Label-free electrochemical biosensors for food and drug application

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ABSTRACT: In food sector, there is a huge demand for rapid, reliable, user & eco-friendly biosensors to analyse the quality and safety of food products. Biosensor based methodology depends upon the recognition of a specific antigens or receptors by corresponding antibodies, aptamers or high-affinity ligands. The first scientifically commercialised sensors were the electrochemical sensors used for the analysis of multiple analytes. An electrochemical sensor is a device that produces analytical electrical signals based on a recognition element with an electrochemical transduction component. Utilization of nanomaterials (e.g. quantum dots, nanoparticles and nanowires) can significantly improve limits of detection of such devices. Electrical methods are ideally suitable for implementation of label-free detection approaches, which give a number of the advantages for the biomedical assays. Nanomaterials and modern microfabrication techniques gives the possibility of miniaturization and multiplex sensing. These make electrical methods more promising for applications in the point-of-care. This review gives an overview of alternative label-free electrochemical nanobiosensors in food analysis, safety and control management. The underlying principles and applications of these biosensors are discussed. Recent developments in biosensor systems with an electrochemical detection are also presented.

Keywords: Amperometric; Electrochemical sensors; Food pathogen; Impedimetric; Potentiometric

INTRODUCTION

Sensors are the devices that are made up of a function-alised sensing material and a signal transducer component system. Role of these two vital components is to transmit the electrical signal without result in any amplification from a selective specific compound or from a variation in a chemical reaction. Sensors based devices can produce any one of the output specialised signals (electrical, optical or thermal) that could be easily converted into the digital electrical signals used for analysis, detection and further processing. Among all these, sensors which are electrochemical in nature, have some advantage over other sensors because; in this case, electrodes equipped in biosensor can sense the substances which are present in the host without resulting in any kind of damage to the host system itself. Broadly, sensors can be categorised into the two-important type: biosensors and chemical sensors. The biosensors deal in the condition of sensing specific aspects, biosensors can detect or analyse biochemical
substances such as nucleotides, certain tissues and biological proteins (Wilson and Gifford, 2005, Sakaguchi, et al., 2007, Chen and Chzo, 2006). Biosensor was first commercialised in the market by Springs Instruments (Yellow Springs, OH, USA) in 1975. It was for the analysis of blood glucose in the patients with diabetes. Various types of biosensor based analytical devices have been refined and commercialised with vast spectrum of usage and applications including detection of food borne pathogens, their toxins and even certain biosensors are relied on the multichannel configuration system (Pohanka, et al., 2007a, 2007b).

In biosensors, active functionalised sensing material present on the electrode act as a catalyst and it must efficiently catalyze biochemical reaction of the compounds to give the output signals (Chaubey and Malhotra, 2002, Simoyi, et al., 2003). Chemical sensors are the device that converts chemical information (chemical activity, composition, particular element or ion, partial pressure) into an analytical useful signal. The consolidation of these two (biosensors and chemical sensors) methodology of classification criteria, results in the development of new arena of sensors referred as the electrochemical biosensors, in which electrochemical techniques are used for the development and working of a biosensor system (Balasubramanian and Burghard, 2006, Wang, et al., 2009, Zhang, et al., 2005). Electrochemical biosensor is an analytical system that integrates a biological recognition element component with a physicochemical signal transducer component which results in an electrical signal that is proportional to a specific single analyte and relates the concentration of the analyte to a measurable response. The working system of biosensor is well established as shown in Fig. 1.

Leland C. Clark known as the “Father of Biosensor” invented the first device to determine the amount of glucose in blood (Clark, et al., 1962). He is well-known as the inventor of Clark electrode, these were called as enzyme electrodes as presented in Fig. 2. Clark electrodes are relied on the fact that glucose oxidase enzyme catalyses the oxidation of glucose to gluconic acid. The consumption of oxygen was followed by electrochemical reduction at a platinum electrode. The concentration of glucose is directly proportional to the change in current, this type of sensor developed known as amperometric based electrochemical biosensor.

An electrochemical biosensor produces an electrical signal proportional to the concentration of a single analyte or group of analytes. Electrochemical biosensors may be categorised based on the type of signal transduction (impedimetric, potentiometric and amperometric) or depending on the nature of the biological recognition process: biocatalytic devices (for example based on enzymes as immobilize biocomponent) and affinity sensors (based on antibodies, membrane receptors, or nucleic acids.

The working principle of an electrochemical biosensor is the potentiality of a specific enzyme in or beneath the biorecognition layer that give electroactive materials for detection and analysis by the physicochemical transducer component which generates the measurable electric signal proportional to the concentration of target analyte as shown in Fig. 3. They are simple, well-established, reliable and stable powerful tools to achieve real-time information by in situ measurements of chemical composition for process control without sampling. This is due to their capability to be miniaturized, to operate in turbid media (unlike optical ones), to have short response time (unlike bioluminescent ones), to reach lower detection limit...
and to be cheaper, compared to the other types of biosensors.

Electrochemical sensors are extensively used in multiple sectors of food industry, traffic, environmental and medical monitoring, for the control of microbial pathogens and their and toxic products contamination. They are industrialised for monitoring the products of bioprocesses (amino acids, yeast, lactic acid, ethanol, etc.), the pollutants in the environment (pesticides, fertilizers, substances estrogenic, CO, CO$_2$, etc.), the relevant substances in clinical diagnostics (glucose, alcohol, DNA, hormones, etc.) and in the forensic field (cocaine, anthrax, nerve agents). Besides all commonly processed industrial enzymes used in biotechnological sector, some enzymes are operated in electrochemical affinity biosensors for the analysis of various metabolites or analytes, some of them are listed in Table 1.

**Table 1.** List of enzymes use as enzyme labels for electrochemical affinity biosensors for a specific clinical metabolite

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Clinical metabolite</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose oxidase, glucose dehydrogenase</td>
<td>Glucose assays</td>
<td>Antiochia, et al., 2007, Kafi, et al., 2006</td>
</tr>
<tr>
<td>Alcohol oxidase</td>
<td>Ethanol</td>
<td>Yildiz and Toppare, 2006</td>
</tr>
<tr>
<td>Urease</td>
<td>Urea</td>
<td>Barhoumi, et al., 2005</td>
</tr>
<tr>
<td>Cholesterol oxidase co-immobilized with cholesterol esterase</td>
<td>Cholesterol assay</td>
<td>Singh, et al., 2007</td>
</tr>
</tbody>
</table>

**BIOSENSING TECHNIQUES AND OTHER CONVENTIONAL METHODS**

Electrochemical biosensors can be operated in the amperometric/ potentiometric/ impedimetric mode. The circumstances of the art and the current development action in sensor based technology and application are defined by:

- Application of the multisensory cells, e.g. for measuring different ions lactate, dissolved oxygen and pH in lab-on-chip based systems.
- Miniaturization of sensors, e.g. for measurement in cell cultures, biological systems, living organisms and cell tissues.
- Application in techniques which requires a data analysis or sophisticated measuring procedure, e.g. impedance measurement.
- Fast sensors development, e.g. for analysis of gas components in ms-time scale by the use of nanostructured based electrodes.
- Development of simple, reliable, improved and stable sensor based materials to increase the stabil-
ity, sensitivity and selectivity, e.g. in solid electrolyte based gas sensors for NOx and hydrocarbons.
- Sensors development based on biological principles and tools.
- All-solid-state sensors development for low temperature operation, e.g. measurement of pH.
- Expansion of sensor based applications respecting temperature, aggressive media and pressure.

As compared to the chromatographic (HPLC, GC), spectrometric (UV–VIS, FTIR) and mass spectrometric (MS) techniques, electrochemical based biosensors are simpler in their configuration as well as in the electronic setup crucial for working activity and for the data acquisition. The skill and cost for calibration and maintenance is low. Signals are directly (in situ) obtained from sensors and give real-time operational information for the process control. Therefore; electrochemical sensors are adopted devices in industrial application and in field application for screenings. On the other side, electrochemical based sensors cannot take over from the above mentioned standard analytical techniques in the terms of detection limit, precision, etc. Due to their simplicity, the electrochemical sensors require details about the impact of measuring experimental factors such as pressure, chemical composition and temperature on the sensor signal, that determine the application limits.

ELECTROCHEMICAL SETUP AND MINIATURIZED ELECTROCHEMICAL SENSORS

The electrochemical setup developed is based on a three-electrode potentiostat system. Fig. 4 shows the schematic diagram of the three-electrode technology. Three-electrode configuration system consists of: RE (reference electrode), CE (counter electrode) and WE (working electrode).

Reference electrode (RE)
Electric potential across the reference electrode is well-known and very much stable. A standard calomel Ag/AgCl is used as reference electrode. The properties of this reference electrode are: Stable potential of 0.242 V vs. normal hydrogen electrode (NHE). The reference electrode has a negligible current to avoid a drop in the potential. For that reason, the reference electrode is built in a way that its environment doesn’t change (inert condition). In the three-electrode system, the use of a counter or auxiliary electrode is used to protect the reference electrode. An alternative electrode is the pseudo reference electrode that has a silver (Ag) wire and it is coated with silver chloride (AgCl). This electrode is appropriate for miniaturization of the electrochemical cell. The pseudo reference electrode shifts the reduction peak potential to more negative potential until it reaches a fixed value.

Counter electrode (CE)
Platinum wire acts as a counter (or auxiliary) electrode. The current passes across the CE (counter electrode) and the WE (working electrode), permitting the RE (reference electrode) to keep its high impedance condition. The electrochemical properties of the platinum wire do not affect the behaviour of the electrode of interest (working electrode) because platinum is an inert material. Usually the counter electrode is much larger than the working electrode. The potential measured is smaller between the WE and the RE than between the WE and the CE.

Working electrode (WE)
All the operations of interest occur on the working electrode. Depending on whether the reaction on the electrode is an oxidation or a reduction, the working electrode is called anodic or cathodic, respectively. Common working electrodes can consist of materials ranging from inert metals such as platinum, silver or, gold, to inert carbon such as glassy carbon or pyrolytic carbon, and film electrodes. Gold-coated Mikromash (CSC12 tipless / without Al) microcantilever acts as a working electrode. This microcantilever allows measuring optically its deflection in parallel with the electrochemical measurements.

Wide range of commercially accessible electrochemical sensors have dimension which do not permit their use in sample media with small volumes (sweat, liquor, blood or directly in contact with human body) because their conventional specified size is standardised. Due to the use of precision engineering in few cases, sensor based devices can be miniatur-
ized as shown in Fig. 5. This method can be utilized in the mm-range, but it cannot miniaturize down to μm-range. In multiple cases, the process of miniaturization is limited due to the short content of operational substances (for e.g. reference electrolyte) are indispensable for minimum life of the sensors.

The ability to establish miniaturized electrochemical sensor devices such as based on lab-on-chip systems and their massive production can be responsible for electrodes in the planar embodiment. Lab-on-a-chip based electrochemical sensors for dissolved biological acting gases like O$_2$ and CO$_2$, dissolved ions and pH were fabricated with small dimensions in planar design so that the analysis under or on plant cells and living animal is possible. The complex impedance is measured in order to monitor the adhesion of those cells.

ELECTROCHEMICAL DETECTION TECHNIQUES

The different types of electrochemical detection techniques in affinity biosensors may be grouped into two categories: label-free and labelled detection cells. In the labelled detection, signal is produced from redox label or reporter reagent that changes upon the receptor-target binding. Label-free techniques are expense; they require extra time and the labelling may transform the receptor affinity to the target analyte. Among the other side, label-free detection techniques analyse the variation in electrical properties of the surface when a targeted bioanalyte molecule interacts with the probe-functionalized surface without any labelling setup. Therefore, label-free based biosensors are much reliable, simpler, stable, lower cost, easier and can ensure the detection of probe-target binding in real time. Electrochemical detection techniques may be categorised based on the measured signal in three major types: potential, current and impedance. Based on the principle of operating system employed in sensors, the electrochemical biosensors are classified into; potentiometric based, amperometric based and impedimetric based transducers that converts the chemical signal or information into a detectable amperometric signal.

Potentiometric based biosensors

Potentiometric based sensors monitor the generation of charge potential at working electrode as compared to the reference electrode in an electrochemical system when there is no current flows between the respected electrodes (Chaubey and Malhotra, 2002, D'Orazio, 2003). It gives the information for ion activity occur-
ring by a reaction inside the electrochemical (Bakker and Pretsch, 2005). For technical measurements, the relation between the potential and concentration of analyte is determined by the use of Nernst equation. For illustration, surface of electrode can be immobilized with glucose oxidase enzyme. Glucose does not cause any severe effect on the pH across working media; yet, gluconate produced from the biochemical reaction by the enzyme causes significant acidification. Earlier, pH based glass electrode was actuated as a physical-chemical transducer element (Buerk, 1993). Nernst potential over the glass electrode is evaluated by the implementation of Nicolsky-Eisenman equation that derives an expression for ion-selective electrode as shown in equation 1:

\[
E = E^0 + \frac{RT}{z_a F} \ln \left[ a_a + \sum_{i=1}^{n} K_{a,i} (a_i)^{Z_a/Z_i} \right]
\]

Nernst equation; the relationship between the concentration and the potential, where E potential, R the universal gas constant, T temperature, F Faraday constant, Z_a followed and Z_i interfering ion valence, a activity of measured and ai activity of interfering ion and K_a,i represents the selectivity coefficient.

Potentiometry can be defined as the direct determination of target analyte concentration using the Nernst equation. Ion-selective electrodes (ISE) are used for the determination of lowest limits for potentiometric based sensor devices. For measuring samples with low concentration, potentiometric sensors are used as they provide the advantage of not chemically affect the sample. As can be seen in Fig. 6, the transducer used is an ion-selective electrode (ISE) that is an electrochemical sensor, based on selective membranes or thin films as their recognition elements. ISEs can detect various ions like NH_4^+, F^-, I^-, CN^-, Na^+, Ca^{2+}, K^+, H^+ or different gas molecules (CO_2, NH_3) in complicated biological matrices by sensing variations in electrode potential when specific ions bind to a relevant ion exchange membrane. The potential difference between these indicator and reference electrodes are proportional to the logarithm of gas fugacity (or concentration) or ion activity, as described by the Nernst equation. Nowadays semi-conductor based physico-chemical transducer components are commonly used. Light addressable potentiometric sensors (LAPS) and Ion-sensitive field effect transistors (ISFETs) based devices are more reliable for the construction of biosensors.

Potentiometric based biosensor fabricated with molecular-imprinted-polymer are developed for the assay of herbicide atrazine that allows detection as low as from sample size 3×10^{-5} to 1×10^{-3} M (Liao, et al., 2006); for the detection of the level of serotonin (neurotransmitter), molecular-imprinted-polymer was also used (Rosseti, et al., 2001). ISFET based sensor immobilized with butyrylcholinesterase was used for glycoalkaloids assay present in commonly consumed vegetables such as peppers, potatoes, eggplants, and tomatoes (Timur and Teloncu, 2004). Glycoalkaloids are toxic to humans if consumed in high concentrations. Here, simple pH based electrode used in sensors was modified with acetylcholinesterase (AChE) enzyme and it was then used for monitoring organophosphate pesticides A concentration (Timur and Teloncu, 2004). Food borne pathogen; Escherichia coli was detected using LAPS that allows the detection limit of pathogen as low as 10 cells/ml when the primary capture antibody was fabricated on LAPS flow through cell, and the secondary antibody used was labelled with urease enzyme for the forma-

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**Fig. 6. Typical schematic diagram of a potentiometric biosensor assays**
tion of sandwich complex setup (Ercole, et al., 2002). Automated 8-channel analysis of biological agents was done using a commercially available Bio-Detector (Smiths Detection, Warrington, UK) based on the LAPS sensor which is there in the mobile laboratories.

**Amperometric based biosensors**

Amperometric based devices are the typical kind of electrochemical sensor, that measure current continuously being produced from the reduction or oxidation of an electro active species in a biochemical reaction. Amperometric based sensors are very sensitive and reliable for mass production in various sectors than the potentiometric based sensors. The working electrode being used in amperometric based biosensor is commonly a particular metal or it can be based on the screen-printed layer that is coated with biorecognition element. Carbon paste equipped with an immobilized enzyme is also an economic cost effective option (Cui, et al., 2005). At the applied input potential, the conversion of electro active species produced in the enzyme layer develops at the electrode and emerging current typically ranging from nA to μA is measured (Mehrvar and Abdi, 2004). The principle of glucose oxidase-based sensor developed for monitoring glucose concentration (Magner, 1998) serves as an example of amperometric biosensor:

\[
\text{GOD(FAD)} + \text{Glucose} \rightarrow \text{GOD(FADH}_2) + \text{gluconolactone} \tag{1}
\]

\[
\text{O}_2 + \text{GOD(FADH}_2) \rightarrow \text{H}_2\text{O}_2 + \text{GOD(FAD)} \tag{2}
\]

\[
\text{H}_2\text{O}_2 \rightarrow 2\text{H}^+ + 2e^- + \text{O}_2 \tag{3}
\]

The above mentioned biochemical reactions (1) and (2) are being driven by the enzyme glucose oxidase (GOD) which contains FAD as its cofactor. Reaction (3) defines the oxidation of hydrogen peroxide species at the potential of about +600 mV.

Oxygen electrodes developed by Clark serve as a ground for the development of the reliable and the simplest type of amperometric based biosensors, in which the electric current generated is directly proportional to the oxygen concentration. This is analysed by the oxygen reduction across the platinum working electrode in reference to the Ag/AgCl reference electrode at a given potential in an electrochemical cell (Chaubey and Malhotra, 2002). Usually, the current is monitored at a constant potential and that is known as amperometric. If this current is monitored during controlled deviations of the potential, it is known as voltammetry. Even though the disadvantage of this often times indirect sensing, it is believed that amperometric sensors keep sensitivity admirable to potentiometric sensors. Aforementioned glucose biosensor is a typical example of amperometric device, which is relied on amperometric detection of hydrogen peroxide. A very distinct application of amperometric device is to monitor the levels of human chorionic gonadotropin β-subunit (β-HCG) in an advanced pregnancy testing, when used in combination with immunosensing techniques (Santandreu, et al., 1999).

Two- or three-electrode based configurations can be assembled for the functioning of amperometric biosensors. Two-electrode system includes working (it has immobilized biorecognition element) and reference electrodes, which can use in the development of label-free electrochemical sensors. Typical disadvantage with the two-electrode based system is the limited defined control of potential across the surface of working electrode of sensor with higher amount of current and due to this, linear range developed could be reduced. This complication can be clear up by using a third auxiliary. Now in this improved system, voltage is given between the working electrode and the reference electrode, and the current flows between the auxiliary and the reference electrodes. Certainly, the common screen printed three-electrode configuration based sensor is well established as can be seen in Fig. 7.

Amperometric based biosensors were generally been

Fig. 7. Example of the three-electrode screen-printed sensor produced by BVT (Brno, Czech Rep.). The sensor body is made from ceramics. A gold working electrode (a) is surrounded by an Ag/AgCl reference electrode (b) and gold auxiliary electrode (c). Letter (d) means silver output contacts. The ruler in the bottom is in millimeter scale.
described for the detection of analytes such as; sialic acid, glucose, lactate, etc (Marzouk, et al., 2007). Biological entities such as microbes like Mycobacterium smegmatis and Bacillus cereus (Yemini, et al., 2007) and detection of nerve agents (Liu and Lin, 2006) and pesticides have also been reported. Amperometric sensors were also reported for carrying out evaluation using nucleic acid which as a biorecognition component or marker. Also various uropathogens evaluated using their specific 16S rRNA sequence (Liao, et al., 2006). Some amperometric based biosensors are commercially available, for example: SIRE P201, Precision Xtra, FreeStyle Freedom Blood Glucose Monitoring System, and GlucoWatch Biographer.

**Impedimetric based biosensors**

Impedimetric based electrochemical biosensors have been certified to be an up-and-coming method for the detection of foodborne pathogens because of their simpler design, sensitivity, portability, rapidity and could be meant for on-the-spot detection as well. Impedance based electrochemical sensors can perform label-free detection and measurement of impedance does not need special components and is responsive to label-free operation. Protein detection can be done commonly by an indirect labeling approach often referred as sandwich assay. Impedance sensing requires no label; this is advantageous for the detection of proteins, while few impedance sensors use a label as mentioned in the literature. Labeling scheme requires expense, sample handling and extra time. Besides the expense and time assistance of omitting the labeling step, label-free action facilitates the detection of target-probe binding. Impedimetric based devices follow either impedance (Z) or its other components including capacitance (C) and resistance (R). Also, inductances usually cause minor influence in an electrochemical cell. Thus, the derivation of impedance measurement has been shown in equation 2.

\[ Z^2 = R^2 + \frac{1}{(2fC)^2} \]

Electrochemical impedance sensors measure the impedance of interface in an AC steady state having constant DC bias circumstances, this is often accomplished by imposing a sinusoidal voltage at an appropriate frequency and measurement of resulting current; process can be done at different frequencies. The ratio of current and voltage determines the impedance. This technique is referring as electrochemical impedance spectroscopy (EIS), it has been used to extensively study and research a variety of an electrochemical phenomena across a wide range of frequency. When the target analyte molecule is trapped by the probe of a sensor, there is a change occurring in the impedance of electrolyte solution interface and this impedance change is detected by EIS technique. Also, the capacitance or impedance of the interface can be analysed at single frequency. EIS approach is relying on the superimposition of a sine-wave potential of small amplitude over a polarisation potential of constant value:

\[ E(t) = E_{polarisation} + \Delta E \sin(\omega t) \]  

(Equation 3)

Here, \( E_{polarisation} \) represents the base potential across the working electrode measured against a suitable reference, \( \omega \) is the frequency of the signal in rad s\(^{-1}\) and the amplitude of the sine wave is denoted by \( \Delta E \). The responsive signal is a sinusoidal current of the same frequency when the system is linear, but different amplitude and phase arising from the voltage:

\[ I(t) = \Delta \sin(\omega t + \phi) \]  

(Equation 4)

Whereas \( I(t) \) represents the instant current value, \( \phi \) is the phase shift angle and the current amplitude is denoted by \( \Delta I \). Impedance is the new phasor ratio as described in equation (5).

\[ Z = \frac{E(t)}{I(t)} = \frac{\Delta E \sin(\omega t)}{\Delta \sin(\omega t + \phi)} \]  

(Equation 5)

Equation (5) transformed into equation (6) by using Euler’s notation for complex numbers is given by:

\[ Z = |Z| e^{j\phi} = |Z| \cos \phi + j|Z| \sin \phi \]  

(Equation 6)

Whereas the term \(|Z|\) denotes the impedance modulus (in \( \Omega \)). This is expressed in Fig. 8. When the potential and the current are in a phase (\( \phi = 0 \)), equation (6) is commonly the expression of Ohm’s law, and the impedance of interface is a resistor. An electrochemi-
cal integral setup is barely this simple and impedance of this system always represents an imaginary element, which may be measured using interfacial phenomena like double-layer charging. This is due to the charge separation occurred across the interface electrode-solution which gives an electrical structure that resembles a capacitor element. However, other techniques such as reaction kinetics and mass transport also assign to the imaginary element of impedance, because of this reason, mostly electrochemical problems are explained in the terms of combinations of resistors and capacitances. Because impedance data may be found in polar coordinates and Cartesian alike, it is necessary to recognize how the various different frameworks relate to each other. Hence:

$$|Z| = \sqrt{(Z')^2 + (Z'')^2} \quad \text{and} \quad \phi = \arctan \frac{Z''}{Z'}$$  \hspace{1cm} (Equation 7)

And further more:

$$Z' = |Z| \cos \phi \quad \text{and} \quad Z'' = |Z| \sin \phi$$  \hspace{1cm} (Equation 8)

Whereas the terms $Z'$ and $Z''$ are used to represent the real and the imaginary elements of the impedance, respectively.

Admittance is simply denoted by the term $Y$. In few cases, it is easier to evaluate the data in terms of the admittance rather than using impedance, because of the fact that networks of admittance and impedance may be calculated by Kirchhoff’s laws. Thus, if multiple impedances are being arranged in series order, then the overall impedance is the sum of all the individual impedances. However, if multiple impedances are being arranged in parallel manner, then it is more appropriate to use admittances, because here the overall admittance can be measured by the sum of individual admittances. Electrochemical cells may act as a network of impedances being arrange in series and parallel order. In this situation, the impedance monitored in EIS experiment represents the impedance of whole integral electrochemical system, $Z_{cell}$, and not commonly that of working electrode alone. Also, electrochemical cell consists of features that can have an impact on the monitored impedance element: for example, the connections and cables used. Electrochemical system processes non-linear dependent relation with potential, so that amplitude of perturbation needs to be significantly chosen to make ensure the linearity of response. This is important for experiments at particularly low frequencies. In an experimental setup, the parasitic noise is used to determine the lower limit of amplitude and the higher limit is determined using the commencement of faradaic nonlinearities. Also, larger amplitudes can be used in the absence of electron transfer processes. Besides the amplitude and the frequency of the disordered signal, another parameter use in order to control in EIS experiments is the polarization potential, because electron transfer rates and interfacial capacitance are potential dependent magnitudes.

Impedimetric based biosensors have certainly been well employed for monitoring and detection of the microbial growth because of the production of conductive toxic microbial metabolites (Liao, et al., 2006). This type of electrochemical sensor has been demonstrated to be a promising technique for the detection of foodborne pathogenic bacteria. The impedance detection methods can be grouped into two basic types based on the presence and absence of specific bio-recognition components. Working of first type is by analysing the change in impedance resulted due to the binding of targets to bioreceptors (nucleic acids and antibodies) that are immobilized onto the surface of electrode, although the principle of detection of second type is relied on the metabolites generated by pathogens (bacterial cells) as a result of microbial growth. Nanoparticles have contributed exclusive advantages for manufacturing of impedimetric based biosensors. Designing of impedimetric sensor has been
carried out by the use bioreceptors (lectins, nucleic acids, bacteriophages and antibodies) immobilization at the surface of a solid electrode. On the basis of type of bioreceptors used, impedimetric biosensors were groups into four different categories as; nucleic acid-based sensors, lectin-based sensors, bacteriophage-based sensors and antibody-based sensors.

Disadvantages with impedimetric biosensors include false positive results arises because of the electrolytes. Impedimetric based biosensors are less recurring as compared to amperometric biosensors and potentiometric; still impedimetric sensors are being used in wide sectors. Amplification of hybridized DNA fragments using the conventional polymerase chain reaction (PCR) has been analysed by impedance assay (MDavis, et al., 2007). Level of ethanol in certain beverages was monitored using impedimetric biosensors immobilized with the Saccharomyces cerevisiae yeast sample (Korpan, et al., 1994). The impedimetric-based commercially available sensor; Malthus 2000 was employed for the detection of Ichthyophonus hofery, pathogenic fungus (Spanggaard, et al., 1994). Polymer coated transducers can act as an alternative platform that can be coupled with biochemical interactions such as; immunoassay systems or enzyme substrate systems, directly or indirectly subjected for deterioration of those employed polymers that can be used as a part of biorecognition component for the designing of electrochemical biosensors. This is a novel concept (Polymer coated transducers) for development of electrochemical biosensors, which has significant advantages over other approaches as it could evolve towards the development of single-use sensor based devices with real potent applications for the detection of a wide variety of molecules. Different types of electrochemical sensors are characterised and compared in Table 2.

**NANOSTRUCTURE MATERIALS IN ELECTROCHEMICAL SENSORS**

The field of science and technology in nano scale (nanotechnology) plays a crucial role in development, fabrication, sensing materials, characterisation and detection of various analytes for food and drug applications. With the integration of nanotechnology, sensitivity and performance of biosensors is significantly enhanced. Advancements in nanotechnology have triggered the establishment of biosensing tech-
niques for detection of hazards compounds associated with food products (Warriner, et al., 2014, Sharma, et al., 2015, Bulbul, et al., 2015), where integration of nanostructures in biosensors have several advantages including (i) target identification; (ii) enhanced output signal through recognition; (iii) increase in sensitivity and selectivity; and (iv) low recovery time. Nanostructures like: metal nanoparticles, nanovials, nanowires, nanostructured materials, carbon nanotubes and nano-composite materials integrated in sensors are playing a significant role in the design and fictionalisation for biosensors.

Nanomaterials act as an ideal element that can be used as a component of transducer. Novel feature of nanostructure is nano scale size that is ranging from few Angstroms to 100 nm, which is under the range of numerous biomolecules such as viruses, small proteins and nucleic acids. This nano scale size lead to a huge increase in surface-to-volume ratio which is critical for expended sensitivity. The extensive advantage of nanostructure elements in label-free electrochemical sensing setup is that the extent of receptors being immobilized on the surface of detector element can certainly be as low as single molecule. Magnetic nanoparticles (MNPs), usually consisting of periodic magnetic elements like: Ni, Co and Fe and their compounds that have been employed for the pre-treatment of materials as well as for target analytes separation from the samples of complicated compositions. Research studies have shown importance of MNPs in the design of rational nanobiosensing (Yao, et al., 2015, Ranjbari, et al., 2015). Recent advancement in the field of nanotechnology has bricked the path for large number of materials and various devices of desirable properties which can be useful for biosensor and electrochemical sensor applications (Hubalek, et al., 2007, Yogeswaran, et al., 2007).

The fabrication of functionalized nanostructure materials could act as a functional aspect of nanotechnology (Hernandez-Velez, 2006). Also, nanostructured array can be used for optical excitation and efficient transport of electrons, and these two parameters make them hypercritical to function and integration of nano system devices (Shie, et al., 2008, Yogeswaran and Chen, 2008). In the designing of electrochemical sensor devices, choice of sensing material for specific analyte in a sample and their major applications are sufficient (Qian and Yang, 2006, Shie, et al., 2008). Among all these devices, use of nanowires in biosensor reveals most interesting and effective studies (Currel, et al., 2005, Roberts and Kelley, 2007, Rosseti, et al., 2001, Zhu, et al., 2006). Nanostructures have specialized chemical and physical properties that are usually absent in bulk structures. Such as the binding of specific analyte to its receptor being immobilized on carbon nanotube (CNT) results in a significant change in the resistance of carbon nanotubes which is used as sensor signal. Such method does not exist in the bulk metals. Also, surface plasmons in nanoparticles and in thin metal films are distinctive properties at nanoscale which are not available in the bulk microscale structures.

These exceptional properties emerge because of the confinement in one to three dimensions. Typical example is enhancement in the electrochemical sensing achieved by nanostructuring of surface of the electrode due to increase in the surface area. Amplification scheme based on nanoparticles have impart enhanced sensitivity of bioelectronics assay by considerable orders of the magnitude. Electronic detection of DNA hybridization using gold nanoparticles was reported (Wang, et al., 2001). This methodology based on capturing nanoparticles to the hybridized targets that is followed by the highly sensitive measurement of metal tracer using the electrochemical anodic stripping. Gold nanoparticle tracers and sandwich immunoassays have been used for bioelectronics measurement of proteins (Dequaire, et al., 2000). Inorganic nanocrystals employ number of electrical tags meant for designing of electronic coding. Among all, three encoded nanoparticles (lead sulphide, cadmium sulphide and zinc sulphide) have been utilized to differentiate signals of the three specific protein targets in relation with stripping voltammetry and the sandwich immunoassay of corresponding metals.

Bioelectronics detection of analytes can be done by using one-dimensional nanostructures which includes; semiconductor or conducting polymer based nanowires and carbon nanotubes (CNT). Due to novel electron transport properties and high surface-to-volume ratio, electronic conductance of these type of nanostructures is greatly influenced by the minor surface
perturbations. Nanostructured based carbon nanotubes (CNT) are typically exciting 1-D nanomaterials that have emerged a considerable importance due to their structure-dependent mechanical and electronic properties. Fig. 9 demonstrating the structure of Carbon nanotube based enzyme biosensor. Carbon nanotubes may be categorized into the single-wall carbon nanotubes (SWCNT) and the multi-wall carbon-nanotubes (MWCNT). SWCNT consist of a cylindrical nanostructure (having a high aspect ratio), which is produced by rolling up a single graphite sheet into a tube. SWCNT can be considered as molecular wires with an every atom on the surface.

Bioelectronics detection can also be done using nanowires that have been more appealing to use in analytical chemistry, chiefly in biosensing technologies. It is due to the novel semiconductive properties linked with nanowires and they are reported in performing sensing for single molecule. Impedimetric based microelectrode immunosensor fabricated with TiO2 nanowire was developed for rapid detection of Listeria monocytogenes (Wang, et al., 2009).

In this, gold microelectrodes were connected to TiO2 nanowires using mask wedding technique and monoclonal antibodies were immobilized on the surface of TiO2 nanowires for specifically capturing the bacteria. Variations in the impedance caused by the complex formation of nanowire-antibody-bacteria were monitored and related to the bacterial population. TiO2 based nanowires exhibited good biocompatibility, good photochemical and chemical stabilities, large surface area and very less extent of protein denaturation. This TiO2 based immunosensor developed also displayed good performance by detecting as low as 102 cfu•mL⁻¹ of Listeria monocytogenes concentration in 1 hour without any significant interference from other pathogens.

Surface of nanostructure may be optimized for the detection of specific analytes by various protocols of chemical and bio functionalization. Besides that, other advantages of functional nanostructures include; enhancement in the detection speed, reduction in sample & reagent consumption and ability to lessen the diagnostic tool. Nanobiosensors can be used to build arrays of biosensors on one substrate. Using this, monitoring of different analytes can be done simultaneously.

**ELECTROCHEMICAL BIOSENSORS FOR FOOD AND DRUG ANALYSIS**

The high-end demand for selective, real-time, low cost and rapid techniques for detection and monitoring of food samples has led to advancements in biosensing system. The integration of micro and nano-structured elements within the biosensing systems (magnetic micro- or nanoparticles, graphite microparticles, gold nanocomposites, nanowires, carbon nanotubes, nanorods, and bioreactors) has provided enhanced analytical improvements and performances in the detection of various food-borne pathogens, allergens (gliadin), food additives (folic acids), and food residues (antibiotics, pesticides). Also, with the integration of magnetic particles, improvement rates in the monitoring of food resides estimated, achieving enhanced and precise electrochemical responses due to the huge size of transducing active area. These simple, user-friendly, hand-held, and sensitive sensors based devices contribute to new analytical paths for in-field detection of food contaminants, mainly pathogens and food residues. An economically important crop such as fundamentally fruits and vegetables can easily be contaminated by gray mold caused by the fungal pathogen Botrytis cinerea. Moreover, some phytopathogenic fungi produces secondary metabolites, known as mycotoxins such as ochratoxin A. Limited number of biosensors employed for food quality control have been reported for fungi determination. Most common food-borne infections are caused by the bacteria Escherichia coli O157:H7, Salmonella (S. enterica and S. typhimurium), Listeria monocytogenes and Campylobacter jejuni. Different electrochemical biosensor applications in food analysis are given in Table 3.

Graphite microparticles in the form of rigid graphite-epoxy composites (GEC) conducting materials have been widely used and characterised to be suitable for biosensing electrochemical system because of their unique electrochemical and physical properties (Alegrè, 1996). An optimal material for biosensing should have surface efficiently immobilized with bioreceptor, a minute nonspecific adsorption of label, a vigorous biological reaction between the target analyte and bioreceptor, and a rapid selective detection of biological process. GECs accomplish all these above-mentioned requirements. Besides that, GEC-based biosensing...
systems are more advantageous over traditional carbon-based materials (CNT) in terms of higher robustness, rigidity, greater simplicity of preparations, and enhanced sensitivity. Unlike glassy carbon and carbon paste, the malleability of the GECs before the curing step allows various configurations with respect to size and shape that are then processed and fixed afterwards the curing step. Moreover, modification can be done on the surface of graphite-epoxy composites by wet and dry adsorption of bioreceptor (proteins, DNAs, antibodies, oligonucleotides), producing a stable and reproducible layer of bioreceptor on the surface transducer, which can be adopted in electrochemical detection as well.

Electrochemical biosensors can detect and monitor microbes in much shorter span of time with high selectivity and sensitivity and also can perform multiple analyses at the same time. These electrochemical based sensors can functional with complex target samples without the use of sample enrichment and without causing any variation in a sample. Since they are high performance and low-cost devices, they are promising to be employed as the stand-alone devices for onsite monitoring.

**ELECTRONIC NOSE AND TONGUE**

Electronic nose or e-nose is a specific type of sensor arrays. It is an odour mapper which can discriminate different volatile compounds due to the electronic response (such as resistance, conductivity and voltage) resulting from the various gas sensors, typically metal-oxide based chemosensors. The sensors in an electronic nose are desired to have a broad selectivity rather than being specific to one type of volatile chemical. The advancement of an electronic nose for detection of pathogen has earned considerable attention in recent years. Conducting polymers has been used as novel detectors in electronic nose systems. As some fungal species are associated with volatile metabolites in this case, electronic nose has been used extensively. Moulds produce secondary metabolites that are mycotoxins, due to ubiquitous nature they contaminate food materials and are of potential risk for human health therefore, prompt-rapid and sensitive detection is critical. Many researchers reported novel efficient cost-effective nanobiosensors for the detection of various mycotoxins. After being contact of volatile compounds by the sensor array, a signal is generated and this can be interpreted with the multivariate analysis. Electronic nose has been reported to classify cereal grains, to discriminate strongly musty and weakly musty oat samples effectively. Development of electronic noses and their application in different food samples such as meat, grains, coffee etc. has been reported (Schaller, et al., 1998).

On the other side, electronic tongue or e-tongue comprising of thirty chemical based sensors and pattern recognition elements for processing of data. It is used for analysis of soft drinks, coffee, mineral water and flesh food. Principle of the electronic tongues function in a similar way to the “electronic nose” as shown in Fig. 10. A sensor array produces signals that are not necessarily specific for any particular chemical species. A pattern of signals is generated, that can be correlated to certain features or qualities of the sample. In this case the sensors operate in an aqueous environment and they have different cross-sensitivities to various chemical species. The most common sensors used are potentiometric in nature, producing an electrical potential characterised by the Nernst equation. The electronic tongue reported to be able to distinguish between; various sorts of beverages, artificial and natural mineral water, commercial brands of coffee, experimental and commercial samples of soft drinks having different sweeteners. Arrays of biosen-
sor can save time by allowing the detection of multiple analytes.

**FUTURE OUTLOOK**

Electrochemical based biosensors being developed, well characterised and it seems to possessed great promising approach for the future timeline. Advantages of electrochemical sensors are their specificity, accuracy, reliability and enhancements with the integration of nanotechnology but major limitation can be the significant high cost of instrumentation. Electrochemical biosensing technology has developed in ev-

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Area of application</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organics: Amino acids, cholesterol, carbohydrates, pesticides, antibiotics, alcohols, vitamins, carboxylic acids, phenols, lipids, lecithin</td>
<td>Common constituents or contaminants in food products</td>
<td></td>
</tr>
<tr>
<td>L-alanine (with Balanine)</td>
<td>Flavor enhancer</td>
<td></td>
</tr>
<tr>
<td>Citrate</td>
<td>Found in several fruits and in all animal and vegetable cells</td>
<td></td>
</tr>
<tr>
<td>Catechins, catechols and tannin</td>
<td>Taste and function of green tea. Quality control in tea processing (substances of astringency)</td>
<td></td>
</tr>
<tr>
<td>Polyphenols</td>
<td>Olive oils (taste and stability of the oil)</td>
<td></td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>Wine, beer, yogurths</td>
<td></td>
</tr>
<tr>
<td>Malolactic acid</td>
<td>Wine quality</td>
<td></td>
</tr>
<tr>
<td>Trimethylamine, putrescine, cadaverine and histamine</td>
<td>Meat spoilage and aging, histamine in red wine</td>
<td></td>
</tr>
<tr>
<td>Nucleotides: hypoxanthine, inosine, inosine-5-monophosphate</td>
<td>Fish freshness, meat aging</td>
<td></td>
</tr>
<tr>
<td>Salicylate</td>
<td>Antimicrobial agent, preservative (now forbid den in most countries)</td>
<td></td>
</tr>
<tr>
<td>Benzoic acid, sorbic acid, tocopherol</td>
<td>Preservatives</td>
<td></td>
</tr>
<tr>
<td>Glutamate</td>
<td>Flavour enhancer</td>
<td></td>
</tr>
<tr>
<td>Lactate</td>
<td>Yoghurt, beer, fruit juices, wine</td>
<td></td>
</tr>
<tr>
<td>Amygdalin</td>
<td>Cyanide-containing sugar in almonds</td>
<td></td>
</tr>
<tr>
<td>Artificial sweeteners (aspartame, saccharin, cyclamate, acesulfame, etc.)</td>
<td>Soft drinks, desserts.</td>
<td></td>
</tr>
<tr>
<td>Glucosinolates</td>
<td>Nitrile-and sulphur-containing heteroglycosides</td>
<td></td>
</tr>
<tr>
<td>Drug and hormone residues</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetracyclines, Sulfonamides, Quinolones, b-lactams, Macrolides, Nitroimidazoles, Amphenicols, Miscellaneous</td>
<td>Poultry muscle, cattle muscle, fish, prawn, honey, milk</td>
<td></td>
</tr>
<tr>
<td>Inorganics: Sulphites, sulphur dioxide</td>
<td>Used as food preservatives, oxidation prevention</td>
<td></td>
</tr>
<tr>
<td>Potassium, sodium, calcium, magnesium, nitrate, nitrite, chloride, sulphate, fluoride, carbonate, and heavy metals</td>
<td>Vinegar, fruit juices, milk, soft drinks, mineral water</td>
<td></td>
</tr>
<tr>
<td>Toxins: Saxitoxin, neosaxitoxin, gonyautoxins, domoic acid, brevetoxin, Protein A, hepatitis A virus, aflatoxin, Ciguatoxin, Ochratoxin A, Patulin, Tetrodotoxin, cholera toxin</td>
<td>Protein A is a product of Staphylococcus aureus. Marine shellfish poisoning</td>
<td>Viswanathan, et al., 2006; Min and Baeumner, 2004</td>
</tr>
</tbody>
</table>
ery field to a great extent but still there is a long way to completely replace the conventional techniques. To achieve this, we need to develop label-free operations that can be able to monitor or detect at very low levels; picomolar to femtomolar of number of analytes in the areas of food sector, environmental monitoring, health care, clinical analysis, etc. and so on. There are still difficulties for fabrication of electrochemical biosensors hence more technical research is needed for finding the alternatives. At present, with the threat of food pathogens, VOC’s, pesticides residues in various food products, the development of accurate, faster, portable, low-cost and reliable biosensors have become significantly important. Increasingly we shall be seeing more and more applications of such systems in “online” or “at line” applications in the food industry ensuring quality and safety of such products.

CONCLUSIONS

Electrochemical sensor based technology gains its practical usefulness from a selective combination of biochemical recognition elements with highly sensitive electrochemical detection techniques. Label-free detection methodology monitors the variations in the electrical properties of surface whenever a target bio-analyte molecule interacts with a probe-functionalized surface without any labelling element. Therefore, label-free biosensors are more reliable, simpler, easier, lower cost, stable and can enable the detection of target-probe binding in real time. Label-free electrochemical biosensors are widely used for monitoring the products of industrial bioprocesses (amino acids, yeast, lactic acid, ethanol, etc.), the pollutants in environment (pesticides, fertilizers, substances estrogenic, CO, CO2, etc...), the relevant substances in clinical diagnostics (glucose, alcohol, DNA, hormones, etc...) and in the forensic field (cocaine, anthrax, nerve agents).

Based on the transducer element, three different types of electrochemical sensor are developed; potentiometric, amperometric and impedimetric. With the integration of nanotechnology, sensitivity and performance of biosensors is significantly enhanced. Nanomaterials like: metal nanoparticles, nanovials, nanowires, nanostructured materials, carbon nanotubes (CNTs) and nanocomposite materials integrated in electrochemical sensors are playing a significant role in the design, fabrication and functionalization. Also, with the integration of magnetic particles, improvement rates in the monitoring of food residues estimated, achieving enhanced and precise electrochemical responses due to the huge size of transducing active area. These devices contribute to new analytical paths for in-field detection of food contaminants, mainly pathogens and food residues. Graphite microparticles in the form of rigid graphite-epoxy composites (GEC) conducting materials have been widely used and characterised to be suitable for biosensing electrochemical system because of their unique electrochemical and physical properties.

Electronic nose and tongue are specific kind of sensor arrays. Electronic noses and tongues have undergone great developments over the last few decades. Recent technological process, like profit of biosensors from the miniaturized electrochemical instrumentation and are more advantageous for some sophisticated applications requiring specific measurement, portability, rapidity, and it requires small volume of target analyte samples to analyse. Diverse commercial applications ensure the demand of electrochemical biosensing devices such as such as to develop analytical electrochemical biosensors for sensitive, rapid, and specific detection of the pathogenic microbes in several sectors such as agriculture, horticulture, food, medical, environmental field and veterinary diagnostics.
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