Review

Relevant Aspects in the Development of Electrochemical Aptasensors for the Determination of Antibiotics—A Review

Daniela Nunes da Silva * and Arnaldo César Pereira *

Natural Sciences Department, Federal University of São João del Rei, 74 Praça Dom Helvécio, São João del Rei 36301-160, MG, Brazil
* Correspondence: daniela.silva.quimica@gmail.com (D.N.d.S.); arnaldo@ufsj.edu.br (A.C.P)

Abstract: Aptamers are three-dimensional structures of DNA or RNA that present high affinity and selectivity to specific targets, obtained through in vitro screening. Aptamers are used as biological recognizers in electrochemical biosensors, the so-called aptasensors, providing greater specificity in recognizing the most diverse analytes. Electrochemical aptasensors have extremely relevant characteristics, such as high sensitivity, low cost compared to other biorecognizers such as antibodies, and excellent compatibility, being considered one of the most promising alternative methods in several areas, such as biomedical diagnosis and monitoring environmental contaminants. In this sense, the present work reviews the relevant aspects of methodologies based on electrochemical aptasensors and their applications in determining antibiotics, seeking to foster innovation in electrochemical biosensors.

Keywords: aptamers; electrochemical biosensor; antibiotic

1. Introduction

Aptamers belong to the class of nucleic acids and are small single-stranded (ss) DNA or RNA molecules. They have the ability to change their three-dimensional conformation in a unique way when binding to a target species. This binding can be accomplished through electrostatic interaction, hydrogen bonding, or van der Waals force. The structure of aptamers allows them to bind to their targets in a way that provides high affinity and specificity, similar to the specificities of monoclonal antibodies; consequently, aptamers are called chemical antibodies [1–3].

Aptamers are stable to variations in pH and temperature and can be stored for extended periods; other recognizers such as antibodies and enzymes can undergo denaturation with these variations [4].

Aptamers can be obtained through the in vitro selection methodology, SELEX (Systematic Evolution of Ligands by Exponential enrichment) [5]. There are different SELEX processes in new selection protocols, but they have the same basic principles. The method consists of amplifying sequences with the highest binding affinity to the species of interest (Figure 1).

The selection generates structures capable of selectively combining/binding the species under analysis. In general, the methodology is based on steps of separation and amplification. The initial part consists of the chemical synthesis of the oligonucleotides used as a starting point. After incubating the oligonucleotides with the species of interest and removing unbound molecules (washing), small amounts of oligonucleotides that interact with the target are amplified by polymerase chain reaction (PCR). In the next step, this new expanded set of oligonucleotides is exposed again to the species of interest in the following cycle. The selected aptamers are cloned, providing individual aptamers with correlated sequences [6,7].
Smaller molecules have a surface area with less access, so the bonds between the aptamer and the species of interest have lower affinity, compromising the specificity of the biological receptor. In this sense, intentional changes are made in the aptamer selection steps to generate species with greater affinities and specificity to the analyte. The literature discusses methods that will contribute to the selection of more suitable sequences according to the purpose [8,9].

Due to the possibility of being synthesized in vitro, and the affinity characteristics, aptamers have been used in several research methods. Aptamers can be developed to detect various analytes, such as organic compounds, metal ions, cells, and proteins. A flexible and cost-effective design allows aptamers to be used in analysis methods with excellent performance [10]. Detection methods using aptamers have been employed in conjunction with colorimetry [11], fluorescence [12,13], surface plasmon resonance [14,15], and chemiluminescent [16] and electrochemical [17–19] techniques. The literature shows a growing number of studies aimed at the development of electrochemical biosensors based on aptamers (Figure 2).

![Figure 1. General representation of the SELEX method.](image1)

**Figure 1.** General representation of the SELEX method.

![Figure 2.](image2)

**Figure 2.** Number of scientific publications on electrochemical aptasensors in the last ten years. Keyword: Electrochemical Aptasensor, Source: Science Direct®, September 2023.
An electrochemical biosensor integrates the following components: the biorecognition element, the transduction element, and the mediators. The recognition element is responsible for identifying the presence of the species of interest in the analyzed environment, and the transduction element transduces the signal detected by the recognition element into a measurable signal. Mediators have the function of providing an interaction between the sample, the biological recognizer, and the transducer. Electrochemical biosensors provide accurate, cheap, and simple detection, thus contributing to the development of miniaturized devices that result in methods with low detection limits, working with reduced sample volumes.

Electrochemical aptasensors are a kind of biosensor that have aptamers as biorecognition elements for the specific detection of analytes. The formation of original three-dimensional structures of aptamers in specific environments favors detection with high precision, high precision. The aptamer recognition event in the electrochemical device produces signals that can be measurable as current or resistance, resulting in voltammetric and impedimetric biosensors, respectively. Impedimetric devices are based on variations in properties' resistance that occur on the electrode surface as a function of biological recognition. In voltammetric detection, the result of the biological interaction at the electrode is transduced as an electrical current. Both impedimetric and voltammetric detection sensors have high sensitivity.

Ikebukuro et al. report the first electrochemical detection system using aptamers. The work consisted of developing an aptasensor aimed at detecting protein with two different antithrombin aptamers capable of recognizing specific parts of the target protein, making it possible to detect thrombin selectively.

Aptasensors can be used in several areas, such as clinical diagnosis, the food industry, and environmental monitoring of contaminating species, with high precision.

In the context of environmental contaminants, the incorrect and exaggerated use of antibiotics has generated concern among researchers because they can provide bacterial resistance. Antibiotic resistance is capable of increasing the severity of diseases, contributing to inefficient treatments, long hospital stays, and increased healthcare costs. In this sense, due to the importance of detecting antibiotics, selective electroanalytical methods using aptamers have stood out as promising alternatives.
This review addresses research from the last ten years about electrochemical aptasensors aimed at determining antibiotics. This study considered antibiotics that are a high risk to the environment and animal health. The relevant aspects in developing aptasensors were also discussed.

2. Antibiotics and Electrochemical Detection

Antibiotics are drugs capable of eliminating or preventing bacterial proliferation and are used in the therapy of various infections in humans and animals [36]. The first antibiotic discovered was penicillin in 1928 by A. Fleming and, later, came other antibiotics produced to treat various bacterial infections [37]. Based on their structure, antibiotics are classified into tetracyclines, macrolides, aminoglycosides, streptogramins, β-lactam antibiotics, quinolones, and peptides [38].

The global consumption of antibiotics has expanded and, due to their widespread use, significant quantities of antibiotics have been released into the environment. They are considered an emerging class of pollutants, due to their presence in high concentrations in water and soil. Many of these compounds show significant persistence and accumulate in solid matrices [39].

In the environment, these residues can cause several negative effects such as ecosystem imbalance. Studies have revealed that ingestion of antibiotics via drinking water increases the risk of serious human diseases [38]. Also, regarding the effects of the excessive use of antibiotics, Singh et al. report that the direct effects are related to the emergence of resistance in human intestinal flora, associated with the consumption of meat products containing antibiotics. The indirect and long-term effects of the indiscriminate use of antibiotics in humans are carcinogenicity, damage to the reproductive system, and teratogenicity [40].

Therefore, monitoring antibiotics is one of the ways to limit the risks due to pollution by these compounds. The main antibiotic detection methods are microbial or microbiological inhibition, immunological, chemoreceptor-based, and chromatography [41]. Electroanalytical methods are alternative methods for detecting antibiotics. These devices have characteristics such as high sensitivity and the possibility of miniaturization at a low cost [42–45].

Due to providing methodologies with high selectivity and robustness, several electrochemical aptasensors were developed to determine antibiotics. The voltammetric, potentiometric, conductometric, and electrochemical impedance spectroscopy (EIS) techniques can be used to detect the signal generated in the biosensor device; therefore, electrochemical aptasensors are classified depending on the technique used [35].

Bai et al. report the construction of an aptasensor for the determination of sulfadimethoxine. On the electrode surface, a hybridization reaction occurred between the probe DNA and the aptamer DNA labeled at its 5′ terminal. There was an improvement in the signal after generating the aptamer-target conjugate. The nuclease present digests the probe’s DNA; thus, the antidsDNA is not free on the electrode surface, providing a greater intensity in the electrochemical signal, and enabling the detection of sulfadimethoxine. Via differential pulse voltammetry, the method showed a linear response range of 0.1–500 nmol L⁻¹ and a detection limit of 0.038 nmol L⁻¹. The applicability of the device was proven through the determination of sulfadimethoxine in drugs and milk samples presenting excellent recovery values [46].

Li et al. describe the construction of a dual-label multiple aptasensor capable of simultaneously detecting the antibiotics, kanamycin (KAN) and tobramycin (TOB). The aptasensor was made up of aptamer filaments, quantum dots, and gold nanoshells. Via differential pulse voltammetry (DPV), the developed aptasensor presented a wide linear response (KAN, 1–4 × 10² nM; TOB, 1–1 × 10⁴ nM) and low detection limits (KAN, 0.12 nM; TOB, 0.49 nM). The application of the aptasensor in milk samples enriched with KAN and TOB was satisfactory [47].

In research by Guan et al., they observed aptamer sequences with high selectivity for penicillin G (PenG). The affinity of the aptameric sequence for PenG was detected using
In research by Guan et al., they observed aptamer sequences with high selectivity for penicillin G (PenG). The affinity of the aptameric sequence for PenG was detected using electrochemical detection. Modifying the surface of the aptasensor with gold nanoparticles, reduced graphene oxide, and further functionalized with tetrahedral DNA nanostructures improved the device’s response (Figure 4). The aptasensor proved to show high specificity in the determination of PenG in a linear range of 0.2 nM to 1 mM with a detection limit of 0.05 nM. When applying the method to milk samples enriched with penG, the aptasensor showed high recovery rates (98–109%). The sample underwent prior treatment to remove fat and provide a more accurate detection method [48].

Naseri et al. report the development of an aptameric biosensor employing a glassy carbon electrode. The base electrode was modified by functionalized carbonaceous nanomaterial to determine tetracycline. The biosensor showed a wide linear range from $1.0 \times 10^{-17}$ to $1.0 \times 10^{-5}$ M and a low detection limit ($2.28 \times 10^{-18}$ M). The proposed device successfully determined tetracycline residues in milk samples [49].

Table 1 presents more methods employing electrochemical aptasensors described in the literature for antibiotic detection in the last ten years.

From the analysis of Table 1, it is possible to notice that different nanomaterials were used in the construction of the devices. Among metallic nanoparticles, gold nanoparticles were the most used. In the applications of the electrochemical aptasensors evaluated, in milk samples, steps such as sample preparation to remove fat and proteins were carried out. The biosensors showed high recovery percentages, proving the ability to detect antibiotics in real samples, and the most-used techniques were DPV and EIE.
Table 1. Application of electrochemical aptasensor in the detection of antibiotics.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Technique</th>
<th>Modification</th>
<th>Sample</th>
<th>Detection Range</th>
<th>Limit of Detection (LOD)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline</td>
<td>DPV</td>
<td>Cu$_2$O@Au</td>
<td>milk</td>
<td>1.0 nM–1000 µM</td>
<td>0.16 nM</td>
<td>[50]</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>EIS</td>
<td>Pencil lead graphite-based electrochemical aptasensor</td>
<td>milk</td>
<td>10$^{-8}$–10$^{-16}$ M</td>
<td>0.8 × 10$^{-18}$ M</td>
<td>[51]</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>DPV</td>
<td>4-carboxyphenyl anchored GCE</td>
<td>milk</td>
<td>1.0 × 10$^{-9}$–1.0 × 10$^{-4}$ g mL$^{-1}$</td>
<td>2.29 × 10$^{-10}$ g mL$^{-1}$</td>
<td>[52]</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>LSV</td>
<td>MWCNTs</td>
<td>milk</td>
<td>1.0 × 10$^{-13}$–1.0 × 10$^{-8}$ M</td>
<td>1.0 × 10$^{-13}$</td>
<td>[53]</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>EIS</td>
<td>TiO$_2$-g-C$_3$N$_4$@Au NPs</td>
<td>wastewater</td>
<td>0.5–3 nM</td>
<td>0.2 nM</td>
<td>[54]</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>DPV</td>
<td>Endonuclease DpnII</td>
<td>milk and water</td>
<td>0.1–100 nM</td>
<td>32 pM</td>
<td>[55]</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>EIS</td>
<td>Co-MOF@TPN-COF</td>
<td>human serum, river water, and milk</td>
<td>1.0 fg mL$^{-1}$–2.0 ng mL$^{-1}$</td>
<td>0.217 fg mL$^{-1}$</td>
<td>[56]</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>SWV</td>
<td>Aptamer cocktail</td>
<td>honey</td>
<td>0.01–1000 ng mL$^{-1}$</td>
<td>0.0073 ng/mL</td>
<td>[57]</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>DPV</td>
<td>rGO/PEI/TiO$_2$</td>
<td>water</td>
<td>0.003–10.0 µM</td>
<td>0.7 nM</td>
<td>[58]</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>DPV</td>
<td>PCNR/GR–Fe$_3$O$_4$–AuNPs</td>
<td>milk</td>
<td>0.05–200 ng mL$^{-1}$</td>
<td>0.028 ng mL$^{-1}$</td>
<td>[59]</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>DPV</td>
<td>MoS$_2$–Au-HE</td>
<td>milk</td>
<td>1.0–1.0 × 10$^{3}$ ng L$^{-1}$</td>
<td>0.8 ng L$^{-1}$</td>
<td>[60]</td>
</tr>
<tr>
<td>Sulfaquinoxaline</td>
<td>DPV</td>
<td>AuPd NPs@Uio-66-NH$_2$/CoSe$_2$</td>
<td>pork</td>
<td>1.0–100 ng mL$^{-1}$</td>
<td>0.547 pg mL$^{-1}$</td>
<td>[61]</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>EIS</td>
<td>Fe$_3$O$_4$-IL</td>
<td>milk</td>
<td>1 × 10$^{-9}$–1 × 10$^{-5}$ M</td>
<td>1 × 10$^{-9}$ M</td>
<td>[62]</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>SWV</td>
<td>PAN@Cu-BTC</td>
<td>meat</td>
<td>10 pM–1 µM</td>
<td>0.32 pM</td>
<td>[63]</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>DPV</td>
<td>phi29 DNA polymerase and nicking endonuclease Nt.AlwI</td>
<td>milk and water</td>
<td>10–200 nM</td>
<td>5.13 nM</td>
<td>[64]</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>EIS</td>
<td>SntOx@TiO$_2$-mC</td>
<td>human serum and human urine</td>
<td>0.01–5 ng mL$^{-1}$</td>
<td>6.7 pg mL$^{-1}$</td>
<td>[65]</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>DPV</td>
<td>SA-AuNPs/OMC-CS</td>
<td>milk</td>
<td>0.1–1000 nM</td>
<td>0.03569 nM</td>
<td>[66]</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>EIS</td>
<td>PdNPs/CNT/Chi</td>
<td>milk</td>
<td>0.10–1500 nM</td>
<td>18 pM</td>
<td>[67]</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>DPV</td>
<td>Si-Fe/NOMC</td>
<td>eye drop</td>
<td>1.0–500 µM</td>
<td>0.03 µM</td>
<td>[68]</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>EIS</td>
<td>POP</td>
<td>milk</td>
<td>1.0 × 10$^{-5}$–5.0 ng mL$^{-1}$</td>
<td>1.33 × 10$^{-6}$</td>
<td>[69]</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>DPV</td>
<td>3D Au-PAMAM/rGO</td>
<td>raw milk</td>
<td>1 µM–1.0 nM</td>
<td>1.0 nM</td>
<td>[70]</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>DPV</td>
<td>GR-TH/HNP-PtCu</td>
<td>pork meat and chicken</td>
<td>5 × 10$^{-7}$–5 × 10$^{-2}$ µg mL$^{-1}$</td>
<td>0.42 pg mL$^{-1}$</td>
<td>[71]</td>
</tr>
</tbody>
</table>
Table 1. Cont.

<table>
<thead>
<tr>
<th>Antibiotic (CAP) and Oxytetracycline (OTC)</th>
<th>Technique</th>
<th>Modification</th>
<th>Sample</th>
<th>Detection Range</th>
<th>Limit of Detection (LOD)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline</td>
<td>DPV</td>
<td>MBCPE/Fe₃O₄NPs/OA</td>
<td>drug, milk, honey, and blood serum</td>
<td>1.0 × 10⁻¹⁰–1.0 × 10⁻⁷ M</td>
<td>2.9 × 10⁻¹¹ M</td>
<td>[72]</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>DPV</td>
<td>C-WO₃@AuNPs</td>
<td>water, milk, honey, and black tea</td>
<td>0.1–100 nM</td>
<td>4.8 × 10⁻² nM</td>
<td>[73]</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>SWV</td>
<td>AuPt@h-CeO₂/MoS₂</td>
<td>water and milk</td>
<td>5.0 × 10⁻⁶–1.0 × 10⁻² ng mL⁻¹</td>
<td>1.02 × 10⁻⁷ ng mL⁻¹</td>
<td>[74]</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>DPV</td>
<td>T7 exonuclease</td>
<td>milk</td>
<td>0.02–40 nM</td>
<td>4.0 pM</td>
<td>[75]</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>SWV</td>
<td>NMOF</td>
<td>milk</td>
<td>10⁻⁴–50 nM</td>
<td>CAP 0.033 pM OTC 0.048 pM</td>
<td>[76]</td>
</tr>
</tbody>
</table>

Gold nanoparticles (AuNPs) on the surface of Cu₂O nanomaterials (Cu₂O@Au); linear sweep voltammetry (LSV); multi-wall carbon nanotubes (MWCNTs); metal–organic frameworks (MOFs); g-C₃N₄ on the surface of TiO₂ microspheres and gold nanoparticles (TiO₂–g-C₃N₄@Au NPs); Co-based metal–organic frameworks (Co-MOFs) and terephthalonitrile-based covalent organic framework (TPN-COF); Square Wave Voltammetry (SWV); polyethyleneimine grafted reduced graphene oxide and titanium dioxide (rGO/PEI/TiO₂) nanocomposite; carbon nanorods (PCNR) formed by porous carbon nanosphere and multifunctional graphene composite (GR-Fe₃O₄–AuNPs); nanosheets of MoS₂ (MoS₂ nanosheets) and a composite composed of MoS₂ nanosheets, Au nanoparticles (AuNPs), and hemin (HE) (denoted as MoS₂-Au-HE); minated zirconium-based MOFs (UiO-66-NH₃); Au and Pd nanoparticles (AuPd NPs@UiO-66-NH₃–CoSe₂); ionic liquid (IL)-ferroferric oxide (Fe₃O₄); polyamiline@copper-1,3,5-benzenetricarboxylic acid (PAN@Cu-BTC); mesoporous carbon nanospheres embedded with SnOₓ (x = 0, 1, or 2) and TiO₂ nanocrystals (SnOₓ@TiO₂@bmC); mesoporous carbon–chitosan (OMC-CS)/gold nanoparticles–streptavidin (AuNPs-SA); palladium nanoparticles decorated on chitosan–carbon nanotube (PdNPs/CNT/Chl); an iron–nitrogen co-doped ordered mesoporous carbon–silicon nanocomposite (Si-Fe/NOMC); novel porous organic polymer (POP); reduced graphene oxide and nanogold-functionalized poly(amiidoamine) dendrimer (3D Au-PAMAM/rGO); thioumine-functionalized graphene (GR-RH) and hierarchical nanoporous (HNP) (GR-RH/HNP-PtCu); magnetic bar carbon paste electrode (MBCPE) with Fe₃O₄ magnetic nanoparticles and oleic acid (OA), (MBCPE/Fe₃O₄NPs/OA); tungsten trioxide-modified with multi-walled carbon nanotubes (MWCNTs) and gold nanoparticles (C-WO₃@AuNPs); composite of Au- and Pt-coated hollow cerium oxide (AuPt@h-CeO₂/MoS₂); Nanoscale metal–organic frameworks (NMOFs).
In antibiotic detection methods, combining the target analyte with the aptamer will lead to the conformational modification of the DNA or RNA molecules, altering the electrochemical signal to achieve target detection. The search for increasingly sensitive methods is a challenge for each proposal developed. The sensitivity of the aptasensor depends on the electrode surface, the interface characteristics of the materials used as modifiers, and the aptamer immobilization [77].

3. Relevant Aspects in the Development of Electrochemical Aptasensors

In the development of an aptameric biosensor with electrochemical detection, the adhesion of the biological material to the surface of the transducer is essential, which requires that the materials used as electrode modifiers present a high surface area and improve electrochemical properties [78]. In this sense, nanomaterials have extremely attractive characteristics and can be used as modifiers on the surface of electrodes [79].

3.1. Nanomaterial-Modified Electrochemical Aptasensors

Nanomaterials have dimensions from 1 to 100 nm, and their properties are different from the properties of molecules and crystalline solids on a larger scale. Carbon-based nanomaterials, such as carbon nanotubes, graphene, and carbon black, show advantageous characteristics, such as a high specific surface area, and excellent electrical conductivity [80].

Structural characteristics of carbonaceous nanomaterials have been widely investigated in methodologies based on biosensors with electrochemical detection. The use of these nanomaterials in aptasensors can lead to more precise and stable methods [81]. The main advantages of using nanoscale materials as modifiers on electrodes are an increased electroactive area, improved surface kinetics, improved electrode selectivity because nanomaterials behave as a stable support for functionalization with specific groups, (this characteristic is important in immobilization of the aptamer), and improved adsorption of analytes on the electrode surfaces, thus increasing sensitivity [82].

Hui et al. present an aptamer-based electrochemical methodology for the detection of streptomycin (STR) in dairy products. To provide greater sensitivity to the aptasensor device, the composite called PANI@N-CNTs, based on carbon nanotubes doped with N and polyaniline (PANI), was used as the substrate. Carbonaceous nanomaterials are excellent electrical conductors, and polyaniline is a conductive polymer with a special conduction mechanism [83].

Metal–organic frameworks (MOFs) are made up of a long network of metallic ions, coordinated to organic molecules. MOFs are considered excellent materials for DNA/RNA immobilization in the construction of methods that employ aptamers. This is because they have functional groups, such as amines and carboxyls, and can be functionalized according to the application. Additionally, they are capable of generating π-stacking and hydrogen interaction with negatively charged aptamers [84].

In several methods using electrochemical biosensors, gold nanoparticles (AuNPs) are part of the sensor construction, because they have electrical conductivity, a high surface area, and are compatible with biological compounds. They can be obtained via electrodeposition on the electrode surface or synthesized and deposited by drop-casting (simple method) [85].

Peng et al. report a biosensor based on aptamers. The device was modified with a nanocomposite formed by metal–organic frameworks, and silver-coated bimetallic gold nanoparticles for the detection of streptomycin (STR). The composite was used to amplify the electrochemical responses. Metal–organic networks have a high porosity and high internal surface area. The noble metal nanoparticles contribute to the better immobilization of the biorecognizer, due to the connection of the thiol group (present in the aptamer sequence) with silver. As a result, the aptasensor presented a fast response and a low detection limit (0.033 nM). The aptasensor was satisfactorily employed to detect STR in food products with high recovery rates (98.2% to 110.1%) [86].

Modifying the aptameric sequences can improve the sensitivity of methodologies employing aptamers. This fact occurs because the modification favors the immobilization
of the biological component and improves resistance to degradation. It is important to highlight that these procedures should not change the specific interaction of the aptamer with the species of interest. Sequence changes are common at the 5′ and 3′ ends and generally consist of the insertion of an active functionality. They promote a greater binding affinity, and consequently more selectivity for the analytes [29,87–89].

Wang et al. describe the development of a printed electrochemical aptasensor, which was modified with carbonaceous nanomaterial and gold nanoparticles for the determination of the antibiotics, kanamycin (KAN) and streptomycin (STR). The sequences used have been changed:

(KAP), 5-NH2-AGATGGGAGGCTAAGCCGA-3; STR aptamer (STP), 5-NH2-GGGTCTGGTGTCTGCTTCTGCTGGTGC-3; complementary single strand of KAP (cKAP), 3-NH2-TCTACCCCCAAACTCCGATTCGGCT-5; complementary single strand of STP (cSTP), 3-NH2-CCCACAGACACAAGACGCAAAACAGCACGCGCAGCA-5.

Due to the nanomaterials used, the aptasensor presented a high electrochemical conductivity and high surface area. The complementary aptamer chains were well inserted into the surface of the transducer. The method for KAN and STR showed excellent limits of detection, 87.3 and 45.0 pM, respectively [90].

Table 2 presents aptameric sequences that were modified in methodologies based on electrochemical aptasensors for antibiotic detection.

![Table 2. Aptameric sequences modified applied in methods based on electrochemical aptasensors for antibiotic detection.](image)

3.2. Configurations of Electrochemical Apatasensors

Several configurations can be used in the process of obtaining an electrochemical aptamer device, according to the analytical purpose. In the detection of electrochemical aptasensors, the labeled (labeled sequences) and unlabeled (unlabeled sequences) approaches are used [97].

In labeled strategies, the aptamer can be signaled by a diversity of species that can undergo oxidation or reduction. These redox flags are linked to the nucleic acid and drive the analytical response when there is biological recognition of the species of interest [Figure 5] [97]. These redox probes are chosen according to the profile, smallest difference between the oxidation and reduction peaks (reversibility), and constant behavior. The response potential range must be considered according to the electrode used [98].
Wang et al. developed an electrochemical biosensor with an aptamer as a recognizer for determining sulfamethazine (SMZ). The working electrode consisted of a glassy carbon electrode modified with an electroactive nanocomposite. A cationic polymer, cationic polyethyleneimine, immobilized the aptamer and favored its interaction with the negatively charged analyte. Using the label-free method, ruthenium complex \([\text{Ru(NH}_3\text{)}_6]^{3+}\) was used as a redox probe. The biosensor showed an excellent LOD of 4.0 pM for SMZ [100].

4. Conclusions

An attenuation or increase in the intensity of the anodic/cathode peak current of the electroactive signaling species may occur at increasing concentrations of the analyte. Thus, the electrochemical signals measured depending on the changes that occur on the surfaces of these biosensors have the possibility of being “signal-on” or “signal-off” (Figure 6), that is, there will be a greater or lesser intensity in the signal measured after the interaction of the biological material with the analyte, depending on the assay format used in the development of the aptasensor. In these strategies, aptasensors are called structure switching [97,98].

**Figure 5.** Illustration of label-based electrochemical aptasensor.

**Figure 6.** Design strategy based on “signal on/off” that occurs when aptamer interacts with an analyte. (A) Electrochemical aptasensor with off to on and (B) on to off (adapted from ref. [7]).
Li et al. report the construction of a biosensor using aptamers for the detection of two antibiotics at the same time. The detection strategy employed metal cations as signal indicators since Cd\(^{2+}\) and Pb\(^{2+}\) cations generated separate peaks in differential pulse voltammetry. The analytes studied were kanamycin and streptomycin. When the analytes were in the evaluated medium, the KAN aptamer (KAP) and the STR aptamer (STP) were released from their complementary chains, generating changes in Cd\(^{2+}\) and Pb\(^{2+}\). At the same time, the complementary chain of each sequence was linked to the poly(A) structure (cSTP-PolyA-cKAP), providing greater structural mobility. In simultaneous electrochemical detection, signal interference may occur from one analyte to another. The proposed aptasensor did not show overlapping responses from the antibiotics, due to the different peaks of the flags used. The method used carbonaceous and metallic nanomaterials as the aptasensor platform and showed detection limits for KAN of 74.50 pM and for STR of 36.45 pM [99].

The development of electrochemical aptasensors with the so-called label-free strategy is carried out by inserting the aptamer on the surface of the transducer and the recognition reaction is monitored through its interference in the electrochemical reaction between the modified surface and a redox probe [98].

Wang et al. developed an electrochemical biosensor with an aptamer as a recognizer for determining sulfamethazine (SMZ). The working electrode consisted of a glassy carbon electrode modified with an electroactive nanocomposite. A cationic polymer, cationic polyethyleneimine, immobilized the aptamer and favored its interaction with the negatively charged analyte. Using the label-free method, ruthenium complex \([\text{Ru(NH}_3)_6\text{Cl}_3]^{2+}\) was used as a redox probe. The biosensor showed an excellent LOD of 4.0 pM for SMZ [100].

4. Conclusions

The present review addressed important aspects in the development of electrochemical aptasensors and showed their applications in the detection of antibiotics. Electrochemical aptasensors provide an approach with high specificity for the detection of antibiotics. They can achieve very high sensitivity, with low detection limits (from ng m L\(^{-1}\) to fg mL\(^{-1}\)). Aptamers can be synthesized in vitro with high sensitivity for specific targets, and they can be modified to demonstrate high precision and stability.

The work showed a growing number of electrochemical aptasensors. Most of the antibiotic detection applications were in milk samples. With advances in material technology, immobilization, and modification of aptamers, there are prospects for the development of portable and low-cost devices, which can be used in situ, such as on dairy farms.

Author Contributions: D.N.d.S.: conceptualization, developed and applied the methodology, and wrote the manuscript. A.C.P.: contributed to writing, review, and editing of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Not applicable.

Acknowledgments: Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and INCT-DATREM.

Conflicts of Interest: The authors declare no conflict of interest.

References

3. Di Mauro, V.; Lauta, F.C.; Modica, J.; Appleton, S.L.; De Franciscis, V.; Catalucci, D. Diagnostic and Therapeutic Aptamers: A Promising Pathway to Improved Cardiovascular Disease Management. JACC Basic Transl. Sci. 2023. [CrossRef]


29. Melinte, G.; Hosu, O.; Cristea, C.; Marrazza, G. Ara H1 peanut allergen detection using a labelled electrochemical aptasensor based on GO-COOH@bimetallic composite platform. *Food Chem.* 2023, 400, 134074. [CrossRef]


32. Zhang, Z.; Karimi-Maleh, H. In situ synthesis of label-free electrochemical aptasensor-based sandwich-like AuNPs/Pt/Ti3C2Tx for ultrasensitive detection of lead ions as hazardous pollutants in environmental fluids. *Chemosphere* **2023**, 324, 138302. [CrossRef] [PubMed]


89. Tı˘g, G.A.; Pekyardımcı, ¸ S. An electrochemical sandwich-type aptasensor for determination of lipocalin-2 based on graphene oxide/polymer composite and gold nanoparticles. Talanta 2020, 210, 120666. [CrossRef]  
92. Li, F.; Wang, X.; Sun, X.; Guo, Y. Multiplex electrochemical aptasensor for detecting multiple antibiotics residues based on carbon quantum dots/tungsten disulfide nanocomposites. TrAC Trends Anal. Chem. 2023, 30, 101499. [CrossRef]  
94. Li, F.; Guo, Y.; Wang, X.; Sun, X. Multiplexed aptasensor based on metal ions labels for simultaneous detection of multiple antibiotics residues. Food Compos. Anl. 2021, 116, 105091. [CrossRef]  
96. Peng, H.; Hui, Y.; Pu, M.; Yang, D.; Zhao, A.; Wang, W.; Wu, S.; Wang, B. Electrochemical aptasensor based on PEI-Fe3O4@Ag NPs nanocomposites for streptomycin detection in dairy products. J. Food Compos. Anl. 2021, 384, 132495. [CrossRef]  

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.