Talanta 148 (2016) 427–438

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Poly(amidoamine) (PAMAM): An emerging material for electrochemical bio(sensing) applications

Elif Burcu Bahadır [a], Mustafa Kemal Sezgintürk [b,*]

[a] Namık Kemal University, Scientific and Technological Research Center, Tekirdağ, Turkey
[b] Namık Kemal University, Faculty of Science, Chemistry Department, Biochemistry Division, Tekirdağ, Turkey

ARTICLE INFO

Article history:
Received 7 September 2015
Received in revised form
4 November 2015
Accepted 6 November 2015
Available online 10 November 2015

Keywords:
Poly(amidoamine)
PAMAM
Electrochemical biosensor
Chemical sensor
Biosensing

ABSTRACT

Poly(amidoamine) (PAMAM) dendrimers have received attention due to their large surface areas with high concentration of functional end groups to bind biological material. They are monodisperse and hyper-branched polymers which include active functional groups outside its surface. These functional groups have been used for immobilizing the biorecognition molecules. They act as bioconjugating reagents and they have various applications in the fields of chemical and biochemical biosensors. Electrochemical techniques (amperometric, impedimetric, potentiometric, electrochemiluminescence) are useful for the determination of target molecules, with high sensitivity and selectivity. Dendrimer-modified enzyme biosensors, DNA biosensors, immunosensors and chemical sensors have been fabricated by using PAMAM dendrimer. This review provides a brief description of PAMAM dendrimers and its applications in biosensors and sensors. These sensors' limit of detection values are also compared in detail. Also this is the first review that assesses PAMAM dendrimers from the point of its usability in biosensor and sensor technologies.

Contents

1. Introduction

Dendrimers are a member of polymers consisting of a central core and branched monomers [1]. The word dendrimer is derived from a Greek term that is dendron, meaning tree, and meros, meaning part [2–4]. A typical dendrimer includes three parts: a central core (C), branched units (B) and surface groups (S). The branched units are arranged in layers named generations and present as the repeating monomer unit [2]. The typical structure of dendrimer is shown in Fig. 1.

Dendrimers have highly-branched structures and are globular shapes with the nanosize ranging from 2 to 20 nm. Due to the unique structural properties such as monodispersity, controllable size and structure, modifiable surface functionalities,

* Corresponding author.
E-mail addresses: msezginturk@hotmail.com, msezginturk@nku.edu.tr (M.K. Sezgintürk).

http://dx.doi.org/10.1016/j.talanta.2015.11.022
0039-9140/

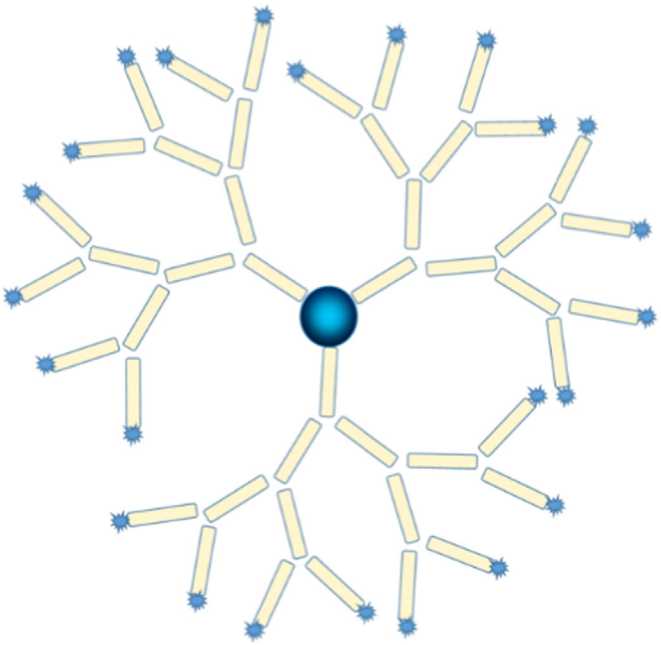

Fig. 1. The typical structure of a dendrimer [2,4].

hydrophilicity, and high mechanical and chemical stability they become favorite synthetic macromolecules in the field of biosensor technology [5,6]. Also they are ideal immobilization surface for biorecognition molecules. These properties contribute biosensors for enhancing their target-capturing ability and sensitive, specific, stabil analysis [5].

Fritz Vogtle and his co-workers developed the first dendrimer in the 1970s. Then, in 1984, Donald A synthesized the primary group of dendrimers. From that day to this day, generation of multiple dendrimer families have been developed in different ways [4]. In this review, we investigated and summarized the recent improvements in PAMAM dendrimer based biosensors and sensors. Most reviews have been organized around biomedical applications such as drug delivery and dendrimer-drug interaction. In this review, not only various characteristic of PAMAM are explained, but also the usability of PAMAM dendrimer in biosensor technology is discussed and LODs of PAMAM dendrimer based biosensors are also compared.

2. Polyamidoamine (PAMAM) dendrimer

In 1984, Tomalia and his co-workers synthesized PAMAM dendrimers for the first time. They are known as the first commercial dendrimers family, which are extensively characterized and commonly used. PAMAM dendrimers have ethylene diamine (EDA) core and an amidoamine repeating monomer unit. Also, a PAMAM dendrimer consists of three architectural structures which are a core, repeating units, active surface groups [4,6].

The size of PAMAM dendrimers ranges from 10 Å to 130 Å in diameter for generation 0 (G0) through generation 10 (G10). It means that the diameter of molecule changes approximately 1 nm as each generation varies (Table 1). Also, dendrimer diameter increases approximately linearly with generation, while the number of functional groups on the surface increases exponentially. Therewith, on the outside of the dendrimer the distance between functional groups and their flexibility decrease with generation [4].

The shape of PAMAM dendrimer is affected by the number of generations. Dendrimer, from G0 to G4 (lower generation dendrimers) have a planar, elliptical shape and from G5 to G10 (higher generation dendrimers) have a spherical shape because their branches are densely packed. In the higher generations the steric hindrance of the branches occur and it can lead to the defective branching structures. The steric hindrance starts with G7 and reduces the synthetic yield and continues until G10, and the higher generations can not be synthesized due to this phenomenon. However, the higher generations of PAMAM dendrimer have larger cavity in their size. The lower generations of PAMAM dendrimer are more polar than its higher generations [4]. The structure of PAMAM dendrimer is shown in Fig. 2.

There are four methods for PAMAM synthesis. In divergent method, synthesis of PAMAM dendrimer begins from a multifunctional core molecule and the reaction occurs between the core and monomer molecules containing one reactive and two dormant groups. The initiator core for PAMAM dendrimer is an ammonia or ethylenediamine molecule. They have three and four possible binding sites for amidoamine repeating units. The surface of the molecule has primary amino groups and two new branches conjugate each of them. In convergent method, dendrimer is synthesized from surface to the core, mostly by “one to one” coupling of monomers, so dendrons are created. Finally, two or more dendrons get together and creat the dendrimer. In combined convergent–divergent synthesis, orthogonally protected branched monomers are used for dendrimer synthesis. In click synthesis method, the copper-catalysed azide-alkyne cycloaddition is mostly used. The click chemistry is known as Cu-catalyzed cycloaddition reaction between an alkyne and an azide forming a 1,2,3-triazole ring. This reaction is a discovery for synthesis of dendrimers [4].

3. PAMAM dendrimer as a construction material for electrochemical biosensor fabrication

A biosensor is an analytical device that consists of two parts, a bioreceptor and a transducer. First part, the bioreceptor is a biorecognition molecule for the spesific target analyte, and second part the transducer transforms the biochemical reaction into a measurable signal [7].

The basic principle of electrochemical biosensors is that biochemical reaction between biorecognition element and specific

Table 1
Approximate diameters (G0–G7) of PAMAM dendrimers [4].

Generation	Number of NH_2 groups on the periphery	Molecular formula	Molecular weight MW	Hydrodynamic diameter (nm)
0	4	$C_{24}H_{52}N_{10}O_4S_2$	609	1.5
1	8	$C_{64}H_{132}N_{26}O_{12}S_2$	1522	2.2
2	16	$C_{144}H_{292}N_{58}O_{28}S_2$	3348	2.9
3	32	$C_{304}H_{612}N_{122}O_{60}S_2$	7001	3.6
4	64	$C_{624}H_{1252}N_{250}O_{124}S_2$	14,307	4.5
5	128	$C_{1264}H_{2532}N_{506}O_{252}S_2$	28,918	5.4
6	256	$C_{2544}H_{5092}N_{1018}O_{508}S_2$	58,140	6.7
7	512	$C_{5104}H_{10212}N_{2042}O_{1020}S_2$	116,585	8.1

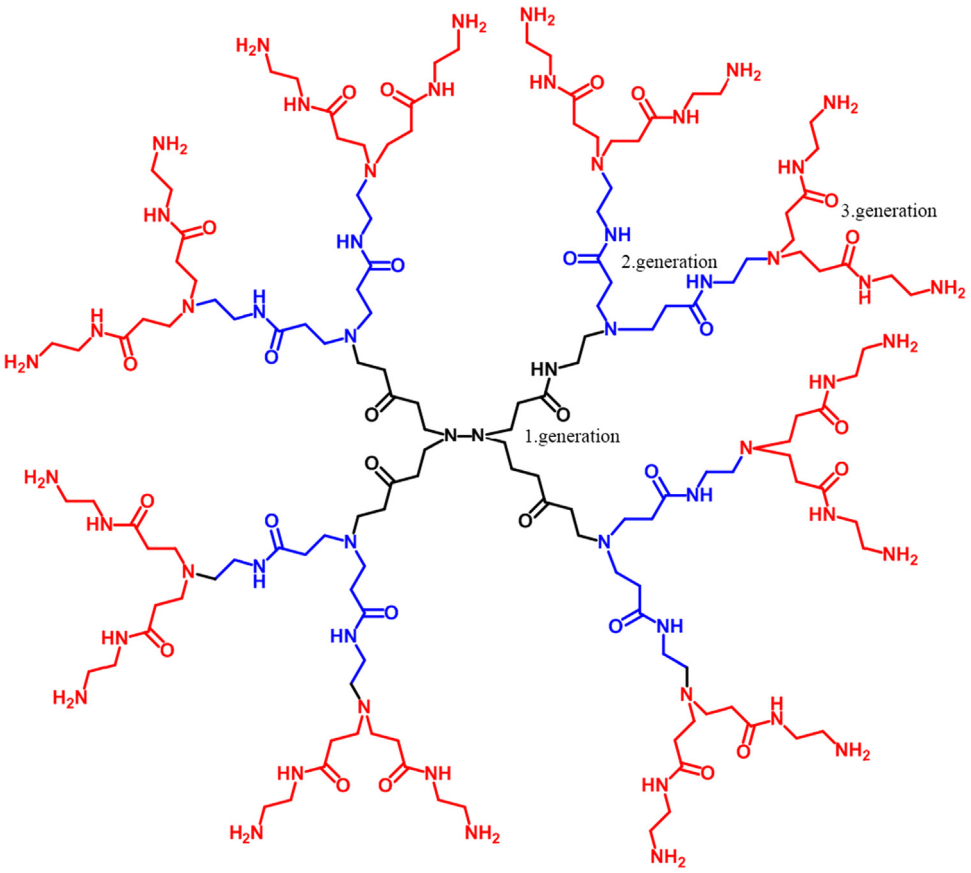

Fig. 2. General structure of a PAMAM.

target analyte producing or consuming ions or electrons, which changes electrochemical properties (electric current or potential) of the solution. The bioelectrochemical reaction generates a current (amperometric), a potential (potentiometric) or alters the impedance of a medium between electrodes [8].

The excellent structural properties of PAMAM dendrimer such as controllable size, high functional and hydrophilic surface area, high mechanical and chemical stability make them a suitable construction material for immobilizing biorecognition elements. The successful use of PAMAM dendrimers for the modification of

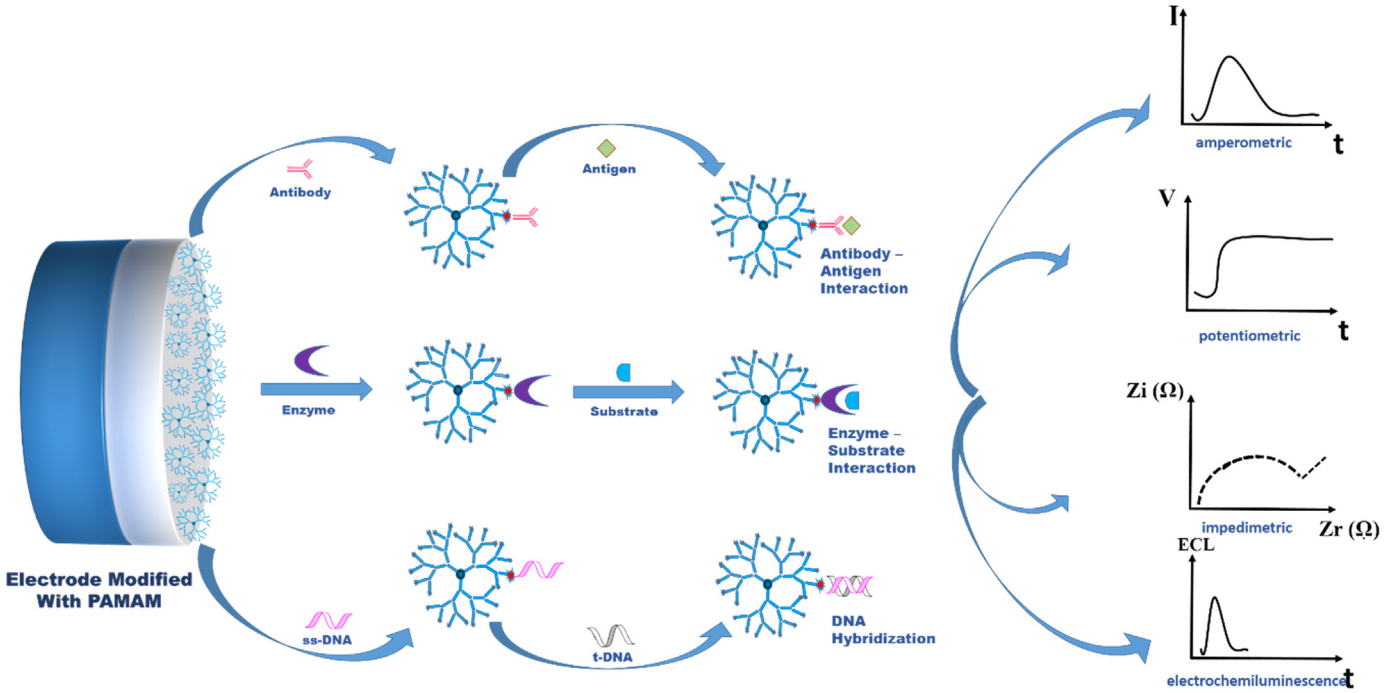

Fig. 3. The use of PAMAM dendrimers in biosensors.

electrode depends on immobilization methods of biorecognition element on the transducer. Biorecognition elements such as enzymes, antibodies, or DNA can be immobilized via physical adsorption and covalent attachment to the PAMAM dendrimer surface, or can be encapsulated into the interior part. In literature, PAMAM dendrimers are coupled up with other nanomaterials such as carbon nanotubes, gold nanoparticles, graphenes and quantum dots to improve the sensitivity of biosensors. In addition, PAMAM dendrimers have been used to fabricate biosensors with high stability, good reproducibility and repeatability, and low limit of detection. And they provide a biosensor operate in extreme conditions [5]. The use of PAMAM dendrimer in biosensors is shown in Fig. 3.

Using PAMAM dendrimers for the construction of biosensors provides several advantages. First, they have multiple conjugation and structurally stable construction sites. Second, they show minimum diffusion limit for the targets and electron-transfering molecules because of their internal void. Third, because of reactive groups on them they provide accessible platform for further modifications for reagentless biosensing. Also, according to literature, PAMAM dendrimer enhances surface immobilizing capacity for detecting target molecule with high selectivity and sensitivity [9].

3.1. Amperometric biosensors based on PAMAM dendrimer

Amperometric biosensors are the most popular class of biosensors. In amperometric measurements, a current occurs as a result of electrochemical oxidation or reduction of an electroactive molecule. This type of measurement is taken by maintaining a constant amplitude voltage at working electrode (gold, platinum, carbon) related to reference electrode, under a fixed potential, current pass through sample. Characteristically, in amperometry, the current is measured at a constant potential. In voltammetry, the current is measured during controlled differences of the potential [9]. In Table 2 studies of amperometric biosensors with PAMAM dendrimer are summarized.

The detection of protein markers with sensitively and selectively is important for diagnosis of diseases. α-1-fetoprotein (AFP) is an oncofetal glycoprotein and important to hepatocellular carcinoma, teroblastoma and especially liver carcinoma diagnosis [49]. PAMAM dendrimers were used for developing of biosensors [14,20,42]. PAMAM dendrimer functionalized graphene and HRP-labeled antibodies were used for the construction of the biosensor. PAMAM functionalized graphene provided large area to the immobilization of a lot of enzyme loaded antibody onto the electrode surface and it amplified signal. In this study, PAMAM dendrimer was bound on the surface of carboxylated graphene via amide bound and HRP-labeled antibodies was crosslinked to the PAMAM dendrimer. Graphene accelerated the electron transfer and amplified the signal because of its high electrical conductivity. The amplified signal was monitored in the presence of H_2O_2. In the presence of hydroquinone and H_2O_2, carboxylated graphene-PAMAM–anti-AFP–HRP immobilized biosensor had larger reduction current than anti-AFP–HRP. In another study for AFP detection, PAMAM dendrimers-capped carbon dots-gold nanocrystal nanocomposite was used for the construction of biosensor. This nanocomposite showed high conductivity, stability and biocompatibility on the electrode surface. Carbon dots are carbon materials, which are soluble and have good electrical conductivity, large surface area. PAMAM dendrimer served as large electrode material for the attaching of carbon dots. This nanocomposite attached on the electrode surface and anti-AFP antibodies immobilized on the electrode via the electrostatic and hydrophobic interactions between gold nanocyrstal and amino groups of antibodies. One different study was performed by using PAMAM dendrimer encapsulated gold nanoparticles as a sensing materials. For the fabrication of biosensing interface, 4-aminothiophenol was self-assembled on the electrode and gold–PAMAM nanocomposite coated the electrode surface by using phtaloyl chloride. This nanocomposite enhanced the electron transfer between the electrode surface and electrolyte solution. Anti-AFP antibodies was bound after ethyleneamine viologen (vio) and phtaloyl chloride binding on the electrode surface. Vio was used as a redox marker and illustrated the antigen–antibody interaction on the electrode surface. It contributed to signal producing and sensitively detection of AFP. The use of gold–PAMAM nanocomposite with high conductivity and large surface area provided to immobilize a large quantity of anti-AFP antibody on the electrode surface and amplification of signal. The response of only anti-AFP antibody modified electrode and Au–PAMAM/anti-AFP antibody modified electrode was compared. Only a small response was observed when only anti-AFP antibody was used. Larger response was found when Au–PAMAM nanocomposite was used. Sulfhydryl viologen and gold nanoparticles was used for the construction of the immunosensor [50]. The LOD's were found as 130 fg/mL [20] and 230 fg/mL [50]. Also, the AuNPs–PAMAM nanocomposite not only increased the electrode surface area and made the electron transfer easy, but also it provided stable matrix for immobilizing biorecognition elements.

The glucose oxidase (GOx) is a member of the oxidoreductase enzymes that catalyses the oxidation of glucose to hydrogen peroxide and D-glucono-δ-lactone [50]. During the oxidation of glucose, FAD is reduced to $FADH_2$ by accepting electrons from glucose. $FADH_2$ then reacts with O_2 to form H_2O_2 and FAD. After addition of glucose, the oxidation peak current increases while the reduction peak current decreases perceptibly. The change of current with the level of glucose has a linear relationship. PAMAM-pyrolle dendrimer–GOx [23], graphene oxide–PAMAM–PtNPs–GOx [31], cysteamine–PAMAM–GOx [34], PAMAM-ferrocene dendrimers–GOx [40], reduced graphene oxide–PAMAM–Ag nanocomposite–GOx [43] were used for the construction of glucose biosensors. Because of graphene's excellent features such as large surface area and high electrical conductivity, low LOD values can be obtained. The stability and reproducibility are two important parameters in any biosensor. Also, these biosensors are stabil under dry conditions and reproducible. In our opinion, this is related with PAMAM dendrimers' good biocompatibility and catalytic activity of GOx in them.

Horseradish peroxidase (HRP) is a member of the heme-containing redox enzymes. The electron transfer transforms Fe(III) to Fe(II) in the heme of HRP. This process catalyzes the reduction of H_2O_2. The electrochemically active centers in HRP are buried in its three-dimensional structure and that obstructs direct electron transfer between HRP and the electrode surface. PAMAM dendrimer is useful for bioconjugating reagents for construction of HRP biosensors. Also it provides suitable environment for HRP. Pyrrole-PAMAM dendrimer copolymers and PAMAM dendrimer/cysteamine/AuNPs composite were used for the construction of H_2O_2 biosensors. Pyrrole-PAMAM dendrimer copolymer is a conductive material and used as enzyme immobilization platform. 3-mercaptopropionic acid (MPA) was self-assembled on the electrode surface. Pyrrole-PAMAM dendrimer was immobilized on the MPA layer. An amide bound was occurred between carboxylic group of MPA and amine group of pyrrole PAMAM dendrimer. The HRP enzyme was immobilized on the modified electrode by electropolymerization. HRP passed good catalytic activity. This might have orginated from effective immobilization of HRP on the pyrrole-PAMAM dendrimer modified electrode and it provided high enzyme loading and stabile electrode. PAMAM dendrimers were linked covalently onto self-assembled monolayer of Au electrode via glutaraldehyde cross-linking. After adsorbing gold

Table 2
Amperometric biosensors based on PAMAM dendrimers.

Target molecule	Fabrication of the biosensor	Bioreceptor	Limit of detection (LOD)	Linear range	Reference
H_2O_2	Au/cysteamine (Cys)/glutaraldehit (GA)/G4-PAMAM/AuNPs/HRP	Enzyme	2 μmol/L	10^{-5}–2.5×10^{-3} mol/L	[10]
Glucose	Au dual electrode/Cys/PAMAM/GA/alcohol oxidase (AOx)+pyranose oxidase (PyOx)	Enzyme	–	0.025–0.5 mM	[11]
Ethanol			–	0.025–0.5 nM	
pO_2			–		
cell density			–		
H_2O_2	Au/3-mercaptopropionic acid (MPA)/pyrrole-PAMAM/HRP	Enzyme	5.7 μM	1–18 mM	[12]
Pencillamine (PCA)	Au/MPA/EDC-PAMAM/AuNPs/Tyrosinase	Enzyme	5.4×10^{-8} mol/L	7.5×10^{-7}–10^{-4} mol/L	[13]
AFP	GCE/AuNPs/anti-AFP/AFP/Gr-PAMAM–anti-AFP-HRP	Antibody	0.45 ng/mL	1-100 ng/mL	[14]
Glucose	ITO/poly(vinyl(sulfonic acid))(PVS)/cobalthexacyanoferrate(CoHCF)/GOx-BSA	Enzyme	17 μmol/L	–	[15]
Tichlorfon	Au/polyaniline (PAN)/camphorsulfonic acid+phenol/AChE+GA/ChO/BSA	Enzyme	100, 6, 700 nmol/L	0.2–25; 0.01–0.2; 1–2.5 μmol/L	[16]
Carbofuran	Au/1-hexadecanethiol/G4-PAMAM/AChE+ChO+GA/BSA	Enzyme	0.003, 0.04,	0.005–0.02; 0.05–0.09;	
Eserine			0.1 nmol/L	0.2–2 μmol/L	
Ethanol	Au/Cys/GA/PAMAM/alcohol oxidase (AOx)+GA	Enzyme	0.016 mM	0.025–1 mM	[17]
Glutamate	GCE/glutamate dehydrogenase (GLDH)/Pt-PAMAM/CNTs nanocomposite	Enzyme	10 nM	0.2–250 μM	[18]
Chloramp-henicol (CAP)	GCE/AuNPs/poly5,2′;5′,2″-terthiophene-3′-carboxylic acid (poly-TTCA)/AuNPs/PAMAM/Cadmium sulfite (CdS)/anti-chloramphenicol acetyl transferase (anti-CAT)/BSA/Hydrazine-CAP	Antibody	45 pg/mL	50–950 pg/mL	[19]
AFP	Au/4-aminonitrophenol/Phtaloyl chloride (Pht)/Au–PAMAM/Pht/Ethylenamine viologen/Pht/anti-AFP/BSA	Antibody	130 fg/mL	0.001–45 ng/mL	[20]
Thrombin	GCE/AuNPs/thrombin aptamerI (TBAI)/hexanethiol/Thrombin/PAMAM-thionine (TH)-hemin-TBA II-BSA	Aptamer	0.1 pM	0.0002–30 nM	[21]
Target DNA	Au/MPA/EDC/NHS/aminoterminated PAMAM (G4.0-NH_2)/Avidin (EDC/NHS)/biotin-labeled ssDNA (capture DNA)/target DNA/reporter DNA with AuNPs on 5′-end	Aptamer	1.4×10^{-14} M	2.7×10^{-14}–1.4×10^{-14} M	[22]
Glucose	Au/MPA/PAMAM-pyrrole dendrimers/GOx	Enzyme	6.3 μM	0.5–4 mM	[23]
H_2O_2	GCE/PAMAM–MWCNTs–AuNPs/Hemoglobin (Hb)	Enzyme	2×10^{-7} mol/L	10^{-6}–2.2×10^{-3} mol/L	[24]
Target DNA	GaN nanowires/1,10-decanedicarboxylic acid/G3-PAMAM/EDC/probe-DNA	Aptamer	0.1 aM	10^{-8}–10^{-9} M	[25]
Human cellular prion protein (PrPc)	Au/polypyrrole and pyrrole functionalized by an active ester (PPy –PyNHP)/PAMAM/ferrocene/biotin hydrazide/streptavidin/biotinylated aptamers	Aptamer	0.8 pM	1 pM–1 μM	[26]
Estradiol	GCE/graphene–polyaniline/PAMAM –Au/estradiol/BSA/HRP-GO-anti-estradiol	Antibody	0.002 ng/mL	0.04–7 ng/mL	[27]
Salbutamol (SAL)	Au/cys/PAMAM–AuNPs doped chitosan-iron oxide nanocomposites (CS-Fe_3O_4–PAMAM)/SAL/HRP/HRP–MWCNTs–Ab bioconjugate	Antibody	0.06 ng/mL	0.11–1061 ng/mL	[28]
t-DNA	SPE/poly(TTCA)/EDC/PAMAM/AuNPs/thiolated DNA probe/1-decanethiol/t-DNA biotinylated probe/avidin-hydrazine	Aptamer	30 fM	50 fM–75 nM	[29]
IgG	SPE/poly(TTCA)/EDC/PAMAM/AuNPs/anti-human IgG/BSA/IgG/biotinylated anti-human IgG/Avidin-Hydrazine	Antibody	25 fg/mL	40 fg/mL–2.5 ng/mL	
t-DNA	Au/MPA/EDC/G1-PAMAM with raphene core/AuNPs/thiolated DNA probe	Aptamer	10^{-12} M	10^{-6}–10^{-12} M	[30]
Glucose	GCE/graphene oxide-(3-glycidyloxypropyl)trimethoxysilane-PAMAM-G4)/PtNPs/GA-GOx	Enzyme	0.8 μM	10 μM–8.1 mM	[31]
Glucose	GCE/CNT–PAMAM+GOx–HRP	Enzyme	2.5 μM	4 μM–1.2 mM	[32]
Carbofuran	GCE/CNTs/PAMAM–AuNPs/AChE	Enzyme	4×10^{-9} M	4.8×10^{-9}–0.9×10^{-7} M	[33]
Glucose	Au/Cys+6-ferrocenyl-1-hexanethiol/GA/PAMAM/GOx/GA+AuNPs	Enzyme	0.6 mM	1–5 mM	[34]
Fructose	Au/cys/GA+PAMAM/GA+Fructose dehydrogenase	Enzyme	–	0.25–5 mM	[35]
CA 125	Mercury film electrode/MB-GOPS/$Ab_{1\text{-}1}$–$Ab_{1\text{-}2}$–$Ab_{1\text{-}3}$/glycin/CA 125-CA 19-9- CA 15-3/PAMAM dendrimer-QD nanocomposites/$Ab_{2\text{-}1}$–$Ab_{2\text{-}2}$– $Ab_{2\text{-}3}$	Antibody	0.005 U/mL	0.01–50 U/mL	[36]
CA 19-9					
CA 15-3					
Dopamine (DA)	Au/Chit-MWCNTs/PAMAM/DNA	Aptamer	0.03 μM	0.2–10 μM	[37]
			0.007 μM	10–100 μM	
Uric acid (UA)				0.5–100 μM	
AFP	GCE/AuNPs/AET/PAMAM/anti-AFP/BSA	Antibody	3 ng/mL	50–500 ng/mL	[38]
Atrazine	GCE/AuNPs/AET/atrazine-BSA/anti-atrazine/anti antrazine-HRP (secondary antibody)	Antibody	1.2 ng/mL	10^{-2}–10^{3} ng/mL	[39]
Glucose	Au/MPA/PAMAM-ferrocene dendrimers/GOx	Enzyme	0.48 mM	1–22 mM	[40]
Glutamate	GCE/MWCNTs/PAMAM/Pt/Glutamate oxidase-polyethyleneimide (PEI)/GA/NAfion	Enzyme	0.5 μM	1–50 μM	[41]
AFP	GCE/PAMAM*carbon dots/Au nanocrystal/anti-AFP/BSA	Antibody	0.025 pg/mL	100 fg/mL–100 ng/mL	[42]
Glucose	GCE/RGO–PAMAM–Ag nanocomposite/GOx/chitosan	Enzyme	4.5 μM	0.032–1.89 μM	[43]
t-DNA	PGE/succinamic acid/G2-PAMAM/calf thymus double stranded DNA (ct DNA) or short DNA oligonucleotide (DNA ODN)	Aptamer	4.2 μg/mL	20–120 μg/mL	[44]
L-tyrosine	GCE/PAMAM–MWCNT film/Hemin	Enzyme	10^{-8} M	0.1–28.8 μM	[45]

Table 2 (*continued*)

Target molecule	Fabrication of the biosensor	Bioreceptor	Limit of detection (LOD)	Linear range	Reference
Hepatitis B virus (HBV) t-DNA	PGE/EDC/PAMAM/DNA oligonucleotide/t-DNA	Aptamer	8.9 μg/mL	25–200 μg/mL	[46]
	GCE/EDC/NHS/PAMAM/ss-DNA/MB-ssDNA	Aptamer	10–9 M	2×10^{-9}–2×10^{-7} M	[47]
Arian influenza virus (AIV) genotype	GCE/MWCNTs–cobaltphtalocyanine (MWCNTs–CoPc)/PAMAM G4/ssDNA	Aptamer	1 pg/mL	0.01–500 ng/mL	[48]

nanoparticles on amino groups of PAMAM dendrimer, HRP was linked to the electrode surface. Because of large surface area of PAMAM dendrimers, the amount of adsorbed gold nanoparticles increased, and as a result immobilized HRP on the electrode surface also increased [10]. Liu et al. [10] found lower LOD value (2 μmol/L) because gold is a conductivite material and enhances electron transfer between electrolyte solution and electrodes.

Carbofuran is a carbamate pesticide, and there are many biosensors for its determination. They are commonly based on enzyme inhibition in the presence of carbofuran. Acetyl cholinesterase (AChE) is an enzyme which is widely used for pesticide (carbofuran) determination. The degree of the inhibition of AChE depends on the level of the carbofuran and the exposure time [33]. PAMAM dendrimer and 1-hexadecanethiol were used for the construction of the biosensor [16]. PAMAM dendrimer increased the mechanical stability of biosensor. Acetyl cholinesterase and choline oxidase was immobilized on the electrode surface. The measurement principle was based on H_2O_2 oxidation.

Glutamate is a neurotransmitter, which has a vital role in the mammalian central nervous system and neuronal pathways in the brain. Glutamate was detected by using enzymatic biosensors which use glutamate dehydrogenase and glutamate oxidase as recognition elements for glutamate detection. PAMAM dendrimer–platinum nanoparticles (PtNPs) conjugate and multiwall carbon nanotubes (MWCNTs) were used for the construction of the biosensor [18,41]. In the biosensor, which used glutamate dehydrogenase as recognition element, the detection principle was based on chemical oxidation of glutamate. After addition of glutamate, an increase in current was observed. The principle of glutamate oxidase biosensor was based on monitoring the reduction current of glutamate. The current was proportional to glutamate level. Another study for glutamate detection was performed by using glutamate oxidase and MWCNTs/AuNPs/chitosan composite film [52]. The detection limit was higher than the PAMAM dendrimer modified biosensor. Because PAMAM dendrimer has large surface area for enzyme to bind, more enzyme causes more signal and low detection limits.

Estradiol (17β-estradiol, E2) is a natural estrogen that has a vital role in various physiological processes and it affects reproductive and sexual functioning [8]. Graphene-polyaniline, HRP–graphene oxide-antibody conjugates and PAMAM–gold nanoparticles (AuNPs) composites were used for the construction of the biosensor [27]. There are a number of surface groups of the PAMAM dendrimer which caused a lot of HRP and anti-estradiol immobilization on the electrode surface and graphene–polyaniline composites were used to facilitate electron transfer between electrode and electrolyte solution, and to increase the stability of the biosensor. The principle of the biosensor was based on the reduction of hydrogen peroxide and the reduction current was monitored as amperometric measurements. This biosensor was successful for the detection of estradiol in water and milk.

Avian Influenza Virus (AIV) infection is a fatal disease in poor countries. A genosensor was fabricated by using MWCNTs–CoPc nanocomposite and PAMAM conjugate [48]. MWCNTs–CoPc nanocomposite was immobilized on the electrode. PAMAM dendrimer provided amino groups on the electrode, these groups formed covalent bounds with carboxyl groups of the activated MWCNTs. Consequently, DNA probes were immobilized on the electrode via covalent coupling between amino groups of PAMAM dendrimer and 50-end phosphate groups of DNA. PAMAM dendrimer gave some freedom to the oligonucleotide for movement and made the effect of steric hindrance easier during the hybridization. So, a stable biosensor was performed. At the same time MWNTs–CoPc nanocomposite increased the oxidation signal of guanine nucleobase and provided sensitive detection of DNA. Another biosensor was constructed by using 16-

mercaptohexadecanoic acid and NHS-EDC chemicals [53]. The LOD values were 5 μg/mL (5×10^6 pg/mL) and 1 pg/mL, respectively. This lower limit of detection can be related with large surface area of PAMAM.

The studies mentioned above, which based on amperometric PAMAM dendrimer biosensors, were investigated and found that PAMAM dendrimer enhances the peak currents and enlarges the electrode surface due to a lot of amine groups on its surface. In most of literature studies, PAMAM dendrimer was used with nanoparticles such as gold nanoparticle. Gold nanoparticles were used mostly for developing a PAMAM dendrimer based biosensor due to its high conductive property. The amine groups on the PAMAM dendrimer surface has high affinity to gold nanoparticles. Because of its spherical structure, the large surface area and density of amine groups on the electrode, the amount of adsorbed nanoparticles on the electrode is increased and as a consequence the amount of immobilized biorecognition element is increased. For binding of biorecognition elements on the PAMAM surface different bioconjugating reagents were used such as glutaraldehyde for immobiling of enzymes and antibodies. They bind to PAMAM dendrimer due to crosslinking property of glutaraldehyde. Thus a suitable environment is formed on the electrode surface. Apart from these properties of PAMAM dendrimer, it supports the enzymatic and catalytic reactions, and increases the lifetime stability of the electrode.

3.2. Impedimetric biosensors based on PAMAM dendrimer

Impedimetric measurements are successful to monitor changes in electrical properties at modified electrode surfaces. These changes are based on biorecognition events such as antigen–antibody interaction, probe DNA-target DNA hybridization. Electrochemical impedance spectroscopy (EIS) is an effective technique for characterization and fabrication of a biosensor. EIS can be effectively used for the quantification of target molecules. The representative Nyquist plots of EIS spectra shows the forming of different layers during the modification of electrode. Characteristically, an impedance spectrum includes semicircle part and a linear part. The semicircle part, at high frequencies associated with the electron transfer limited process, whereas the linear part shows the diffusion limited process. Also, each reaction process is shown by an electric circuit which includes resistance, capacitors, or constant phase elements arranged in parallel or in series. The Randles–Ershler electric equivalent circuit model was mostly used for monitoring electrochemical reaction. It consists of an ohmic resistance (R_s), a charge transfer resistance (R_{ct}), a double layer capacitance and a warburg impedance (Z_w). Ohmic resistance shows the resistance of the electrolyte solution between working and reference electrode. The charge transfer resistance is known as the diameter of semicircle. The double layer capacitance is the capacitance of the complex bioactive layer. Warburg impedance shows the normal diffusion between the electrode and the complex layer. In an electrochemical composition, the resistance of electrolyte solution depends on the bulk features of the electrolyte solution [8]. Table 3 summarizes impedimetric biosensors with PAMAM dendrimer in literature.

Prostate-specific antigen (PSA) is one of the first tumor biomarkers for screening and diagnosis of prostate cancer [49]. High-branched PAMAM dendrimer has adequate functional groups for chemical bonding. These groups increase the amount of antibodies on the electrode surface, and thus it increases the sensitivity and the selectivity of electrochemical analysis. A PSA immunosensor was fabricated by using MWCNT-ionic liquid chitosan (Chit), thiol terminated PAMAM–AuNPs composite and HRP-labeled anti-PSA bioconjugate [54]. PAMAM dendrimer provided large surface area but it decreased the conductivity of modified electrode and limited

Table 3
Impedimetric biosensors based on PAMAM dendrimers.

Target molecule	Fabrication of the biosensor	Bioreceptor	Limit of detection (LOD)	Linear range	Reference
PSA	GCE/MWCNTs-ionic liquid (IL) chitosan (Chit)/thiol terminated PAMAM–AuNPs/anti-PSA/BSA	Antibody	0.5 ng/mL	5–25 ng/mL	[54]
S. typhimurium	GCE/PAMAM–MWCNT-Chi/AuNPs/mercaptoacetic acid (MACA)/EDC/NHS/anti-Salmonella/BSA	Antibody	5×10^2 CFU/mL	10^3–10^7 CFU/mL	[55]
Nitrite	GCE/MWCNT–PAMAM-Chit/Cyt-C	Enzyme	0.01 μM	0.1–29 μM 29–254 μM	[56]
Thrombin (TB)	Au/Cys/GA/PAMAM G4-NH_2/GA/TB aptamer	Aptamer	0.01 nM	1–50 mM	[57]
Vascular endothelial growth factor (VEGF)	Au/Cys/PAMAM+GA/VEGFR-1/BSA	Antibody	5 pg/mL	5–125 pg/mL	[58]
Activated protein C (APC)	SPE/carboxylated poly(amidoamine) (PAMAM)/aminolinked APC aptamer	Aptamer	0.74 μg/mL	5–12.5 μg/mL	[59]
APC	8-channel screen printed electrode/PAMAM dendrimer with 16 succinamic acid surface groups/amino linked DNA aptamer	Aptamer	1.81 μg/mL in buffer	0–7.5 μg/mL	[60]
Target DNA	Au/2-aminoethanthiol (AET)/GA+ PAMAM/GA/probe DNA	Aptamer	3.8×10^{-12} M	10^{-11}–10^{-8} M	[61]
α-synuclein (α-SYN)	GCE/poly-o-aminobenzoic acid (poly-o-ABA)/EDC/NHS/PAMAM–Au/thionine/α-SYN/BSA/Ab_1/HRP-Ab_2-AuNPS	Enzyme	14.6 pg/mL	20 pg/mL–200 ng/mL	[62]
benzo[a] pyrene (BaP)	Au/2-amino-5,2′:5′2″-terthiophene (poly-ATT)/GA/PAMAM/GA/BaP/HRP/Ab_1/multi-HRP–SiO_2–Ab_2 bioconjugates	Enzyme	6 pg/mL	0.01–2 ng/mL	[63]
t-DNA	GCE/MWCNTs/G2-PAMAM/Probe DNA-EDC	Aptamer	0.1 pM	0.5–500 pM	[64]

Table 4
Electrochemiluminescence biosensors with PAMAM dendrimers.

Target molecule	Fabrication of the biosensor	Bioreceptor	Limit of detection (LOD)	Linear range	Reference
Thrombin (TB)	GCE/Chi-Graphene/AuNPs/thrombin aptamer (TBA)/MCH/TB/PAMAM–PtNPs/Ru-prolidase (GPDA)–TBA bioconjugate	Aptamer	5 fmol/L	0.01 pmol/L–10 nmol/L	[67]
DNA	Au/poly(diallyldimethylammonium chloride) (PDDA)-CNTs/AuNPs/thiolated DNA/6-mercapto-1-hexanol (MCH)/t-DNA/PAMAM/CdSe–CdS QD-DNA Probe	Aptamer	3.2 fmol/L	0.01–10 pmol/L	[68]
Cancer cells	Fe_3O_4@Au magnetic nanoparticles (MNPs)/thiol-modified aptamer/PAMAM-QDs-DNA probe/cell/HNO_3		89 cells/mL	160–15360 cells/mL	
AFP	GCE/Nafion–Ru–PtNPs/Ab_1/BSA/Ab_2 conjugate (Ab_2/AuNPs–PAMAM/PEI–rGO)	Antibody	3.3 fg/mL	0.01 pg/mL–10 ng/mL	[69]
t-DNA	GCE//AuNPs/hairpin DNA molecular beacon (MB) probe with a thiol group at 5′ end and an amino group at 3′ end/t-DNA/GA+CdSQDs-PAMAM	Aptamer	2.5 aM	5 aM–0.1 nM	[70]
IgG	ITO/SWCNTs/NHS-EDC/$[Ru(byp)_2PICNH_2]^{2+}$/anti-IgG/casein/IgG-G1.5 PAMAM-$[Ru(byp)_2PICNH_2]^{2+}$–SiNP bioconjugates	Antibody	1.1 ± 0.1 pM	20 pM–300 nM	[71]
CEA	GCE/AuNPs/anti-CEA(Ab_1)/BSA/CEA/PAMAM-Cysteine/hollow gold nanospheres (HGNPs)–Ab_2 conjugates	Antibody	6.7 fg/mL	20 fg/mL–1 ng/mL	[72]
Mercury ion	GCE/SWCNTs/EDC/NHS/spacer/Hg^{+2} spesific oligonucleotide (MSO)/$Ru(bpy)_2^{+3}$ loaded G4-PAMAM	Aptamer	2.4 pM	7 pM–50 nM	[73]
CA15-3	GCE/AuNPs/anti-CA 15-3/BSA/CA 15-3/ZNs–PAMAM–luminol–Ab_2 bioconjugate	Aptamer	0.033 U/mL	0.1–120 U/mL	[74]

electron transfer. To overcome this handicap, gold nanoparticles were used in this study. Before measurement, immunosensor was incubated with different concentrations of PSA and excessive HRP-labeled PSA antibody. After addition of H_2O_2 the reduction peak current of thionine increased due to electrocatalytic activity of immobilized HRP toward H_2O_2 reduction while related oxidation peak current decreased. These results indicated that the attached HRP retained its catalytic activity toward H_2O_2 reduction and thionine was a suitable electron transfer mediator. The immunosensor response was based on the redox process. The biosensor response was compared with ELISA and there was no difference between the results. Also the proposed immunosensor can be successfully applied for PSA detection in real samples. In another study, 16-mercapto-1-hexadecanoic acid, 11-mercapto-1-undecanol, gold nanoparticles were used for the construction of sandwich immunosensors [65]. When compared the detection limits of these biosensors, PAMAM modified immunosensor had lower detection limits. PAMAM dendrimer has a high density of terminal group for antibody immobilization.

Salmonella typhimurium (*S. typhimurium*), a gram-negative bacteria, causes food-borne illnesses. A biosensor was developed by attaching anti-Salmonella antibodies onto electrode which include AuNPs and PAMAM dendrimer-MWCNTs-Chit nanocomposite [55]. MWCNT, PAMAM and AuNPs increased the sensitivity of the biosensor. Gold nanoparticles bonded to PAMAM dendrimer via amido-gold affinity. Because of large surface of PAMAM dendrimer, the loaded quantity of AuNPs increased. So the amount of antibody on the electrode increased. MWCNTs increased the conductivity of the biosensor and enhanced electron transfer between electrode surface and electrolyte solution. The immobilization of anti-Salmonella on the electrode surface and the interaction with Salmonella cells caused an increase in the electron transfer resistance (R_{ct}). A linear relationship between R_{ct} and Salmonella concentration was obtained.

Vascular endothelial growth factor (VEGF) has a vital role in regulation of angiogenesis and vascular permeability and is used as a biomarker for breast cancer detection [49]. A biosensor was fabricated for VEGF determination and PAMAM dendrimers were used for the construction of their biosensor [58]. The PAMAM dendrimers were bound to self-assembled monolayer via covalent coupling. PAMAM dendrimer had amino groups on its surface and vascular endothelial growth factor receptor-1 (VEGF-R1) was attaced on the surface via glutaraldehyde cross-linking. An impedimetric immunosensor was developed for VEGF determination and VEGF-R1 was covalently attached by coupling with 11-mercaptoundecanoic acid on gold electrodes [66]. The biosensors' LOD values were 5 pg/mL and 1 ng/mL. Because of high surface area of PAMAM dendrimer, higher amount of VEGF-R1 was loaded on the electrode surface and thus the LOD value of the first biosensor was found to be lower.

Based on the studies, it can be concluded that, impedimetric PAMAM dendrimer biosensors enlarge the electrode surface owing to primary amine groups on its surface, thus the amount of biorecognition molecules increases and this increment causes lower detection limit. PAMAM dendrimer is positively charged and a redox probe can access to the immobilized PAMAM, so it improves the speed of electron transfer between electrolyte and electrode, which causes a small Nyquist plot diameter and charge transfer resistance.

3.3. Electrochemiluminescence (ECL) biosensors based on PAMAM dendrimer

Electrochemiluminescence (ECL) is a sensitive and selective detection technique and mostly used in clinical diagnosis, environmental and food analysis. It has wide dynamic concentration

response range. Generally, for the ECL techniques, three ECL reagents including luminol, quantum dots and tris(2,2′-bipyridyl) ruthenium (II)($Ru(bpy)_3^{2+}$) or their analogues have been adopted. Among them, $Ru(bpy)_3^{2+}$ and their analogues are widely used due to their low oxidation potential, cheap reagent consumption, and the high emission yields [67]. Table 4 summarizes electrochemiluminescence biosensors with PAMAM dendrimer in literature.

Carcinoembrionic antigen (CEA) is used as a biomarker for clinical diagnosis of colon tumors, breast tumors, ovarian carcinoma, gastric, pancreatic, lung carcinomas [49]. An ECL biosensor was fabricated by using PAMAM dendrimer-L-cysteine-hollow gold nanospheres nanocomposite for CEA detection. L-cysteine was bound to the carboxyl-terminated PAMAM dendrimers by using EDC/NHS coupling. The hollow gold nanospheres (HGNPs) coupled on cysteine modified electrode via thiol-gold bonding. Then anti-CEA (Ab_2) conjugated onto PAMAM-L-Cys-HGNPs. After addition of CEA, the sandwich immunosensor was formed between the anti-CEA (Ab_1) and the Ab_2-PAMAM-L-Cys-HGNPs. And ECL response was obtained. In this sensor, PAMAM dendrimer was used in ECL reactions and it amplified the signal, thus a sensitive biosensor was fabricated [72].

A novel solid-state $Ru(bpy)_3^{2+}$ ECL immunosensor was constructed by Xiang and co-workers for AFP detection. The structure of biosensor included poly(ethylenimine) (PEI) functionalized reduced graphene oxide (PEI–rGO) and AuNPs on PAMAM dendrimers. PEI was used as co-reactant and it enhanced the ECL response. Graphene oxide was used for increasing the amount of PEI. Also the larger surface area increased the loading amount of PEI, thus graphene oxide was chosen. This PEI–rGO composite increased the ECL signal and the conductivity of the biosensor. PAMAM dendrimer was another co-reactant. Because of its large surface area, it increased the amount of immobilized antibody. For increasing the conductivity, gold nanoparticles was used. AuNPs–PAMAM conjugate enhanced the ECL signal. The capture antibody (Ab_2) was attached on the Nafion–Ru–PtNPs complex. Nafion–Ru–PtNPs complex, which was an ECL substrate, enhanced the ECL stability. The detection antibody (Ab_2) immobilized on AuNPs–PAMAM/PEI–rGO nanocomposite. In the presensce of AFP, the sandwich immunoreaction was occurred between capture antibody and detection antibody labeled AuNPs–PAMAM/PEI–rGO. There was a relationship between ECL intensity and AFP level [69].

Thrombin has important role both of procoagulant and anticoagulant reactions [57]. An ultrasensitive electrochemiluminescence (ECL) aptasensor was developed for thrombin (TB) determination. PAMAM dendrimer combined with PtNPs was used as platform to assemble substantial Ru, prolidase (GPDA) and avidin labeled thrombin aptamer (avidin–TBA) to form PAMAM–PtNPs–Ru–GDPA–TBA bioconjugate. The bioconjugate was successfully modified on the electrode surface. The proposed aptasensor had three attractive advantages: Firstly, PAMAM dendrimer acted as a matrix for PtNPs, prolidase (GPDA) and avidin–TBA immobilization. Secondly, it was used as a co-reactant of Ru to amplify the ECL signal. Thirdly, using the co-reactant and luminescence reagent together onto the electrode surface could effectively shorten the reaction time, increased the electron transfer, and enhanced the ECL response [67].

Carbohydrate antigen 15-3 (CA 15-3) is used as a biomarker for breast carcinoma patients with distant metastases [49]. A novel electrochemiluminescence (ECL) biosensor was fabricated for sensitive determination of CA 15-3. In this study, gold nanoparticles was used as the sensor platform which had large surface area and ZnO nanorods (ZNs)–PAMAM–luminol-anti-CA 15-3 secondary antibody (Ab_2) was used as signal probe. In the presence of CA 15-3 antigen an immunocomplex was occurred between the ZNs–PAMAM–luminol–Ab_2 onto the electrode surface and a signal is formed through this reaction. ZNs–PAMAM dendrimer was used as a carrier. Because of highly-branched structure of PAMAM dendrimer, a vast of luminol was bound to the carboxylated PAMAM dendrimers via luminol's amino groups. Also, a sensitive ECL immunosensor was developed. The catalytic activities of ZNs, a vast of luminol and excellent conductivity should have been good effect on the sensitivity of the biosensor [74].

It is apparent that, electrochemilunescence PAMAM dendrimer biosensors are not only a good support material onto which biorecognition element are immobilized, but also they are a good co-reactant for enhancing the ECL signal of ECL reactants. Addition of a coreactant on the electrode surface causes short reaction time, enhances the electron transfer kinetics and amplifies the ECL signal.

3.4. Potentiometric biosensors based on PAMAM dendrimer

In potentiometric measurements, the potential variation between both a working and a reference electrode is determined by a voltammeter when there is insignificant current flowing through them. The potential difference is measured due to the oxidation and reduction of the species in sample solution. Also, potentiometry gives data about the flowing ions in an electrochemical reaction. The transducer may be an Ion Selective Electrode (ISE) based on thin film or selective membranes as biorecognition units. Analytical information is obtained when the ISE converts the biorecognition process into a potential signal [8]. Table 5 summarizes potentiometric biosensors with PAMAM dendrimer in literature.

Yao and co-workers fabricated a biosensor based on ENFETs for direct glucose determination [75]. They used dendrimer encapsulated PtNPs and GOx for the construction of their biosensor. GOx was immobilized on amine groups of PAMAM dendrimer via covalent coupling. The sensitivity was increased by using Pt nanoparticles and dendrimers. Siqueira et al. developed field-effect biosensors with layer-by-layer films containing SWCNTs and PAMAM dendrimers for penicillin determination. For penicillin determination penicillinase enzyme was used and it was attached onto the electrode surfaces, via constant-capacitance measurements [76–79].

Table 5
Potentiometric biosensors based on PAMAM dendrimers.

Target molecule	Fabrication of the biosensor	Bioreceptor	Limit of detection (LOD)	Linear range	Reference
Glucose	ISFET/poly(allylamine) hydrochloride (PAH)/GOx/PAMAM–PtNPs	Enzyme	0.15 mM	0.25–2 mM	[75]
Penicillin	SiO_2 layer/Ta_2O_5/PAMAM–SWCNT film/Penicillinase	Enzyme	5 μM	5 μM–25 mM	[76]
Penicillin G	P–Si–SiO_2–Ta_2O_5 chip/SWCNT–PAMAM layer by layer films/ penicillinase	Enzyme	–	25 μM–10 mM	[77]
Penicillin G	P–Si–SiO_2–Ta_2O_5 chip/SWCNT–PAMAM layer by layer films/ penicillinase	Enzyme	10^{-4} M	10^{-4}–10^{-3} M	[78]
Penicillin G	Light addressable potentiometric sensor/SWCNT–PAMAM layer by layer films/penicillinase	Enzyme	0.5×10^{-3} M	0.5×10^{-3}–5×10^{-3} M	[79]

In the studies which based on potentiometric PAMAM dendrimer biosensors, PAMAM dendrimers were used with other nanomaterials such as platinum nanoparticles, single walled carbon nanotubes. This nanocomposites act as a proper porous membrane with larger surface area. So, the amount of biorecognition molecules increases and more biorecognition molecules provide a low detection limit.

4. Chemical sensors based on PAMAM dendrimer

According to IUPAC, chemical sensor is identified as an apparatus that converts chemical data into measurable signal. The chemical data originates from a chemical reaction of the analyte or from a physical feature of the system [80]. An electrochemical sensor mostly consists of a working electrode, a counter electrode and a reference electrode. Enzymes, antibodies, aptamers and other biorecognition elements are not used for the construction of chemical sensors [81]. Table 6 summarizes sensors with PAMAM dendrimer in literature.

Dopamine is a neurotransmitter, which has a vital role in the regulation of the central nervous, cardiovascular, renal and hormonal systems [94]. Amperometric sensors were fabricated by using hydroxyl-terminated PAMAM dendrimers and metal nanoparticles such as Pt nanoparticles for dopamine determination [88,94]. The synthesis of Pt nanoparticles in PAMAM dendrimers were performed with chemical reactions. Metal nanoparticles were used as a catalyst for the electro-oxidation of dopamine and PAMAM acted as excellent electrode matrix. Another sensor was developed by using sulfonated β-cyclodextrin doped polypyrrole [96]. When compared the detection limits of these biosensors, PAMAM dendrimer modified biosensor had lower LOD. This might be metal nanoparticle's conductivity and PAMAM dendrimer's large surface area.

Bisphenol A (BPA) is one of the endocrine disruptor, which interferes the endocrine system of living organisms, increase cancer disease, defect immune system and damage reproduction system. For the fabrication of sensors for bisphenol determination, nanoparticles such as quantum dots and Fe_3O_4 nanoparticles and PAMAM dendrimer were used [83,97]. Nanoparticles increased the conductivity of the electrode and enhanced the electron transfer, PAMAM dendrimers provided large surface area for immobilization of BPA. The principle of biosensors was based on the enhanced oxidation peak current and the lowered oxidation potential. Under the optimum circumstances, the oxidation currents increased linearly with the increasing concentrations of BPA. Both of these biosensors' specificity, sensitivity and reproducibility were investigated. The recovery test illustrates that these biosensors could be used for BPA analysis in food such as milk. Another sensor was modified with polyglutamate acid and amino-functionalized carbon nanocomposite for BPA detection. Due to its large surface area, it provided more BPA immobilization onto the electrode, which led to an increase in the sensitivity of the sensor [97].

Nitrite is a source of nitrogen in green plants and used as preservatives and fertilizing agents. But, nitrite can react irreversibly with hemoglobin thus producing methemoglobin which reduces blood capacity to transport oxygen. A biosensor was developed for nitrite determination by using Ag–PAMAM nanocomposites [93]. The Ag–PAMAM modified electrode had a wonderful electrocatalytic performance for the oxidation of nitrite. They found that the oxidation current was related with nitrite concentration. Another study was performed by using polythionine/carbon nanotube nanocomposite for modification of the biosensor [98]. Polythionine and carbon nanotube served as a mediator and conductive material, respectively. They accelerated the electron transfer. When the detection limits of these biosensors were compared, lower detection limit was found in PAMAM dendrimer modified biosensor. Large surface area, prominent electrical conductivity and good stability of PAMAM dendrimer affected the detection limit positively. Also it acted as an electrochemical sensing interface.

When the studies which based on chemical PAMAM dendrimer biosensors are inspected it can be concluded that PAMAM dendrimer enlarges the surface area and provides excellent electrocatalytic performance. The catalytic redox reactions are increased due to active sites on PAMAM dendrimer. The using of the PAMAM dendrimer in chemical determining of a negative charged molecule on the electrode surface is based on the interaction between negative charged molecule and positive charged PAMAM dendrimer. PAMAM dendrimer can be immobilized easily onto the electrode surface via polyvalent interactions such as surface coating or cross-linking. In the presence of dendrimer, metal nanoparticles do not aggregate and the metal nanoparticles overcome the conductivity limitation of the dendrimers. Thus, the electrode surface and conductivity of electrode is increased and lower sensitivity is obtained.

Table 6
Sensors based on PAMAM dendrimers.

Target molecule	Fabrication of the biosensor	Transducer	Limit of detection (LOD)	Linear range	Reference
H_2O_2	Au/MPA/PAMAM + EDC/AuNPs/Prussian Blue (PB)	Amperometric	–	6×10^{-6}–10^{-2} M	[82]
Bisphenol A (BPA)	GCE/CoTe QDs/PAMAM	Amperometric	10^{-9} M	0.013–9.893 μM	[83]
Chlortetracycline (CD)	Au/β-cyclodextrin (CD)-MWCNTs with amine functional group/Au–PAMAM/CTC–MIPs	Amperometric	4.954×10^{-8} mol/L	5×10^{-9}–5×10^{-2} mol/L	[84]
Dopamine	Au/AuNPs/CdS–PAMAM	ECL	0.012 μmol/L	0.05–10 μmol/L	[85]
BPA	GCE/Fe_3O_4/PAMAM	Amperometric	10^{-8}–3.07×10^{-6} M	0.005 μM	[86]
Potassium	Au/MPA/PAMAM/NiHCF	Potentiometric	0.024 mol/L	–	[87]
	Au/AET/PAMAM/NiHCF		0.014 mol/L		
Dopamine	GCE/Phthalic anhydride/PAMAM–Pt	Amperometric	19 ppb	–	[88]
humidity	Polyester substrate (PET)/G1 PAMAM–NH_2–AuNPs	Impedimetric	–	30–90 (%)	[89]
Oxalic acid	GCE/PAMAM–MWCNTs/PdNPs	Amperometric	0.02 mM	0.03–5 mM	[90]
Cu^{2+}	GCE/MWCNTs/PAMAM/dithiobis[succinimidylpropionate] (DSP-AuNPs)/Cys-AgNPs	Amperometric	0.48 nM	1–1000 nM	[91]
Dopamine	Pencil lead/PAMAM G4.0-64OH	Amperometric	6.67 nM	0–20 μM	[92]
Nitrite	GCE/Ag–PAMAM nanocomposite	Amperometric	0.40 μM	4–1440 μM	[93]
Dopamine	GCE/PAMAM–Ir	Amperometric	0.97 μM	–	[94]
	GCE/PAMAM–Pt		0.82 μM	–	
	GCE/PAMAM/Rh		0.51 μM	–	
Uric acid	GCE/AuNPs@Cys/PAMAM-EDC	Amperometric	1.7×10^{-4} mg/dL	–	[95]

5. Conclusions and future outlooks

PAMAM dendrimers have received considerable attention in recent years owing to their unique structure, excellent features for application in clinical, food and environmental analysis. Also they are hyper-branched and monodisperse polymers and have three dimensional and globular structure. They have good structural homogenity, controllable size and surface functionality. They have a vast number of active groups outside the terminal surface. Because of these properties, they increase the surface area of electrode and they are ideal matrix for immobilizing biomolecules. As a PAMAM dendrimer has multiple sites to bind to antibody, enzyme and DNA. Thus, by using PAMAM dendrimer the signal of biosensor is amplified. The conductivity of PAMAM dendrimer is not good, and that obstructs its wide application in electrochemical biosensors and sensors. Metal nanoparticles such as gold, platinum, silver, conductive polymers such as poly(3,4-ethylenedioxythiophene) (PEDOT) enhances the conductivity of electrodes, so it facilitates the electron transfer between electrode and electrolyte solution. Also, the sensitivity, specificity and stability and reusability of biosensors are enhanced. Graphene, which is the thinnest material in our universe, is an ideal support material for electrochemical biosensors and sensors. Also, high electrical conductivity and large surface area make them potential matrix for development of a biosensor. The large surface area of graphene enhances the surface loading of desired biomolecules such as antibodies, enzymes. Thus, the electrochemical detection response amplifies, detection limit decrease and the sensitivity of biosensor is increased. Other support materials such as mercoptoacids (16-mercaptohexadecanoic acid, 11-mercaptoundecanoic acid), 1-hexadecanol, 11-mercapto-1-undecanol, polypyrrole, polythionine have been used for development of biosensors. When compared with these biosensors in which these materials are used as a support, PAMAM dendrimer based biosensors have lower detection limits and high sensitivity. PAMAM dendrimer has a lot of functional groups outside its surface, so it causes a decrease at the detection limits and an increase at the sensitivity of measurement.

This review shows recent improvements in the application of PAMAM dendrimer for electrochemical biosensing and sensing (e.g., amperometric, impedimetric, potentiometric, electrochemiluminescence). PAMAM dendrimer not only improves the sensitivity of electrochemical techniques but also provides a series of applications. PAMAM dendrimers are also important structures in biosensor technology for immobilization of biorecognition molecules and for increasing biosensor stability. Also, electrochemical biosensors and sensors based on PAMAM dendrimer show excellent promise for future applications in clinical diagnosis, environmental monitoring and food process control. We hope that this review has illustrated the success of PAMAM dendrimer-based electrochemical biosensors and sensors in literature and it will guide the researchers to study on new, easy-to use biosensors and sensors development.

Taking into consideration the properties of PAMAM dendrimers, which is discussed above, and its applications in electrochemical and chemical sensor, we conclude that PAMAM dendrimer-based electrochemical biosensors and sensors are applicable for the determination of biochemical molecules such as cancer and cardiac markers, metabolites, proteins, target DNA of diseases and chemical compounds such as anticancer drugs, pesticides and heavy metals. The innovations in PAMAM dendrimer-based biosensing and sensing devices will provide portable biosensors detecting biological and chemical molecules in situ and in real time.

Acknowledgment

Support from TÜBİTAK (The Scientific and Technological Research Council of Turkey, Project number: 113 Z 678) is greatly acknowledged.

References

[1] P. Su, S. Wang, Y. Shi, Y. Yang, Process Biochem. 48 (2013) 614–619.
[2] F. Aulenta, W. Hayes, S. Rannard, Eur. Polym. J. 39 (2003) 1741–1771.
[3] P. Kesharwani, K. Jain, N.K. Jain, Prog. Polym. Sci. 39 (2014) 268–307.
[4] N.T.P. Azar, P. Mutlu, R. Khodadust, U. Gunduz, J. Hacettepe, Biol. Chem. 41 (2013) 289–299.
[5] M. Hasanzadeh, N. Shadjou, M. Eskandani, J. Soleymani, F. Jafari, M. Guardia, Trends Anal. Chem. 53 (2014) 137–149.
[6] A. Bosnjakovic, Wayne State University Dissertations, 2011, pp. 1–10.
[7] A. Sassolas, L.J. Blum, B.D. Leca-Bouvier, Biotechnol. Adv. 30 (2012) 489–571.
[8] E.B. Bahadır, M.K. Sezgintürk, Biosens. Bioelectron. 68 (2015) 62–71.
[9] H.B. Harris, B.L. Turner, Nova Science Publishers, New York, 2013.
[10] Z. Liu, Y. Yang, H. Wang, Y. Liu, G. Shen, R. Yu, Sens. Actuators B 106 (2005) 394–400.
[11] M. Akin, A. Prediger, M. Yuksel, T. Höpfner, D.O. Demirkol, S. Beutel, S. Timur, T. Scheper, Biosens. Bioelectron. 26 (2011) 4532–4537.
[12] M. Şenel, E. Çevik, Curr. Appl. Phys. 12 (2012) 1158–1165.
[13] N.B. Li, J. Kwak, Electroanalysis 19 (2007) 2428–2436.
[14] G. Shen, X. Hu, S. Zhang, J. Electroanal. Chem. 717–718 (2014) 172–176.
[15] F.N. Crespilho, M.E. Ghica, M. Florescu, F.C. Nart, O.N. Oliveira Jr., C.M.A. Brett, Electrochem. Commun. 8 (2006) 1665–1670.
[16] M. Snejdarkova, L. Svobodova, G. Evtugyn, H. Budnikov, A. Karyakin, D. P. Nikolelis, T. Hianik, Anal. Chim. Acta 514 (2004) 79–88.
[17] M. Akin, M. Yuksel, C. Geyik, D. Odaci, A. Bluma, T. Höpfner, S. Beutel, T. Scheper, S. Timur, Biotechnol. Prog. 26 (2009) 896–906.
[18] L. Tang, Y. Zhu, L. Xu, X. Yang, C. Li, Talanta 73 (2007) 438–443.
[19] D. Kim, M.A. Rahman, M.H. Do, C. Ban, Y. Shim, Biosens. Bioelectron. 25 (2010) 1781–1788.
[20] B. Kavosi, R. Hallaj, H. Teymourian, A. Salimi, Biosens. Bioelectron. 59 (2014) 389–396.
[21] Y. Yuan, G. Liu, R. Yuan, Y. Chai, X. Gan, L. Bai, Biosens. Bioelectron. 42 (2013) 474–480.
[22] G. Li, X. Li, J. Wan, S. Zhang, Biosens. Bioelectron. 24 (2009) 3281–3287.
[23] M. Şenel, C. Nergiz, Synth. Met. 162 (2012) 688–694.
[24] X. Liu, X. Zhang, Y. Zhao, W. Liu, B. Wang, Y. Zhang, Z. Zhang, Chem. Res. Chin. Univ. 26 (5) (2010) 723–728.
[25] P. Sahoo, S. Suresh, S. Dhara, G. Saini, S. Rangarajan, A.K. Tyagi, Biosens. Bioelectron. 44 (2013) 164–170.
[26] A. Miodek, G. Castillo, T. Hianik, H. Korri-Youssoufi, Biosens. Bioelectron. 56 (2014) 104–111.
[27] J. Li, S. Liu, J. Yu, W. Lian, M. Cui, W. Xu, J. Huang, Sens. Actuators B 188 (2013) 99–105.
[28] S. Liu, Q. Lin, X. Zhang, X. He, X. Xing, W. Lian, J. Huang, Sens. Actuators B 156 (2011) 71–78.
[29] M.J.A. Shiddiky, M.A. Rahman, C.S. Cheol, Y. Shim, Anal. Biochem. 379 (2008) 170–175.
[30] K. Jayakumar, R. Rajesh, V. Dharuman, R. Venkatasan, J.H. Hahn, S.K. Pandian, Biosens. Bioelectron. 31 (2012) 406–412.
[31] E. Araque, C.B. Arenas, M. Gamella, J. Reviejo, R. Villalonga, J.M. Pingarrón, J. Electroanal. Chem. 717–718 (2014) 96–102.
[32] Y. Zeng, Y. Huang, J. Jiang, X. Zhang, C. Tang, G. Shen, R. Yu, Electrochem. Commun. 9 (2007) 185–190.
[33] Y. Qu, Q. Sun, F. Xiao, G. Shi, L. Jin, Bioelectrochemistry 77 (2010) 139–144.
[34] M. Karadağ, C. Geyik, D.O. Demirkol, F.N. Ertas, S. Timur, Mater. Sci. Eng. C. 33 (2013) 634–640.
[35] K. Damar, D.O. Demirkol, Talanta 87 (2011) 67–73.
[36] D. Tang, L. Hou, R. Niessner, M. Xu, Z. Gao, D. Knopp, Biosens. Bioelectron. 46 (2013) 37–43.
[37] X. Liu, Y. Peng, X. Qu, S. Ai, R. Han, X. Zhu, J. Electroanal. Chem. 654 (2011) 72–78.
[38] M. Giannetto, L. Mori, G. Mori, M. Careri, A. Mangia, Sens. Actuators B 159 (2011) 185–192.
[39] M. Giannetto, E. Umiltà, M. Careri, Anal. Chim. Acta 806 (2014) 197–203.
[40] M. Şenel, C. Nergiz, E. Çevik, Sens. Actuators B 176 (2013) 299–306.
[41] Y. Yu, Q.Y. Sun, T. Zhou, M. Zhu, L. Jin, G. Shi, Bioelectrochemistry 81 (2011) 53–57.
[42] Q. Gao, J. Han, Z. Ma, Biosens. Bioelectron. 49 (2013) 323–328.
[43] Z. Luo, L. Yuwen, Y. Han, J. Tian, X. Zhu, L. Weng, L. Wang, Biosens. Bioelectron. 36 (2012) 179–185.
[44] G. Congur, E. Eksin, F. Mese, A. Erdem, Sens. Actuators B 201 (2014) 59–64.
[45] Q. Ma, S. Ai, H. Yin, Q. Chen, T. Tang, Electrochim. Acta 55 (2010) 6687–6694.
[46] F. Mese, G. Congur, A. Erdem, J. Electroanal. Chem. 719 (2014) 92–97.
[47] S. Wei-Jie, A. Shi-Yun, L. Jin-Huan, Z. Lu-Sheng, Chin. J. Anal. Chem. 36 (3) (2008) 335–338.
[48] X. Zhu, S. Ai, Q. Chen, H. Yin, J. Xu, Electrochem. Commun. 11 (2009)

1543–1546.
[49] E.B. Bahadır, M.K. Sezgintürk, Talanta 132 (2015) 162–174.
[50] W. Liang, W. Yi, S. Li, R. Yuan, A. Chen, S. Chen, G. Xiang, C. Hu, Clin. Biochem. 42 (2009) 1524–1530.
[52] B. Batra, C.S. Pundir, Biosens. Bioelectron. 47 (2013) 496–501.
[53] M.F. Diouani, S. Helali, I. Hafaid, W.M. Hassen, M.A. Snoussi, A. Ghram, N. Jaffrezic-Renault, A. Abdelghani, Mater. Sci. Eng. C. 28 (2008) 580–583.
[54] B. Kavosi, A. Salimi, R. Hallaj, K. Amani, Biosens. Bioelectron. 52 (2014) 22–28.
[55] J. Dong, H. Zhao, M. Xu, Q. Ma, S. Ai, Food Chem. 141 (2013) 1980–1986.
[56] Q. Chen, S. Ai, X. Zhu, H. Yin, Q. Ma, Y. Qiu, Biosens. Bioelectron. 24 (2009) 2991–2996.
[57] Z. Zhang, W. Yang, J. Wang, C. Yang, F. Yang, X. Yang, Talanta 78 (2009) 1240–1245.
[58] M.K. Sezgintürk, Z.O. Uygun, Anal. Biochem. 423 (2012) 277–285.
[59] A. Erdem, G. Congur, Colloids Surf. B 117 (2014) 338–345.
[60] A. Erdem, G. Congur, Sens. Actuators B 196 (2014) 168–174.
[61] A. Li, F. Yang, Y. Ma, X. Yang, Biosens. Bioelectron. 22 (2007) 1716–1722.
[62] Y. An, X. Jiang, W. Bi, H. Chen, L. Jin, S. Zhang, C. Wang, W. Zhang, Biosens. Bioelectron. 32 (2012) 224–230.
[63] M. Lin, Y. Liu, C. Liu, Z. Yang, Y. Huang, Biosens. Bioelectron. 26 (2011) 3761–3767.
[64] N. Zhu, H. Gao, Q. Xu, Y. Lin, L. Su, L. Mao, Biosens. Bioelectron. 25 (2010) 1498–1503.
[65] L. Huang, G. Reekmans, D. Saerens, J. Friedt, F. Frederix, L. Francis, S. Muyldermans, A. Campitelli, C.V. Hoof, Biosens. Bioelectron. 21 (2005) 483–490.
[66] E.B. Bahadır, M.K. Sezgintürk, Artif. Cells Nanomed. Biotechnol. (2014), http://dx.doi.org/10.3109/21691401.2014.962743.
[67] Y. Yuan, X. Gan, Y. Chai, R. Yuan, Biosens. Bioelectron. 55 (2014) 313–317.
[68] G. Jie, J. Zhang, G. Jie, L. Wang, Biosens. Bioelectron. 52 (2014) 69–75.
[69] C. Xiong, H. Wang, Y. Yuan, Y. Chai, R. Yuan, Talanta 131 (2015) 192–197.
[70] F. Divsar, H. Ju, Chem. Commun. 47 (2011) 9879–9881.
[71] A. Venkatanarayanan, K. Crowley, E. Lestini, T.E. Keyes, J.F. Rusling, R.J. Forster, Biosens. Bioelectron. 31 (2012) 233–239.
[72] Y. Zhuo, G. Gui, Y. Chai, N. Liao, K. Xiao, R. Yuan, Biosens. Bioelectron. 53 (2014) 459–464.
[73] F. Ma, Y. Zhang, H. Qi, Q. Gao, C. Zhang, W. Miao, Biosens. Bioelectron. 32 (2012) 37–42.
[74] X. Jiang, H. Wang, R. Yuan, Y. Chai, Biosens. Bioelectron. 63 (2015) 33–38.
[75] K. Yao, Y. Zhu, X. Yang, C. Li, Mater. Sci. Eng. C. 28 (2008) 1236–1241.
[76] J.R. Siqueira Jr., M.H. Abouzar, A. Poghossian, V. Zucolotto, O.N. Oliveira, M. J. Schöning, Mater. Sci. Eng. 25 (2009) 497–501.
[77] J.R. Siqueira Jr., M. Backer, A. Poghossian, V. Zucolotto, O.N. Oliveira, M. J. Schoning, Phys. Status Solidi A 2074 (2010) 781–786.
[78] J.R. Siqueira Jr., M.H. Abouzar, M. Bäcker, V. Zucolotto, A. Poghossian, O. N. Oliveira, M.J. Schöning, Phys. Status Solidi A 206 (3) (2009) 462–467.
[79] J.R. Siqueira Jr., C.F. Werner, M. Backer, A. Poghossian, V. Zucolotto, O. N. Oliveira, M.J. Schöning, J. Phys. Chem. C. 113 (2009) 14765–14770.
[80] A.D. McNaught, A. Wilkinson, Blackwell Scientific Publications, Oxford, 1997.
[81] D.E. Garcia, ProQuest LLC, USA, 2009.
[82] N.B. Li, J.H. Park, K. Park, S.J. Kwon, H. Shin, J. Kwak, Biosens. Bioelectron. 23 (2008) 1519–1526.
[83] H. Yin, Y. Zhou, S. Ai, Q. Chen, X. Zhu, X. Liu, L. Zhu, J. Hazard. Mater. 174 (2010) 236–243.
[84] W. Lian, J. Huang, J. Yu, X. Zhang, Q. Lin, X. He, X. Xing, S. Liu, Food Control 26 (8) (2012) 620–627.
[85] F. Sun, F. Chen, W. Fei, L. Sun, Y. Wu, Sens. Actuators B 166–167 (2012) 702–707.
[86] H. Yin, L. Cui, Q. Chen, W. Shi, S. Ai, L. Zhu, L. Lu, Food Chem. 125 (2011) 1097–1103.
[87] J.A. Lo´pez, J. Manrı´quez, S. Mendoza, L.A. Godı´nez, Electrochem. Commun. 9 (2007) 2133–2139.
[88] M.G. García, G.M.E. Armendáriz, L.A. Godínez, J. Torres, S. Sepúlveda-Guzmán, E. Bustos, Electrochim. Acta 56 (2011) 7712–7717.
[89] P.G. Su, C. Shiu, Sens. Actuators B 165 (2012) 151–156.
[90] H. Ahmar, A.R. Fakhari, M.R. Nabid, S.J.T. Rezaei, Y. Bide, Sens. Actuators B 171–172 (2012) 611–618.
[91] L. Cui, J. Wu, J. Li, Y. Ge, H. Ju, Biosens. Bioelectron. 55 (2014) 272–277.
[92] G. Armendariz, J. Manríquez, A. Santamaría, A. Herrera-Gómez, E. Bustos, OmniaScience (Chapter 6) ⟨http://dx.doi.org/10.3926/oms.122⟩, 2014.
[93] D. Ning, H. Zhang, J. Zheng, J. Electroanal. Chem. 717–718 (2014) 29–33.
[94] E.B. Bustos, M.G.G. Jim´enez, B.R. D´ıaz-S´anchez, E. Juaristi, T.W. Chapman, L. A. God´ınez, Talanta 72 (2007) 1586–1592.
[95] A.S. Ramírez-Segovia, J.A. Banda-Alemán, S. Gutiérrez-Granados, A. Rodríguez, F.J. Rodríguez, L.A. Godínez, E. Bustosa, J. Manríquez, Anal. Chim. Acta 812 (8) (2014) 18–25.
[96] C.C. Harley, A.D. Rooney, C.B. Breslin, Sens. Actuators B: Chem. 150 (2010) 498–504.
[97] Y. Lin, K. Liu, C. Liu, L. Yin, Q. Kang, L. Li, B. Li, Electrochim. Acta 133 (2014) 492–500.
[98] C. Deng, J. Chen, Z. Nie, M. Yang, S. Si, Thin Solid Films 520 (2012) 7026–7029.