-9}–4.17×10^{-7} M D: 6.30×10^{-9} M.	T: 30 days R.S.D.: 4.1%	E: cholesterol oxidase (ChOx) N: AuNPs/ILs/HTNS/ITO	Cholesterol detection	[153]
Electrochemical Amperometric	L: 4 μM–0.7 mM D: 1 μM S: 1.55 μA/mM	R: 13 s T: 35 days R.S.D.: 1.6%	E: apo-cholesterol oxidase (apo-ChOx) N: PTBA/FAD/apo-ChOx	Cholesterol detection	[154]

Electrochemical Amperometric	L: 0.05–7.4 mM, D: 10 μM S: 139.5 $\mu AmM^{-1}\ cm^{-2}$	R: 4 s T: 6 weeks R.S.D.: 4.82%	E: cholesterol oxidase (ChOx) N: $Bi_2O_2CO_3$nanoplates/Au electrode	Cholesterol detection	[155]
Electrochemical Potentiometric	L: 0.5–48 μM D: 0.26 ± 0.015 μM S: 4460 $\mu A\ mM^{-1}\ cm^{-2}$	T: 14 days R.S.D.: 4.87%	E: cholesterol oxidase (ChOx) N: Nafion/ChOx/MoS_2-AuNPs/GCE	Cholesterol detection	[156]
Electrochemical Amperometric	L: 2.0–510.0 μM D: 0.8 μM S: 109.9 $\mu A\ mM^{-1}$	R: 5 s T: 45 days R.S.D.: 3.9%	E: cholesterol oxidase (ChOx) N: MWCNT-PANI	Cholesterol detection	[157]
Optical/calorimetric	L: 50–700 mg/dL D: 11.8 mg/dL	R: 200 s T: 7 days R.S.D.: 3.17%	E: cholesterol oxidase (COD) N: PMIGC and immobilized COD	Cholesterol and glucose detection	[158]
Electrochemical Amperometric	L: 35.3–500 μM D: 10.5 μM S: 14.3 $mA\ M^{-1}\ cm^{-2}$	R: 8 s T: 60 days R.S.D.: 7.4%	E: cholesterol oxidase (ChOx) N: Au-SPE/Pt/PDA-ChOx	Cholesterol detection	[159]
Chemiluminescent	L: 0.08–300 $\mu mol\ L^{-1}$, D: 35 $nmol\ L^{-1}$	T: 3 months R.S.D.: 3.2–4.3%	E: cholesterol oxidase (ChOx) N: MoS2 QDs and graphene QDs	Cholesterol detection	[160]

4.4 Phospholipase Biosensors

Phospholipases are enzymes that hydrolyze phospholipids [32]. Phospholipids are present in all living microorganism as a major component of all biological membranes. Phospholipases are important markers in pancreatic and coronary diseases [161]. Diverse biosensors to detect phospholipids are reported in the literature. A biosensor to detect phospholipase D activity in rapeseed was developed. A Clark-type oxygen sensor was used to co-immobilize choline oxidase and catalase using glutaraldehyde and cyclohexyl isocyanide. The sensor had a linear response in the range 3.34×10^{-3}–0.167 mM and was used for 600 assays over an 18-month period under different pH conditions [162]. A biosensor to monitor phospholipids in serum samples was developed. Phospholipids are hydrolyzed by the enzyme phospholipase D to choline which was analyzed by a fiber-optic biosensor based on choline oxidase and oxygen transduction. The linear range for the biosensor was 0.08 ± 3.00 mg mL^{-1} phosphatidylcholine [163]. Another choline biosensor based on the determination of H_2O_2 generated at the electrode surface by the enzyme choline oxidase was developed. This biosensor was used to detect choline released from phosphatidylcholine by phospholipase D. Choline oxidase retained onto a horseradish peroxidase was immobilized in a solid carbon paste electrode. Amperometric detection of choline oxidase was detected in Ag/AgCl. The sensor has a linear behavior over the range of 5.0×10^{-7}–7.0×10^{-5} M, with a detection limit of 1.0×10^{-7} M [164]. Amperometric detection of choline was realized at an applied potential of −0.2 V versus saturated calomel electrode in 1/15 M phosphate-buffered solution (pH 7.4) with a linear response range between 5.0×10^{-6} and 6.0×10^{-4} M choline and a response time of 15 s. Choline oxidase and horseradish peroxidase were cross-linked onto a HRP-immobilized carbon paste electrode [165]. Quantitative determination of total, free, and phosphatidyl-bound choline in milk and a dietary supplement using phospholipase D packed bioreactor coupled to a choline oxidase-based amperometric biosensor was reported in [166]. The response for choline and phosphatidylcholine was linear up to 0.5 and 1 mM, respectively, and the detection limits were 0.02 and 0.03 mM.

A panel of monoclonal antibodies was raised against recombinant bovine p85α to probe its multi-domain structure in relation to function using a Biacore biosensor instrument. Phosphatidylinositol 3-kinase, which generates putative novel second-messenger phospholipids, is a heterodimer composed of regulatory adaptor 85-kDa and catalytic 110-kDa subunits. Epitopes for nine monoclonal antibodies were mapped in relation to the main structure of p85a using recombinant protein fragments expressed in bacteria. Real-time binding experiments using phospholipid-containing

vesicles showed that p85α by itself could specifically bind certain phospholipids [167]. A capacitive biosensor was used to determine phospholipase activity. The sensing electrodes have a structure $Au/S(CH_2)_{17}CH_3$/substrate/electrolyte. Hydrolysis of the substrate by phospholipase A_2 leads to the formation of water-soluble products from the insoluble substrate. Desorption of these products into aqueous phase increases the electrode capacitance. The detection limit of the biosensor is 0.5 ng/mL (500 μunits/mL) [168]. A sensor was used to study the role of Ala426 and Lys438 of phospholipase D from *Streptomyces septatus* TH-2 (TH-2PLD) in substrate recognition. Liposomes were captured on the surface of a Sensor Chip L1 (Biacore AB) as "ligand". By substituting Ala426 and Lys438 with Phe and His, respectively, the inactive mutant showed stronger interaction with phosphatidylcholine and weaker interaction with phosphatidylglycerol than the inactive TH-2PLD mutant. We demonstrated that Ala426 and Lys438 of TH-2PLD play a role in sensing the head group of phospholipids [169]. A biosensor for quantification of lipoprotein-associated phospholipase A_2 was achieved using an iridium-modified carbon-based biosensor. The biosensor was based on the hydrolysis of short-chain carboxylic esters, produced as products, organic acids, and alcohol. The alcohol was then oxidized by alcohol oxidase producing hydrogen peroxide, which was detected electrochemically and was quantified using the iridium-modified carbon-based biosensor. The detection of the lipoprotein-associated phospholipase A_2 was linear in the concentration range 0–150 UmL^{-1}, with a sensitivity of 1.45 nAU^{-1} in bovine serum [170]. A liquid crystal-based sensor for real-time identification of phospholipase like toxins was designed. The sensor can selectively identify beta-bungarotoxin, when it hydrolyzes a phospholipid monolayer self-assembled at aqueous liquid crystal interface, through orientational responses of liquid crystal. As a result, optical signals that reflect the spatial and temporal distribution of phospholipids during the hydrolysis can therefore be generated in a real-time manner. The sensor is sensitive and requires less than 5 pg of beta-bungarotoxin for the detection [171].

By integrating enzyme-triggered liposome release with controllable Au nanoparticle aggregation, it was possible to design a sensor system which makes use of a natural nonimmobilized lipid substrate to detect phospholipase activity. With this sensor enzyme, concentrations as low as 700 pM were detected in real time [172]. The Förster resonance energy transfer sensor is capable to measure intracellular phospholipase A_2 in living cells and small organisms. The probe was modified to detect particular isoforms. Arachidonic acid-mimicking fatty acids were prepared by copper-mediated coupling reactions. Probes with a single double bond in

the 5-position exhibited favorable substrate properties for secretory phospholipase A_2. In vitro experiments with the novel unsaturated doubly labeled phosphatidylethanolamine derivatives showed preferred cleavage of the sensor by the physiologically group V sPLA2 [173]. A biosensor named phosphatidic acid indicator (Pii) was developed based on the principle of Förster resonance energy transfer (FRET). In the biosensors, a lipid binding is sandwiched with fluorescent proteins which are tagged with the plasma membrane-targeting sequence of K-Ras. The addition of synthetic phosphatidic acid, or the activation of phospholipase Dor diacylglycerol kinase at the plasma membrane, changed the level of FRET in Pii-expressing cells, demonstrating the response of phosphatidic acid indicator to phosphatidic acid. The biosensor detected divergent phosphatidic acid content among various cell lines as well as within one cell line and even phosphatidic acid distribution within the cell [174]. Table 3 displays a list and characteristics of diverse biosensors related to phospholipids detection.

5 New Trends in Biosensor Design

Enzymatic biosensors have shown potential to detect cholesterol and triglycerides in blood samples with an excellent response time, repeatability, adequate linearity, and proper detection limits, although lifetime still is an issue that will require further improvement. Electrochemical biosensors incorporating nanomaterials, such as CNTs, MWCNTs, ZnO nanorods, AgNPs, AuNPs, PtNPs, SnO_2 NPs, ZnO NPs, Cu_2O NPs, CNTs–PtNPs, etc., will improve the specificity, sensitivity, response time, dynamic range, robustness, and miniaturization of enzyme biosensors.

Novel devices and applications will appear as the technology advances, i.e., in biotechnology, there is an increasing trend to downscale bioprocesses in order to study biotransformations in more detail in microreactors; in this sense biosensors will be a proper tool to monitor metabolites at low scale. The reduced size of biosensor will increase the development of commercial portable devices such as intelligent packing in food industry. In vivo biosensors, lab on chip implanted in the patient's body, and nano tattoos will be used to continuously monitor the concentration of specific substances. Definitively, we will hear more frequently about lipase, esterase, phospholipase, and other enzyme biosensor applications in diverse knowledge areas.

Table 3
Description of diverse phospholipase biosensors

Biosensor transducer	Linearity (L) Detection limit (D) Sensitivity (S)	Response time (R) Timelife (T) Repeatability (R.S.D)	Enzyme involved (E) Novelty (N)	Application	Reference
Electrochemical Amperometric	L: 3.34×10^{-3}–0.167 mM S: 10 nmol	R: 10 s T: 18 months R.S.D.: 2%	E: choline oxidase N: choline oxidase and catalase co-immobilized on oxygen sensor.	Phospholipase D activity	[162]
Piezoelectric Capacitive	L: 1.0–1.1 μF cm^{-2} D: 50 pg mL^{-1}		E: lipase, phospholipase N: Au/$S(CH_2)_{17}CH_3$/substrate/electrolyte.	Activity of lipase phospholipase A_2	[175]
Chemiluminescent	L: 3–150 μM D: 1.2 μM	R: 15 samples/h T: 2 weeks	E: choline oxidase (ChOx) and horseradish peroxidase (HRP) N: FIA ChOx and HRP polymer bead	Phospholipase D activity	[176]
Optical	L: 0.08 ± 3.00 mg mL^{-1} D: 0.08 mg mL^{-1}	R: 20 min T: 40 days R.S.D.: 5.4%	E: choline oxidase N: FIA nylon membrane onto an oxygen sensitive layer	Phospholipids in serum, phosphatidylcholine	[163]
Piezoelectric Capacitive	L: 1.0–1.1 μF cm^{-2} D: 500 μunits/mL	–	E: phospholipase A N: Au/$S(CH_2)_{17}CH_3$/substrate/electrolyte	Phospholipase A activity	[168]
Electrochemical Amperometric	L: 5–50 μmol/L	R: 3–4 min R.S.D.: 4–5%	E: choline oxidase (ChOx) N: MW coupled to O_2/choline oxidase	Choline in foods	[177]
Electrochemical Amperometric	L: 5.0×10^{-7}–7.0×10^{-5} M D: 1.0×10^{-7} M. S: 0.1 M	R: 80 s T: 23 days R.S.D.: 8.5%	E: choline oxidase (ChOx) N: HRPsCPE	Choline from rat glands	[164]
Electrochemical Amperometric	L: 5.0×10^{-6}–6.0×10^{-4} M D: 3 μM	R: 15 s T: 1 month R.S.D.: 7.4%	E: choline oxidase (ChOx) and horseradish peroxidase	Phosphatidylcholine in serum Samples	[165]

(continued)

Table 3
(continued)

Biosensor transducer	Linearity (L) Detection limit (D) Sensitivity (S)	Response time (R) Timelife (T) Repeatability (R.S.D)	Enzyme involved (E) Novelty (N)	Application	Reference
			(HRP) N: PLD, CHOD, HRP		
Electrochemical Amperometric	L: 0–0.1 mM, D: 0.12 μM	R.S.D.: 2.9%	E: choline oxidase (ChOx) N: FIA-Pt/PPYox/BSA-GLU-ChO	Determination of choline in milk	[178]
Electrochemical Amperometric	L: Choline up to 0.5 mM and phosphatidyl choline up to 1 mM, D: Choline 0.02 mM and phosphatidyl choline 0.03 mM	T: 1 month R.S.D.: Choline 2.8% and phosphatidyl choline 3.2%	E: choline oxidase N: FIA-PLD-PBR ChO	Major choline fractions in milk	[166]
Electrochemical Amperometric	L: 5×10^{-6} to 1×10^{-4} M D: 1×10^{-7} M	R: 8 s T: 31 days R.S.D.: 4.8%	E: choline oxidase (ChOx) N: ChOx/MWCNT/Pt	Choline released from lecithin by phospholipase D	[179]
Electrochemical Amperometric	L: 5×10^{-7} M–1×10^{-4} M D: 5×10^{-7} M S: 88.6 μA mM^{-1} cm^{-2}	R: 10 s T: 2 months	E: choline oxidase (ChOx) N: LBL-Pt-PB-EACC.	Phosphatidylcholine in serum samples	[180]
Electrochemical Amperometric	L: 0–150 UmL^{-1} D: 1.45 nAU^{-1}	R.S.D.: 2–59%-15.59%	E: short-chain carboxylic esters, alcohol oxidase, Lp-PLA_2 N: iridium nanoparticle	Lipoprotein-associated phospholipase A_2	[170]
Optical Fluorescence	L: 0.025–1000 U/L D: 0.010 U/L	R.S.D.: 3.0%	E: phospholipids N: phospholipid graphene nanoassembly	Phospholipase D activity	[181]
Optical chemiluminescence	L: 0.006–2 mM D: 0.0005 mM	T: 30 days R.S.D.: 3.51%	E: choline oxidase and peroxidase N: ZnONR films	Choline analysis	[182]

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