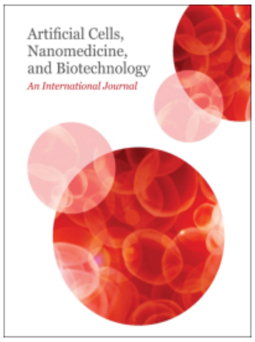

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A review on impedimetric biosensors

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Abstract

Electrochemical impedance spectroscopy (EIS) is a sensitive technique for the analysis of the interfacial properties related to biorecognition events such as reactions catalyzed by enzymes, biomolecular recognition events of specific binding proteins, lectins, receptors, nucleic acids, whole cells, antibodies or antibody-related substances, occurring at the modified surface. Many studies on impedimetric biosensors are focused on immunosensors and aptasensors. In impedimetric immunosensors, antibodies and antigens are bound each other and thus immunocomplex is formed and the electrode is coated with a blocking layer. As a result of that electron transfer resistance increases. In impedimetric aptasensors, impedance changes following the binding of target sequences, conformational changes, or DNA damages. Impedimetric biosensors allow direct detection of biomolecular recognition events without using enzyme labels. In this paper, impedimetric biosensors are reviewed and the most interesting ones are discussed.

Keywords: biosensor, electrochemical impedance spectroscopy, impedance-based biosensor

Introduction

The pioneering study of Clark and Lyons, more than four decades ago, shed light on some analytical researches in designing of biosensors, which perform as economical and fast tools for clinical, chemical, environmental, and pharmaceutical studies. Because of its simple use and portability in relatively complex samples, biosensors offer a potential alternative to advanced bioanalytical systems (Jin et al. 2011).

Electrochemical impedance spectroscopy (EIS) appears to be an excellent technique for the investigation of both bulk and interfacial electrical properties of electrode systems which can be used to determine quantitative parameters of electrochemical processes. If biorecognition events occur at the modified surfaces, the interfacial properties change. Shortly, EIS provides a fingerprint of the interfacial region (Maalouf et al. 2007).

Electrochemical reactions, known as electron transfers at the electrode surface, involve electrolyte resistance, adsorption of electroactive species, charge transfer at the electrode surface, and mass transfer from the bulk solution to the electrode surface. Each reaction process is represented by an electrical circuit that consists of resistance, capacitors, or constant phase elements combined in parallel or in series. The most favorite model of electrical circuit for a simple electrochemical reaction is the Randles–Ershler electrical equivalent circuit model, consisting of electrolyte resistance (R_s), charge-transfer resistance (R_{ct}) at the electrode/electrolyte interface, double-layer capacitance (C_{dl}), and mass transfer resistance (R_{mt}), also Warburg impedance (W). The solution resistance is determined by the solution conductivity and the geometry of the reaction cell, namely the distance between the electrodes and the cross-sectional area of solution linking the electrodes. The double layer capacitance displays the electrostatic interplay between the electrode and the electrolyte and depends on the electrode area, nature, the electrolyte ionic strength, and permittivity. Together, R_{ct} and W make up the faradaic impedance. R_{ct} reflects the charge transfer kinetics and can be thought of as the ratio of overpotential to current in absence of mass transfer limitation. Equivalent circuit models can be partially or completely empirical, each circuit component comes from a physical process in the electrochemical cell and has a characteristic impedance behavior (Yuan et al. 2010).

The impedance spectrum of an electrochemical system which is a function of frequency can be displayed in Nyquist or Bode plots. Nyquist plots usually include a semicircle region lying on the axis followed by a straight line. The semicircle portion (at higher frequencies) corresponds to the electron-transfer-limited process, and the straight line (at low-frequency range) represents the diffusion-limited process. This spectrum can be used to extract the electron transfer kinetics and diffusional characteristics. In very fast electron transfer processes the impedance spectrum

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includes only the linear part, while very slow electron transfer processes are characterized by a large semicircular region (Yuan et al. 2010, Wang 2006). The diameter of the semicircle equals to the electron transfer resistance (Wang 2006). Bode plot is logarithm of the absolute value of impedance (Z) and the phase (Φ) are plotted against the logarithm of frequency (f).

EIS is a powerful tool for investigating the interfacial properties related to bio-recognition events occurring at the modified surfaces. One of the advantages of EIS is the small amplitude perturbation from steady state, which makes it a non-destructive technique. The impedance can be measured in the presence or absence of a redox couple, which is referred to faradic and non-faradic impedance measurement, respectively. The faradic biosensors detect biorecognition events which occur at the modified electrode by measuring the change in the faradaic current (interfacial electron transfer resistance) owing to steric hindrance caused by the biomolecular interaction and/or by the electrostatic repulsion between the free charges of the target molecules and the electroactive species in the supporting electrolyte. Redox probe selection depends on various parameters such as the charge, hydrophobicity/hydrophilicity, size of the redox couple, and the chemical and physical properties of the modified electrodes (Elshafey et al. 2013a).

The limitations of the EIS technique are the several requirements to obtain a valid impedance spectrum. Theoretically, there are three basic requirements for AC impedance measurements: linearity, stability, and causality. The accuracy of EIS measurement depends not only on the technical precision of the instrumentation but also on the operating procedures (Yuan et al. 2010).

Biosensors based on impedance

Biosensors are designed for monitoring a biological reaction at the surface of transducers (Helali et al. 2006). A variety of biomolecules such as enzymes, antibodies, cells, and microorganisms are the basic detection elements. For the development of an electrochemical biosensor, the key requirement is reproducibly immobilizing these biomolecules on to the sensor surface with keeping their biological activity (Rezaei et al. 2014). According to literature, various strategies have been used to design impedimetric biosensors. Among these strategies are formation of surface functional groups (carboxyl, amino, etc.) through various chemistries such as silane on glass or indium tin oxide, and alkanethiol monolayers on Au to covalently link biomolecules to surfaces; the physical entrapment of biomolecules by various electrochemically formed polymers (i.e., polypyrrole and polyaniline) or gel coatings; immobilizing biomolecules within Langmuir–Blodgett (LB) films based on amphiphilic polyelectrolytes; and the layer-by-layer assembly of alternately charged polyions.

Nanostructured materials, which are very attractive owing to their unique optical, electrical, catalytical, and magnetic properties, have been used in the field of biosensors and nanoelectronic devices.

Antibody-antigen based impedimetric immunosensors

Impedimetric immunosensor is a sensitive technique for simultaneous, label-free detection of antigen–antibody binding. In impedimetric immunosensors antibodies are immobilized on the electrodes, optical fiber, or semiconductor chips, and they have attracted great interest in recent years because of their promising applications in various areas. Antibodies and antigens are bound to each other and thus immunocomplex is formed and the electrode is coated with a blocking layer. As a result of that, electron transfer changes. After antigens binding to the antibodies, the access of the redox probes is hindered to a higher degree than in the absence of antigens. Since the Faradic reaction of a redox couple hinders increasingly, the electron transfer resistance will increase and the capacitance will decrease (Hou et al. 2013).

The most important step in the preparation of impedimetric immunosensors is immobilization of biomolecules on the electrode surface, because it is essential to generate stable, reproducible, and selective biosensors. For example, antibodies are physically adsorbed on the gold surface; but the adsorption of antibodies directly onto the bulk metal surface leads to the denaturation and the reduction of their bioaffinity. When antibodies are adsorbed on the surface of noble metal nanoparticles can retain their activity due to the biocompatibility of these nanoparticles mainly AuNPs (Chullasat et al. 2011). Table I summarizes the impedimetric immunosensors in literature.

Antibody-binding proteins such as protein A and protein G are generally used to immobilize oriented antibodies. Elshafey et al. (2013a) developed an electrochemical impedance immunosensor based on the detection of cancer marker epidermal growth factor receptor (EGFR) in brain plasma. Anti EGFR antibodies bind to protein G modified electrode via their nonantigenic Fc regions.

Canbaz et al. (2014) quantified HER-3 for the first time by using anti-HER-3 antibody as biorecognition element. Self-assembly of hexandithiol on a gold electrode was successfully performed and then gold nanoparticles immobilized on SAMs to enhance the surface area. Cysteamine monolayers were self assembled on the gold nanoparticles. After performing SAMs, glutaraldehyde was used as crosslinking agent to anti-HER-3 immobilization. By using BSA, active ends of the electrode surface are blocked. Finally, artificial serum samples were spiked with HER-3 and detected by the biosensor. They used the single impedance technique, which is firstly used to characterize the binding between HER-3 and anti-HER-3.

Hou et al. (2014) constructed a sandwich immunosensor for PSA detection. The signal was amplified based on the Ab_2–-AuPd–DNA toward the catalytic precipitation of 4-choloro-1-naphthol (4-CN). DNAzyme catalyzed the oxidation of 4-CN, while AuPd hybrid nanostructures not only provided a large surface coverage for immobilization of biomolecules but also promoted 4-CN oxidation to some extent. The produced insoluble benzo-4-chlorohexadienone via 4-CN was coated on the electrode surface, and hindered the electron transfer between the solution and the electrode, thus increasing the Faradaic impedance of the base electrode.

Table I. Impedimetric immunosensors.

Target molecule	Immobilization step	Assay principle	Limit of detection	References
Sulfate reducing bacteria (SRB)	Foam Ni/AuNPs/11 mercaptoundecanoicacid (MPA)/Ab/BSA/SRB	Antibody–bacteria interaction	2.1×10^1–2.1×10^7 cfu/ml	Wan et al. (2010)
Aflatoxin M1 (AFM1)	Ag wire/11-mercaptoundecanoic acid (MPA)/primary monoclonal antibody (anti-AFM1 mAb)/EDC/NHS/AFM1	Antibody–antigen interaction	1 pg/ml	Bacher et al. (2012)
Pathogenic bacteria	Anodized alümina membrane/(3-glycidyloxypropyl)-trimethoxysilane/(GPTMS)/hyaluronic acid (HA)/EDC/sulfo-NHS/anti-E.coli O157:H7 antibodies/E.coli O157:H7	Antibody–pathogen interaction	83.7 cfu/ml	Joung et al. (2013)
Semicarbazide (SEM)	GCE/chitosan-cystein(chi-cys)/EDC/NHS/AuNPs/SEM-MCAb/BSA/SEM	Antibody–antigen interaction	1 ng/ml	Jin et al. (2013)
Doxorubicin	Au/3-(trimethoxysilyl)-1-propanethiol (MPTs)AuNPs/doxorubicin monoclonal antibody/BSA/doxorubicin	Antibody–antigen interaction	10.09 pg/ml	Rezaei et al. (2014)
Interleukin-6-antigen	SiO_2/Si/SWCNTs/AuNPs/mercaptoacetic acid/EDC/NHS/anti-IL-6-antibody-BSA/IL-6 antigen	Antibody–antigen interaction	0.01 pg/ml	Yang et al. (2013c)
Campylobacter (CJ)	GCE/o-carboxymethylchitosan Fe_3O_4 nanoparticles/anti-Fla A monoclonal antibodies 2D12(2D12 McAbs)/BSA/CJ	Antibody–bacteria interaction	1×10^3 cfu/ml	Huang et al. (2010)
Ketamine	Au/3-mercaptopropionic acid(3-MPA)/EDC/NHS/ketamine antibody/ketamine	Antibody–antigen interaction	0.41 pmol/L	Chen et al. (2013b)
Hemoglobin	Silicon/Au/1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-biotinyl(biotinyl–PE and 6-mercaptohexadecanoic acid (MHDA)/neutravidin/biotinylated anti-hemoglobin/hemoglobin	Antibody–antigen interaction	20 ng/ml	Hleli et al. (2006a)
Bisphenol A (BPA)	GCE/5i2:5′2″-terthiophene-3′-carboxylic acid (TTCA)/EDC/polyclonal (PAb) antibody/PBSTG/BSA/BPA	Antibody–antigen interaction	0.3 ng/ml	Rahman et al. (2007)
VEGF	Au/cysteamine/PAMAM/glutaraldehyde/VEGFR1/BSA/VEGF	Receptor–factor interaction	5 pg/ml	Sezgintürk and Uygun (2012)
C-reactive protein (CRP)	Au/polyethylene glycol (PEG)/EDC/NHS/anti CRP/BSA/CRP	Antibody–antigen interaction	176 pM	Bryan et al. (2013)
Troponin specific antibody	Au/cys/synthetic peptide (epitope cTnC-89–98 of troponin C) C-antibody	Antibody–peptide interaction	–	Mantzila et al. (2007)
Atrazine	Gold deposited silicon/biotinyl-PE/MDHA/IgG/neutravidin/biotinylated-Fab fragment K_{47} antibody/atrazin	Antibody–antigen interaction	20 ng/ml	Hleli et al. (2006b)
Ochratoxin A (OTA)	Indium tin oxide (ITO)/chitosan(CS)-polyaniline(PANI)/IgG/BSA/OTA/OTA	Protein–antigen interaction	1 ng/ml	Khan and Dhayal (2009)
5-Morpholino-3-amino-2-oxalidone (AMOZ)	Au/1,4-benzendithiol/AuNPs/AMOZ-McAb/BSA/AMOZ	Antibody–antigen interaction	1 ng/ml	Jın et al. (2011)
Penicillin G	Thioctic acid/EDC/NHS/anti-penicillin/1-dodecanethiol/penicillin	Antibody–protein interaction	3×10^{-15}	Thavarungkul et al. (2007)
Anti-avidin	Ti/TiO2 electrode/avidin/BSA/anti-avidin	Antibody–antigen interaction	–	Mantzila and Prodromidis (2006)
Salmonella typhimurium	Au/tyramine/glu/anti-Salmonella antibodies (SA)/BSA/S.typhimurium	Antibody–bacteria interaction	10 cfu/ml	Pournaras et al. (2008)
TREM-1 MMP9 HSL	Gold screen printed electrode/thiolated antibody (MCH-Ab)DL-dithiothreitol (DTT)	Antibody–antigen interaction	3.3 pM 1.1 nM 1.4 nM	Ciani et al. (2012)
HCCR-1	Au/Prolinker B/MCH/anti-HCCR-1/HCCR-1	Antibody–antigen interaction	–	Chen et al. (2013a)
Anti-hemogglutinin antibodies	GCE/EDC/NHS/ethanolamine/anti-His IgG monoclonal antibody/recombinant His tagged hemogglutinin (His_6-H5HA)/BSA/Anti-hemoggutinin antibodies	Antibody–antigen interaction	2.1 pg/ml	Jarocka et al. (2014)
EGFR	Au/AuNPs/Cys/1,4-phenylene diisothiocynate (PDITC)/protein G/ethanolamine/anti-EGFRantibody/EGFR	Antibody–antigen interaction	0.34 pg/ml in PBS; 0.88 pg/ml in human plasma	Elshafey et al. (2013a)
IGF-1	Au/AuNPs/1,6-hexandithiol (HDT)/monoclonal anti IGF-1/BSA/IGF-1	Antibody–antigen interaction	0.15 pg/ml	Rezaei et al. (2011)

(*Continued*)

Table I. (*Continued*)

Target molecule	Immobilization step	Assay principle	Limit of detection	References
Okaidaic acid (OA)	Screen printed electrode (SPE)/4-carboxyphenyl film/NHS/EDC/anti-okaidaic acid monoclonal antibody (anti-OA-mAb)/ethanolamine/OA	Antibody–antigen interaction	0.3 μg/L	Hayat et al. (2012)
Atrazine	Niobium/niobium oxide(Nb/NbOxHy)/APTES/Fb fragment K47/atrazine	Protein–antigen interaction	50 μg/ml	Helali et al. (2008)
E.coli	AuSPEs/crosslinker 3,3′-dithiobis (sulfosuccinimidyl propionate) (DTSSP)/anti E.coli antibody/cell culture/ethanolamine AuSPEs/2-pyridyl disulfide activated E.coli antibody/cell culture	Antibody–cell interaction	10^4 CFU/ml 3.3 CFU/ml	Escamilla-Gómez et al. (2009)
CEA	GCE/AuNP/Ab_1/CEA/HRP-GO-Ab_2	Sandwich type	0.64 pg/ml	Hou et al. (2013)
α-fetoprotein (AFP)	GCE/Au-Gra/anti-AFP(Ab_1)/BSA/AFP/SWCNHs-HRP-GOD-Ab2	Sandwich type	0.33 pg/ml	Yang et al. (2013a)
Aflatoxin M1 (AFM_1)	SPE/BSA + AFM_1/gold labelled anti-AFM_1 antibody/AFM_1	Sandwich type	15 ng/ml	Vig et al. (2009)
Chagas' disease	Au/anti-CRA + anti-FRA/CRA/FRA	Antibody–antigen interaction	–	Diniz et al. (2003)
E.coli O157:H7	Graphene oxide paper(rGOPE)/AuNPs/streptavidin/biotinylated anti E.coli O157:H7 antibody/BSA/E.coli O157:H7	Antibody–cell interaction	1.5×10^2 cfu/ml	Wang et al. (2013b)
SRB	GCE/reduced graphene sheets(RGSs)/anti-SRB antibody/BSA/SRB cell	Antibody–cell interaction	10^2 cfu/ml	Wan et al. (2011)
Low density lipoprotein (LDL^-)	Au/polyvinylformal(PVF)/AuNPs/anti-LDL^-Mab/BSA/LDL^-	Antibody–antigen interaction	3.50 μg/ml	Oliveira et al. (2011a)
Human serum albümin (HSA)	Silicon nitride (SiN_4)/SiO_2/aldehyde functionalized Si-P/triethoxysilane aldehyde(TEA)/monoclonal anti-HSA/BSA/HSA	Antibody–antigen interaction	10^{-14} M	Caballero et al. (2012)
CD 14 or CD 16	Au/MUA/MCH/EDC/NHS/protein G/BSA/CD 14 or CD 16 antibody/monocytes	Protein–cell interaction	1000 cells	Montrose et al. (2013)
Hapten/herbicide	SPE/AuNPs/anti-diuron antibody/hapten/herbicide	Antibody–antigen interaction	5.46 ng/ml	Bhalla et al. (2012)
CRP	H-terminated nanocrystalline diamond (NCD)/anti-CRP antibodies/BSA/CRP	Antibody–antigen interaction	10 nm	Vermeeren et al. (2011)
Ciproflaxin	Au/pyrrole-NHS/anticiproflaxin antibody/BSA/ciproflaxin	Antibody–antigen interaction	10 pg/ml	Ionescu et al. (2007)
Digoxin BSA	SPE/aniline/biotin-sulfo-NHS/neutravidin/biotinylated anti-digoxin antibody/HSA/digoxin or BSA	Antibody–antigen interaction	0.1 ng/ml 1.5 ng/ml	Barton et al. (2009)
atrazine	Au/polypyrolle film/nitrilotriacetic acid (NTA)+ Cu^{+2}/histidine-tagged IgG atrazine antibody/BSA/atrazine	Antibody–antigen interaction	10 pg/ml	Ionescu et al. (2010)
OTA	Au/4-carboxyphenyl (4-CP)/EDC/NHS/OTA antibody/ethanolamine/OTA	Antibody–antigen interaction	0.5 ng/ml	Radi et al. (2009)
Fenvalerate	GCE/CS/glu/fenvalerate monoclonal antibody/rabbit ovalbumin/fenvalerate	Antibody–antigen interaction	0.8 μg/L	Wang et al. (2013a)
Murine double minute 2 (MDM2)	Polycrystalline Au/cys/PDITC/MDM2 antibody/ethanolamine/MDM2	Antibody–antigen interaction	1.3 pg/ml	Elshafey et al. (2013b)
Human chorionic gonadotropin (hCG)	Screen printed carbon ink electrode/polypyrrole-pyrole-2-carboxylic acid copolymer/NHS/EDC/hCG antibody/ethanolamine/hCG	Antibody–antigen interaction	2.3 pg/ml	Holford et al. (2013)
Psoriasin	SPE/polyaniline/biotin-sulpho-NHS/neutravidin/biotinylated anti-psoriasin/BSA/psoriasin	Antibody–antigen interaction	250 pg/ml	Truong et al. (2011)
Cardiac troponin (cTnI), Soluble lectin like oxidized LDL receptor-1 (sLOX-1)	Au/MHDA + biotin caproyl-DPPE neutravidin/biotinylated anti-sLOX-1 or anti-cTnI/cTnI or sLOX-1	Antibody–antigen interaction	10^{-13} M	Billah et al. (2012)
Anti-myelin basic protein (anti-MBP)	Pt/gelatin-titanium dioxide TiO2/MBP/BSA/anti-MBP Pt/gelatin/MBP/BSA/anti-MBP	Antibody–antigen interaction	0.1495 ng/ml 0.1528 ng/ml	Derkus et al. (2013)
OTA	ITO/TiO_2/CS/IgG/BSA/OTA	Antibody–antigen interaction	10 ng/ml	Khan and Dhayal (2008)
OTA	ITO/nano-ZnO/rabbit-immunoglobulin antibodies (r-IgGs)/BSA/OTA	Antibody–antigen interaction	0.006 ng/dm^3	Ansari et al. (2010)

(*Continued*)

Table I. (*Continued*)

Target molecule	Immobilization step	Assay principle	Limit of detection	References
Adenovirus (Ads)	Au/PANI + 2-mercaptoethylamine(2-ABA)/sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC)/reduced IgG/Ads	Antibody–adenovirus interaction	1000 virus particles/ml	Caygill et al. (2012)
Atrazine	İnterdigitated μ-electrode (IDμE)/antigen/atrazine antibody	Antibody–antigen interaction	5.76 ppb	Rodríguez et al. (2008)
Doxorubicin	Stainless stell(SS)/APTES/AuNPs/doxorubicin-specific monoclonal antibody/BSA/doxorubicin	Antibody–antigen interaction	1.7 pg/ml	Rezaei et al. (2013)
Chloramphenical (cap)	Au/SATUM/AuNPs/MSA/EDC/NHS/anti-cap antibody/ethanolamine/cap	Antibody–antigen interaction	10^{-16} M	Chullasat et al. (2011)
E.coli O157:H7	Au/11-mercapto-1-undecanol/epichlorohydrin/HA/EDC/NHS/anti E.coli O157:H7/E.coli O157:H7	Antibody–bacteria interaction	7 cfu/ml	Joung et al. (2012)
Stress-induced-phosphoprotein-1 (STIP-1)	Nanowell (NWA)/11-mercaptoundecanoic acid/EDC/NHS/streptavidin/ethanolamine/biotinylated anti-STIP-1 antibody/STIP-1	Antibody–bacteria interaction	10 pg/ml	Lee et al. (2013)
PSA	GCE/β-cyclodextrin (CD)/anti-mouse antibody PSA (Ab_1)/PSA/Ab_2-AuPD-DNA/4-CN + H_2O_2	Sandwich type	0.73 pg/ml	Hou et al. (2014)
Histidine tagged proteins	Au/HDT/gold nanorods/anti-his (C-term) mouse monoclonal antibody (AH-IgG)/BSA/rSPR-His_6	Antibody–antigen interaction	5 pg 7ml	Wasowicz et al. (2008)
Multiple Sclerosis	Disposable printed circuit board (PCB) electrodes/16-MHDA/EDC/sulfo-NHS/anti-IL-12-IgG/etanolamin/.interleukin-12 (IL-12)	Antibody–antigen interaction	<100 fM	Bhavsar et al. (2009)
Multiple Sclerosis	Gold-coated silver ribbon electrode/16-MHDA/EDC/NHS/anti-IL-12-IgG/etanolamin/.interleukin-12 (IL-12)	Antibody–antigen interaction	<100 fM	La Belle et al. (2007)
Salmonella typhimurium	GCE/MWCNT/PAMAM/AuNPs/MACA/NHS/EDC/anti Salmonella typhimurium antibodies/BSA/Salmonella typhimurium	Antibody–cell interaction	5×10^2 CFU ml^{-1}	Dong et al. (2013)
Hepatitis B	Pt/polyvinylbutyral(PVB)-Au-hepatitis B surface antibody(HBsAb)/HBsAg	Antibody–antigen interaction	7.8 ng/ml	Tang et al. (2004)
Insulin	Au/polyethylene glycol/EDC/NHs/anti-insulin/BSA/insulin	Antibody–antigen interaction	1.2 pM	Xu et al. (2013b)
Atrazine	Au/magnetic beads coated with streptavidin/biotinyl-FAB fragment K47 antibody/atrazine	Antibody–antigen interaction	10 ng/ml	Helali et al. (2006)
Tumor necrosis factor (TNF-α)	Silicon wafer/Ti-Au layer/dithiobis-succinimidyl-propionate (DSP)/anti-TNF-α/ethanolamine/TNF-α	Antibody–antigen interaction	~57 fM	Pui et al. (2013)
HSA	Au/APTES/anti-HSA/%5 milk powders/HSA	Antibody–antigen interaction	~2×10^{-4} mg/ml	Chuang et al. (2011)
E.coli O157:H7	Au/MHDA/EDC + AFP + DIEA/anti-E.coli/2-(2-amino etohxy)ethanol (AEE)/E.coli O157:H7	Antibody–cell interaction	2 CFU/mL	Barreiros dos Santos et al. (2013)
MCF-7 cells	GCE/polypyrrole-NHS film/anti c-erbB-2/BSA/MCF-7 cells	Antibody–cell interaction	100 cell/mL	Seven et al. (2013)
Deep venous thrombosis biomarker (D-dimer)	Silicon wafers/Ti/Au/11-amino-1-undecanethiol (amino thiol)/SWCNT-COOH/NHS/EDC/anti-D-dimer/D-dimer	Antibody–antigen interaction	0.1 pg/ml	Bourigua et al. (2010)
S-100 protein	Au/12-mercaptododecanoic acid (12-MCA)/5′-(mercaptomethyl)-2,2′bithiophene-5-carbaldehyde (S2TA)/antiS100/ethanolamine hydrochloride (ETA-HCl)/S100	Antibody–antigen interaction	10 ng/ml	Chen et al. (2011)
17β-estradiol	Au/3-mercaptopropionic acid (3-MPA)/EDC/NHS/esterogen receptor-α/BSA/17β-estradiol	Receptor–factor interaction	10^{-13} M	Kim et al. (2012)
E.coli	Au/PANI/Glu/anti-E.coli antibody/E.coli	Antibody–antigen interaction	10^2 CFU/mL	Chowdhury et al. (2012)
HER-3	Carbon nanotube modified screen printed electrode/EDC/NHS/anti-HER-3/BSA/HER-3	Antibody–antigen interaction	2 fg/ml	Asav and Sezgintürk (2014)
Cancer-associated sialyl-Tn (STn) antigen	SPE/Au/MHDA/EDC/NHS/Sambucus nigra agglutinin type I (SNA-I)/ethanolamine/STn antigen	Antibody–antigen interaction	20 ng	Silva et al. (2014)

(*Continued*)

Table I. (*Continued*)

Target molecule	Immobilization step	Assay principle	Limit of detection	References
HER-3	Au/HDT/AuNPs/Cys/glu/anti-HER-3/BSA/HER-3	Antibody–antigen interaction	0.2 pg/ml	Canbaz et al. (2014)
HER-3	ITO/APTES/glu/anti-HER-3/BSA/HER-3	Antibody–antigen interaction	40 fg/ml	Canbaz and Sezgintürk (2014)
Buprenorphine hydrochloride (BN)	GCE/MWCNTs/BSA/BN	Antibody–antigen interaction	1.5 nM	Gholivand et al. (2014)
Human influenza virus hemagglutinin (HA)	Au (MACS)/coiled-coil peptide (CCP) modified microelectrode array/HA-antibody/HA	Antibody–antigen interaction	1 pg/ml	Arya et al. (2014)
Protein troponin T	Printed circuit boards (PCB)/zinc oxide thin films (ZO)/dithiobis succinimidyl propionate (DSP)/anti-troponin T capture antibody/Superblock/protein troponin-T	Antibody–antigen interaction	10 fg/ml in PBS, 100 fg/ml in serum	Jacobs et al. (2014)
HER 3	Au/4-aminothiophenol/glu/anti-HER3/BSA/HER3	Antibody–antigen interaction	0.28 pg/ml	Sonuç and Sezgintürk (2014)
Carcinoembriyonic antigen (CEA)	Au/1,6-haxandithiol (HDT)/Staphylococcal protein A (SPA)/anti-CEA/BSA/CEA	Antibody–antigen interaction	0.1 pg/ml	Zhou et al. (2014)
Gram − bacteria Gram + bacteria Fungus Human cervical cancer cells	Au/3-mercapto-1-propanesulfonic acid (MPS)/polycation (PAH)/polyanion (PSS)/PAH/concanavalin A (Con A) or ricinus agglutinin (RCA)/BSA/bacteria or mammalian cells	Lectin–bacteria or cells interaction	10^3 cfu/ml	Xi et al. (2011)
Lipopolysaccharide (LPS) Lipoteichoic acid (LTA)	Steel electrode/PANI/glu+ Con A/BSA/LPS Steel electrode/PANI/glu+ Con A/BSA/LTA	Con A lectin–toxin interaction	-	Da Silva et al. (2014)

Xi et al. (2011) developed a label free electrochemical impedimetric biosensor based on lectin–bacteria interaction. *S. cerevisiae* was captured by concanavalin A (Con A) interface through the interaction of Con A with the glycosyl complexes on cellular surface. In the presence of mannose (Man) on the electrode surface or in the reaction solution, the R_{ct} was decreased. The resulting electrode also showed a major decrease of the R_{ct}, indicating the decreased amount of bound *S. cerevisiae* and efficient competition between Man and *S. cerevisiae*.

Aptamer-based impedimetric biosensors

Aptamers are artificial single-stranded DNA or RNA oligonucleotides (typically smaller than 100 mer). They are selected from large randomized oligonucleotide libraries by SELEX (systematic evolution of ligands by exponential enrichment). Different types of target molecules including proteins, small molecules, cells, viruses and bacteria, and amino acids can be bound specifically to aptamers with high affinity. Aptamers have presented some superior features such as high specificity of binding affinity, better stabilization, and longer shelf life when compared to antibodies (Erdem et al. 2014).

Electrochemical nucleic acid sensors (genosensors) provide sensitive and inexpensive nucleic acid detection in complex samples, and they require a reduced number of PCR-based amplification steps without the need for target purification (Regan et al. 2014).

In impedimetric techniques, changes in current, resistance or impedance following the binding of target sequences (hybridization), conformational changes or DNA damages can be monitored. After target binding a measurable change occurs with these modes (1) direct detection of hybridization (label-free), (2) labeling of the target nucleic acid sequences with redox active substances/nanoparticles or (3) signal probes (indirect labels) that intercalate within the stacked base pairs, electrostatically bind to the phosphate backbone or sit within the grooves of the double helix (Regan et al. 2014). Table II summarizes the impedimetric aptamer-based biosensors in literature.

Various materials such as gold nanoparticles, quantum dots, carbon nanotubes (Bonanni and Del Valle 2010), ZnO (Yumak et al. 2011), FePt/ZnS nanocore–Shell (Chang and Wu 2010), a mercaptoacetic acid-modified cadmium sulfide (Sun et al. 2007) and carbon-nanotubes/nano zirconium dioxide/chitosan (Yang et al. 2007), graphene oxide/gold nanoplatform (Gupta et al. 2013) have demonstrated that they amplify the changes in charge transfer resistance (R_{ct}) and increase the sensitivity of DNA hybridization detection. Zhang et al. (2013b) used an adjunct probe for signal amplification. An adjunct probe, thiol-modified DNA sequence with 14 bases, functioned as a fixer to immobilize the dissociative element of reporter probe to form loop structure. It blocked charge transfer and amplified EIS signal. An increase of R_{ct} in the presence of the adjunct probe was more than ten times that of R_{ct} without adjunct probe. Bonanni et al. (2009) used streptavidin modified gold nanoparticles for signal amplification of impedimetric detection of DNA hybridization. It permitted rapid formation of an easily detected conjugate with biotinylated DNA through a streptavidin–biotin

Table II. The impedimetric antibody-antigen biosensors.

Target Molecule	Immobilization step	Assay principle	Limit of detection	References
DNA	Au/DNA+ MCH/complementary DNA/[Co(GA)$_2$(aqphen)]Cl	hybridization	–	Regan et al. (2014)
Target DNA	GCE/GO+ EDC/p-aminothiophenol (ATP)/AuNPs/ssDNA/target DNA	hybridization	1.1×10^{-14} M	Gupta et al. (2013)
Target DNA	Au/capture prob+ adjunct probe/6-mercapto-1-hexanol/reporter probe/target DNA	hybridization	6.3 pM	Zhang et al. (2013b)
Thrombin	Au/aptamer 15 mers/mercaptopropanol/Thrombin Au/aptamer 29 mers/mercaptopropanol/Thrombin	Aptamer–thrombin complex	3.1 ng/ml	Meini (2012)
Thrombin	Au/cysteamine hydrochlorise (CH)/PAMAM/amino modified thrombin aptamer(15-mer single stranded DNA) (TBA)/Thrombin	Aptamer–thrombin interaction	0.1 nM	Zhang et al. (2009)
Poly-G	Cocarbon nanotube microelectrode arrays/EDC/sulfo-NHS/poly-C-NH$_2$ oligonücleotide/ SDS+ hydroxylamine/poly(ethylene glycol complementary strand (Poly-G)	hybridization	100 pM	Pacios et al. (2012)
Thrombin	Au/thiolated thrombin-binding aptamers (TBA)/thrombin/TBA and rhodamin 6G (R6G) functionalized AuNPs	hybridization	0.02 nM	Li et al. (2008)
PML/RARA fusion gene	GCE/FePt/CNTs/ssDNA/target DNA (cDNA)	hybridization	2.1×10^{-13} M	Zhang et al. (2013a)
Dengue serotype	Au/AuNpPANI with SH-terminal group/specific primer/complementary target	hybridization	7 pM	Nascimento et al. (2011)
catechol	Pencil graphite electrode (PGE)/MWCNT/DNA/catechol (CA)	hybridization	–	Ensafi and Rezaei (2014)
Target DNA	Au/probe DNA/dithiothireitol (DTT)/MCH/target DNA/reporter DNA-AuNPs	hybridization	30 aM	Wang et al. (2012)
Lysozyme	PGE/CHIT-GO/amino linked DNA aptamer (APT)/lysozyme	Aptamer–protein interaction	0.38 µg/ml	Erdem et al. (2014)
Aflatoxin M1	Au/cys/AuNPs/ss-HSDNA/aflatoxin M1	Aptamer–mycotoxin interaction	0.36 ng/ml	Dinçkaya et al. (2011)
80 bases long ss DNA	SiO2/S4N4/LDH/ODN/20 bases-DNA probe/100-bases long DNA/complementary 80 base long DNA	hybridization	1.8 ng/ml	Baccar et al. (2012)
Lysozyme	GCE/graphene/Fe$_2$O$_3$/lysozyme-binding aptamer (LBA)/lysozyme	Aptamer–protein interaction	0.16 ng/ml	Du et al. (2013)
Target DNA	GCE/polyaniline/ERGNO probe DNA/t-DNA	hybridization	2.5×10^{-16} M	Yang et al. (2013b)
PML/RARA fusion gene	Carbon ionic liquid electrode (CILE)/TiO$_2$/probe ss DNA/PML/RARA fusion gene	hybridization	2.8×10^{-14} M	Zhang (2013)
Target DNA	SPCE/MWCNT/aminated DNA probe/biotinylated target DNA/strept-AuNPs/silver	Streptavidin–biotin interaction	22 fmol	Bonanni et al. (2009)
Immunoglobulin E	GCE/MWCNT/ionic liquid (IL)/chitosan nanocomposite (CHIT)/IGE-specific 5′-amino terminated aptamer/BSA/methylene blue (MB)/IGE	Aptamer–protein interaction	37 nm	Khezrian et al. (2013)
Cytochrome c (cyt c)	Graphite-epoxy composite electrode (GEC)/aptamer/PEG/cyt c	Aptamer–protein interaction	69.2 pM	Ocaña et al. (2014)
lysozyme	SPE/MWCNT/amino-linked DNA aptamer/lysozyme	Aptamer–protein interaction	12.09 µg/ml	Rohrbach et al. (2012)
Thrombin	Pt/poly(pyrrole-nitrilotriacetic acid)(pyrrole-NTA)/Cu^{+2}/histidine tagged thrombin-binding aptamer (His TBA)/thrombin	Aptamer–protein interaction	4.4×10^{-12} mol/L	Xu et al. (2013b)
Ig E	Nanocrystalline diamond (NCD) film/EDC/amino (NH$_2$)-terminated IgE aptamer/BSA/IgE	Aptamer–protein interaction	0.03 µg/ml	Tran et al. (2011)
Thrombin	Au/DTT/thiol modified anti-thrombin binding aptamer (TBA)/MCH/thrombin	Aptamer–protein interaction	0.3 ng/ml	Qi et al. (2013a)
Target DNA	GCE/MWNT/G$_2$-PAMAM/probe DNA (ssDNA)/target DNA	hybridization	0.1 pM	Zhu et al. (2010)
Thrombin (Thr)	Avidin-graphite epoxy composite electrode (AV-GEC)/biotinylated aptamer of thrombin (AptThrBio1)/Thr/AptThrBio2/gold-streptavidin nanoparticles (strep-AuNPs)/silver	Sandwich type	0.2 pM	Ocaña and Valle (2014)
Target DNA	Au/MUA/MCH/3′SH-prob ss DNA-NH$_2$-5′/AuNPs/DDT/target DNA	hybridization	0.3 fM	Wang et al. (2013c)
H5 target DNA	Complementary metal oxide semiconductor (CMOS) silicon dioxide thin film/APTES/ glutaraldehyde/H5 amino modified capture DNA probe/H5 target DNA	hybridization	1 fM	Lai et al. (2012)
Hepatit B virüs (HBV) DNA	PEGs/AuNRs (gold nanorods)/thiol linked DNA probe ss ODNs/target ssODNs	hybridization	0.5 µg/ml	Congur et al. (2013)
Tombramycin	Polycrystalline gold electrode/MPA/EDC/NHS/tombramycin/ethanolamin/anti-tombramycin aptamer	Aptamer–protein interaction	0.7 µM	González-Fernández et al. (2011)
E.coli outer membrane proteins (OMPs)	Au/E.coli DNA aptamer I (ECAI) functionalized with a thiol group/MCH/OMPs Au/E.coli DNA aptamer II (ECAII) functionalized with a thiol group/MCH/OMPs	Aptamer–protein interaction	10^{-7} M	Queirós et al. (2013)

(*Continued*)

Table II. (*Continued*)

Target Molecule	Immobilization step	Assay principle	Limit of detection	References
dt DNA	ITO/Au film/complementary DNA (cDNA)/MCH/selected DNA/dt DNA	hybridization	10 aM	Wun and Yang (2013)
Acetamiprid	Au/AuNPs/aptamer/MCH/acetamiprid	Aptamer–protein interaction	1 nM	Fan et al. (2013)
Bleomycin (BLM)	Pencil graphite electrode (PeGE)/ds DNA/bleomycin (BLM)+ cis-platin (Cis-DPP)+ mitomycin C (MC)	Aptamer–antigen interaction	1.63 µg/ml	Erdem and Congur (2013)
Vibrio cholerate	GCE/ITO/nanostructured magnesium oxide (nMgO)-cMWCNTs/NHS/EDC/NH_2-ssDNA/target DNA	hybridization	~21.7 ng/µL	Patel et al. (2013)
Activated protein C	Multi-SPE8/G2-PS/DNA-APT/BSA/APC	Aptamer–protein interaction	1.81 µg/ml	Erdem and Congur (2014)
Cocaine	Au/Triangular Pyramid Frustum Nanostructure (TPF_{DNA})/cocaine	Aptamer–antigen interaction	0.21 nM	Sheng et al. (2014)
Hepatitis B virus (HBV)	Pencil graphite electrode (PGE)/G4-PAMAM/EDC/HBV-DNA probe/complementary DNA (target)	hybridization	8.9 µg/ml	Mese et al. (2014)

interaction, thus avoiding the need of synthesizing DNA modified with nanoparticles, which is a more time consuming procedure. Due to signal amplification it was possible to increase the sensitivity of the method, obtaining a comparable signal with a DNA amount 40 times lower.

The charge transfer resistance (R_{ct}) of the electrode increased with the concentration of the target DNA hybridized with the target ss-DNA (Gupta et al. 2013, Zhang 2013). The single-base mismatched DNA sequence and double-base mismatched DNA sequence were recognized via comparing the increase of their R_{ct} values. The results demonstrated that this DNA biosensor displayed high selectivity for the hybridization detection (Zhang 2013).

Thrombin was selected as target molecules for several studies in literature, because it is a major stimulus of both procoagulant and anticoagulant reactions, and thus is a key element in various pathogenesis, including leukemia, arterial thrombosis, and liver disease (Xu et al. 2013a). The most extensively investigated prototype thrombin-binding aptamer (TBA) is a 15-mer single-stranded DNA. After binding to thrombin, TBA forms an intermolecular quadruplex structure and restricts the activity of thrombin. The assembly of the TBA monolayer film onto the electrode surface led to a further increase in the R_{ct} value, owing to the abundance of negative charges on TBA sugar-phosphate backbone. After incubation in thrombin, the formation of TBA–thrombin complex on the electrode surface contributed to a significant increase in the R_{ct} value. Also, TBA–thrombin complex insulates the electron transfer between the electrode surface and electrode solution (Zhang et al. 2009).

Another strategy for DNA detection involves a three-component "sandwich" assay, in which the redox label is attached to a synthetic sequence specifically designed to bind an overhang portion of the probe and target DNA. These strategies need an extra chemical labeling step either in the target DNA or in the synthetic oligonucleotide which makes the process more expensive and effortful (Drummond et al. 2003).

Cell-based impedimetric biosensors

In cell-based biosensors, bacterial or prokaryotic cells have been used for detection systems. Higher eukaryotic and mammalian cells are higher detection systems. Using cell for biorecognition events have advantages. They are capable of external stimuli *in situ* analysis, most tissue types of cell lines and a wide range of growth media are commercially available and they provide more comprehensive and complex functional information than nucleic acid and immunochemical methods (Banerjee and Bhunia 2009).

Biological cells have very different electrical properties because of the cell membranes which consist of a lipid bilayer containing many proteins, where the lipid molecules are oriented with their polar groups facing outward into the aqueous environment, and their hydrophobic hydrocarbon chains pointing inward to form the membrane interior. In the inside of a cell is present membrane covered particulates, such as mitochondria, vacuoles and a nucleus, and many dissolved charged molecules. While the cell membrane is highly insulating, the interior of the cell is highly conductive.

The conductivity of the cell membrane is around 10^{-7} S/m, whereas the conductivity of the interior of a cell can be as high as 1 S/m (Pethig and Kell 1997, Yang and Bashir 2008).

Bacterial biosensors coupled to impedimetric detection can be classified into two types, depending on the location of the bacterial cells in the experimental set up. One type deals with measuring the impedance change caused by the interaction between the analyte and bacteria-modified electrodes. Another type deals with measuring metabolites produced by bacterial cells as a result of growth in the presence of the analyte. Table III summarizes the impedimetric biosensors based on cells in literature.

Qi et al. (2013b) constructed a biosensor, in which bacteria mediated bioimprinted films were used for selective bacterial detection. Chitosan (CS) doped with reduced graphene sheets (RGSs) was electrodeposited on an indium tin oxide electrode, and the resulting RGSs–CS hybrid film served as a platform for bacterial attachment. After depositing RGSs–CS nanocomposite film onto the ITO electrode, the R_{ct} decreased when compared with the result obtained with bare ITO electrode. As a result of graphene facilitating the charge transfer process of $[Fe(CN)_6]^{4-/3-}$. A much larger resistance was observed when sulfate reducing bacteria (SRB) attached onto the conductive films—because the bacteria hindered the charge transfer process. After the nonconductive CS film was formed to embed the bacteria, the R_{ct} increased again. Subsequently, fluorine silane coupling agent was coated on the functionalized ITO electrode and the SRB were washed away by acetone to leave bioimprinted cavities. Consequently, the charge transfer resistance decreased sharply.

Enzyme-based impedimetric biosensors

Effective immobilization of enzyme onto electrode surface is one of the key steps in the fabrication of biosensors. Various immobilization strategies such as physical adsorption, covalent attachment, entrapment, cross-linking, or affinity have been used. Each immobilization method presents advantages and disadvantages. The choice of the most appropriate technique also depends on the enzyme nature, the transducer, and the associated detection mode. The best method of enzyme immobilization is related to biosensor application which requires maximum sensitivity or stability. If immobilization method causes enzyme denaturation or conformational change or if the enzyme on its active site modifies, sensitivity decreases. Enzyme denaturation and blocking of active sites cause loss of activity. The solution of this drawback is using spacer arm between the enzyme and the support under covalent binding. Techniques based on affinity interactions between enzymes and (strept)avidin molecules, lectins, or sugars allow to immobilize enzymes in an ordered and site-specific manner, thus developing efficient biosensors. Likewise, self-assembled monolayer-based immobilization reduces the number of random orientations, generates uniform, reproducible and stable structures with high coverage (Sassolas et al. 2011).

Table IV summarizes the impedimetric biosensors based on enzymes and proteins in literature.

Oliveira et al. (2011a) constructed a biosensor for bacterial lipopolysaccharide determination. The deposition of poly(vinyl chloride-vinyl acetate maleic acid) (PVM) on the electrode surface formed a film with a large surface area for the assembly of cysteine-coated gold nanoparticles (AuNpCys) and further immobilization of CramoLL lectin. As a result of the AuNpCys inorganic–organic composite containing an amine group with a positive charge was adsorbed on a negatively-charged PVM-modified gold electrode. Finally, negatively charged CramoLL was immobilized on the AuNpCys–PVM composite film based on an electrostatic interaction between oppositely charged species.

Cortina et al. (2006) developed a urea impedimetric biosensor using Röhm Pharma Eudragit S-100 ehteric polymer,

Table III. The impedimetric biosensors based on cells.

Target molecule	Immobilization step	Assay principle	Limit of detection	References
Exogenous agents	Au/cys/laminin/EDC/NHS/PC 12 cells/exogenous agents	Calcium exocytosis and ion modulation	–	Slaughter and Hobson (2009)
E.coli	Nitride silicon wafer/gold layer/E.coli	E.coli and its metabolites	–	Kim et al. (2009)
Acetylcholin (ACh)	Electrode/bactoagar gel and neuroblatoma (N_2a)cells/ACh	Conductivity	–	Valero et al. (2009)
E.coli	Platinum surface/E.coli	Conductivity	10 CFU/ml	Muñoz-Berbel et al. (2008)
Sulfate reducing bacteria (SRB)	ITO/reduced graphene sheets (RGSs)-CHIT film/CS/fluorinated silane coupling solution/SRB	Binding SRB change impedance of biosensor	0.7×10^4 CFU/ml	Qi et al. (2013b)
Trichloroethylene (TCE)	Gold micro electrode/aminothiol/SWCNT/anti-pseudomonas antibody/casein/P.putida FI (PpF1)/TCE	Toulenedioxygenase of PpF1 degraded TCE	20 μg/L	Hnaien et al. (2011)
Non-lytic M13 bacteriophage infection	Au/mercaptoacetic acid (MACA)/EDC/NHS/anti-E.coli antibody (IgG)/E.coli/M13 phage	Pathogen-cell interaction	–	Cheng et al. (2013)
Bacteriophage PhiX174	Platinum disc electrode/E. Coli WG5	Bacterial infection caused lysis of host strain	–	García-Aljaro et al. (2009)
m.o.	Silicon wafer/Au/agar	Bacterial growth	–	Choi et al. (2009)
Organochlorine pesticides (OCPs)	Steel/starch-casein-agar (SCA)/streptomyces strain M7 (SM7)/OCP	Chloride ion release	–	Rodriguez et al. (2014)
ZD6474	Au/RPMI 1640/T47D/ZD6474	Cytotoxic effect of anticancer drug	–	Pradhan et al. (2014)

Table IV. The impedimetric biosensors based on enzymes and proteins.

Target molecule	Immobilization step	Assay principle	Limit of detection	References
H_2O_2	GCE/MWCNTs/1-butyl-3-methylimidazolium hexafluorophosphate ([bmim][PF6]/catalase (CAT)	Enzymatic degradation	0.025 nM	Shamsipur et al. (2012)
Glucose	Au/MPA/glucose oxidase (GOx) SAMs	Enzymatic degradation	15.6 μM	Shervedani et al. (2006)
Urea	Pt/ppy (polypyrole)	Concentration of urea	50 μM	Mondal and Sangaranarayanan (2013)
Amyloid-beta oligomers (AβO)	Au SPE/polytramine/3-(4-hydroxyphenyl) propionic acid (POPA)/NHS-biotin/neutrAvidin/biotin-LC-PrPc (95–110)/amyloid-beta oligomers (AβO)	Concentration of AβO	0.5 pM	Rushworth et al. (2013)
Glutamate	GCE/methyl viologen/nafion/glutamate oxidase (GLOD)/L-glutamate	Enzymatic degradation	20 μM	Maalouf et al. (2007)
Creatinine (Cre)	Creatinine (Cre)-methylacrylate (MA)-molecular imprinted polymer (MIP) based CPE/creatinin	Concentration of creatinine	20 ng/ml	Reddy and Gobi (2013)
PCR products	Au/MCH/immobilized probe DNA-SH/streptavidin–alkaline phosphate conjugate/enzymatic substrate solution	İnteraction between DNA-SH and target oligo	1.2 pmol/L	Lucarelli et al. (2005)
Cyanide	Silicon wafers/gold top layer/BSA + glycerol+ catalase/poly(vinyl alcohol)-photopolymerizable styrylpyridinium groups (PVA-SbQ)/cyanide	Enzymatic inhibition	4 μM	Bouyahia et al. (2011)
H_2O_2	Pt disc/α-COOH-PPy/Cyt-C	Reaction between Cyt C and H_2O_2	0.25 μM	Shamsipur et al. (2008)
Dopamine	GCE/PEGlated arginine magnetic functionalized Fe_3O_4 nanoparticles/dopamine	Slow electron transfer kinetic	14.1 μM	Chandra et al. (2012)
Bacterial lipopolysaccharide (LPS)	Au/poly(vinyl chloride-vinyl acetate maleic acid (PVM)/L-cysteine-gold nanoparticle (AuNpCys)/CramoLL/BSA/LPS	CramoLL-LPS	–	Oliveira et al. (2011b)
Dopamine	Skeleton Nickel/poly(aniline boranic acid) (PABA)/dopamine	Dopamine–boronic acid interaction	–	Plesu et al. (2013)
Urea	Carbon and silver SPE/Eudragit S-100 solution (Eudrogit+ NaCl+ urease+ carbodiimide)/urea	Enzymatic degradation	0.02 M	Cortina et al. (2006)
Rennet	Au/Dithiobis-N-succinimidyl propionate (DTSP)/k-casein/rennet (in imidazole)	Between para-k casein and chymosin interaction	–	Panagopoulou et al. (2012)
Alcohol	Gold coated microscope slides/polydiaminobenzene film/aniline+ alcohol oxidase/alcohol	Enzymatic degradation	–	Myler et al. (2005)

which is a copolymer of methyl methacrylate and methacrylic acid that undergo a breakdown process at pH values higher than 7. In this enzymatic biosensor, urea hydrolysis by urease, which causes an increase of pH, triggers the degradation of polymer and generates an increase in capacitance, which is followed by EIS.

Conclusion

According to IUPAC, biosensor is defined as a self-contained integrated device which is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor) which is in direct spatial contact with a transducer element. A biosensor should be clearly distinguished from a bioanalytical system, which requires additional processing steps, such as reagent addition. Furthermore, a biosensor should be distinguished from a bioprobe which is either disposable after one measurement, i.e., single use, or unable to continuously monitor the analyte concentration. Impedimetric biosensors have been designed by immobilizing bioreceptors such as antibodies, nucleic acids, enzymes, lectins, cells, and bacteria at the surface of the electrode. After binding target molecule to the electrode surface; a shift in impedance, a change in capacitance or admittance at the bulk of the electrode interface owing to the insulating properties are observed. The most important step in developing biosensors is to immobilize bioreceptors on electrode surface appropriately. In literature; covalent and affinity immobilization, physical adsorption, entrapping bioreceptors in films or gels, layer-by-layer deposition, cross-linking are used for the immobilization of receptors. Nanomaterials, thanks to their unique physical and chemical properties have recently become an interest for the design of electrochemical impedimetric biosensors. Nanomaterials, that are formed from metals, carbon, or polymeric species, exhibit high sensitivity, stability, and conductivity. Electrochemical impedance spectroscopy has been widely used by many research groups to detect DNA hybridization, antibody-antigen reactions, and enzyme reactions. It is a quite sensitive label free electrochemical technique for monitoring biorecognition events at the electrode surface. Because of this it provides low detection limit. Determination is rapid and requires short detection time. Impedimetric biosensors are reproducible, when the biorecognition elements are immobilized on the electrode surface by using strong chemical bonds such as SAM immobilization method. Many studies on impedimetric biosensors are focused on immunosensors and aptasensors. In immunosensors, antibodies and receptors are immobilized on the electrode surface. After the formation immuno reaction between antibody and antigen or receptor and factor, a change in impedance occurs. One way to enhance the response of a label-free immunosensor is to increase the amount of the immobilized antibody on the electrode surface, and another way is to immobilize antibody on an electrode surface incorporated with gold or silver nanoparticles, carbon nanotubes, and polymers such as chitosan and polyaniline. In impedimetric aptasensors, hybridization between probe and target DNA, conformation changes, or DNA damages lead to change in the impedance. High specificity of binding affinity, better stability, and larger shelf life are advantages of aptamer-based biosensors. Cell-based impedimetric biosensors are useful in external *in situ* analysis. Bacterial, procaryotic, and mammalian cells have been used to monitor the amount and activity of microorganisms. Few studies on enzyme-based impedimetric biosensors have been reported. These biosensors act based on detection of substrate or product of an enzyme reaction.

Declaration of interest

The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

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