Chapter 4
Impedimetric Sensors in Environmental Analysis: An Overview

Sunil Bhand and Gautam Bacher

Abstract In recent years, there has been a great need for rapid, reliable, specific and sensitive techniques for environmental monitoring. Conventional analytical techniques for environmental monitoring involves high cost, skilled personnel and also often are not available for online detection. On the other hand, impedance-based electrochemical sensing has the advantages of low cost, ease of use, portability and ability to perform both screening and online monitoring. Impedance-based detection technique is very powerful tool for the analysis of interfacial properties related to biosensing and chemical sensing at the modified electrode surfaces. Impedance method is less destructive as compared to other electrochemical methods for bio and chemical analysis. Impedance sensing gives direct electrical signals and does not require a label or other pre-treatment process. Label-free detection for biological and chemical analysis has been widely reported to detect environmental toxins. This chapter describes basic concepts in sensor design and construction and also covers recent developments in the field of impedimetric sensing applied to environmental analysis. Selected examples are discussed with respect to mycotoxins (aflatoxin M1, aflatoxin B1, ochratoxin A), antibiotic and pesticide residue analysis.

Keywords EIS · Biosensor · Electrodes · Antibody · Aptamer
Mycotoxin · Immunosensor

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1 Introduction

Biosensors have emerged as a promising toll to the traditional methods for the detection of pathogens and toxins (Alocilja and Radke 2003; Arora et al. 2006). Biosensors cover the wide range of analyte detection in complex matrices and have shown great potential in areas such as clinical, food analysis, bioprocess and environmental monitoring. Biosensors are of great interest because of their several advantages over the conventional techniques in the field of environmental analysis. The main advantages in the use of biosensors as compared to conventional methods are the short analysis time, low-cost detection, their suitability for automated analysis and the possibility to perform in real time. Direct monitoring of antibody-antigen interaction facilitates label-free detection, which will have many advantages such as high sensitivity, ease of detection, lower sample cost and short analysis time. Among the several types of transducers, label-free biosensors based on impedance have received considerable attention in recent years. These sensors have various attractive features associated with the use of specifically the electrochemical transducers, such as scalability of production, low cost and scope for miniaturization. Electrochemical impedance spectroscopy (EIS)-based sensors are considered as promising candidates for real-time applications. Moreover, the label-free nature of EIS has major advantages over amperometric and potentiometric sensors. Hence, impedimetric biosensors have potential for simple, rapid, label-free and low-cost detection of biomolecules in the environmental analysis.

2 Impedimetric Biosensor

Impedimetric detection is predominantly based on affinity biosensors (Van Emon 2007). It is used to capture and quantify immunological binding events such as antibody-antigen interaction on an electrode surface. This binding results into impedance change which is proportional to the concentration of the target analyte specific to the receptor. Lorenz and Schulze in 1975 (Lorenz and Schulze 1975) explained EIS by measuring resistive and capacitive properties of materials, when a small amplitude sinusoidal ac excitation signal typically of 2–10 mV is applied to a system (Bartlett 2008; Suni 2008). The impedance response is obtained by varying frequency over a wide range. In impedance measurement, a suitable ac voltage is applied to the electrode system and the corresponding response current is measured. The resistive and capacitive components of impedance are extracted from in-phase and out-of-phase current. Impedance methods are very useful because it can capture electron transfer process at high frequency and mass transfer activity at low-frequency range. The change in electron transfer resistance or change in capacitance is observed at the electrode/electrolyte interface as a result of antibody-antigen binding. The electron transfer resistance or capacitance change depends on faradaic or non-faradaic impedance measurement.

2.1 Electrochemical Impedance Spectroscopy (EIS)

EIS is an efficient tool for the analysis of bio-interfacial properties associated with bio-recognition occurring at the modified electrode surfaces. EIS is less destructive method for analysis of biological interactions (Bogomolova et al. 2009) compared to other electrochemical methods such as cyclic voltammetry (CV) or differential pulse voltammetry (DPV) and facilitates easy diagnostics (Daniels and Pourmand 2007). EIS analyses in terms of electrical components such as resistance and capacitance change that occurs at the electrode surface. Such components enable capturing of very sensitive biological binding events such as antibody-antigen interaction. Till date, several immunosensors based on EIS have been reported (Lisdat and Schäfer 2008; Qi et al. 2010). Specifically, label-free detection method for biological and chemical analysis has been reported for detection of marine bio-toxins (Syaifudin et al. 2009) and characteristics of food products (Li et al. 2011).

2.1.1 Fundamentals of EIS

The impedance (Z) of an electrochemical system is determined by applying a suitable voltage with small amplitude and measuring the corresponding current response. Thus, Z is the ratio of the voltage–time function $V(t)$ to the resulting current–time function $I(t)$ as given by Eq. 2.1

$$Z = \frac{V(t)}{I(t)} = \frac{V_0 \sin(2\pi ft)}{I_0 \sin(2\pi \mathrm{ft} + \varphi)} \tag{2.1}$$

where

V_0 and I_0	are the amplitude of voltage and current signals
f	is the applied frequency
t	is time and
φ	is the phase shift between the voltage and current

In an electrochemical cell, the impedance 'Z' represents the total opposition offered by all the components (resistors, capacitors and or inductors) to the flow of electrons and ions present. In an ac circuit, the electrode kinetics, redox processes, diffusion phenomena and other molecular interactions at the electrode surface also oppose the flow of electrons (Macdonald 1987). Impedance measurement gives information in terms of resistance/capacitance, upon changes at the electrode surface. However, the contribution from inductance is significant at high frequency in impedance spectra. Whereas, in EIS-based sensors, the contribution from inductance is of less significance.

Generally, Impedance is a complex number, with ohmic resistance as real component and the capacitive reactance as imaginary component. The data are

usually analysed using Nyquist and Bode plots. In the Nyquist plot, the imaginary component Z'' (out-of-phase) of impedance is plotted against the real component Z' (in-phase) of impedance for each excitation frequency as presented in Fig. 1a. A distinctive shape of faradaic impedance spectrum is presented with Nyquist plot (Fig. 1b). This comprises of a semicircle region and a straight line. The straight line (linear part, $\phi = 45°$), observed at the low-frequency range, represent a mass transfer limited process and the semicircle portion observed at high frequency range, denotes a charge transfer limited process. Whereas, in Bode plot, the absolute impedance data is plotted against frequency as shown in Fig. 1c. In non-faradaic impedance measurement, redox probe is absent in the electrolyte and electrode surface is covered only with dielectric layer resembling perfect insulator. Hence electrode setup works as a capacitor. Thus, in such situation the antibody-antigen interaction causes change in capacitance resulting in impedance change. Usually, Bode plot is used to represent impedance data against frequency for analysis of Ab–Ag interaction and quantification in terms of impedance '*Z*'.

Capacitive immunosensors are mainly based on measurement of the change in dielectric properties and/or thickness of the dielectric layer. An electrolytic capacitor is represented by two plates, where the first plate is represented by sensing

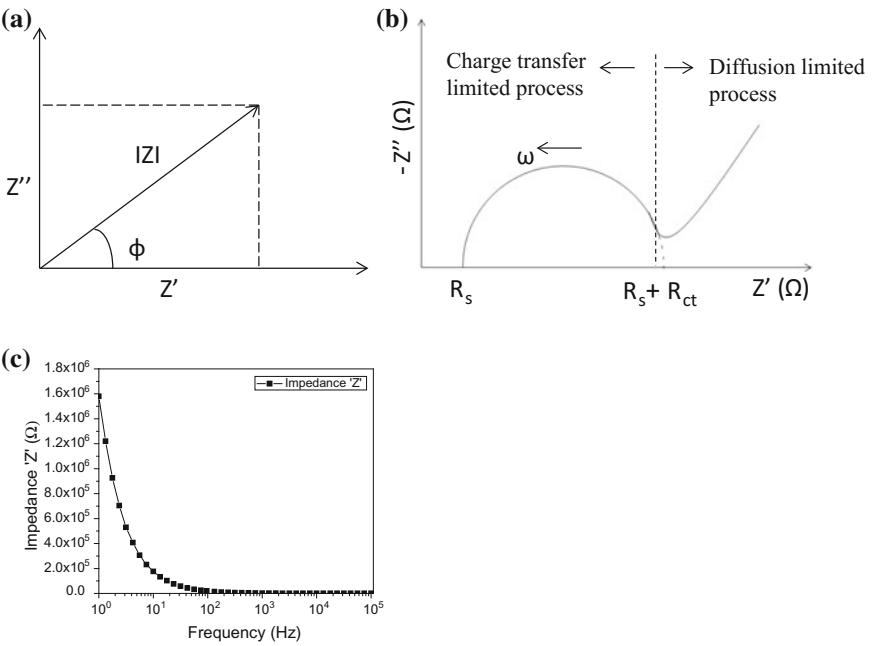

Fig. 1 **a** Phasor diagram of impedance **b** Nyquist plot for faradaic EIS (R_s: electrolyte resistance, R_{ct}: charge-transfer resistance, C_{dl}: double-layer capacitance, ω: angular frequency, Z': real component of Z and Z'': imaginary component of Z) **c** Bode plot for faradaic EIS

electrode and the second plate is represented by electrolyte. This allows the detection of an analyte specific to the bioreceptor that has been already immobilized on the insulating dielectric layer (Berggren et al. 2001; Katz and Willner 2003; K'Owino and Sadik 2005). Generally, this configuration resembles as capacitor having charge storage ability, and hence, the capacitance between the sensing electrode and the electrolyte is represented by Eq. 2.2.

$$C = \frac{\varepsilon_0 \in A}{d} \tag{2.2}$$

where

'ε' is the dielectric constant of the medium between the plates,
'ε_0' is the permittivity of free space,
'A' is the surface area of the plates in square metre, m^2,
'd' is the thickness of the insulating layer in metre, m.

2.1.2 EIS Measurement

EIS measurement is generally carried out using either three electrodes or a two electrode setup, which is a part of an electrochemical cell. In a three-electrode setup, the working electrode should be chemically stable, conductive and solid materials such as Pt, Ag, or graphite is used. Conventionally, the reference electrode is a silver metal coated with a layer of silver chloride (Ag|AgCl) and the auxiliary electrode a platinum wire. The advantage of a three electrode system is its stable half cell potential due to the greater charge flow through auxiliary instead of the reference electrode; this in turn helps in maintaining its half-cell potential (Ronkainen et al. 2010). A two-electrode system consists of a working and reference electrode. In two-electrode system, the need for a counter electrode is eliminated since the reference electrode can carry the charge with no adverse effects when current density is low. Both three-electrode systems as well as two-electrode systems are reported for biosensing. Since a disposable biosensor system does not require long-term stability, hence two electrode system may be preferable. In impedance biosensors, the applied voltage should be quite small, usually up to 10 mV in amplitude for several reasons. First, the current–voltage relationship is linear only for small perturbations (Barbero et al. 2005), and impedance is strictly defined for linear region only. The second reason for using a small perturbation is to avoid disturbing the probe layer since the covalent bond energies are usually in the order of 1–3 eV (Daniels and Pourmand 2007), but probe–target and electrode–probe binding energies can be much less. Correctly performed EIS does not damage or even disturb the biomolecular probe layer; this is a major advantage over voltammetry or amperometry where extreme voltages are usually applied. A schematic of EIS measurement setup is shown in Fig. 2a–f.

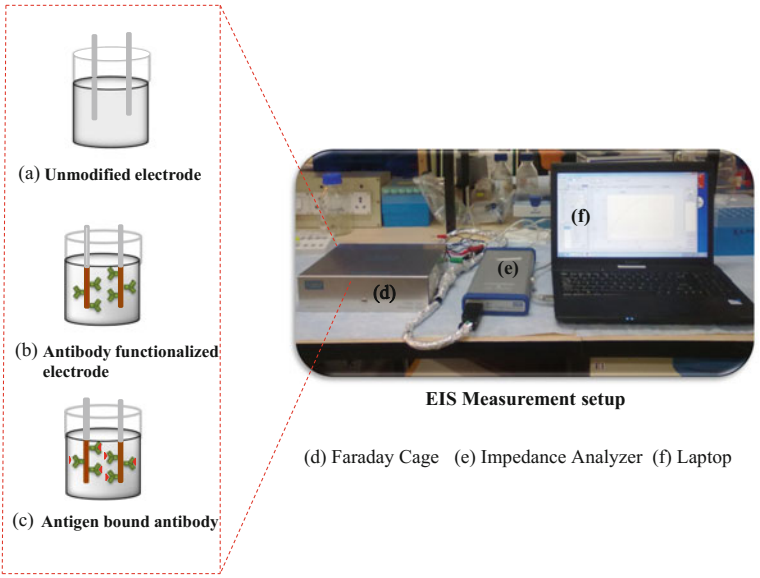

Fig. 2 Schematic diagram of EIS measurement setup

2.1.3 Interpretation of EIS Data

In EIS technique, a sinusoidal voltage is applied across the electrode and corresponding current signal is measured. The impedance is calculated by applying Ohm's law as given in Eq. 2.3.

$$Z = \frac{V}{I} = Z' + jZ'' \tag{2.3}$$

where Z is impedance, real term Z' (real component of Z) and Z'' (imaginary component of Z). An equivalent circuit is used to analyse impedance data. An equivalent circuit is the representation of the physical system parameters in terms of electrical components, mainly resistors, capacitors or constant phase elements. These electrical components are connected in series or in parallel depending on how and when different events occur in the system under study. The data interpretation and analysis by equivalent circuits is broadly accepted.

2.1.4 Labelled Versus Label-Free Detection

In labelled biosensors, a label is attached to the target and the amount of label detected corresponds to the number of bound targets (Daniels and Pourmand 2007). The labels mostly used are fluorophores or an enzyme. Labelling needs extra steps for sample preparations and hence adds extra time and cost. However, labelling a

target can significantly alter its binding properties and also might result in highly variable coupling efficiency (Haab 2003). These problems are very serious when a protein target is used. Therefore, for protein target, an indirect labelling is used. Indirect labelling requires two probes that bind to the target. The first probe is immobilized on the solid support, the analyte is introduced and then a secondary probe is introduced after washing. This second probe is labelled or can be detected by introducing yet another labelled probe that binds to all the secondary probes. This method increases selectivity with higher development cost and hence limited use in research. In label-free system, the interaction between target molecule and probe results changes in electrical properties of the surface due to the presence of the target molecule only. Moreover, label-free operation facilitates real-time detection of target-probe binding (Skládal 1997) which is not possible with labelled systems.

2.1.5 Faradaic Versus Non-faradaic Response

EIS can be divided into two categories: faradaic and non-faradaic EIS (Yang et al. 2004). In faradaic measurement, the charge is transferred across the electrode/electrolyte interface. Whereas, in a non-faradaic EIS, transient currents can flow without actual charge transfer (e.g. as in charging a capacitor). For electrical circuit analysis, a non-faradaic interface is characterized by a capacitor, and a faradaic interface is characterized by a resistor. Actual electrode–solution interfaces can have both faradaic and non-faradaic components. Faradaic impedance measurement requires a redox probe, while non-faradaic impedance measurement can be performed in the absence of a redox probe. When an electrode is immersed in electrolyte without having redox probe, the interfacial capacitance at the electrode can be used as a sensitive function of surface change associated with a binding event (Berggren et al. 2001). The change in capacitance due to binding of analyte is mainly because of changes in dielectric constant, charge distribution; electrolyte or water penetration. This is practically simple since no redox probe needs to be added to the electrolyte and hence very useful from an end-user perspective. A non-faradaic impedance biosensor using high-density microelectrode array was reported for *E.coli* O157:H7 detection (Radke and Alocilja 2005). AFM1 detection in the ppt range was reported by non-faradaic impedance measurement (Vig et al. 2009). The capacitive biosensor usually defines a sensor based on non-faradaic system and refers to one that makes measurements at a single frequency.

2.1.6 Equivalent Circuit Analysis for EIS

The interfacial phenomena of electrochemical cell are represented by an equivalent circuit and behave same as the real cell under given excitation (Yang et al. 2003). The electrochemical phenomena at the electrode/electrolyte interface can be modeled with electrical components used in equivalent circuit corresponding to

Table 1 Reported equivalent circuit design for impedimetric biosensor

Recognition element	Analyte	Circuit design	Detection limit	References
Anti-serum	Serum with Johne's disease	RC	10 $ngmL^{-1}$	Li et al. (2014)
Anti-HSA antibody	HSA	R(RC)	$2.4 \pm 0.1 \times 10^{-8}$ to $84.3 \pm 1.2 \times 10^{-8}$ M	Wongkittisuksa et al. (2011)
Antibody	AFM1	R_s, $C_{dl(ol)}$ ($R_{dl}C_{dl\ (il)}$)	6.25–100 pg mL^{-1}	Bacher et al. (2012)
IgG antibodies	Ciprofloxacin	R(RC)(RC)	10 $pgmL^{-1}$ to 100 $ngmL^{-1}$	Ionescu et al. (2007)
Anti-salmonella antibodies	Salmonella	R(C(RW))	100–10,000,000 $cfumL^{-1}$	Dong et al. (2013)
Anti-IL-6 antibodies	IL-6	(RC)(C(RW))	0.01 $fgmL^{-1}$	Yang et al. (2013)
Anti-CEA antibodies	CEA	R_DC_DL	10 μM	Jin et al. (2016)
Anti-OTA antibody	Ochratoxin A (OTA)	R(C(RW))	0.01–5 $ngmL^{-1}$	Malvano et al. (2016)

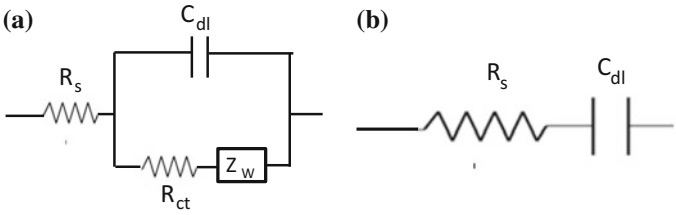

Fig. 3 Electrical equivalent circuit models for **a** faradaic impedance measurement and **b** non-faradaic impedance measurement

experimental impedance data. The electrical components used in equivalent circuit include the ohmic resistance of electrolyte solution R_s, the Warburg impedance Z_W, which represent diffusion of ions from bulk electrolyte to the electrode, the double-layer capacitance C_{dl}, and charge-transfer resistance R_{ct}, when redox probe is present in the electrolyte solution. Usually, more than one circuit model can fit the experimental data, but a unique equivalent circuit is selected based on the one best describing the physical characteristics of electrochemical cell. A simple equivalent circuit was reported having resistor and capacitor in series to show the behaviour of the impedance test using two electrodes setup (Yang and Bashir 2008). A summary of reported equivalent circuits for impedimetric immunosensors is presented as Table 1. In case of faradaic impedance measurement, Randles circuit (Fig. 3a) is commonly used to fit the EIS experimental data. A simple electrical equivalent circuit model was reported for non-faradaic impedance measurement to detect *Salmonella typhimurium* (Yang et al. 2003). For non-faradaic impedance measurement, a simple equivalent circuit consists of a series combination of medium resistance (R_s) and the double-layer capacitance (C_{dl}) was presented as shown in Fig. 3b.

2.1.7 Double-Layer Capacitance (C_{dl})

When the electrodes are polarized as compared to electrolyte, it attracts ions of opposite charge. This attractive tendency is countered by the randomizing thermal motion of the ions but results in a build-up of ions with opposite charge near the surface. This local charge imbalance prevents the electric field generating from the charged surface from penetrating very far into solution. The characteristic length of this spatial decay of the electric field is called the Debye length. The locally enhanced populations of ions act like the second plate of a capacitor; hence, it is termed as double-layer capacitance or diffuse layer capacitance (Fig. 4).

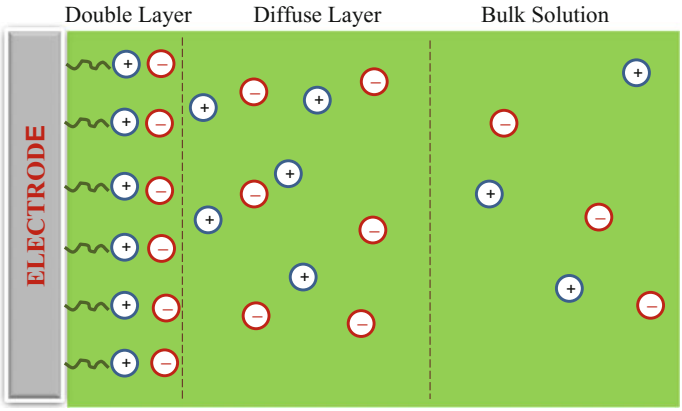

Fig. 4 Schematic diagram of electric double layer with distribution of +ve and −ve charge species

2.1.8 Constant Phase Element (CPE)

The electrode| electrolyte impedance change is also influenced by the frequency dispersion. This frequency dispersion is generally attributed to a 'capacitance dispersion' expressed in terms of a constant phase element (CPE). CPE behaviour is generally attributed to distributed surface reactivity, surface inhomogeneity, roughness or fractal geometry, electrode porosity, and current and potential distributions associated with electrode geometry (Jorcin et al. 2006). CPE is generally used to model double-layer capacitance of electrodes over simple capacitor (Gawad et al. 2004). The complex impedance of a CPE is given by Eq. 2.4.

$$Z_{\mathrm{CPE}} = \frac{1}{(j\omega)^m A} \tag{2.4}$$

where 'A' is analogous to a capacitance, ω is the frequency expressed in rad/sec and m is the CPE phase parameter. It can easily be seen that $m = 1$ corresponds to a capacitor.

2.2 Impedimetric Immunosensor Constructions

An impedimetric biosensor consists of a pair of bio-functionalized electrodes, integrated with an insulating cross-linking layer connected to a transducer. The selection of electrode material and the appropriate surface modification of electrode are crucial for achieving the desired function and performance.

2.2.1 Selection of Electrode Material

The choice of electrode material is crucial in construction of impedimetric immunosensor for better performance in terms of sensitivity and selectivity. Metals such as Pt, Au (Kim et al. 2000), Ag (Brunelle 2001) and stainless steel have been reported as electrode materials owing to their excellent electrical and mechanical properties. The choice of materials was based on biocompatibility, low cost and ease of construction or fabrication of sensors.

2.2.2 Micro-interdigitated Electrode (μ-IDEs)

μ-IDEs are widely used for the development of biosensors. They present advantages in terms of low ohmic drop and increased signal-to-noise ratio (Maruyama et al. 2006). μ-IDEs have been investigated in varying shapes, size and structures and number of alternate fingers. Since μ-IDEs does not require a reference electrode, the measurement setup is quite simple and easy to perform compared to a conventional electrochemical setup (Nebling et al. 2004). μ-IDEs are generally fabricated by using photolithography techniques on silicon (Si) substrates with a features size varying from nanometres (nm) to tens of microns. Most commonly used metals for the fabrication of electrodes are Au, Ti and Pt. A summary of μ-IDEs with various metals with dimensions is presented as Table 2.

2.2.3 Self-Assembled Monolayers (SAMs)

Self-assembled monolayers (SAMs) of organic cross-linkers are widely used to attach specific bioprobes to the electrode. In impedance-based sensors, SAMs also act as an insulating layer. The common linkers are based on thiols (–SH) bound to electrode surfaces (Love et al. 2005). The use of proper SAMs helps in oriented and repeatable immobilization of biomolecules (Cheng et al. 2008). SAMs are also used

Table 2 Summary of reported fabricated μ-IDEs with various metals

Material	Substrate	Fabrication techniques	References
Gold (Au)	Silicon	Photolithography	Baccar et al. (2014)
Titanium (Ti), nickel (Ni) and gold (Au)	Pyrex	Photolithography	Laczka et al. (2008)
Platinum (Pt)	Borosilicate glass	Photolithography	Yang et al. (2011)
Silver (Ag)	Epoxy-glass fibre PCB	Photolithography	Cortina et al. (2006)
Aluminium (Al)	Silicon	Photolithography	Moreno-Hagelsieb et al. (2007)

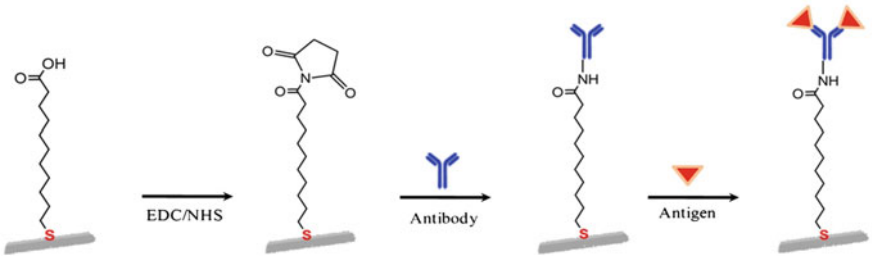

Fig. 5 Schematic showing functionalization of electrode surface by SAMs and binding of antibody-antigen on a metal substrate

to prevent protein denaturation at electrode surface and for improving stability of biomolecules (Kafi et al. 2007). A tightly packed (high leakage resistance, R_{leak}) SAM is desired for non-faradaic sensor, in contrast with faradaic sensors where the electrode surface needs to be accessible to the redox species (Lai et al. 2006).

2.2.4 Immobilization of Receptor

The bioreceptor is an integral component of the biosensor device. It is essential that the bioreceptor should be selective and sensitive towards specific target analyte. It should prevent interference by another substance from the sample matrix. For construction of a biosensor, immobilization of a biomolecules on a SAMs surface is an important requirement (Arya et al. 2009). Different immobilization strategies were employed and compared for selective and sensitive detection of biomolecule (dos Santos et al. 2009). An affinity biosensor is prepared by immobilizing antibodies onto a substrate of conducting or semiconductor material. A typical example of surface modification of a metal electrode and antibody coupling via SAMs is presented in Fig. 5. Noble metal substrates are very suitable for this purpose (Hou et al. 2004). The immobilization method also affects the immune-recognition event of antibodies towards antigens (Sassolas et al. 2012).

3 Materials and Instrumentation

All the chemicals used were of analytical grade and were used as received. Ag wire (diameter = 0.25 mm) was procured from ACROS Organics, USA. Anti-AFM1 and AFB1-fractionated anti-serum primary monoclonal antibody (mAb) were purchased from AbCam (UK). AFM1 standard, AFB1 standard, Tween20, 11-MUA, 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), certified reference material (CRM) ERM-BD 282 (AFM1 in whole milk powder < 0.02 μg kg^{-1}) were purchased from

Sigma–Aldrich, USA. Ethyl alcohol 200 proof was purchased from TEDIA, USA. Hydrogen peroxide (H_2O_2) 30% (w/v), acetonitrile (ACN) HPLC grade, disodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4) from MERCK (Germany) and sodium hypochlorite (4%) solution were purchased from Fisher Scientific (India). For sample handling, micropipettes (eppendorf®, Germany) were used. Centrifugation of milk sample was done by minispin (eppendorf®, Germany). Shaking and filtration of the samples were done by Spinix shaker (Tarsons, India). For the handling of AFM1 standard solution, glove box (Cole Parmer, USA) was used. For preparing all the solutions, water produced in a Milli-Q system (Millipore, Beford, MA, USA) was used. Impedance measurements were carried out using IVIUM CompactStat impedance analyser, Netherland. The required protocols and detailed procedures for the preparation of solutions, reagents and standards are as described (Bacher et al. 2012).

4 Experimental Setup for Aflatoxin Analysis

The measurement setup consisting of a pair of functionalized metal electrode with an appropriate diameter was immersed in the analyte solution confined in glass cell and separated by an optimal distance. The measurement setup is kept in an enclosed chamber. The anti-aflatoxin M1 monoclonal antibodies (mAb) were covalently coupled on a metal wire electrode through SAMs. The functionalized wire electrodes were connected to an impedance analyser. The schematic diagram of measurement setup is presented in Fig. 6.

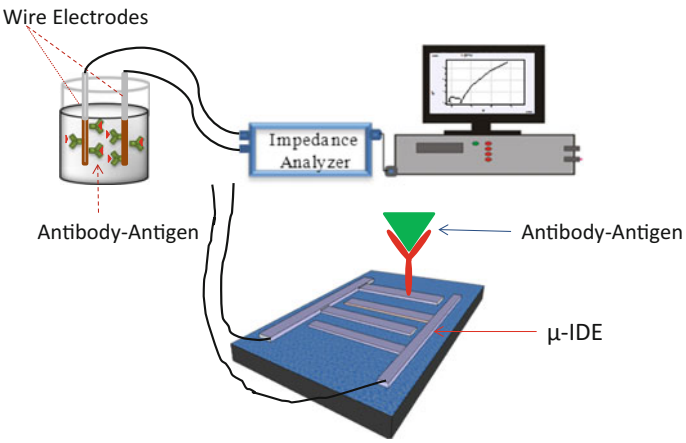

Fig. 6 Schematic connection diagram of impedance measurement setup

4.1 EIS Study for Aflatoxin M1 Analysis

The quantification of the interaction of anti-AFM1 and AFM1 was carried out using EIS. For non-faradaic impedance measurement, the impedance of the interface is measured at a single frequency. Figure 7 shows the impedance response of antibody-antigen interaction on for a blank (without AFM1 and a sample with 50 ppt AFM1). A distinctive change in impedance was observed over the blank which can be correlated with the presence of AFM1 in the low-frequency region. Such response can be measured for higher concentrations of the analyte under consideration.

4.2 Construction of Calibration Curve (AFB1 Analysis)

As another typical example for analytical purposes, non-faradaic impedance measurements were conducted using functionalized electrodes for AflatoxinB1 (AFB1) with increasing concentration (0.1–100 $pgmL^{-1}$). The impedance data were recorded at a frequency of 1 Hz with applied ac potential of 5 mV. A significant impedance change was observed at 1 Hz frequency. The specific interaction of anti-AFB1 and AFB1 at the electrode/electrolyte interface results in an overall increase in impedance change from baseline response for AFB1. The percentage

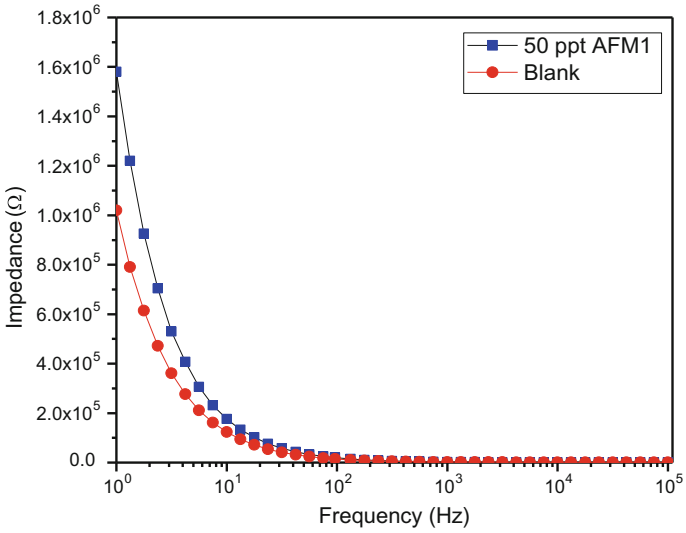

Fig. 7 Impedance response of the immunosensor before and after antibody-antigen interaction (50 ppt AFM1) at room temperature (0.01 M PBS medium, frequency range 1 Hz to 100 kHz at ac potential of 10 mV)

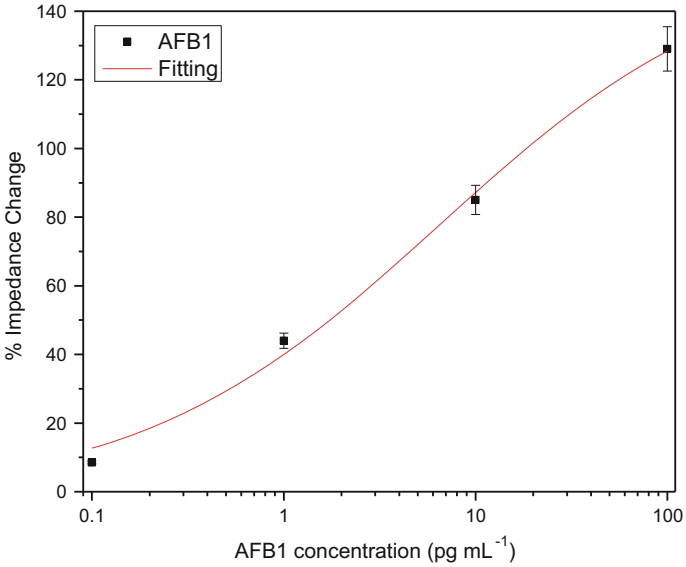

Fig. 8 Calibration curve of AFB1 (0.1–100 pgmL^{-1}) at a frequency range of 1–100 kHz), 5 mV ac potential

impedance change was determined corresponding to various concentrations of AFB1. The resulting calibration curve is presented in Fig. 8. A linear range for AFB1 detection 1–100 pgmL^{-1} with SD = 0.15 and R^2 = 0.99 was achieved. Limit of detection (LOD) was found to be 0.1 pg mL^{-1}.

5 Recent Developments in Impedimetric Sensing

It has been established that impedimetric immunosensor provides a platform for detection of various contaminants including food toxin in the environment. One of the important challenges in the analytical sciences is to develop the devices and methods capable of detecting carcinogenic or highly toxic chemical contaminants requiring detection at levels as low as 1–50 ngL^{-1} range or below. The development of impedimetric sensors has gained advances with application in various environmental matrices spanning from water to food and even to the cellular level. For instance, impedimetric immunosensor for detection of aflatoxin M1 (AFM1) is reported with a detection limit of 1 pgmL^{-1} within 20 min. This technique has also been extended to milk product such as flavoured milk and yogurt (Kanungo et al. 2014). In the recent years, the use of screen-printed three electrode as well as interdigitated electrodes in impedimetric sensors has gained significant attention in environmental analysis (Rivas et al. 2015; Istamboulié et al. 2016; Li et al. 2016; Gu et al. 2015; López Rodriguez et al. 2015; Liu et al. 2015; Sharma et al. 2017).

Table 3 Recently reported impedimetric biosensor for different analytes

Analyte	Matrix	Transducer	LOD	References
Ochratoxin A (OTA)	White wine	Screen-printed carbon electrode (SPCE)	5.65 ng kg^{-1}	Rivas et al. (2015)
AFM1	Milk	SPCE	1.15 ng L^{-1}	Istamboulié et al. (2016)
AFB1	Rice	Screen-printed interdigitated microelectrodes	5 ng mL^{-1}	Li et al. (2016)
Deoxynivalenol (DON)	Cell toxicity	Gold microelectrode	0.03 μg mL^{-1}	Gu et al. (2015)
Lindane	Bacterial culture	Stainless steel elctrodes	120 μg L^{-1}	López Rodriguez et al. (2015)
Carbofuran	Water	Gold electrode	0.1 ng mL^{-1}	Liu et al. (2015)
Kanamycin	Milk	SPCE	0.11 ng mL^{-1}	Sharma et al. (2017)
DNA molecules	Deionized water	Gold microelectrode	Nano molar	Liu et al. (2008)

In addition to the use of bioreceptor (antibodies, enzymes and aptamer) as sensing element, the impedimetric sensors devices based on interdigitated electrodes have also been demonstrated for the detection of DNA concentration and length in deionized water (Liu et al. 2008). Further developments in label-free detection using EIS incorporated integration of nonmaterial in detection of DNA sequences as well as to confirm the signal amplification in peptide-based nucleic acid and as genosensors (Bonanni and Del Valle 2010). The use of nucleic acid receptors such as aptamer is also gaining significant attention owning to their selectivity and better stability over antibody-based receptors. Recently reported impedimetric biosensors for antibiotic residue analysis in milk and pesticide residue analysis as well as mycotoxins analysis are summarized in Table 3 with the reported limit of detection achieved using EIS.

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