REVIEW

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# Enzyme-based electrochemical biosensors for food safety: a review

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Electrochemistry Laboratory, Department of Chemistry, Chaudhary Devi Lal University, Sirsa, Haryana, India **Abstract:** In recent years, food storage environment safety has been a major concern for food and health scientists. There is growing interest in electrochemical biosensors due to their high sensitivity and rapid response. The aim of this review article is to provide details regarding the development of enzyme-based electrochemical biosensors, and their use in the detection of a range of chemical and biological compounds in the food industry. We have focused on the basic principle, generation, classification, and application of electrochemical biosensors for food safety. **Keywords:** electrochemical biosensor, bioreceptor, transducer, food-borne pathogens

#### Introduction

Food is a basic requirement of all human beings. Microorganisms such as pathogens, viruses, and bacterium are present in food, and partially or completely destroy the nutritional quality of food. Food-borne pathogens are a major threat to food safety. Pathogens can cause serious diseases that lead to 40% of the total 50 million deaths annually in the world.<sup>1</sup> Food production, preservation, and prevention from chemical and microbial contamination, insect infection, and pathogenic contamination have become very essential in today's fast life style.<sup>2</sup> For food safety and quality, various conventional analytical techniques are used such as polymerase chain reaction, culture- and colony-based methods, and immunology-based methods. These methods are more time consuming, laborious, and require a well-trained person. There is an increasing demand for developing a fast and sensitive technique to monitor food-borne pathogens.

A biosensor is a device or instrument that comprises a biological sensing material combined with a chemical or physical transducer, which converts a chemical or biological signal into an electrical signal.<sup>3</sup> A typical biosensor is shown in Figure 1.

Biosensors have many applications in food (pathogen, additives), environmental monitoring (toxic pollution), clinical diagnoses (glucose in blood, cholesterol), and biodefense (biowarfare) due to their selectivity, sensitivity, stability, and quick response time.<sup>4</sup> The capacity of a biosensor is enhanced by using nanomaterials such as carbon nanotubes (CNTs), nanowires, magnetic nanoparticles (NPs), nanorods, and quantum dots.<sup>5</sup> These nanomaterials have a high capacity for charge transfer and make the sensor suitable for higher sensitivity values and lower detection limits.<sup>5</sup> Keeping in mind the above facts, an extensive literature survey was carried out on electrochemical biosensors for food safety. This review provides information regarding the history, classification, and application of electrochemical biosensors for food safety.

Nanobiosensors in Disease Diagnosis 2016:5 29-39

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http://dx.doi.org/10.2147/NDD.S64847

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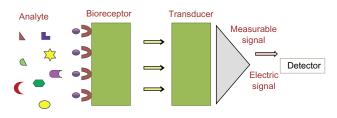


Figure I A typical representation of a biosensor.

### **Historical perspective**

The first glucose sensor was proposed by Clark and Lyon<sup>6</sup> from Cincinnati Hospital in 1962. An oxygen electrode is entrapped in a thin layer of glucose oxidase (GOx) via a semipermeable dialysis membrane in which the oxygen consumption is monitored by the enzyme-catalyzed reaction.<sup>7</sup>

Glucose + 
$$O_2 \xrightarrow{GOx} Gluconic acid + H_2O_2$$
 (1)

Updike and Hicks<sup>8</sup> modified the oxygen background by using two working oxygen electrodes in which one contained the enzyme and the other calculated the differential current. In 1969, Guilbault and Montalvo reported the first enzymebased potentiometric biosensor in which urease enzyme was immobilized on an ammonia electrode for the detection of urea. In 1973, Guilbault and Lubrano developed an amperometric enzyme electrode for the determination of glucose in blood samples and monitored the amount of liberated hydrogen peroxide.<sup>9</sup>

$$2H_2O_2 \rightarrow O_2 + 2H^+ + 2e^-$$
 (2)

### First-generation glucose biosensor

The first-generation glucose biosensor is based on the use of natural oxygen and determination of the amount of liberated hydrogen peroxide.<sup>7,10</sup> Glucose is converted into gluconolactone by GOx with simultaneous reduction of flavin adenine dincleotide (FAD) to FADH<sub>2</sub>, and finally, FAD would be regenerated as FADH, to produce

 $H_2O_2$ . When voltage is applied, the oxidation of  $H_2O_2$  takes place, which results in an electric signal.<sup>7</sup> Several shortcomings, such as oxygen dependence are present in the first-generation biosensor.

$$GOx (FAD) + Glucose \rightarrow GOx (FADH2) + Gluconolactone (3)$$

$$GOx (FADH2) + O2 \rightarrow GOx (FAD) + H2O2$$
(4)

The active site and the FAD prosthetic group are buried deep within the enzyme, which restricts the diffusion of reagents,<sup>11</sup> and second, there is limited solubility of  $O_2$  in aqueous media.<sup>10,12</sup> Marcus theory shows that the electron transfer decreases exponentially with increasing distance.<sup>13,14</sup> The diagrammatic representation of the first-generation glucose biosensor is shown in Figure 2.

#### Second-generation glucose biosensor

The first-generation biosensor needs further improvement due to the oxygen deficiency. The limitation of the first-generation glucose biosensor is removed by replacing the oxygen with redox (electron) mediators that carry electrons from enzyme to electrode.<sup>15,16</sup> Mediators like poly(vinylpyridiene) and poly(vinylimidazole) combine with osmium complex electron and minimize the distance between the center of polymers and FAD of enzyme, which results in the rapid response of sensor and high current. The diagrammatic representation of the second-generation glucose biosensor is shown in Figure 3. The second-generation biosensor has the defect of leaching the mediator.<sup>12,17</sup> Hence, there was a demand for the production of the third-generation biosensor.

$$GOx-FADH_{2} + 2Mediator_{(Ox)}$$
  

$$\rightarrow GOx-FAD + 2Mediator_{(Red)} + 2H^{+}$$
(5)

$$2\text{Mediator}_{(\text{Red})} \rightarrow 2\text{Mediator}_{(\text{Ox})} + 2e^{-}$$
(6)

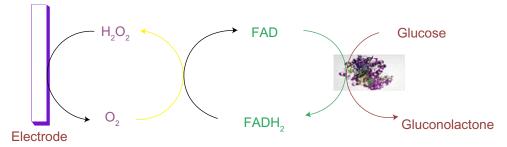


Figure 2 Diagrammatic representation of the first-generation of a glucose biosensor. Abbreviation: FAD, flavin adenine dinucleotide.

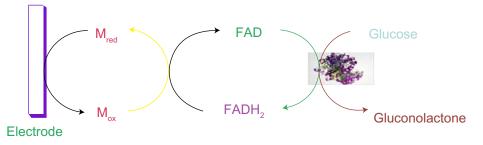


Figure 3 Diagrammatic representation of the second-generation of a glucose biosensor. Abbreviation: FAD, flavin adenine dinucleotide.

# Third-generation glucose biosensor

The third-generation glucose biosensor is advantageous due to the absence of a mediator and involves direct electron transfer between the enzyme and electrode. Conducting organic salts like tetrathiafulvalene–tetracyanoquinodimethane were used for direct electron transfer from enzyme to electrode.<sup>18,19</sup> Mesoporous electrode materials with increased electrode surface and dynamics are gaining more interest without the difficulties in the usage of mediators and the deficiency of oxygen.<sup>20,21</sup> The diagrammatic representation of the third-generation glucose biosensor is shown in Figure 4.

# Electrodes used in electrochemical biosensors

Electrodes play an important role in electrochemical biosensors. The detection capacity of the electrochemical biosensor can be affected by the electrode surface dimension, its material, and modifications.<sup>22</sup> Three different types of electrodes are used in the electrochemical biosensor, ie, reference electrode, working electrode, and auxillary (counter) electrode.

# Reference electrode

Reference electrode is an electrode that resists the changes in the value of its potential and has negligible current.

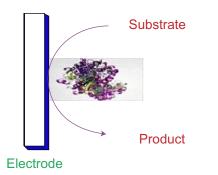


Figure 4 Diagrammatic representation of the third generation of a glucose biosensor.

A good reference electrode is nonpolarizable and has constant potential during the passage of low current.

## Working electrode

It is the electrode on which all the processes of interest take place in an electrochemical system. Working electrode can be referred to as either cathodic or anodic depending on the reaction that takes place at the surface of the working electrode.<sup>23-25</sup>

# Auxiliary (counter) electrode

A platinum wire is generally used as a counter (or auxiliary) electrode because platinum is an inert material and does not affect the behavior of the working electrode.

# Important characteristics of the electrochemical biosensor

Sensitivity of the electrochemical biosensor must be high. Sensitivity is defined as the electrochemical response of the biosensor for a very small change in the analyte concentration, pH, temperature, and so on. The electrochemical biosensor must also be selective in nature. Electrochemical response of the biosensor should be specific for a particular target analyte. Response time of the electrochemical biosensor should be very small so that it can very quickly record any changes that take place in the target analyte. The detection limit of the electrochemical biosensor is such that it can easily detect very low concentrations of the analyte.

# Classification of electrochemical biosensor

Electrochemical biosensors are classified into two types, depending on the type of bioreceptor and transducer used.

# On the basis of bioreceptor

Bioreceptors are the protein molecules that are embedded in the cellular membrane to which target analytes specifically bind. Bioreceptors can be classified into five different types.

The classification of different types of biosensors is shown in Figure 5.

#### Enzyme-based bioreceptor

An enzyme is a large, complex macromolecule consisting largely of proteins that act as powerful catalysts to convert substrates into products. The enzymes used in the biosensor and their mode of action, which involve oxidation or reduction, can be detected electrochemically.

The main reason for the popularity of bioreceptors is the catalytic activity of enzymes and their specific binding capacity. These biosensors utilize enzymes that are specific for the desired molecules. Different types of enzymes were used for the fabrication of biosensors. For example, fructose dehydrogenase enzymes were used for fructose,<sup>26</sup> alcohol oxidase enzyme for alcohol,<sup>27</sup> amino acid oxidase for amino acid,<sup>28</sup> and glucose dehydrogenase for glucose.<sup>29</sup> The lifetime of a sensor is limited by the stability of the enzyme. The five basic methods of enzyme immobilization are adsorption, microencapsulation, entrapment, cross-linking, and covalent bonding.<sup>30</sup>

#### Adsorption

Adsorption is a simple and quick method for manufacturing enzymatic biosensors. It is of two types: physical adsorption (physisorption) and chemical adsorption (chemisorptions). Physisorption is usually weak and involves the formation of van der Waals bonds. Chemisorption is much stronger and involves the formation of covalent bonds.

#### Microencapsulation

This method uses an inert membrane to trap the biomaterial for the transducer. The membrane includes cellulose acetate (which excludes proteins and the transportation of interfering species like ascorbate), polycarbonate (a synthetic material that is nonpermselective), collagen (a natural protein), Teflon (a synthetic polymer that is selectively permeable to gases like oxygen), nafion, and polyurethanes.

#### Entrapment

A polymeric gel is prepared in a solution containing biomaterial. The enzyme is entrapped within the gel matrix. This can cause barriers to the diffusion of substrate and slowing down of the reaction. The most commonly used gel is polyacrylamide. Conducting polymers (polypyrroles) are used for the electrode.

#### Cross-linking

In this method, bifunctional reagents such as glutaraldehyde bind the biomaterial that is chemically bonded to solid supports. It can be a useful method for stabilizing adsorbed biomaterials.

#### Covalent bonding

Some functional groups can be covalently bonded to the support matrix (transducer or membrane). The advantage of this method is that the enzyme will not be released during use. In order to protect the active site, the reaction is carried out in the presence of substrate.

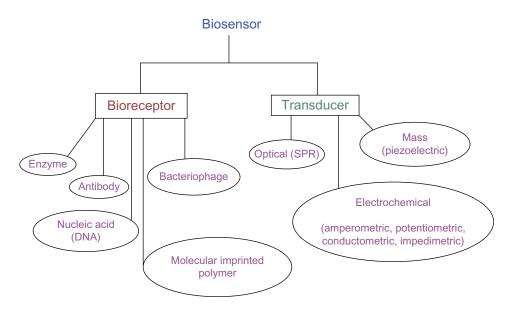


Figure 5 Biosensor classifications and methods. Abbreviation: SPR, surface plasmon resonance.

Antibodies are common bioreceptors used in biosensors. The antibodies may be monoclonal, polyclonal, or recombinants depending on the properties and synthesis.<sup>31</sup> Antibody–antigen-based biosensor is also known as immunosensor. Figure 6 shows a typical antigen–antibody interaction. This type of interaction is similar to lock-and-key interaction in which the antigen will bind to the antibody if it has correct conformation.<sup>32</sup> Some of the disadvantages of using antibodies in the biosensors are the binding capacity of antibodies affected by the pH and temperature conditions; and the irreversible antibody–antigen interaction binding they may interrupt.<sup>33</sup>

#### Nucleic acid-based bioreceptor

A biosensor that uses nucleic acid as a bioreceptor is known as genosensor.<sup>34</sup> Nucleic acid analysis has become an important tool for the identification of microorganisms such as pathogens, bacterium, and so on, which are commonly present in food and the environment.<sup>35–39</sup> The process is based on the principle of complementary base pairing, adenine-thymine, and cytosine-guanine in DNA. If the target nucleic acid sequence is known, complementary sequences can be synthesized, labeled, and then immobilized on the sensor. The hybridization probes can then base pair with the target sequences, generating an optical signal.<sup>40</sup>

#### Molecular-imprinted polymers-based bioreceptor

It is a technique of producing artificial recognition sites by forming a polymer around a molecule that can be used as a template. Molecular-imprinted polymers (MIPs) can be synthesized for any analyte molecule and are capable of binding target molecules with affinities. MIPs possess many disadvantages such as the fact that it is very difficult to completely remove the template from MIPs and the imprinted polymer is insoluble.<sup>41</sup>

#### Bacteriophages-based bioreceptor

Bacteriophages are viruses that are made of an outer protein coat and inside genetic material (DNA or RNA).

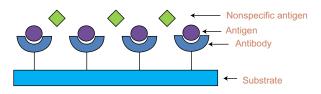


Figure 6 Antigen-antibody interaction taking place at the surface of the transducer.

Bacteriophages are considered as biorecognition elements for the identification of various microorganisms present in food and environment. The viruses can bind to specific receptors and inject their genetic material inside the bacteria. Researchers reported the use of phages as a biorecognition substance for the identification of various pathogens such as *Escherichia coli*,<sup>42</sup> *Staphylococcus aureus*,<sup>43</sup> and *Bacillus anthracis*<sup>44,45</sup> on sensing platforms.

# On the basis of transducers

Transducer plays an important role in the detection process of the biosensor and converts the biological or chemical signal into an electrical signal. Depending upon the type of transducer used, they are classified into optical-, electrochemical-, and mass-based biosensors.

#### Optical biosensor

These types of biosensors are based on measuring the changes in the intensity of light and convert light signal into an electrical signal that can be recorded in the form of current or potential. Optical biosensors have gained considerable interest for bacterial pathogen detection due to their sensitivity and selectivity. The most commonly used technique of optical detection is surface plasmon resonance (SPR) for pathogen detection.

#### SPR-based optical biosensor

SPR is based on the phenomena of optical illumination of metal surface for the detection of food-borne pathogens. To capture the optical illumination, certain antibodies were immobilized on a thin gold film. The interaction of light with the electrons present in the metal leads to the generation of strong resonance. When the pathogen is bound to the metal surface, there is a shift in the resonance to the higher wavelength and the concentration of bound pathogen is directly proportional to the amount of shift in the resonance.<sup>46</sup> Figure 7 shows a typical SPR-based biosensor. With the help of SPR technique, diagnosis of pathogens such as E. coli O157:H7 and S. aureus using bacteriophage as bioreceptor has been reported.<sup>47</sup> A modified technique was developed for the detection of E. coli O157:H7 at a low concentration of 50 colony forming unit (CFU)/mL called long-range surface plasmons in which magnetic NPs were used.48 Many researchers use commercially available SPR biosensor for the identification of food-borne pathogens. BIACORE 3000 was used for the detection of Listeria monocytogenes<sup>49</sup> and Salmonella,<sup>50</sup> and Spreeta<sup>TM</sup> was used for E. coli O157:H7.51,52

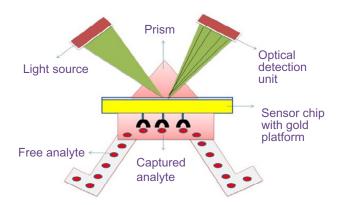


Figure 7 Surface plasmon resonance-based optical biosensor.

#### Electrochemical biosensor

The present popularity of the electrochemical biosensor over other biosensors is due to advantages such as low cost, good sensitivity and selectivity, use in turbid media, and miniaturization potential for the detection of food-borne pathogens.<sup>53</sup> In the electrochemical biosensor, when there is an interaction between the sensor electrodes and the sample analyte, then the changes in the current and potential values are measured. They are classified into amperometric (current), potentiometric (potential), and impedimetric (impedance), which depend on the type of transducer used.<sup>54</sup>

Amperometric transduction is most common in the electrochemical method for the detection of food-borne pathogens.<sup>55</sup> In an amperometric biosensor, current is produced by the oxidation or reduction of electroactive species at the working electrode (ie, gold, carbon, platinum). The value of current magnitude produced at the surface of working electrode is proportional to the quantity (concentration) of analyte present in the test solution.<sup>56</sup> Figure 8 shows the pictorial

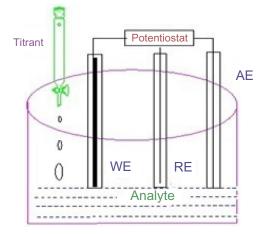


Figure 8 Amperometric biosensor.

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Abbreviations: AE, auxiliary electrode; RE, reference electrode; WE, working electrode.

representation of a amperometric-based electrochemical biosensor.

Kong et al<sup>57</sup> fabricated an amperometric glucose biosensor in which they immobilized GOx on the ZnO nanotubes using cross-linking method and further detected the amount of glucose in blood samples. Concentration of glucose was found to be 50  $\mu$ M–12 mM within 3 seconds response time. The sensitivity of the biosensor was found to be 21.7  $\mu$ A/mM cm<sup>2</sup>, and its experimental detection limit was found to be 1.0  $\mu$ M.

Caib et al<sup>58</sup> used two enzymes along with CNTs for the detection of cholesterol for the clinical diagnosis of diseases such as brain thrombosis, arteriosclerosis, and coronary heart disease<sup>59,60</sup> based on CNTs that were fabricated through layerby-layer method by using bienzyme biosensor (horseradish peroxidase and cholesterol oxidase) and obtained the linear range of cholesterol from 0.18 to 11 mM, with a detection limit of 0.02 mM.

Yanping et al<sup>61</sup> developed an amperometric biosensor for the organophosphate pesticide with a detection limit of 0.5 ng/mL by modifying glassy carbon electrode with acetylcholinesterase immobilized on porous reduced graphene oxide. For the detection of *Campylobacter jejuni* in chicken wash matrix, an amperometric biosensor was developed with a detection range of 10<sup>3</sup>–10<sup>7</sup> CFU/mL.<sup>62</sup> Detection of algal toxins, such as domoic acid and microcystin-LR, in food chain was reported.<sup>63–65</sup> Lata et al<sup>28</sup> fabricated an amperometric biosensor by covalently immobilizing an L-amino acid oxidase onto carbon nanotube/zinc oxide nano particles/ polyaniline (MWCNT/ZnONPs/PANI)/Au electrode that gives a broad range from 0.001 to 70 mM with a detection limit of 0.35 µm and a response time of 4 seconds.

Jesus et al<sup>66</sup> fabricated an amperometric glucose biosensor based on layer-by-layer film formed between the silsesquioxane polyelectrolyte and phthalocyanine that was modified with GOx and a film of nafion. Current shows linearity with a glucose concentration range of 1–10 mmol/L with a detection limit of 0.16 mmol/L and a sensitivity of  $1.397 \times 10^{-7} \mu A \text{ (mmol/L)}$ .

In a potentiometric biosensor, the biorecognition process is converted into a potential signal. This sensor uses ion-selective field effect transistors (ISFETs) and lightaddressable potentiometric sensors (LAPSs).

In order to increase electrical conductivity in ISFETs, an electric field is used to generate excess charge in semiconductor substrate.<sup>67</sup> In a potentiometric immunosensor, enzyme-labeled antibodies such as GOx, urease, or alkaline phosphatases are used, which are able to change either pH or ionic strength during the detection of microorganisms present in food sample.<sup>68</sup> LAPS evolved from ISFET by combining potentiometry with optical sensor for the detection of food-borne pathogens. Figure 9 shows a typical potentiometric biosensor having working electrode composed of CNT, polyvinylpyrilidon, NP, and potassium chloride (KCl) with asbestos membrane.

Due to simultaneous detection of several analytes, semiconductor-based LAPS was used because of its small size and multichannel arrangement.<sup>69</sup> LAPS measures a photocurrent produced by a light-emitting diode.

Singh et al<sup>69</sup> used LAPS for the detection of *Yersinia pestis* and *Bacillus globigii* spores with a limit of detection (LOD) of 10 cells/spores per sample, and later Dill et al<sup>70</sup> used LAPS for the detection of *Salmonella typhimurium* at a low level of colony forming unit, ie, 119 CFUs.<sup>70</sup>

Ahuja et al<sup>71</sup> developed a potentiometric urea biosensor based on bovine serum albumin embedded on the surface of modified polypyrrole film. The electrode shows a linear response of  $6.6 \times 10^{-6}$  to  $7.5 \times 10^{-4}$  M urea in 70–90 seconds.

Conductometric biosensor is generally based on the conductance measurement, that is, whenever a change in the ionic concentration of an analyte occurs, there is a subsequent change in the electrical conductivity of the solution or changes in the flow of current.<sup>72</sup> The microbial metabolism changes occur in the medium or analyte, which result in an increase in both capacitance and conductance, causing a decrease in impedance.<sup>73</sup> Therefore, conductance, capacitance, impedance and resistance are interrelated with each other, but they only differ in ways of monitoring the test system.<sup>74,75</sup> Tahir and Alocilja<sup>76</sup> developed a conductometric

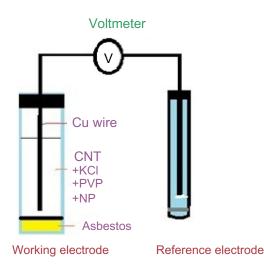


Figure 9 Potentiometric biosensor.

Abbreviations: Cu, copper; CNT, carbon nanotube; KCl, potassium chloride; PVP, polyvinylpyrilidon; NP, nanoparticle.

biosensor for the detection of *E. coli* O157:H7 and *Salmonella* food pathogens in 10 minutes with an LOD of 81 CFU/mL.

# Nanomaterials-based electrochemical biosensors

Nanomaterials, such as magnetic NPs, carbon nanostructures, and quantum dots, have important components to enhance performance in terms of lower detection limit, and higher sensitivity and faster electron transfer. The use of metal NPs and graphene is common practice to increase surface area and conductivity of the electrochemical biosensor. The addition of metal NPs in the working electrode increases sensitivity and current signal response time of the electrochemical biosensor. Huang et al<sup>77</sup> have reported the application of CNTs for crystallization of proteins and building of bioreactors and biosensors. When titanium dioxide NPs combined with CNTs significantly increase disinfectant properties against *Bacillus cereus* spores,<sup>78</sup> Ali et al<sup>79</sup> have reported a gold NPs sensor for the detection of *E. coli* O157:H7 in feed samples.

# Application of electrochemical biosensors for food safety

The essential requirement of the food industry is that the food supplied in the market should be 100% safe and of very good quality. For this, the food industry should perform different food analysis methods and different stages of quality checks to ensure the quality and safety of foods. Other concerns of the food industry include increasing the product yield, optimizing energy input, monitoring the food processing, and to raise the food processing automation level. Proper packing of the food is also essential in order to avoid environmental contamination during transport and storage of the food. Determination of chemical and biological contaminants in food is of importance for ensuring healthy nutrition for people. Salmonella, L. monocytogenes, C. jejuni, B. cereus, Vibrio cholerae, and E. coli, and so on are the common pathogens that contaminate food. Hence, it is very important to detect these microbial contaminants using rapid, sensitive, specific, and inexpensive methods of analysis. This goal of the food industry can be achieved by the use of electrochemical biosensors for the detection of chemical and biological contaminants in foods. Electrochemical biosensors provide rapid, specific, and inexpensive food sample analysis.

Table 1 summarizes the response time, LOD, sensitivity, and response time reported by different researchers for the detection of analyte present in food samples. An amperometric detection of *E. coli* O157:H7 was reported in 25 minutes

Table I Some examples of electrochemica	al biosensors used in food analysis
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Electrochemical biosensor	Analyte	Samples	Limit of detection	Sensitivity	Response time	Reference
Amperometric	Fructose	Honey, apple juice, orange juice	-	0.62±0.10 nA/µM	_	26
Electrochemical	Tryptamine	Real samples	7.4×10 <sup>-8</sup> mol/L	_	-	85
Electrochemical	Glucose	Real samples	I–10 mM	5.03 μA/mM	3.0 seconds	86
Amperometric	Glucose	-	0.14 μm	2.9 mAcm/mM	_	87
Amperometric	Polyphenols	Red wine	I.0×I0 <sup>-</sup> 6 M	0.0566 mA/M	_	88
Conductometric	Heavy metal ions (Zn <sup>2+</sup> and Cd <sup>2+</sup> )	Water	10 ррb	30 m	-	89
Electrochemical	Escherichia coli	Surface water	3 CFU/10 mL	_	_	90
Electrochemical	Staphylococcus epidermidis	-	1.0×10 <sup>6</sup> CFU/mL	-		91
Electrochemical	Salmonella typhimurium	Chicken carcass wash	10 <sup>2</sup> CFU/mL	-	15 minutes	92
Electrochemical	Listeria monocytogenes	Milk	10 <sup>2</sup> CFU/mL	_	3–4 hours	93
Electrochemical	Campylobacter jejuni	Culture and chicken carcass wash water	10 <sup>3</sup> CFU/mL	-	2–3 hours	62

Notes: Real samples of Tryptamine used were tomato juice and banana pulp. Real samples of glucose used were grape, honey and watermelon; hyphens (-) represent data not available.

Abbreviation: CFU, colony forming unit.

with an LOD of 5,000 cells/mL.<sup>80</sup> Xiang et al<sup>81</sup> fabricated an electrochemical immunosensor for the detection of *Salmonella* with the help of gold NPs dispersed in chitosan and found a low detection limit of 5.0 CFU/mL. Another important application of the electrochemical DNA biosensor for the detection of *S. aureus* nuc gene sequence was found in the concentration range of  $1.0 \times 10^{-13}$ – $1.0 \times 10^{-6}$  mol/L with an LOD of  $3.23 \times 10^{-14}$  mol/L ( $3\sigma$ ).<sup>82</sup> Girousi et al<sup>83</sup> fabricated a mitochondria-based amperometric biosensor for the determination of L-glutamic acid in the range of  $10\pm100$  mM with a sensitivity of  $0.021 \mu$ A/mM. Girousi et al<sup>84</sup> again fabricated a mitochondria-based amperometric biosensor for the determination of L-succinic acid in the range of 0.05–0.4 mM with a sensitivity of  $0.388 \mu$ A/mM, and the detection limit was found to be 0.02 mM.

### **Future perspective**

Much has been achieved in the field of electrochemical biosensors for food safety, however more needs to be done in the near future. Future work should be focused on the development of a novel biosensor in which power consumption must be reduced and more efficient power sources (batteries, capacitors, and so on) must be developed and fixed into biological detection systems to reduce the size and weight of the system and to increase system utility. A handheld and easily portable and smart electrochemical biosensor is needed so that detection of chemical and biological toxins can be made in the field of actual production so that proper monitoring of the food samples can be done. Future research should focus on the development of biosensors that may help fight against the disease-causing food-borne pathogens. Nanocomposites are receiving increasing interest for sensor construction in recent years. Handling of biosensors should be made simple so that even someone without specialized knowledge can use it without the help of qualified persons. Multifunctional and versatile biosensing systems are required for the analysis of multiple analytes using a single device. A more sensitive biosensor that is capable of detecting the nanomolar ranges in the field of food industry, environmental monitoring, and medical diagnosis will certainly prove fruitful.

# Conclusion

Biosensor-based devices have become an important part of the equipment used in laboratories to detect biological response. In spite of having developed a number of biosensors for detecting food-borne pathogens, it is still a challenge to create biosensors for the reliable and effective determination of microorganisms in real food samples. Conventional methods (enzyme-linked immunosorbent assay, polymerase chain reaction) for detecting food-borne pathogens are good but need well-trained persons, involve tedious procedures, and take a long time to show results. Ideal biosensors will have great potential to achieve better results and detect multiple pathogens in a very short time. Electrochemical biosensors have great potential in the future following further improvements.

### Disclosure

The authors report no conflicts of interest in this work.

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