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Nanomaterials based electrochemical nucleic acid biosensors for environmental monitoring: A review

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ABSTRACT

The ecosystem delivers natural services to humans and other living beings that are crucial for health, quality of life and existence. The purpose of environmental monitoring is to realize whether the quality of the environment is getting better or worse. Different types of techniques are used for environmental monitoring. Traditional methods are mostly time-consuming, involve expensive equipment and skilled personnel. Biosensors represent an exciting and pertinent approach to overcome the limitations of existing devices used in the diverse areas of environmental monitoring. It can complement laboratory-based techniques and can also be applied for remote testing. Electrochemical nucleic acid (NA) biosensor integrates the sensitivity of electroanalytical methods with the inherent bio-selectivity of NA. The acceptance of NA biosensors depends on specificity, sensitivity, small molecule detection as well as cost-effectiveness. Considering these factors, a huge number of nanomaterials have been employed to facilitate immobilization of NA as well as to enhance conductivity and sensitivity. The NA component in the biosensor detects the analyte(s) based on signal generation owing to binding or catalytic event, which is proportional to the concentration of the analyte(s). This review compiled the components and strategies of building electrochemical NA biosensors, methods of transducing NA, the process of enhancing conductivity with nanomaterials and recent progress and innovative tactics used in the field. Besides these, applications of electrochemical NA biosensors for qualitative and quantitative analysis of environmental pollutants, steroid compounds, mycotoxins, heavy metals, antibiotic residues and pesticides have been discussed with illustrations along with limitations, challenges, future directions for the electrochemical NA biosensor applications and development.

1. Introduction

Since the inception of the industrial revolution, environmental pollution has become one of the most vital issues in the world. Air pollution, water pollution, and soil contamination are the key types of environmental problems caused by numerous emerging contaminants [1]. The increasing environmental pollution owing to emerging harmful agents is a serious problem, and the release of harmful pollutants such as toxins, toxic heavy metals, pesticides, and pharmaceutical xenobiotics to the ecosystem is a global concern. In environmental monitoring, the inability to detect low sample concentration and the scarcity of selectivity and sensitivity are the substantial obstacles of traditional methods. Furthermore, a long and specialized sample pretreatment process is time-consuming. Rapid and reproducible detection of these pollutants is thus necessary considering their global impact. There is a crucial necessity to design and develop monitoring methods that will offer higher efficacy and accuracy to detect a broad spectrum of various pollutants [2,3]. In this situation, electrochemical NA biosensors have been demonstrated to be valuable tools to sense little sample, less concentration of elements by small analytical devices with simplicity, portability, ease of application, high sensitivity & selectivity [4,5].

Nevertheless, the application of biosensors has not been extended as much as expected for analytical applications, principally due to the instability of the biological recognition elements [6] and low sensitivity. A biosensor integrates a biologically active component with a suitable transducer to produce a quantifiable signal relational to the concentration of target analyte present in the sample [7]. Among the biosensors, electrochemical biosensors offer more advantages due to its robustness, easy miniaturization, a lower limit of detection, also with little analyte sizes, and competence to be employed in turbid fluids [8].

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NA possesses remarkable structural and functional characteristics [9] with high stability. Recently, the NA has emerged as an influential and multipurpose tool in the construction of biosensors [10], which offer a promising alternative for cheaper, quicker, and simpler environmental pollutants detection. NA biosensor constitutes an essential class of point-of-care analytical devices because it can convert recognition signal into an interpretable analytical signal in quicker time as compared to other methods, and thereby producing precise and sensitive results. Biosensors are composed of three parts: (a) biorecognition elements such as a NA or other components that serve as a mediator, (b) detector/transducer element, which converts a biological signal to a readable output, and (c) the signal processor, which displays the user-friendly signal way [11].

For three decades, rapid detection and monitoring have paved the way for expanding electrochemical NA biosensors. For its flexibility, ease of application in comparatively complex samples, and transportability, the electrochemical NA biosensors nowadays are one of the backbones in analytical chemistry. Mainly, electrochemistry has played a vital role in developing fabrication methods for biological processes and biosensors. Along with these, the explosion of activity in nanoparticles and nanotechnology and its huge potential has dramatically affected the biosensor technology, opening novel paths of research for the electrode components and transduction [12]. Different kinds of nanomaterials are available for NA immobilization, including gold, silver, silicon, and copper nanoparticles. Meanwhile, carbon originated materials, like graphite, graphene, and carbon nanotubes, have found application in NA immobilization on the electrode surface and facilitate sensitivity, specificity, conductivity, and selectivity [13–21].

Various categories of highly sensitive and selective NA biosensors have been established over the period. Among them, nanomaterials based electrochemical NA biosensors have attracted significant consideration for the recognition of pollutants. The high sensitivity, adaptability with recent microfabrication technologies, cost-effectiveness, and movability make these suitable candidates for a widespread of applications in the area of environmental monitoring [22]. The electrochemical NA biosensor field is currently a multidisciplinary area of study that linked the principles of chemistry, physics, and biology with the fundamentals of nanotechnology and nanoelectronics.

Works of literature are available on the application of fluorescence-based sensors for the recognition of poisonous heavy metals in the ecosystem [23]; Electrochemical biosensors using screen printed electrodes (SPE) & nanowire [2] and aptamers, DNAzymes, aptazymes for environmental pollution monitoring [24]. The existing review literature is deficient in the application of electrochemical NA biosensors for the recognition of environmental pollutants with the recent development of nanomaterials as sensitivity and selectivity enhancer. Owing to the emerging contaminants alarm and the deficiency of sensitive and selective detection procedures, diverse electrochemical NA biosensors have been advanced recently. This review aims to represent the exclusive potential of electrochemical NA biosensors with specific emphasis on the strategies of NA in electrochemical sensing, the process of improvement of electron transfer and conductivity, innovative approaches and applications of electrochemical NA biosensors for detection of steroids, mycotoxins, heavy metals, pesticides, insecticides, antibiotics. Finally, the work has been wrapped up with limitations of existing sensors, concluding remarks, and future perspectives.

2. Strategies for the building of NA-based biosensors

A biosensor consists of a biological element fabricated on a transducer surface to measure a chemical or biological quantity. The components comprise a biorecognition part, a transducer, and an electronic device consisting of an amplifier, processor, and display. The biorecognition element, basically a bioreceptor, can interact with a specific analyte; The transducer assesses this interaction and produces a signal proportional to the concentration of the analyte. The generated signal is then amplified and processed by the electronic system, enabling low concentration detection [25]. The primary detection techniques involve electrochemical biosensors, including potentiometry, amperometry, voltammetry, and electrochemical impedance measurements [26,27]. NAs or its derivatives like aptamers are extensively used as recognition components for biosensor construction, particularly in recognition of DNA, proteins, or small target molecules, and regarded as auspicious substitutes for antigen-antibodies based assay. Design strategies can be divided into two basic groups based on dynamics of NA employed for analyte(s) recognition.

2.1. Strategies used in DNA base-pairing hybridization

The electrochemical DNA biosensor is designed based on NA hybridization dynamics. The pairing of a DNA sequence with its precisely complementary sequence is based on all types of NA hybridization-based detection approach. The electrochemical detection of this hybridization typically involves monitoring the current response under potential controlled conditions [28–31]. The electrode modified with probe generally comes in contract in the solution containing the target NA, whose sequences to be detected. When the solution contains a target DNA sequence complementary to the fabricated probe, a duplex DNA structure is formed on the electrode surface. In some cases, this hybridization affair is recognized directly through the change in electrical signals. In other cases, specific electroactive indicator molecules are conjugated with DNA duplex, which changes electrochemical parameters, such as capacitance or conductance. In both cases, the amount of analytes can be measured based on signal intensity. In the development of suitable electrochemical DNA hybridization biosensors, the preparation of DNA modified electrodes and the selection of target sequence and the selectivity of electrochemical probes are of great importance [31,32]. In NA biosensors, the classical biorecognition component is a short single-stranded DNA, called probe DNA, having a unique nucleotides sequence, which is capable of hybridizing with a complementary DNA. In general, the end of the DNA probe is modified with amino, thiol, hydrazide, phosphorothioates, or biotin groups to facilitate immobilization on the electrode surface or deposited nanoparticles electrode surface. End modification introduces a site-specific group in DNA probe to facilitate covalent attachment as well as allows insertion of a spacer molecule between the probe and the surface. The inserted perpendicular spacer improves the immobilized probe's flexibility and facilitates the accessibility of the target sequence. This also extends the probe DNA sequence away from the electrode surface, decreasing the adsorption and steric effects as depicted figure (Fig. 1) [33].

Two principal requirements must be satisfied in the operation of DNA biosensors. These are: (a) high sensitivity in recognition of the nucleotide sequence and (b) high specificity for identification of complementary sequence, including reflection of a single nucleotide alteration like point mutation and single-base mismatch [34]. Hybridization based detection may apply one of the forms discussed below:

Label-free or indicator-less sensing commonly applies guanine residues in the target DNA as the analytical signal source. The guanine residues can be directly electrooxidized or oxidized by applying redox mediators [34,35]. In indicator-based sensing, noncovalent redox indicators such as groove binders, DNA intercalators, and interacting species have been used to differentiate between the ssDNA probe and the hybrid dsDNA electrode surface. These indicators can respond to the alteration of DNA quantity based on negative charge density or can identify hybrid DNA structures based on intercalators selectivity toward the duplex DNA [34,35]. Sandwich hybridization assay uses a covalently labeled reporter probe into two steps NA recognition (Capturing probe-target DNA, target DNA-reporter probe). The reporter probes are selected to hybridize with the target DNA after the sequence identified by the capturing probe to confer efficient electronic communication between the label and the electrode [34,35].

On the other hand, electrochemical molecular beacons employ hairpin-forming probes. The signal on-off is achieved by a change in con-

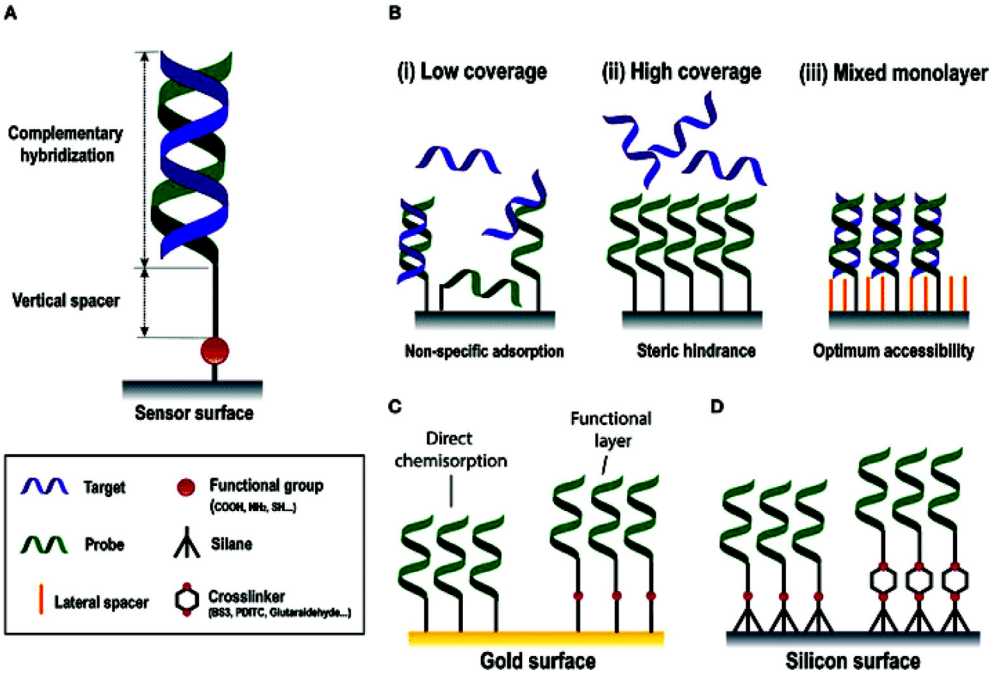

Fig. 1. NA immobilization and biosensors surface activation process. (A) Scheme of a standard DNA probe hybridization with a complimentary stand showing vertical spacer. (B) Different surface coverage patterns of electrode: (i) low, (ii) high, and (iii) mixed monolayer. (C) NA fabrication tactics on the gold electrode surface using direct chemisorption (left) and the introducing of a functional layer (right). (D) Fabrication strategies on silicon surface through silanes without (left) or with (right) crosslinkers (Reprinted from Ref. [33] with permission under Creative Commons Attribution License (CC BY)) Frontiers.

formation of a probe-bearing fluorophore and quencher at the opposite ends. Within hairpin assemblage, the label is positioned adjacent to the electrode surface and produces a distinctive electrochemical response [34,35]. In the presence of taget analyte, fluorophore and quencher separate from each other upon hybridization and show opposite electrical characteristics of its native form, which is based on the detection.

2.2. Strategies used in affinity-based NA biosensor

The affinity-based NA biosensor is known as Aptasensor. The aptamer is a short ssDNA or ssRNA molecule that can selectively bind to specific target elements, like carbohydrates, peptides, proteins, small molecules, toxins, and cells. Aptamers form unique three-dimensional conformations after interacting with its targeted ligand. Based on the relative three-dimensional orientations of ligand and aptamer in the ligand-aptamer complex, the ligand can be classified into two basic groups: (1) embedded group and (2) outside-binding group.

The ligands belong to the embedded group, are typically concealed within a minor capturing pocket formed by distinct nucleotides sequence of the aptamer. For example, small molecules, like ATP [36] and cocaine [37] are typical ligands of this group. The design plans principally concern the manipulation of the aptamers aiming embedment of the ligands. On the other hand, ligand biomacromolecules with complicated spatial structures such as proteins belong to the outside-binding group. Some proteins, like thrombin [38], platelet-derived growth factor-BB (PDGF-BB) [39], comprise more than a single aptamer-binding spot that might offer various designs in recognizing plans. Up to now, many research works have been reported connecting with the immobilization of aptamer-based biosensors. Those biosensors were built by a diversity of techniques, including optical, electrochemical, and mass-sensitive sensing. Most of those biosensors' design approaches are grouped into 4 styles: Ligand-induced structural switching on mode; sandwich or sandwich-like mode; ligand-induced displacement mode and competitive replacement mode [40]. These modes are graphically presented in Fig. 2.

In target-induced structure switching mode, the targets directly bind to its aptamers, leading to the conformational change of aptamers to define patterns, followed by the changes of measurable properties, such as the quantity of the signal (Fig. 2(I)) [40]. The sandwich mode strategies consist of a minimum of three fragments, like a piece of meat inserted within two pieces of bread. Some proteins in nature pollutants have double binding sites, which gives them the capability to attach two identifiable molecules and form sandwich-like complexes. The sandwich structures may take patterns like aptamer-pollutant-aptamer, aptamer-pollutant-antibody (Fig. 2(II)) [40]. Both the target-induced displacement mode and the sandwich mode belong to structure-dependent measurement. The assembly of this target-induced displacement mode aptamer sensors depends on the distinct conformations of the aptamers like hairpin confirmation or the selective target-aptamer complex (Fig. 2(III)). But, in the design of some biosensors, particularly for the electrochemical aptamer-based biosensors, a target induced displacement might be replaced with a structure-independent strategy [36]. In competitive replacement mode, aptamers might have similar features to antibodies. Many identification techniques that take benefit of antibodies can be developed into aptamer-based approaches. For example, most immunoassays for tiny compounds detection are competitive assays that depend on the replacement of surface-attached antibodies by the analyte in solution (Fig. 2(IV)). This replacement strategy could be pragmatic to the aptamer-based assays [40].

A simplified example for affinity mode electrochemical NA biosensor for the identification of *Salmonella* was depicted in the figure (Fig. 3) where glassy carbon electrode (GCE) was modified with Graphene oxide (GO) and gold nanoparticles (GNPs) to enhance electron transfer characteristics [43].

3. Role of nanomaterials in NA based biosensor development

Nucleic acids play vital roles in biosensing. Especially, the conjugation of functional NA probe and nanomaterials has given rise to a significant development in the field of biomolecular detection. Through its exclusive physico-chemical features, nanomaterials facilitate the sensing method and magnify the signal of detection events. Thus, the linking of NAs with various nanomaterials unlocks a promising future for molecular detection. The current developments in biosensing techniques by employing various nanoplatforms with NAs, particularly gold nanoparticles, silica nanoparticles, quantum dots, and carbon nanotubes [44] have opened up many doors for environmental pollutants detection.

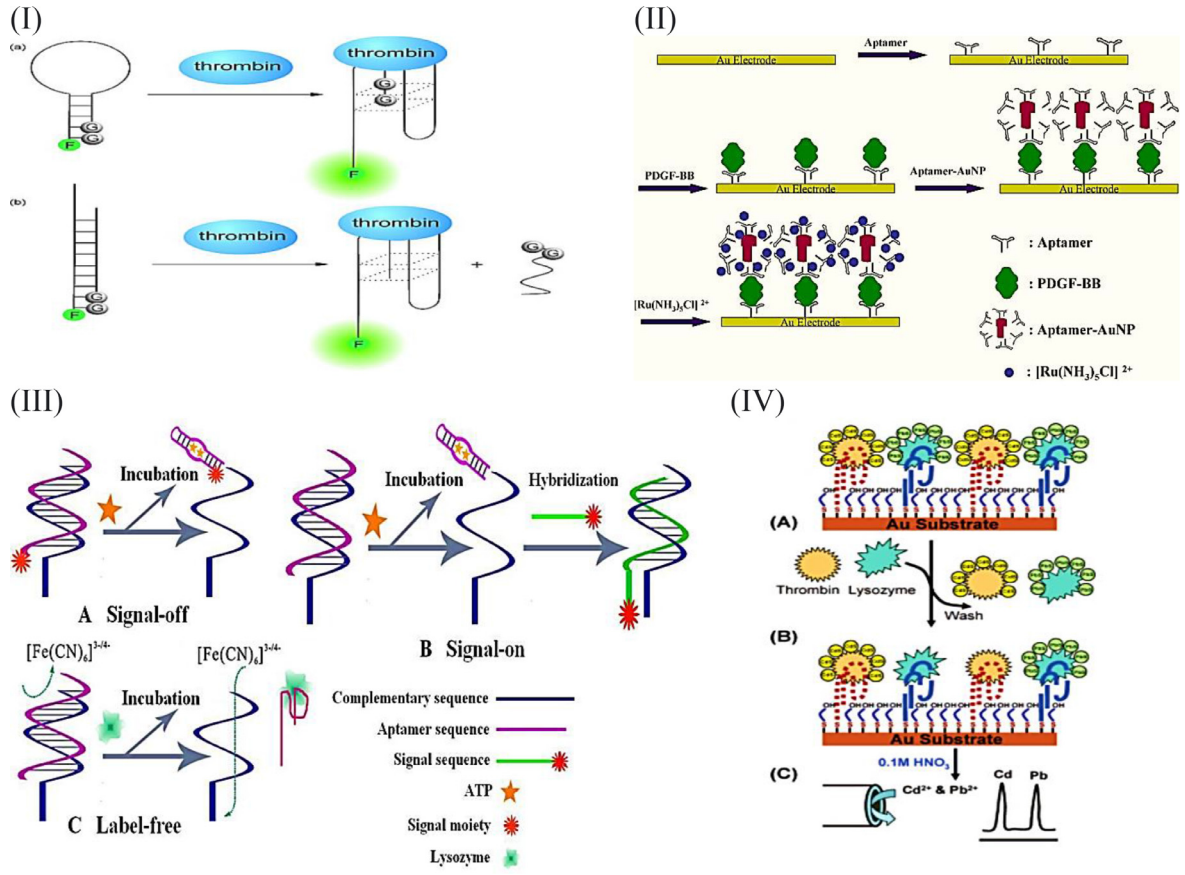

Fig. 2. Graphical illustration of (I) Ligand-induced structural switching on mode with thrombin capturing process; (II) sandwich or sandwich-like mode with platelet-derived growth factor-BB(PDGF-BB) detection process; (III) ligand-induced displacement mode with APT & Lysozyme and (IV) competitive replacement mode with thrombin & lysozyme. Reprinted (I) from Ref. [41], (II) from Ref. [39], (III) under CCBY (http://creativecommons.org/licenses/by/3.0/) from Ref. [40] and (IV) from Ref. [42], respectively with permission.

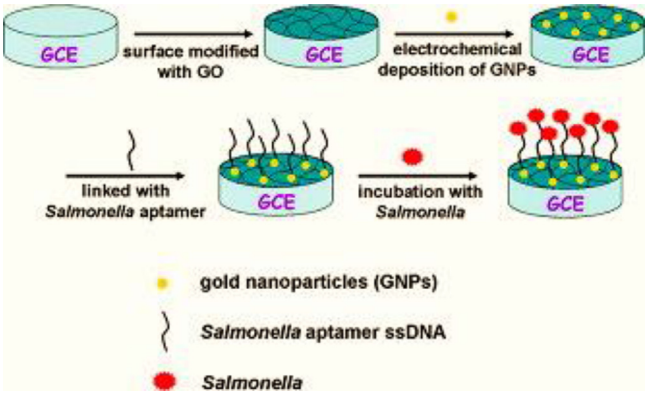

Fig. 3. An affinity-based DNA biosensor (aptasensor) for electrochemical detection of *Salmonella* (Reprinted from Ref. [43] with permission. Copyright (2014) Elsevier).

Environmental monitoring can benefit from different modes of DNA recognition besides base-pairing hybridization events. In particular, interactions of fabricated dsDNA with low molecular weight pollutants can be used for the identification of those substances. Since the poisonous characteristics of many pollutants such as carcinogens and mutagens are correlated with their interaction with DNA, it is rational to exploit interaction for designing noble environmental biosensors. The careful modification of a transducer surface, through the thoughtful fabrication of NAs detection layer, can thus form the basis for novel sensing device development and provide solutions to environmental pollutants detection [45].

Besides these, nanomaterials-based elements offer excessive sensitivity and selectivity toward developing biosensors, especially electrochemical NA sensors. Nanoparticles have found incredible importance in biosensing by serving as building blocks in nanosensing devices for various practical applications. Different nanomaterials show different performances in the biosensing process. Within the metal's nanoparticles, gold found more feasible application due to its resistance against oxidation [46] and have no toxicity. But the other nanoparticles like silver is susceptible to oxidation and have toxic manifestation when applied as theranostics. Therefore, potential challenges like bio incompatibility and toxicity of nanomaterials for biosensors application needs to be addressed before application in theranostics [47].

Also, nanoparticle-grounded signal magnification tactics have potential advantages and disadvantages [48]. Nonetheless, nanoparticles are considered vital elements in bioanalytical tools to improve the sensitivity and reduce detection limits as low as single-molecule recognition [49]. In this situation, it is valuable to mention platinum-based nanoparticles' development for intensification of the electrochemical signal to identify low DNA concentration [50]. Likewise, semiconductor quantum dots and iron oxide nanocrystals have optical and magnetic characteristics [51]. The use of different size cantilever biosensors such as nano-, micro-, and milli-cantilevers are critically examined owing to their application opportunity in numerous arenas.

Besides the element of nanoparticles, the size and shape also have a great influence on the sensing of electrochemical biosensors through the modulation of catalytic performances of oxidation and reduction

process. Studies showed that nanoparticles having the same structural and chemical parameters except size or shape have variable influences in functionality, selectivity, catalytic activity, and robustness. With the alteration in size and shape, surface chemistry and structure are also change, which modulates the catalytic behavior and sensitivity. With the reduction in size, more atoms become available on the topmost surface layer, including the portions of atoms at the corner and edge, and thus increase specific surface area. Other than this size-dependent coordination, the electronic state of a metal nanoparticle could differ or even transit from a metallic to molecular state along with the reduction of size. Nanoparticles with dissimilar shapes could have diverse crystallographic structures, density, packing, and electronic state of atoms. These size and shape-dependent features are closely linked with the surface structure and chemistry of a catalyst/sensing nanoparticle, which regulates performance [52].

Nanobiotechnology has a striking space of research in the conjugation of NAs with nanoparticles [53]. The synthesized nanoparticles should have heterogenicity on their surface for reactivity. Carbon allotrope-based nanomaterials are disfigured by a lack of surface heterogenic reactivity, which is vital for the surface fabrication of bioprobe. To improve the biomolecule addon to the functionalized surface as well as the succeeding recognition, precise synthesis of nanomaterial interface is essential.

Nanomaterials can be prepared by two main approaches: the top-down approach and the bottom-up approach. In the top-down process, a macroscale molecule is designed and controlled to construct a precise replica with a reduced dimension. This process is repeated until desirable nanoscale sizes are obtained. The bottom-up tactic is the opposite, in which larger structures are made by the assemblage of individual atoms or molecules with the help of supramolecular chemistry [54]. Though both methods play a vital role in the preparation of nanomaterial-based biosensors, the bottom-up approach have better application. At this stage, the majority of the nanomaterials need covalent or non-covalent modifications [55] to facilitate the attachment of NA probe for biosensor application. Finally, the development of hybrid nanomaterials integrates selective catalytic and recognition features to biomaterials with unique photonic, electronic, and catalytic characteristics [53], which might enhance biosensing capability.

4. Nanomaterials for enhancement of molecular recognition

Translating biological information to an easy process electronic signal is difficult owing to the limitations of linking an electronic device directly to the biological setting. The electrochemical NA biosensor offers a striking way to analyze a biological sample's elements by the direct conversion to an electronic signal [32]. The aptamers are oligonucleotide or peptide derivatives that able to capture low molecular-weight target molecules. As compared to the traditional antibodies to antigens, the aptamers own numerous exceptional characteristics such as comparatively easy to synthesis, enduring storage, high selectivity, and widespread applicability [56,57]. A huge number of nanomaterials have been proposed for the immobilization of NAs or aptamers to facilitate conductivity and sensitivity [58–60] due to many advantages. These are: (1) Nanomaterials can facilitate signal transduction. The detection signal can be amplified up to some scale orders when appropriate nanomaterials are employed as reporters [61]. (2) Nanomaterials can make detection events further effective. Nanomaterials can be adapted according to the purpose of the designed NA probes. In addition, cooperative interaction between nanomaterials and NA plays a vital role in molecular recognition [62]. (3) the unusual interactions between nanomaterials and NA make the application of nanomaterials more realistic for molecular recognition. Thus, a combination of NA design and different nanomaterials will improve innovative roles in molecular recognition. Numerous nanomaterials such as gold nanoparticles, quantum dots, silica nanoparticles, magnetic nanoparticles, and carbon nanotubes have been extensively applied in molecular recognition [44] and signal enrichment.

The gold NPs (AuNPs) or it's transformed conducting polymer was applied as a substance for immobilization on the electrode surface [63,64]. Moreover, the electroactive indicators such as ferrocene, ruthenium complexes, methylene blue (MB), ferrocene-bearing polymers, and $Fe(CN)_6^{4-/3-}$ are applied for the signal transduction [65–69]. Also, the one-dimensional nanostructured materials called single-walled nanomaterials have been more widely studied as resources for aptamers' immobilization. This is due to its exclusive characteristics, for instance, superior conductivity, rich surface chemistry, large surface/volume ratio, and excellent electrocatalytic properties [70–73]. Another approach is to use bimetallic nanowires having nanoporous and dendritic structures that provide rigorous roughness to the surface, hence induces higher electrocatalytic properties [74].

Carbon nanotubes (CNTs) are other stimulating materials for enhancing electrical conductivity in electrochemical NA biosensors. The CNTs consist of a single layer or multiple layers of sp^2 hybridized carbon atoms folded into nano-cylinders [75]. The 2 core forms of CNTs are the single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) [76]. The CNTs have achieved significant attention owing to its unique features such as good chemical, mechanical and thermal stability, high electrical conductivity, high tensile strength, and elasticity [77]. Between the two types of CNTs, the MWCNTs has gathered more attention from researchers due to its unique features such as extended surface area, sharp electrochemical response, good adsorption capacity, better chemical stability, significant electrical conductivity, and higher tensile strength [78]. Typically carbon is insoluble in water, and incorporating functionalized-MWCNTs to electrodes provides a larger surface area, higher mechanical strength, and better electrical conductivity [79].

The structure of nanoparticles is a crucial characteristic for their performance in biosensing. Nanomaterials introduce variability to the sensing platforms and permit flexibility between different detection mechanisms depending on the shape and composition. For example, nanorods may have significantly diverse characteristics from nanospheres of the same material [80]. Surface area is also related to structural properties, which result from the structural pattern of nanomaterials. The increment of surface area per unit mass results in an increment in biosensing capability due to the chemical reactivity enhancement [81].

Due to the diversity of structural characteristics, carbon allotrope-based nanomaterials have found enormous research interest in the sector of biosensing. These nanomaterials are highly valued due to the availability of a diversity of carbon allotropes, such as fullerenes, diamonds, graphite, as well as novel patterns like graphene, nanotubes, and nanohorns [82]. Each of these allotropes owns unique and inimitable structures, which lead to widespread exploitation for diverse biosensing applications. For example, fullerene displays excellent biocompatibility, structural stability, good affinity, and inert behavior towards numerous biomolecules like NA [55].

Inorganic nanomaterials may have various anisotropies, for instance, spherical, triangular, and nanohole [83]. They also appear in multiple forms, like bimetallic alloys, metal-organic framework (MOFs), core–shell structures, nanowires, and nanotube. These can improve biosensors' biocompatibility and transduction behaviors with the help of attractive interface and surface structures. They function as fabrication platforms, augment refractive index, catalyse reactions, amplify mass changes, and speed electron transfer in biosensors [55].

For enhancement of electron transfer and improvement of conductivity of electrochemical NA biosensor, lots of electrode modification evidence are available in the literature. Zheng et al. (2014) reported an electrochemical pseudo-tri-enzyme aptasensor based on Pt-Pd nanowires amplification and hemin/G-quadruplex [84]. An electrochemical aptasensor for recognizing thrombin was established based on the thrombin-binding aptamer (TBA) as a molecular detection component and MWCNTs as a support of the electrochemical capture probe.

The amine-conjugated 12-mer capture probe was bound to the MWCNTs fabricated on GCE. The 21-mer target aptamer probe contains 15-mer TBA labeled by ferrocene, which was hybridized with the capture probe and precisely identified thrombin [85]. A sensitive aptasensor was made as an electrochemical nano tool based on the structural switching of an aptamer for analyzing cocaine. The aptamer bound on GCE surface covalently which were previously modified with cadmium telluride (CdTe) quantum dots (QDs). After capturing the cocaine by the aptamer, a three-way junction complex was formed. The complex amplified the steric interference of the modified GCE platform and caused a difference in the corresponding electrical signal in the presence of a redox indicator [86]. Another Ag-Pt nanoparticle reinforced on reduced graphene oxide nanosheets (Ag-Pt-rGOs) composite was erected and applied as a novel sensing platform to detect tumor necrosis factor-alpha (TNF-α) in the presence of catechol indicator. Gold screen-printed electrode (SPE) fabricated with Ag-Pt-rGOs and applied to enhance enzyme-free and label-free electrochemical aptasensor for the recognition of TNF-α [87]. The carbon-supported Ag-Pt nanoparticles with diverse Ag to Pt ratios were effectively made through a facile step by step reduction method and employed as electrocatalysts to detect methanol fuel cell [88]. Another aptasensor for sensitive assay of the cocaine was established utilizing the electrochemical transduction approach. The aptasensor was built by covalently immobilizing the aptamer-activated AgNPs on the MWCNTs/ionic liquid/chitosan nanocomposite. These nanoparticles improve the biosensor's electrical conductance and performance properties and augment the stacking capacity of the aptamer DNA [89]. The graphene, a single-atom-thick two-dimensional carbon sheet, has inspiring applications for its exclusive morphology and characteristics. Graphene was reported as a novel electrochemical biosensing platform by benefiting the ultra-high electron transfer capacity and exceptional interaction with ssDNA. Adenosine triphosphate binding aptamer (ABA) fabricated on the gold electrode surface could strongly adsorb graphene owing to the robust $\pi-\pi$ contact and caused in a significant reduction of the charge transfer resistance (R_{ct}) of the transducer, which is the basis for sensitive detection [90]. Signal-on electrochemical aptasensor for the acute recognition of lipopolysaccharide (LPS) by joining the 3-way DNA hybridization method and nanomaterials-based signal augmentation has been reported by Bai et al. (2014). With the assistance of DNA1, the capture probe hybridized with the assistant probe to unwind its loop structure and develop a ternary "Y" shaped junction assembly. The DNA1 could be free from the junction upon accessibility of nicking endonuclease to start the subsequent hybridization process. At that point, a lot of sliced capture probe was generated in the systematic process, which was able to conjugate with DNA2-nanocomposite followed by the toluidine blue (TB) binding as an electroactive compound which amplifies the signal in the presence of graphene and AuNPs [91].

The development of the mechanism for DNA probe fabrication on the transducer surface and electrochemical recognition are two crucial steps for the success of electrochemical DNA biosensor. Selection of the appropriate immobilization technique is vital to ensure the immobilized DNA probes monolayer on the working electrode. Consequently, selecting suitable fabricating nanomaterials and designing unique detection elements between probes and targets, surface transducer alteration to augment the electrical conductivity, the introduction of nanofabrication process, and nanomaterials for signal intensification are parameters affecting the sensing process. Strategies used for probes DNA immobilization on electrochemical transducer surface are adsorption, covalent attachment, and avidin/streptavidin-biotin interaction [92]. A variety of factors may affect these sensing of probe DNA into electrodes surface as well as electrochemical detection of pollutants. Identification of proper nanomaterials, fabrication, and modification of nanomaterials with attaching groups moieties to facilitate conjugation with NA and attachment of suitable moieties with NA is to be considered wistfully [92] for appropriate detection and enhancement of molecular recognition.

5. Applications of NA biosensor in environmental monitoring

Environmental monitoring is one of the most prioritized areas in the ecosphere due to its close connection between the environment and human well-being [93]. The release of detrimental contaminants like pesticides, insecticides, heavy elements, and pharmaceutical xenobiotics to the environment is a global concern. Traditional analytical approaches for determining environmental contaminants comprise numerous chromatographic approaches like gas chromatography, high-performance liquid chromatography, and mass spectrometry. But these techniques need costly chemicals, time-consuming sample preparation, sophisticated equipment [93–95], skilled human resources, and not appropriate for point of application monitoring. In this field, less expensive, fast, in situ, easy to handle, portable, and real-time analytical techniques like biosensors are urgently required to monitor such contaminants to overcome the further magnification of ecological problems [93]. The recent applications of electrochemical NA biosensors in various sectors are discussed in the following sections and summarized in Table 1.

We have discussed the importance of qualitative and quantitative detection of pollutants in Section 5.1, emphasizing the benefits and basic strategies used to capture and identify environmental pollutants. Section 5.2 presents pollutant detection mechanisms using an affinity-based detection process under different subheading such as steroids, mycotoxin, heavy metals, pesticides, and antibiotics. In Section 5.3, we have briefly stated environmental pathogens detection based on DNA hybridization technique though limited literature is available in environmental pollutants detection using this technique.

5.1. Qualitative and quantitative analysis of pollutants

The qualitative and quantitative analysis of environmental pollutants is a crucial topic in understanding and dealing with the hazards to the environment. In this situation, aptamer or NA based electrochemical biosensors have attained significant attention because of the ability to solve many difficulties and challenges in environmental pollution [96] and other systems. Target analytes such as toxins, steroids, insecticides, heavy elements, and antibiotics can exist in ecological samples in various forms [96]. Electrochemical NA biosensors have been testified to recognize and monitor many environmental contaminants employing aptamers and NAs as detection components [97]. These NA biosensors have merits over the photosensitive, piezoelectric, or thermal process. Electrochemical transduction is fast, very sensitive and selective, disposable, vigorous, well-suited with new micro-fabrication technology, simple to miniaturize, high throughput, and not influenced by sample turbidity. Electrochemical reactions normally deliver an electronic response directly, without a costly signal transduction device [96]. Hence, we focus on electrochemical DNA biosensors or aptasensor.

Two key tactics have been established in the field of NA based electrochemical biosensors. One of them is based on the hybridization of NAs. A single-stranded oligonucleotide attached to a transducer surface can hunt for complementary strands in a sample and hybridize to form a double strand. However, to monitor environmental samples that lack NA, the second category of DNA biosensors termed aptasensor is useful. Biosensors sense the interaction of small contaminants with NA conjugated to a transducer. These biosensors might be applied as a general indicator of contamination since they can provide fast and easy to assess information upon such compounds' availability [98]. Electrochemical recognition of small compound binding to aptasensor has lately been established on diverse platforms for environmental monitoring. Among those, label-free EIS has seemed like a promising approach for characterizing biomolecule activated substrates and a sensitive procedure to monitor aptamer-target conjugation happening on the transducer surface. More notably, EIS is non-destructive, which made it very attractive for aptamer-based small pollutants recognition [99,100].

Table 1
Summary of recent applications of NA biosensors for environmental pollution detection.

Pollutants	Recognition Element	Electrode platform	Sensing method	Linear Range	Limit of Detection	Ref.
Chlorpyrifos	Aptamer	Carbon black/GO/Fe_3O_4	Electrochemical-voltammetric	0.29 nM to 0.29 mM	94 pM	[142]
Botulinum neurotoxin	Aptamer	Gold/ polypyrrole/ streptavidin/biotin-aptamer	Electrochemical	40 pg mL^{-1} to 100 ng mL^{-1}	40 pg mL^{-1}	[143]
Acetamiprid	Aptamer	AuNPs/MWCNTs/rGO/nanoribbons	Electrochemical-impedimetric	50 fM to 10 μM	17 fM	[144]
	Aptamer	AgNPs/nitrogen-doped GO	Electrochemical -impedimetric	0.1 pM to 5 nM	33 fM	[145]
	Aptamer	PtNPs/ Aptamer	Electrochemical-impedimetric	10 pM to 100 nM	1 pM	[123]
Atrazine	Aptamer	PtNPs/ Aptamer	Electrochemical-impedimetric	22 pg mL^{-1} to 0.22 μg mL^{-1}	2.2 pg mL^{-1}	[123]
Tetracycline	Aptamer	GCE/ Aptamer	Electrochemical	0.1–100 ng. mL^{-1}	1 ng. mL^{-1}	[146]
	Aptamer	Skin printed gold/ streptavidin/biotin-aptamer	Electrochemical-CV, SWV	10 nM to 10 μM	10 nM	[147]
Murcury	Nucleic acid	Gold/SWCNTs	Electrochemical-voltammetric	10 fM to 1 μM	3 fM	[148]
Lead	DNA	C-TiO_2 NTs	Amperometric, DPASV	0.01–160 nM	3.3 pM	[149]
	Aptamer	Thionine/AuNPs	Amperometric, DPV and CV	0.6–50 nM	312 pM	[150]
	DNA	Gold electrodes	EIS	0.05–1μM	34.7 nM	[151]
	DNA	Gold electrodes	EIS	10μM to 10 pM	10 pM	[152]
Brevetoxin-2	Aptamer	Gold/cysteamine SAM	Electrochemical-impedimetric	0.01–2000 ng mL^{-1}	106 pg mL^{-1}	[103]
17β-estradiol	Aptamers	CdSe nanoparticles/TiO_2 nanotubes	Photo-electrochemical	0–80 pM	33 fM	[105]

Notes: GO: graphene oxide, MOF: metal-organic framework, MWCNT: multi-walled carbon nanotubes, QD: quantum dots, rGO: reduced graphene oxide, SAM: self-assembled monolayer, SERS: surface enhancement Raman spectrum.

5.2. Pollutants detection based on affinity for NA

5.2.1. Detection of steroid compounds

Steroidal estrogens have become a serious and emerging concern in the environment since the start of global industrialization. Steroid compounds such as estrone, estradiol, and estriol pose severe threats to soil, water resources, plants, and humans. Especially, several studies have revealed that higher amounts of natural and artificial estrogens feminized male fish. The effects include diminishing testes dimensions, affecting reproductive aptness, reducing sperm count, inspiring the production of vitellogenin (VTG) in males, and changing other reproductive features [101]. One of the first electrochemical aptasensor for the recognition of 17β-estradiol was described by Kim, Jung [102]. They found a linear range of detection for 17β-estradiol from 0.01 to 1 nM, and the detection limit was 0.1 nM. For the detection of Brevetoxins (BTXs), another label-free competitive electrochemical aptasensor was reported based on competition within the BTX-beads and BTX-Horse redox peroxidase (HRP) bioconjugate. The detection limit was observed 106 pg/mL for BTXs in this aptasensor [103]. Elshafey et al. stated an aptamer to determine the smallest potent neurotoxin, anatoxin-a (ATX), embedded in an electrochemical impedance spectroscopy (EIS) aptasensor. This aptasensor showed a detection limit of 0.5 nM with a linear detection range from 1 nM to 100 nM [104].

Lately, electrochemical aptasensors combined with nanostructures compounds have enormous potential for recognizing small combinations for environmental monitoring. Fan et al. testified a very sensitive photoelectrochemical (PEC) aptamer platform based on TiO_2 nanotube adapted with CdSe QDs for the detection 17β-estradiol (E2). 1st CdSeNPs were deposited electrochemically on the internal and external surface of the TiO_2 nanotubes, followed by the E2 aptamer was fabricated on the CdSeNPs conjugated with the TiO_2 nanotubes (Fig. 4) [105]. The aptamer complex amplified steric hindrance after attachment of the aptamer to E2, which choked the dissemination of ascorbic acid and subsequently reduced signal. The PEC aptasensor showed a linear detection range from 0.05 pM to 15 pM and a detection limit of 33 fM for 17β-estradiol.

Bisphenol A (BPA) is a compound that interferes with the endocrine system. BPA might mimic the function like the oestrogen hormone and interrupt the oestrogen-oestrogen receptor conjugation process in humankind and animals. This BPA can upsurge cancer rate, decline semen power, and decrease immunity [106]. An ultrasensitive voltammetric aptasensor was designed employing screen-printed carbon electrode modified with gold nanoparticles by Hassani et al. (2020). The thiolated aptamer was immobilized on a gold modified surface to determine Bisphenol A in an environmental sample. The cyclic voltammetry and electrochemical impedance spectroscopy were applied to characterize the electrochemical properties of the aptasensor. Detection of Bisphenol A was carried out using differential pulse voltammetry in the presence of $[Fe(CN)_6]^{3-/4-}$ in the electrolyte (Fig. 5). The aptasensor exhibited linear detection range from 1 pM to 10 nM with limit of detection 0.113 pM [107].

Another simplified and label-free electrochemical aptasensor was developed based on AuNPs, dotted graphene nanocomposite, and GCE for the monitoring of BPA. An anti-BPA aptamers function as an entry of the long tunnels. When BPA not available, the anti-BPA aptamers to keep on unfolded, and the entrance persisted unlocked, thus permitting the passage of electrons to the electrode surface. However, with BPA availability, the aptamer was altered to a G-quadruplex shape that blocked the entry of long tunnels. Then gates shut down, resulting in the obstruction of electron movement to the electrode surface. The ferricyanide was appointed as an electrochemical indicator to examine the contacts within aptamer and BPA. The resulting GNPs/GR deposit exhibited well current response for BPA detection (Fig. 6). The peak electricity variation of ferricyanide was linear concerning BPA concentration ranging from 0.01 nM to 10 nM [106].

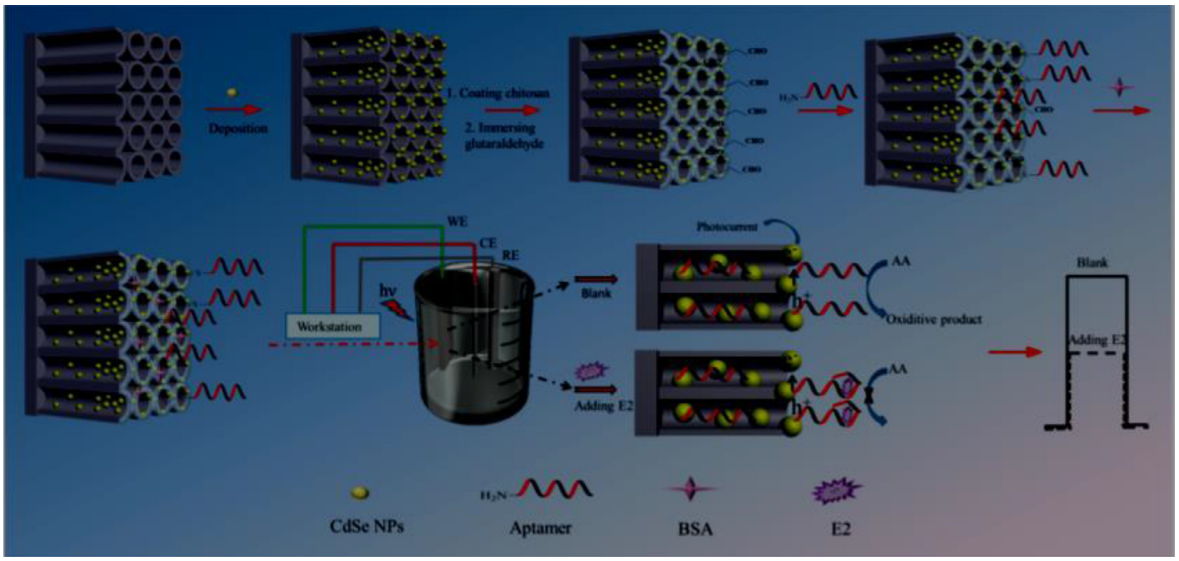

Fig. 4. Diagrammatic representation of a 17β-estradiol photoelectrochemical aptasensor and the detection mechanism (Reprinted from Ref [105] with permission. Copyright (2014) American Chemical Society).

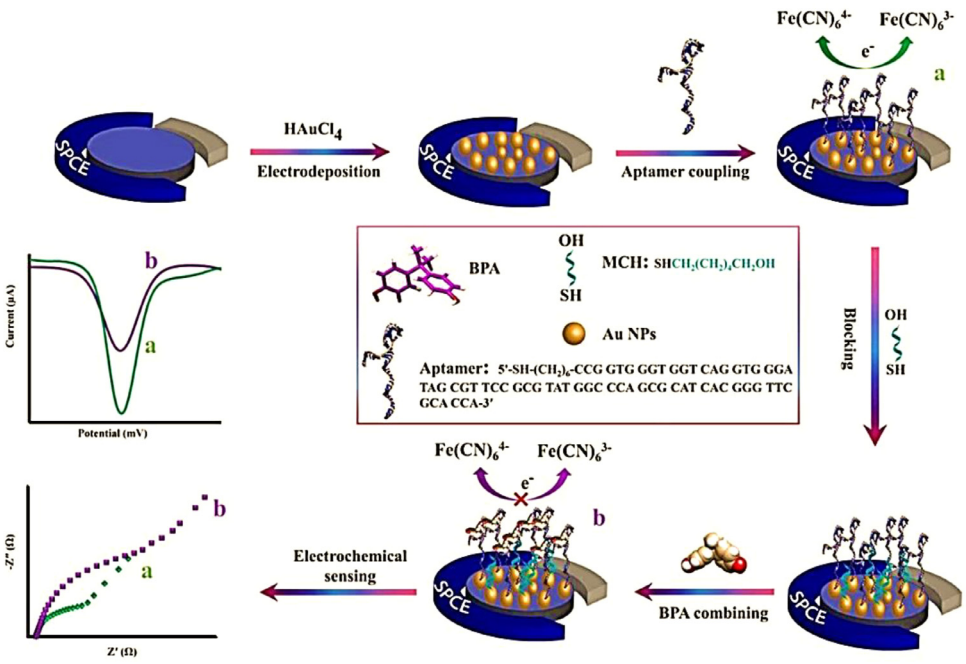

Fig. 5. The step by step schematic representation of high-performance electrochemical aptasensor for ultrasensitive detection of Bisphenol A as an environmental pollutant. (Reprinted from Ref [107] with permission under Creative Commons Attribution License (CC BY). Copyright (2020) frontiers in Bioengineering and Biotechnology)

5.2.2. Detection of mycotoxins

Mycotoxins are a group of nature made substances produced by mould living on crops, are the major toxins that contaminate food. Although natural, these have some adverse effects on human health, including kidney damage, stomach ailments, and immune clampdown. Ochratoxin A (OTA) is one of the most abundant food-polluting mycotoxins, potentially carcinogenic to humans [96,108]. For the recognition of Ochratoxin A (OTA), the Wang group described an AuNPs-conjugated reduced graphene oxide (AuNPs-rGO) as a signal magnification substance. The capture DNA was attached to the gold electrode, and the reporter DNA was immobilized with AuNPs-rGO, where AuNPs-rGO-DNA acted as a carrier for reporter DNA. This was an impedimetric aptasensor that could sense as low as 0.74 pM of OTA [109]. Details process was depicted in Fig. 7.

An electrochemical aptasensor was reported based on the AuNPs-conjugated guanine-DNAs sandwich model. The conjugate permit binding with methylene blue was used as a signal magnification nanocarrier. The AuNPs-DNAs were replaced after adding OTA, subsequently reduction of the signal [110]. Alternative structures of electrochemical aptasensor might be polyamidoamine (PAMAM) dendrimers as fabrication podium [111], AuNPs attached with enzyme-linked podium [112], or united rGO and magnetite (Fe_3O_4) NPs [113]. Until now, a limited number of mass-based aptasensor has been testified for the assessment of tiny elements, except for the surface plasmon resonance (SPR) aptasensor for the recognition of OTA and the tobramycin antibiotic [114]. More expansion of aptasensor will rely on exclusive nanomaterials and signal intensification methods to sense small targets with high sensitivity and specificity. Some investigations exhibited AuNPs-based SPR aptasensor to recognize adenosine, with a limit of detection in the range of pM to fM [115].

Anatoxin-a (ATX) is the minutest but powerful neurotoxin. High-affinity NA aptamers were chosen against ATX using the systematic evo-

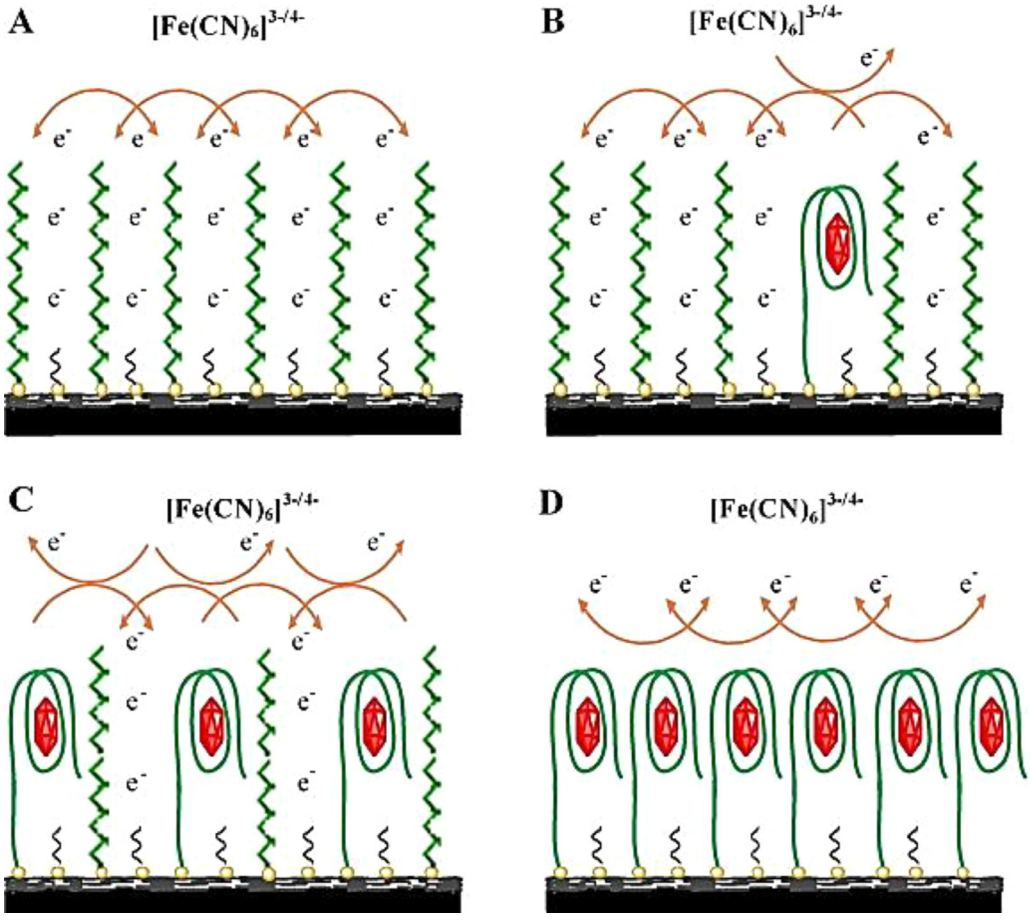

Fig. 6. The designed approach for the detection of BPA; the channel gates remain open when BPA is absent (A); Some of the gates became closed upon addition of lower concentration BPA, owing to aptamer conformational deviations (B); addition of BPA, further increase the closed gates number (C), and finally, at higher concentration, most of the gates became closed (D) (Reprinted from Ref. [106] with permission. Copyright (2014) Elsevier).

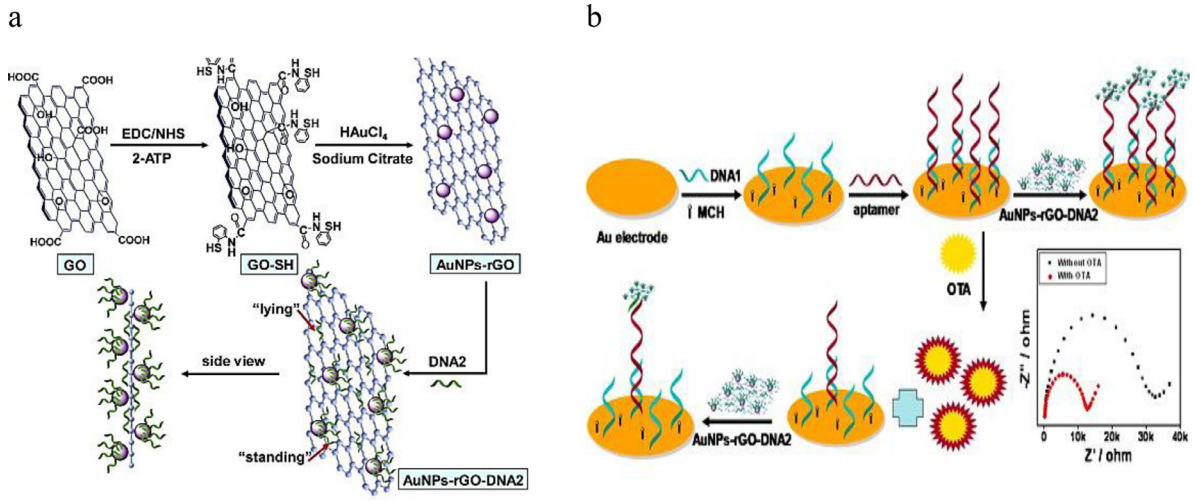

Fig. 7. a Stepwise synthesis of AuNPs-rGO (top) and the preparation of AuNPs-rGO-DNA2 bioconjugation (bottom) [109]. b. Graphical presentation of the impedimetric aptasensor with AuNPs-rGO as a signal amplifier (Reprinted from Ref. [109] with permission. Copyright (2014) Elsevier).

lution of ligands by exponential enrichment (SELEX). The aptamer sequence of maximum affinity was designing for the label-free recognition of ATX. The self-assembled aptamer monolayer (SAM) was formed on a gold electrode surface via a disulfide bond characterized using CV and EIS. Upon binding ATX to the immobilized aptamer, a substantial reduction in the electron-transfer resistance was noticed due to the aptamer's structural alteration (Fig. 8). The aptasensor showed a linear detection range from 1 nM to 100 nM and a detection limit of 0.5 nM for ATX. The Kd value anti-ATX aptamer was estimated by both the electrochemical and the fluorescence methods. Remarkably, the Kd calculated from the aptasensor signal displayed a lower value than fluorescence, suggesting that the aptamer's attaching to the Gold electrode has improved its

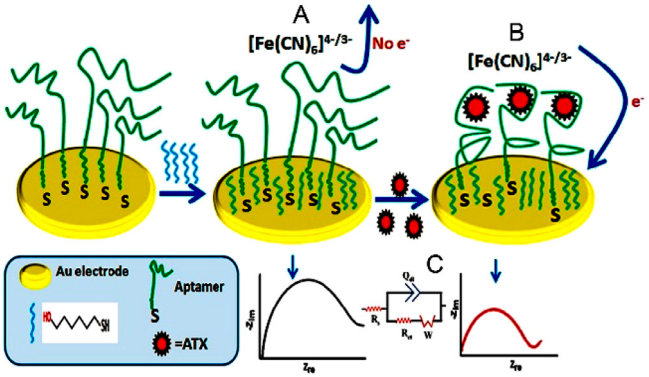

Fig. 8. Assembly of the label-free impedimetric Anatoxin-a aptasensor. In the absence of ATX, the $[Fe(CN)_6]^{4-/3-}$ redox probe was expelled from the electrode surface, and the redox reaction was deterred (A). In the presence of ATX, the aptamer tempted switching into a compact structure, permitting access of the $[Fe(CN)_6]^{4-/3-}$ to the electrode surface, enabling electron movement (B). Equivalent circuit Rs (Qdl[RetW]) was employed to fit the rate scans along with an impedance spectrum (C) (Reprinted from Ref. [104] with permission. Copyright (2015) Elsevier).

affinity for ATX. Also, the ATX aptasensor exhibited high constancy and high specificity toward common cyanobacterial toxins [104].

5.2.3. Detection of heavy metals

Pollution with heavy elements might have adverse consequences on human health and the ecosystem. For example, heavy elements, like mercury and arsenic, cause severe toxicity to the neurons and endocrine system, along with heart complications, skin damage, and cancer [96]. Arsenic is a noxious carcinogen extensively distributed in various parts of the earth. Humans can be exposed to arsenic by direct and indirect ingestion, i.e., swallowing arsenic-laden water and eating crops cultivated in arsenic-accumulated lands, respectively. While arsenic can provoke severe and enduring health effects, the more severe effects of chronic ingestion or long-term arsenic exposure can cause various cancers, skin lesions, arsenicosis, and cardiovascular diseases. Single-stranded DNA aptasensor has been reported by Kim et al. group for the recognition of arsenic as a potential device to be applied in an alarming process [116].

Exposure to a trace amount of lead (Pb) can cause neurological, cardiovascular, reproductive, and developmental disorders [117]. An electrochemical DNAzyme biosensor was established based on catalysis of a DNAzyme for precise and sensitive determination of lead ion (Pb^{2+}). Upon binding of Pb^{2+} with DNAzyme, DNA-Gold bio-bar codes to attain signal increment. A unique DNAzyme for Pb^{2+} was fabricated on a Gold electrode surface thru a thiol-Gold interface. The DNAzyme hybridizes with a precisely designed opposite substrate strand containing overhang. The overhang hybridizes to the DNA-gold bio bar code. A redox compound, $Ru(NH_3)_6{}^{3+}$ binds to negatively charge phosphate moiety of DNA by electrostatic interface and functions as an electrochemical signal transducer. The DNAzyme catalyzes the hydrolytic breakdown of the substrate after conjugation of Pb^{2+} with the DNAzyme. As a result, the substrate strand, the DNA bio-bar code as well as the $Ru(NH_3)_6{}^{3+}$ are removed from the gold electrode surface. The electrochemical signal of $Ru(NH_3)_6{}^{3+}$ was decreased due to the removal of $Ru(NH_3)_6{}^{3+}$ from the surface. Differential pulse voltammetry (DPV) pulse of $Ru(NH_3)_6{}^{3+}$ offers numerical measurement of the Pb^{2+}, where linear detection range was from 5 nM to 0.1 μM. DNA-gold bio-bar codes' usage increases the sensitivity of detection by 5 times, allowing recognition of as low as 1 nM of Pb^{2+}. The DPV signal of this DNAzyme biosensor was insignificant for other metal ions, suggesting the selectivity for Pb^{2+} detection (Fig. 9) [118].

Heavy metals are non-degradable and persist for a long time in nature. That is why detection of heavy metal pollution like copper is of particular concern. Therefore, a pressing demand to develop precise ion

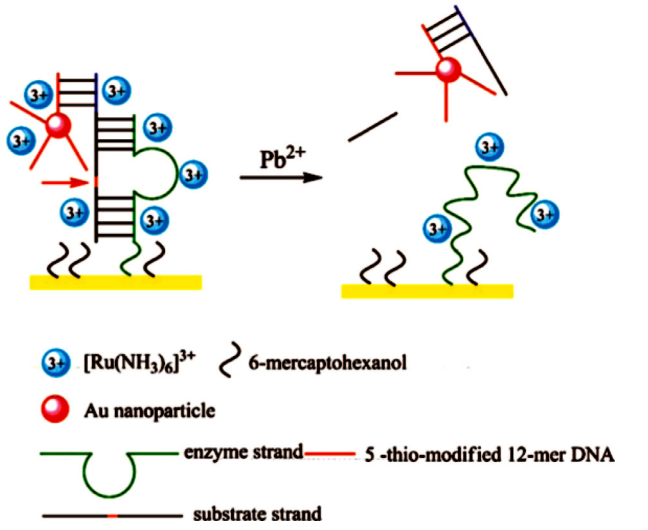

Fig. 9. The principle and architecture of the electrochemical DNAzyme biosensor for Pb^{2+} detection (Reprinted from Ref. [118] with permission. Copyright (2008) American Chemical Society).

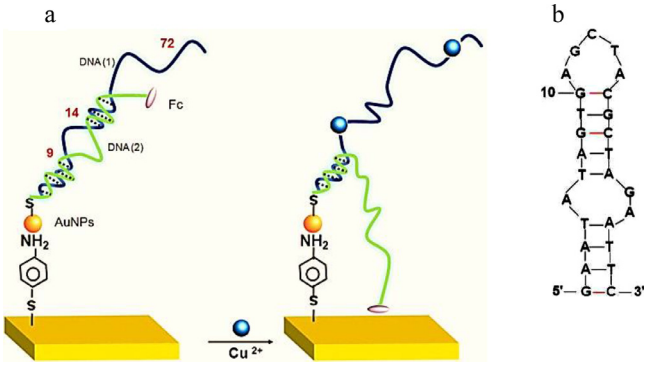

Fig. 10. a Graphical representation of the DNAzyme-based Cu^{2+} electrochemical biosensor (Reprinted from Ref. [119] with permission. Copyright (2011) Elsevier). b. Favorable secondary assembly of DNA for aptamer (Reprinted from Ref. [119] with permission. Copyright (2011) Elsevier).

biosensors for the quick determination of toxic heavy elements. A very sensitive and selective electrochemical aptasensor for the determination of Cu^{2+} ions based on AuNPs was mentioned. Here, AuNPs provided a great surface area to fabricate an excessive quantity of aptamers and useful electrochemical signal transduction. It showed high sensitivity, a lower detection limit, and a broad detection range as Cu^{2+} aptasensor. The electrical signal amplified proportionately with the concentration of Cu^{2+} from 0.1 nM to 10 μM and a detection limit of 0.1 pM. The other divalent cations did not interfere with the detection of Cu^{2+} ion, indicating high selectivity of the sensor (Fig. 10) [119].

5.2.4. Detection of pesticides

Pesticides or insecticides are used to control pests and insects. However, the concerns are the effects on non-target organisms. Pesticides are chemical formulations, apply to destroy mycological or animal pests. More than 98% of applied pesticides and 95% of herbicides spread to an endpoint other than its target animals due to spraying or spreading through whole agronomic areas [120]. Atrazine is one of the most extensively applied herbicides used to prevent the growth of weeds.

Similarly, acetamipride, an insecticide also use in crops to guard them against pest attack. After leaching into the ecosystem, these can cause toxicity in people and wildlife. Wang et al. developed an

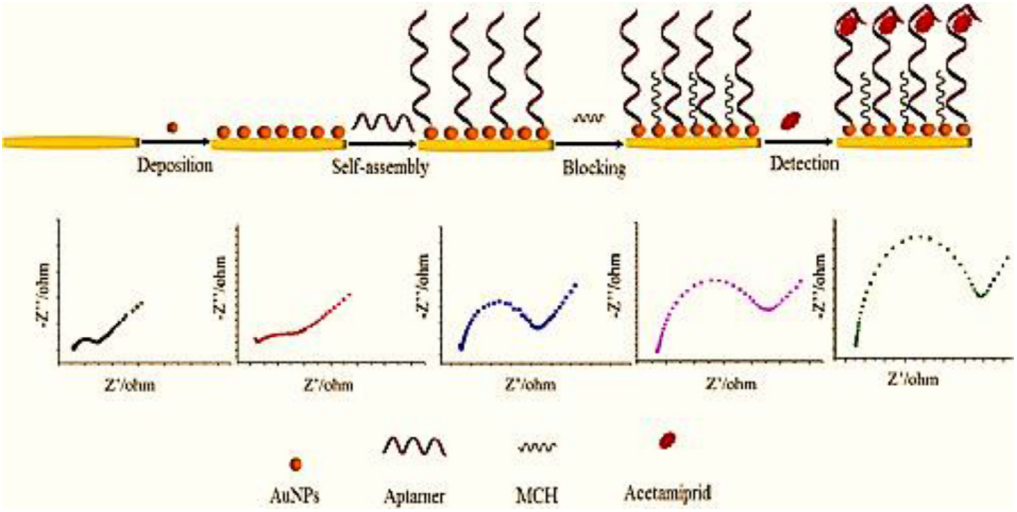

Fig. 11. Step by step aptasensor fabrication and acetamiprid detection procedure (Reprinted from Ref. [121] with permission. Copyright (2013) Elsevier).

oligonucleotide aptamer capable of sensing up to 4 extremely toxic organo-phosphorous insecticides, including isocarbophos and profenofos omethoateas, and phorate [118]. Fan et al. designed another aptasensor for sensitive and specific identification of acetamiprid based on EIS. AuNPs were deposited electrochemically on the gold electrode by CV to increase the sensitivity of the aptasensor. The modified electrode was acted as a platform for aptamer fabrication. The acetamiprid on the AuNPs-deposited electrode surface formed the acetamiprid-aptamer complex, which increased electron transfer resistance (R_{et}). This variation is of R_{et} values proportional to the concentration of acetamiprid. The NA aptasensor displayed high sensitivity and specificity for acetamiprid recognition, which was authenticated by a low K_d value for acetamiprid-aptamer conjugate and by real sample analysis (Fig. 11) [121].

Rapini et al. (2016) reported a signal off electrochemical oligo aptasensor to detect acetamiprid grounded on a competitive arrangement and screen-printed arrays. To increase the aptasensor performance, the polyaniline and AuNPs were gradually deposited electrochemically on the surface of graphite SPE by CV. The AuNPs were acted as a platform for thiol-modified DNA aptamer conjugation. Diverse acetamiprid solutions were analyzed, comprising a fixed quantity of biotinylated complementary DNA sequence by this DNA aptasensor. Streptavidin-alkaline phosphatase complex was added, and the enzyme-catalyzed the breakdown of 1-naphthyl phosphate to 1-naphthol, which was recognized by DPV. A reduction of the signal was observed upon the rise of pesticide concentration, confirming that the sensor work as signal off approach. A dose-response curve was drawn in the concentration range from 0.25 to 2.0 μM of acetamiprid after parameters optimization, and the detection limit was calculated to 0.086 μM. The specificity of the aptasensor was validated by atrazine analysis (Fig. 12) [122].

An impedimetric biosensor was established for concurrent recognition of acetamiprid and atrazine, which are the two widely used insecticides. In this biosensor, platinum (Pt) nanoparticles (NPs) were fabricated in a bridge-like pattern within interdigitated electrodes (IDEs) by applying the sputtering and e-beam lithography techniques. The resulting Pt-NPs microwires were chemically activated to permit the covalent binding of aptamers against the acetamiprid and atrazine onto the biosensor surface. The bare biosensing platform enabled electron transmission via the microwire-bridged IDEs, but upon analyte attachment to the aptamers, charge transfer was hampered, leading to an upsurge in the electrochemical cell's impedance. The aptamers allowed the sensitive and specific recognition of acetamiprid with a linear detection range from 10 pM to 100 nM and detection limit (LOD) of 1 pM, and atrazine having a linear detection range from 100 pM to 1 μM with LOD of 10 pM, correspondingly (Fig. 13). Its rationality was verified against several commonly used pesticides and with actual water samples [123].

5.2.5. *Detection of antibiotics*

Antibiotics are used for farming animals, as prophylactic in animal feed, and as therapeutic agents. A very small fraction of the ingested antibiotics is metabolized by the faunas, leaving a significant proportion to be either amassed in tissues or excreted and discharged into the environment. Antibiotics in the ecosystem may result in antibiotic resistance with the consequent probability of transferring to the human body via the food chain [96,124,125]. Recently, Metha et al. designed and developed DNA based electrochemical aptasensor for the identification of chloramphenicol. They immobilized the aptamers on the gold electrode surface through the SAM approach and built an aptasensor that was very sensitive and specific for the recognition of chloramphenicol [126].

Tetracyclines are a class of broad-spectrum antibiotics that protect us from pathogens by inhibiting their translation. This antibiotic has been found as hepatotoxic to pregnant mothers. A label-free electrochemical aptasensor for the recognition of tetracycline was reported using Prussian blue (PB) as an indicator. To increase the sensitivity of the aptasensor, a PB-chitosan-glutaraldehyde (PB-CS-GA) system was employed. At first, the PB-CS-GA was immobilized on the GCE surface, followed by colloidal AuNPs drop-casting on the electrode to facilitate immobilization anti-tetracycline aptamer. The step by the step fabrication method of the aptasensor was characterized by scanning electron microscope (SEM) and CV. The target tetracycline captured onto the aptasensor induced the electrode's electrical signal owing to the non-conducting biomolecules. The response of DPV was used for the detection of tetracycline concentration at optimum conditions. The aptasensor exhibited high sensitivity and a linear detection range from 10^{-9} to 10^{-5} M and 10^{-5} to 10^{-2} M having correlation coefficients of 0.994 and 0.992, respectively. The limit of detection was found 3.2×10^{-10} M (RSD 4.12%) (Fig. 14) [127].

Oxytetracycline (OTC), another broad-spectrum antibiotic, has extensive application in livestock as a food preservative. For electrochemical biosensing and detection of oxytetracycline in mouse blood and urine, Zheng et al. (2013) developed a gold electrode-based sensing platform. The gold electrode surface was functionalized with an Fc-labeled DNA-1 and OTC aptamer DNA-2. After capturing OTC by DNA-2, redox-active electrochemical probe Fc was released and generated signals based on the detection (Fig. 15) [128].

Kanamycin is an aminoglycoside antibiotic. Animal-derived food residues with kanamycin cause severe side effects. A simple aptamer-based detection method was developed for the label-free electrochemi-

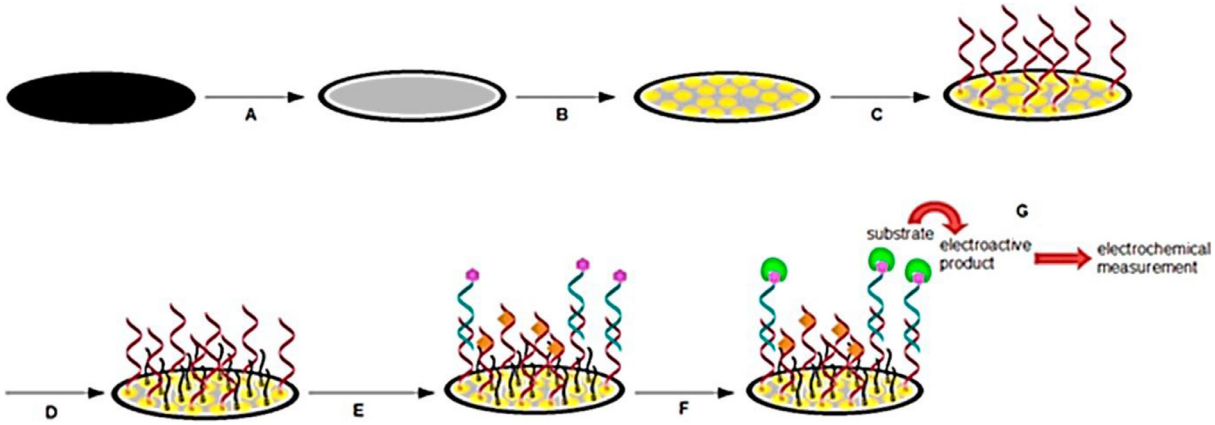

Fig. 12. Graphical representation of the aptasensor for the determination of acetamiprid: A) aniline electropolymerization; B) AuNPs electrodeposition; C) oligo1 immobilization; D) SAM formation with 6-mercapto-1-hexanol; E) competitive response within acetamiprid and complementary oligo2; F) streptavidin-alkaline phosphatase conjugate coupling; G) electrochemical recognition of end product by DPV after incubation with 1-naphtylphosphate (Reprinted from Ref. [122] with permission. Copyright (2016) Elsevier).

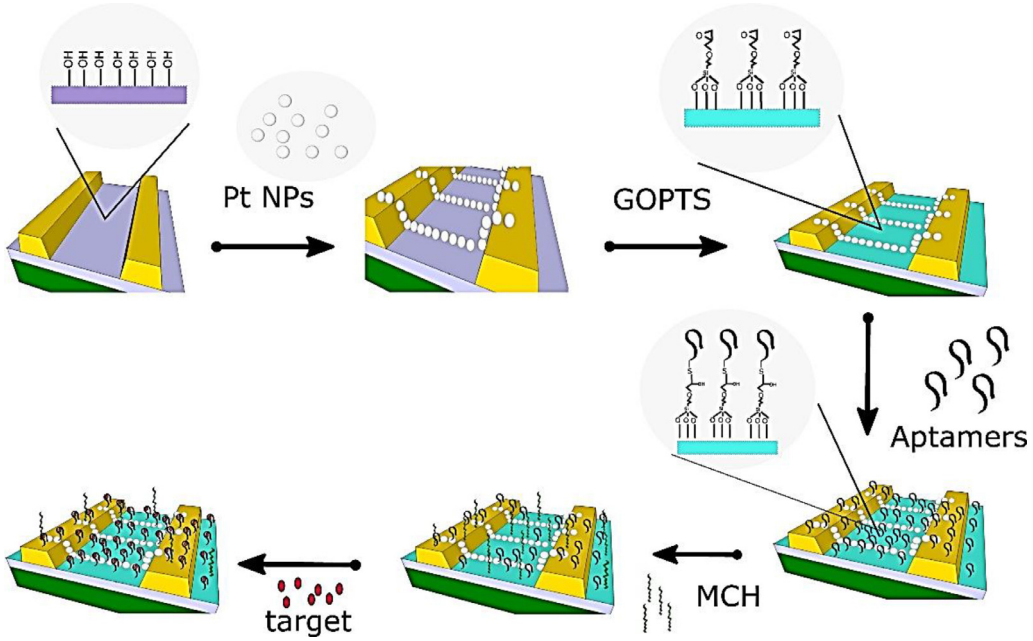

Fig. 13. Scheme of surface functionalization strategy. After fabrication of the Platinum nanoparticles (Pt NPs) microwires, the surfaces were coated with glycidoxypropyltrimethoxysilane (GOPTS). Thiol-conjugated aptamers were covalently bound onto the surfaces, and non-specifically attached aptamers were detached after incubation with MCH (Reprinted from Ref. [123] with permission. Copyright (2018) Elsevier).

cal recognition of kanamycin. A 5′ thiol conjugated kanamycin-targeted aptamer was assembled onto the gold electrode surface via Au-S interface. The configuration of the aptamer was altered upon binding to kanamycin. Concurrently its coverage of the electrode surface increased due to the creation of a stem-loop assembly. Consequently, the electron transmission resistance increased among the solution and the electrode. Utilizing $[Fe(CN)_6]^{3-/4-}$ as an electrochemical indicator, the sensor's SWV response was determined to measure kanamycin concentration. The recognition range of the aptasensor was 10–2000 nM. The method was effectively applied to recognize kanamycin in milk products and was found similar response and recognition limit as standard (Fig. 16) [129].

Another electrochemical sensor film made up of MWCNTs, a room-temperature ionic liquid (RTIL) of 1-butyl-3-methylimidazolium hexafluorophosphate ($BMIMPF_6$), and amino-functionalized graphene (GR–CO–NH–CH_2–CH_2–NH_2) was built for the identification of kanamycin. At first, MWCNTs-$BMIMPF_6$ nanocomposites were immobilized onto the GCE surface. The film was then modified with GR–CO–NH–CH_2–CH_2–NH_2 for substantial improvement of electrode conductivity. The electrochemical aptasensor displayed a broad linear detection range for kanamycin, beginning from 0.001 to 100 μM with a detection limit of 0.87 nM under the optimal settings. Lastly, the developed electrochemical aptasensor was effectively used for kanamycin identification in a real sample with high sensitivity, reproducibility, and constancy (Fig. 17) [130].

Xu et al. (2014) also reported a sandwich-type electrochemical aptasensor for the recognition of kanamycin. The aptasensor was made by assembling polyamidoamine dendrimer-AuNPs (PAMAM-Au) and graphene-polyaniline (GR-PANI) composites on the GCE surface. The electrode modified materials displayed high charge-transport characteristics, as well as high biomolecules, filling capacity. It exhibited that the conventional approach had a broad linear detection range from 5×10^{-6} to 4×10^{-2} μg mL^{-1} with a limit of detection of 4.6×10^{-6} μg mL^{-1} (Fig. 18) [131].

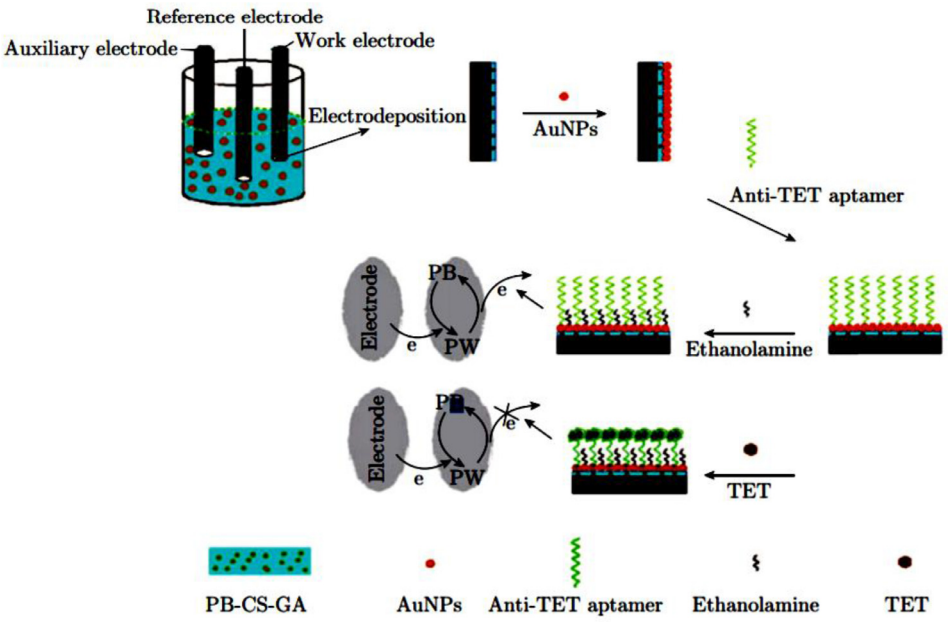

Fig. 14. The stepwise assembly process of the aptasensor for the label-free detection of tetracycline (Reprinted from Ref. [127] with permission. Copyright (2014) Springer.)

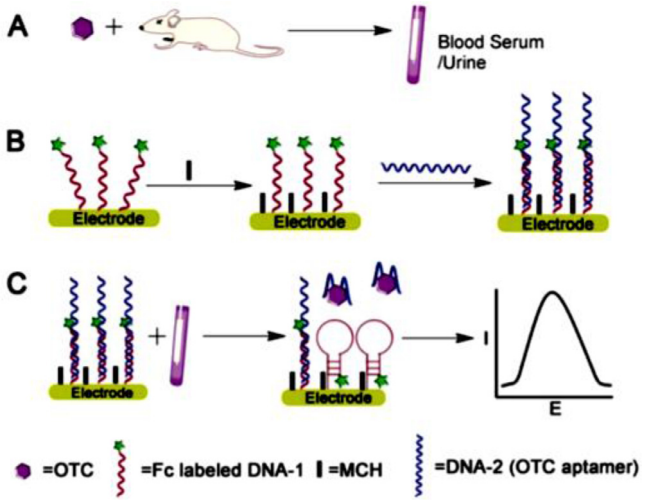

Fig. 15. A schematic presentation of the principles of OTC aptasensor. (A) Mouse blood serum and urine samples collection, (B) A gold electrode surface functionalization with a dsDNA monolayer (formed from Fc-labeled DNA-1 and OTC aptamer DNA-2). (C) Blood serum or urine OTC was seized by DNA-2, generating Fc signals at 0.37 V (Reprinted from Ref. [128] with permission. Copyright (2013) The Royal Society of Chemistry)

Streptomycin (STR) is an aminoglycoside antibiotic prescribed for humans and animal to treat gram-negative bacterial infections. STR residue causes severe side effects on human health [132]. A very sensitive and selective electrochemical aptasensor for recognizing STR was developed based on a signal enhancement tactic. The aptasensor was made using porous carbon nanorods (PCNR) derived from porous carbon nanosphere and multifunctional graphene nanocomposite (GR-Fe_3O_4–AuNPs) as sensing materials. GR-Fe_3O_4–AuNPs functioned as labels to attain higher sensitivity and lower detection limit. The developed aptasensor showed broader linearity to STR ranging from 0.05 to 200 ng mL^{-1} with a detection limit of 0.028 ng mL^{-1}. The aptasensor was effectively used to recognize STR in real samples with reproducibility, high selectivity, and constancy (Fig. 19) [133].

In another study, AuNPs and thiol-GQDs (GQDs-SH) were used as the nanomaterial for the sensitive determination of STR. GQD-SH was first fabricated on the GCE surface, followed by immobilization of the AuNPs with the SH moieties of GQDs through a covalent bond (Au-S). The aptamer was conjugated on the electrode surface via the contact between the thiol moiety of the aptamer. STR was presented on the surface of the prepared aptamer/AuNPs/GQD-SH/GCE as a target. As a result, nanoaptasensor/STR complex was formed, which altered the electrochemical signal, and the signal was assessed with the EIS method. The reported aptasensor displayed a widespread linear detection range from 0.1 to 700 pg.mL^{-1}. The developed aptasensor was effectively used to detect STR in real samples (Fig. 20) [132].

The high-throughput biosensors appropriate for both the hydrophilic and lipophilic compounds are extremely looked-for environmental contaminants monitoring, especially antibiotics detection. Yang et al. (2017) reported the next cohort of signaling-probe displacement electrochemical aptasensor (SD-EAS II) by standardizing the surface chemistry for the determination of lipophilic antibiotics such as ampicillin and sulfadimethoxine (SDM). SD-EAS II with HS-$(CH_2)_2$-$[OCH_2CH_2]_6$-OCH_3 formed SAM, which permitted the detection of ampicillin and SDM with a very low detection limit (10 pM and 1 nM, respectively), wide dynamic detection range (100 pMto 1 mM and 1 nM to 1 mM, respectively), and higher selectivity (>100 and 10,000 fold, respectively). The real usages of SD-EAS II were confirmed by the determination of ampicillin/SDM spiked in lake water with a limit of detection of 0.28 and 7 nM, respectively (Fig. 21) [134].

5.3. Pollutants detection based on DNA hybridization

Nucleic acid hybridization biosensors can be used to direct pollutants when DNA/RNA is the analyte(s). This kind of NA biosensor is known as genosensor. Genosensor is formed by incorporating a short synthetic oligonucleotide sequence (probe) onto a transducer surface that generates a signal. The immobilized probe serves as the biorecognition element and identifies the target DNA or RNA. The key steps to develop a genosensor are probe design and fabrication, followed by formation and identification of the hybridization response [24]. In these sorts of biosensors, DNA base-pairing between complementary stands is the biological recognition event (Fig. 22). Fabrication of a single-stranded DNA probe onto supporting materials such as nanoparticles or nanocomposites allows sequence-specific enhanced recognition of DNA hybridization by these sensors [135].

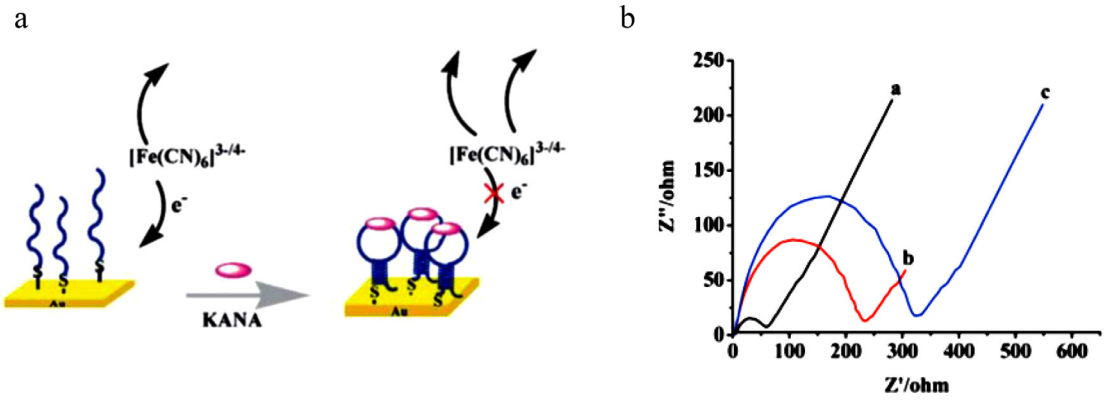

Fig. 16. a. Schematic diagram of the aptasensor based electrochemical detection of kanamycin (Reprinted from Ref. [129] with permission. Copyright (2015) The Royal Society of Chemistry). b. Different EIS spectra: electrochemical EIS of the bare gold electrode (a), aptamer-activated gold electrode (b), and aptamer-activated gold electrode upon incubation in 1 mM kanamycin solution (c) in the presence of 10 mM $[Fe(CN)6]^{3-/4-}$ (Reprinted from Ref. [129] with permission. Copyright (2015) The Royal Society of Chemistry).

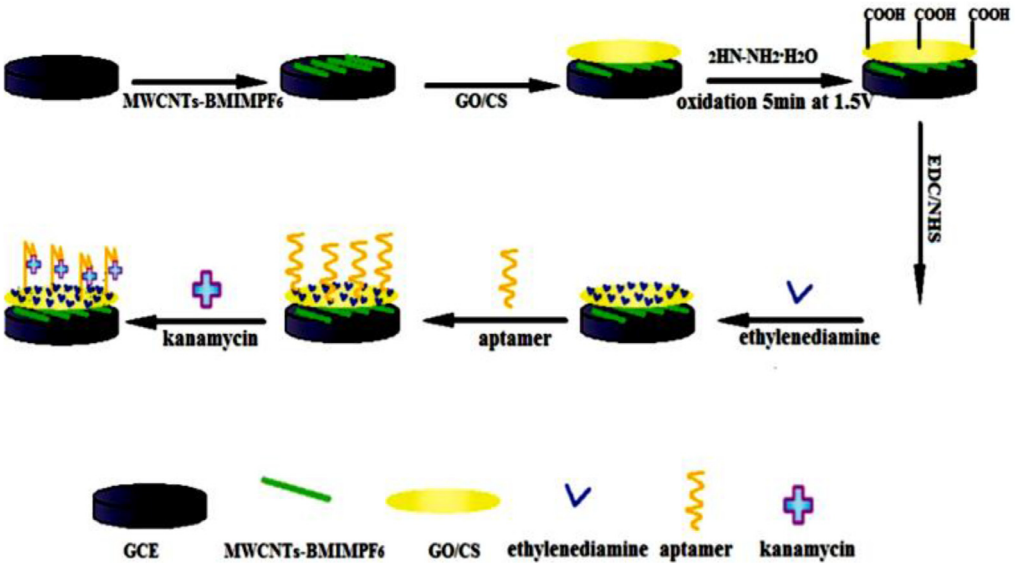

Fig. 17. Step by step fabrication process of the aptasensor for the detection of kanamycin (Reprinted from Ref. [130] with permission. Copyright (2015) The Royal Society of Chemistry).

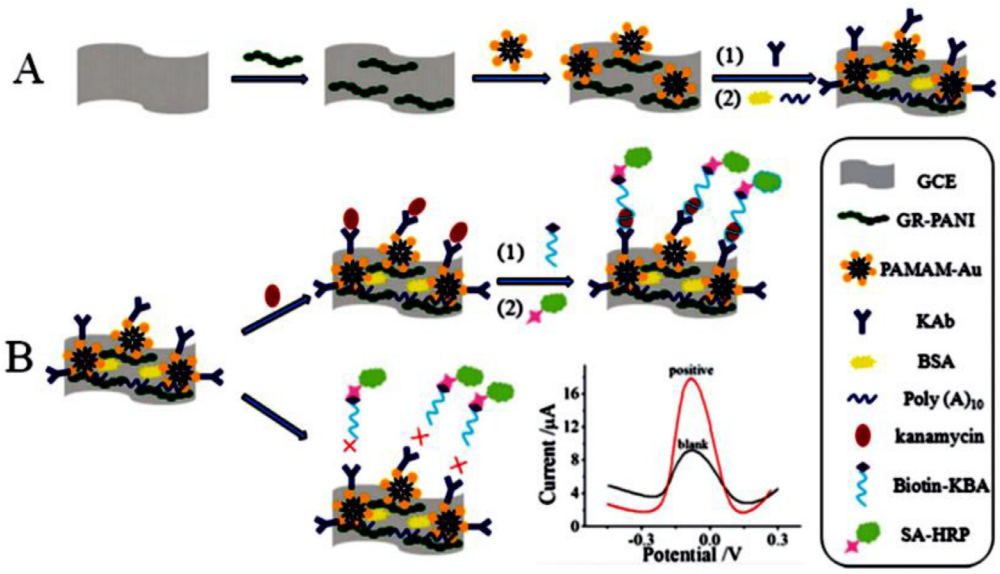

Fig. 18. (A) Graphical presentation of a sandwich-type electrochemical aptasensor and the fabrication process of GO-PANI and PAMAM-Au nanocomposite on the electrode surface. (B) application of the aptasensor for the detection of kanamycin (Reprinted from Ref. [131] with permission. Copyright (2014) The Royal Society of Chemistry).

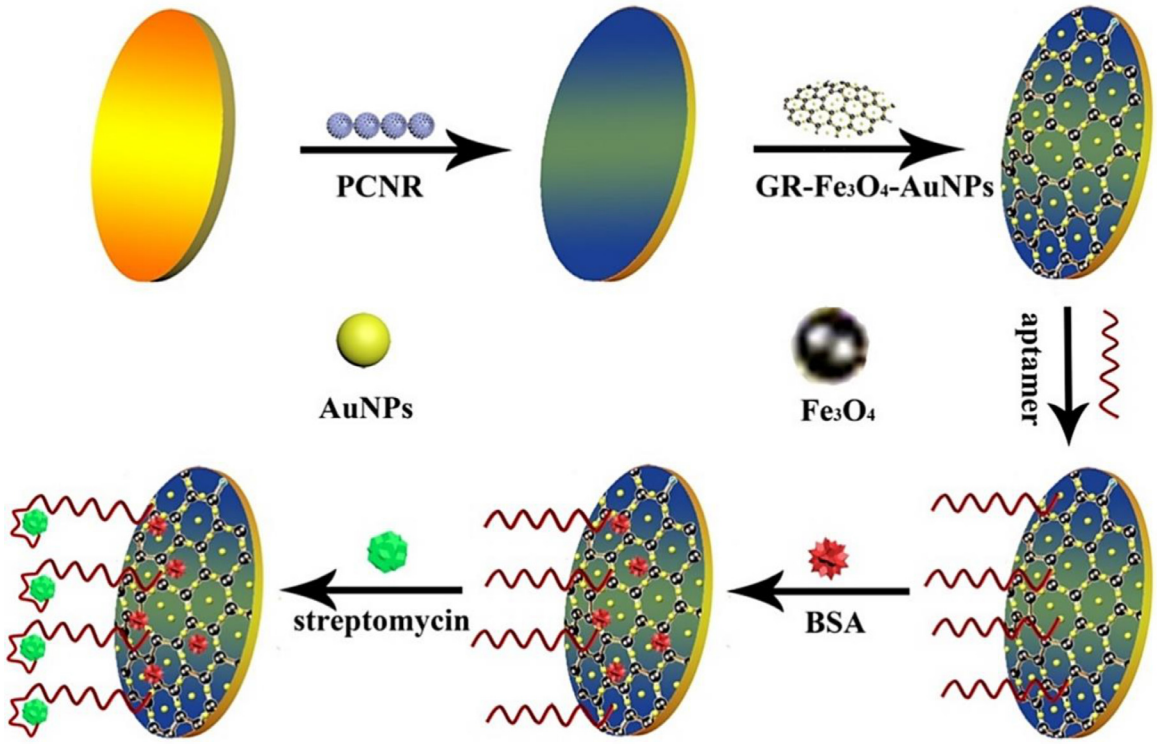

Fig. 19. Schematic representation of the fabrication and detection process of streptomycin aptasensor (Reprinted from Ref. [133] with permission. Copyright (2017) Elsevier).

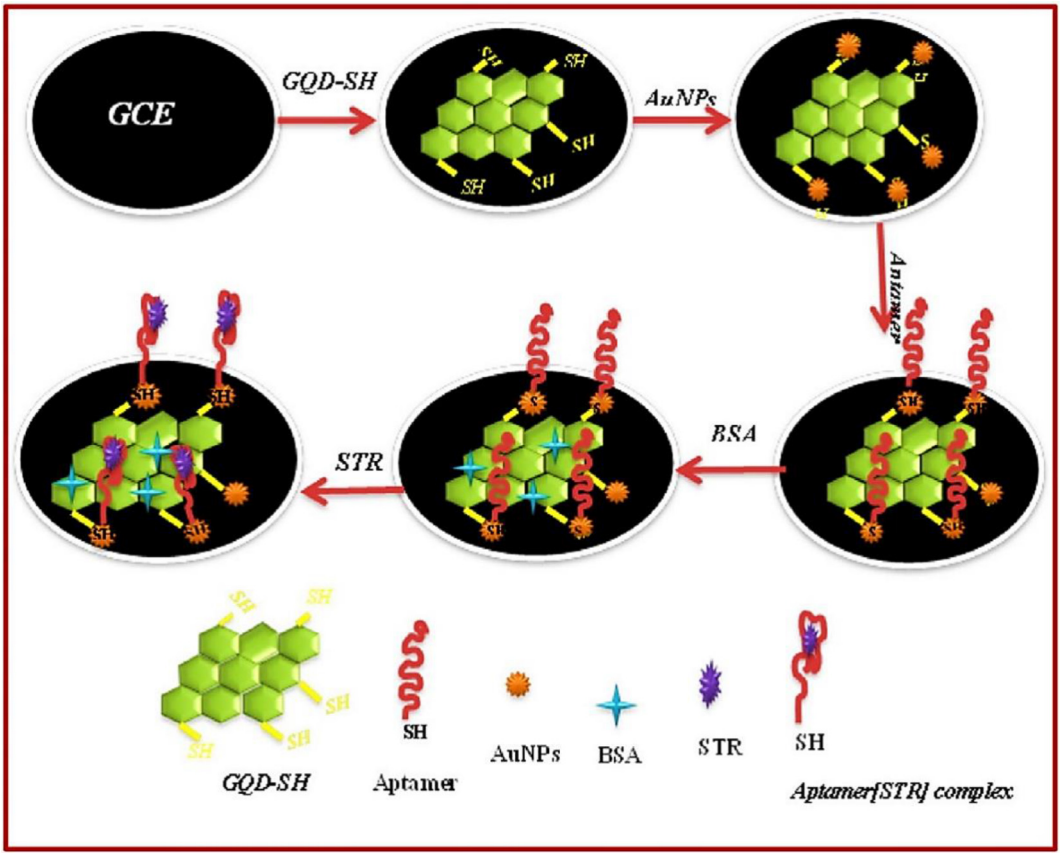

Fig. 20. Graphical presentation of the aptamer/AuNPs/GQD-SH/GCE electrochemical nanoaptasensor for the determination of STR (Reprinted from Ref. [132] with permission. Copyright (2018) Elsevier).

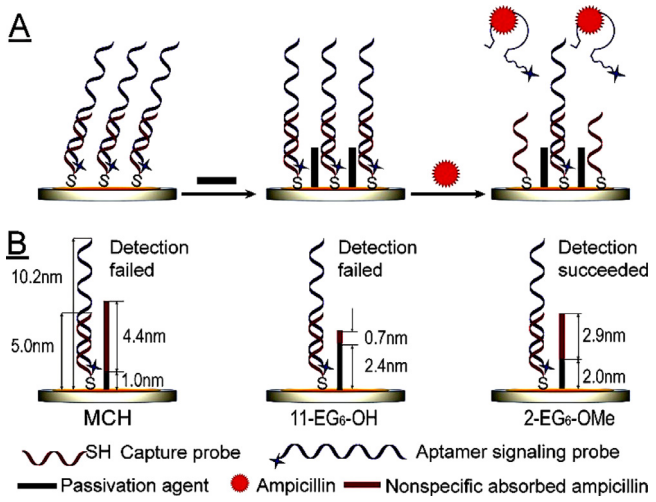

Fig. 21. Graphical presentation of SD-EASs for Ampicillin determination (A); the measured depths of SAMs (MCH, 11-EG_6-OH, and 2-EG_6-OMe) and non-specific assembled of Ampicillin on the surface (B). Only SD-EAS/2-EG_6-OMe (SD-EAS II) was capable of the quantitative and reproducible determination of Ampicillin (Reprinted from Ref. [134] with permission. Copyright (2017) Elsevier).

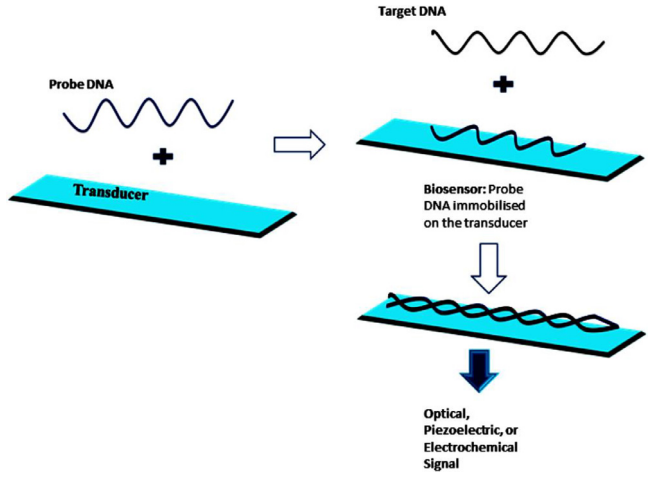

Fig. 22. Graphical presentation of the fundamental principle in NA hybridization biosensors (Reprinted from Ref. [135] with permission. Copyright (2019) Elsevier).

DNA hybridization-based biosensors are adaptable in scrutinizing an individual's genetic facts based on an NA sequence of pathogens. Owing to its simplicity, the DNA hybridization method is more recurrently applied in the detection process rather than direct DNA sequencing technique. Genosensors were widely applied for the recognition of pathogenic microorganisms related to environmental pollution. Sometimes, genosensors are coupled with PCR, as a precise detection method. Using a disposable electrode array, the concurrent electrochemical determination of *Staphylococcus aureus, Listeria monocytogenes, Salmonella enterica*, and *E. coli 0157:H7* amplicons was reported [136]. Wang et al. reported electrochemical genosensor for identifying *Cryptosporidium, E. coli, Giardia,* and *Mycobacterium tuberculosis* [137]. Baeumner's group also established a genosensor combining the microfluidic system with a potentiostat to detect Dengue virus RNA [138]. DNA and RNA molecules were measured through a sandwich hybridization method similar to the one earlier stated, with an electrochemical transduction mechanism [139,140]. Another quick method was reported by Elsholz et al. for detecting and quantifying *Pseudomonas aeruginosa, E. coli, S. aureus, Enterococcus faecalis*, and *S. epidermidis pathogens* based on 16S rRNA-specific oligonucleotide microarrays [141].

6. Current limitations and future challenges

The contemporary approaches for biosensing are integrating multiple pieces of knowledge, including application nanomaterials for bioconjugation and signal enrichment as well as sensing techniques ranging from optical, electrochemical to electromechanical. Physico-chemical characteristics NA makes these more exciting. Aptamers/NAs are easy to synthesize and functionalize with functional groups that facilitate their immobilization on the electrode surface. In the case of the electrochemical biosensor, the redox chemical groups allow the transformation of molecular interactions such as DNA hybridization or pollutants capturing electrical signals correlating a target analyte(s). These biosensors have wonderful applications in environmental monitoring due to its rapid analysis, low cost, a fabrication that makes way to direct detection of contaminants with low detection limit and high precision for as low as single-molecule detection.

However, there are still some limitations that NA based electrochemical biosensors need to overcome, such as the development of a methodical approach for NA or aptamer selection process for biosensors, immobilization of molecules on the conductive surface, quality of the semiconductor substance, and perfection of the signal-to-noise ratio. Besides, the Electrochemical NA biosensors should be focused on employing multiplex and complex sample conditions. The next level of advancement should aim at vigorous, regenerative, and durable sensing elements for long-lasting application. A combination of nanomaterials, nanocomposites, and nanopolymers may offer hybrid devices for improved usage in various types of biosensors. Though a handful of NA-based electrochemical biosensor has transitioned from research laboratory benchtop to portable point-of-care devices, most of these biosensors' sensitivity and selectivity are not satisfactory.

At the current stage, generally, electrochemical NA biosensor application is the single target-oriented approach, and the modified transducer is not reusable. However, it is amenable to parallelization, hence holding great promise for multiple targets' simultaneous monitoring. Altogether, NA biosensors' application in electrochemical biosensing is still in the near middle stage of development. Therefore, several problems should be overcome to make electrochemical aptasensor applicable for detecting multiple analytes in complex samples. Several homologue/analogue molecules may interact with DNA aptamer non-specifically, such as binding to the sugar-phosphate group spine of DNA and hence competitively inhibit the precise conjugation of the target analytes. Besides these, non-target NA in the biological fluids may be hybridized with NA probe, affecting the biosensor configuration for sustaining its appropriate binding site. So, emphasis should be given, so that aptamer or NA can target in the real sample. The electrochemical biosensor is vulnerable to the salt or ions composition; Thus, the solvent composition may also affect the biosensor characteristics.

7. Conclusions

This review demonstrates recent developments and applications of electrochemical NA biosensors for environmental monitoring and biosensing approaches, including an overview, presenting key signs of progress in the field and showing the extent of biomolecular sensing tactics growth of nanotechnological methods in biosensing. NA biosensor discovery like aptamers almost three decades ago and a combination of aptamers with the electrochemical process is an exciting event that opened novel perspectives in various fields. Nowadays, biosensors are prevalent in various fields of environmental monitoring, which has been described with illustrations. A broad range of techniques can be applied for the innovation of NA biosensors. Its conjugation with high-affinity biomolecules permits the sensitive and precise recognition of a range of pollutants. The conjugation of NAs/aptamers with enzymes, various

electrochemical indicators, and different NPs have opened new perspectives in the innovation of electrochemical aptasensors. NA/aptamers' flexibility and the possibility of its chemical modifications allowed us to exploit various aptamers' immobilization methods and apply various techniques of aptamer-analyte detection. Therefore, over the last decade, a tremendous effort has been observed to expand electrochemical NA biosensors.

A limited number of electrochemical NA-based biosensors is currently available, while the number of different counterpart detection techniques are much more significant where electrochemical NA biosensors may be applicable. Therefore, despite NA biosensors' advantages or aptasensors over antibodies or allied techniques, further effort is vital for widespread electrochemical aptamer/NA-based technology in real-world applications. This review may provide an idea to find out space and scope for the expansion of miniaturized, easy-to-applicable electrochemical NA biosensors for large scale applications.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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