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Review

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

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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

Citation: da Silva, D.N.; Pereira, A.C. Relevant Aspects in the Development of Electrochemical Aptasensors for the Determination of Antibiotics—A Review. *Electrochem* **2023**, *4*, 553–567. https://doi.org/10.3390/electrochem4040035

Academic Editor: Masato Sone

Received: 17 November 2023
Revised: 6 December 2023
Accepted: 7 December 2023
Published: 12 December 2023

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].

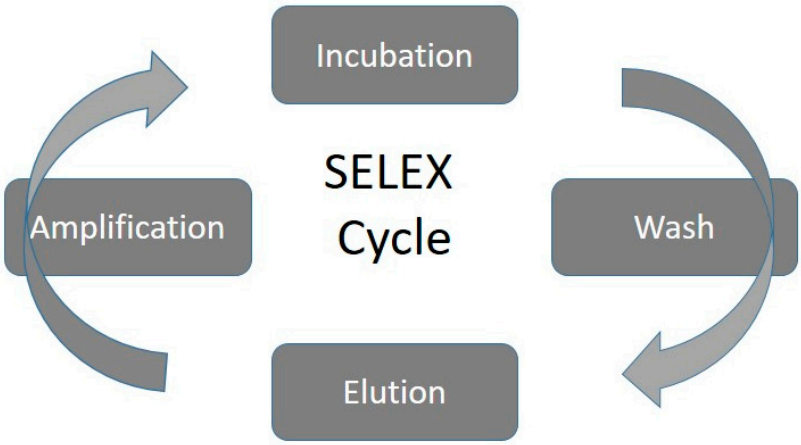

Figure 1. General representation of the SELEX method.

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).

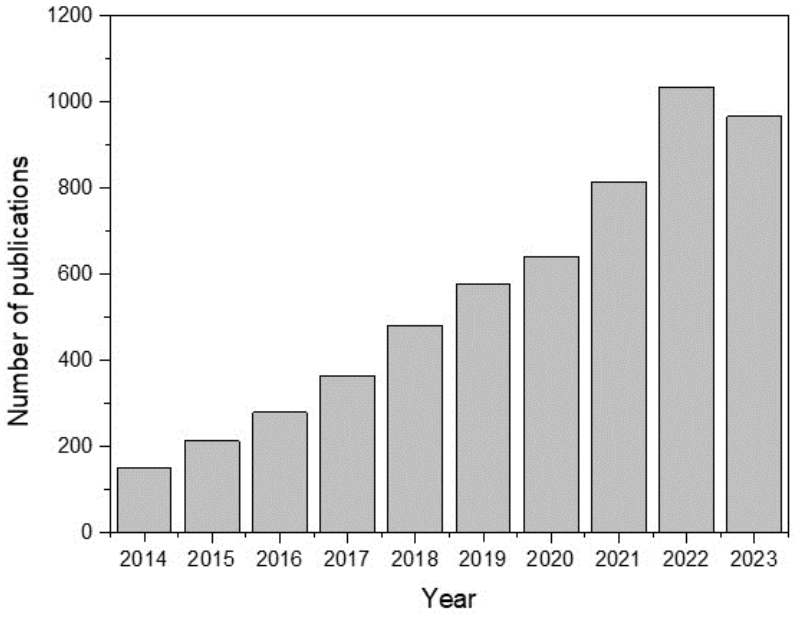

Figure 2. Number of scientific publications on electrochemical aptasensors in the last ten years. Keyword: Electrochemical Aptasensor, Source: Science Direct®, September 2023.

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 [20]. 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 (Figure 3). 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 [21,22].

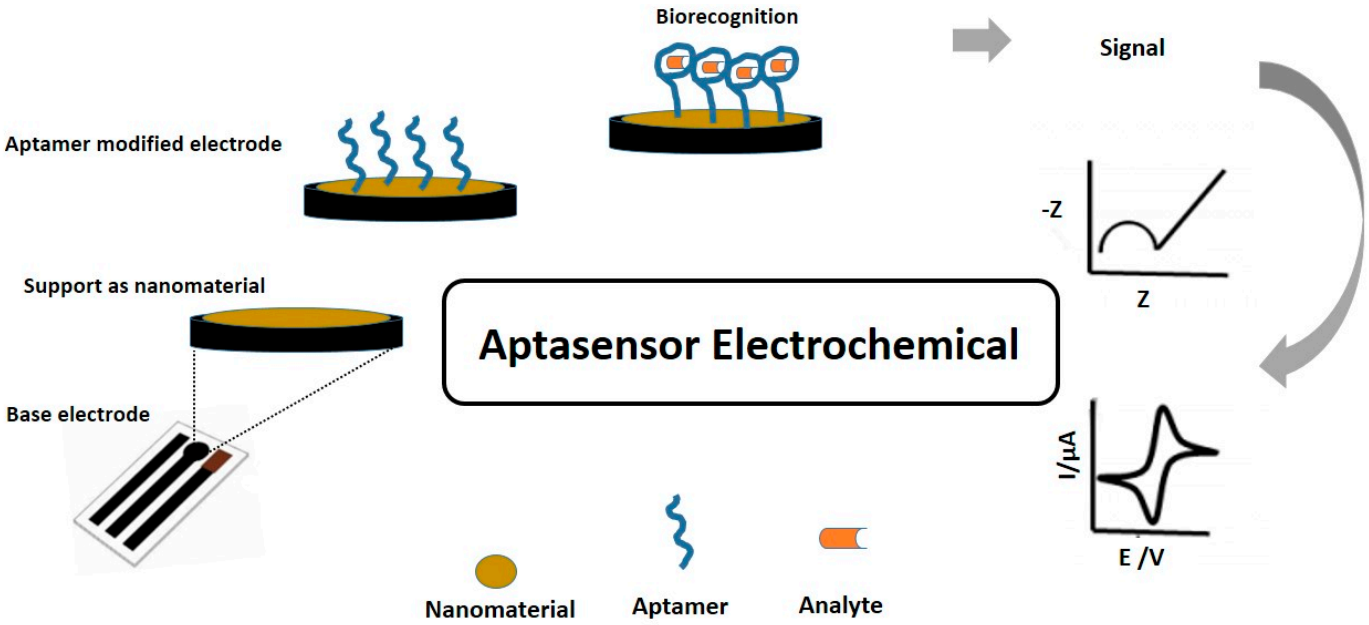

Figure 3. Electrochemical aptasensor illustration.

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 [23].

Aptasensors can be used in several areas, such as clinical diagnosis [24–27], the food industry [28–30], and environmental monitoring of contaminating species [31–34], 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 [35].

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 × 10^2 nM; TOB, 1–1 × 10^4 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

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].

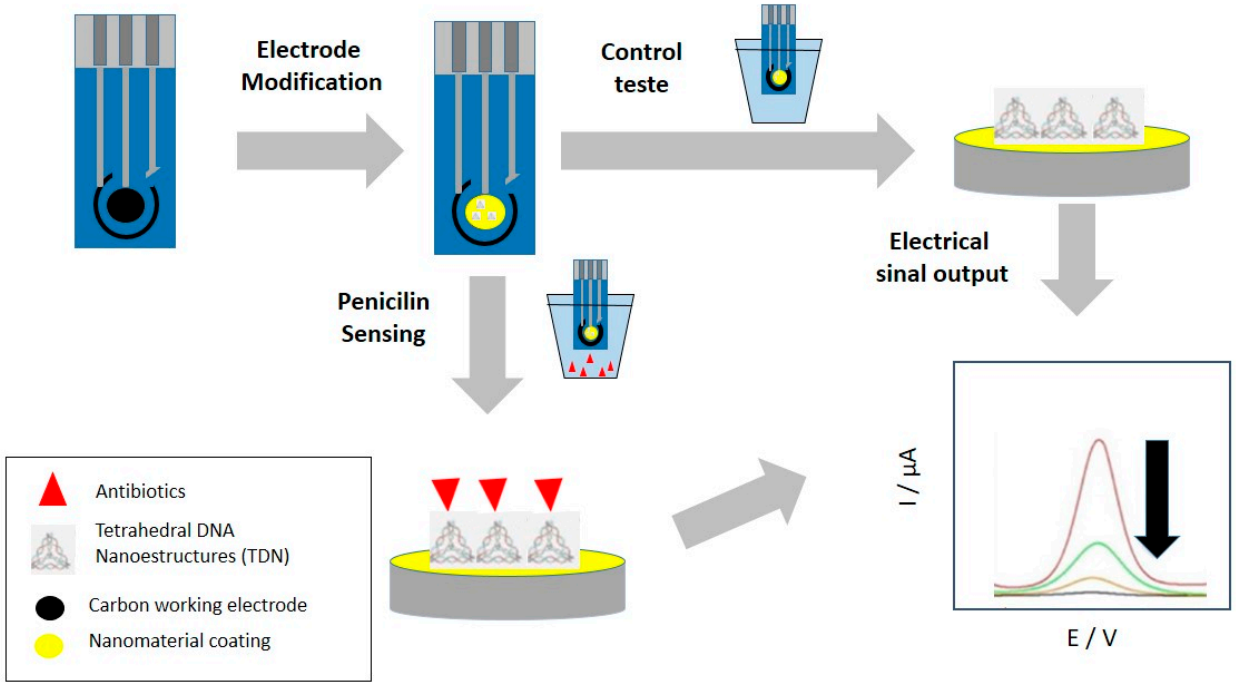

Figure 4. Illustration of penicillin detection with the electrochemical aptasensor (adapted from ref. [48]).

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 1.0×10^{-17} to 1.0×10^{-5} M and a low detection limit (2.28×10^{-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.

Antibiotic	Technique	Modification	Sample	Detection Range	Limit of Detection (LOD)	Ref.
Tetracycline	DPV	Cu_2O@Au	milk	1.0 nM–1000 µM	0.16 nM	[50]
Streptomycin	EIS	Pencil lead graphite-based electrochemical aptasensor	milk	10^{-8}–10^{-16} M	0.8×10^{-18} M	[51]
Oxytetracycline	DPV	4-carboxyphenyl anchored GCE	milk	1.0×10^{-9}–1.0×10^{-4} g mL^{-1}	2.29×10^{-10} g mL^{-1}	[52]
Ampicillin	LSV	MWCNTs	milk	1.0×10^{-13}–1.0×10^{-8} M	1.0×10^{-13}	[53]
Amoxicillin	EIS	TiO_2-g-C_3N_4@Au NPs	wastewater	0.5–3 nM	0.2 nM	[54]
Ampicillin	DPV	Endonuclease DpnII	milk and water	0.1–100 nM	32 pM	[55]
Ampicillin	EIS	Co-MOF@TPN-COF	human serum, river water, and milk	1.0 fg mL^{-1} –2.0 ng mL^{-1}	0.217 fg mL^{-1}	[56]
Tetracycline	SWV	Aptamer cocktail	honey	0.01–1000 ng mL^{-1}	0.0073 ng/mL	[57]
Ciprofloxacin	DPV	rGO/PEI/TiO_2	water	0.003–10.0 µM	0.7 nM	[58]
Streptomycin	DPV	PCNR/GR–Fe_3O_4–AuNPs	milk	0.05–200 ng mL^{-1}	0.028 ng mL^{-1}	[59]
Kanamycin	DPV	MoS_2-Au-HE	milk	1.0–1.0×10^5 ng L^{-1}	0.8 ng L^{-1}	[60]
Sulfaquinoxaline	DPV	AuPd NPs@UiO-66-NH_2/$CoSe_2$	pork	1.0–100 ng mL^{-1}	0.547 pg mL^{-1}	[61]
Tetracycline	EIS	Fe_3O_4-IL	milk	1×10^{-9}–1×10^{-5} M	1×10^{-9} M	[62]
Tetracycline	SWV	PAN@Cu-BTC	meat	10 pM–1 µM	0.32 pM	[63]
Tobramycin	DPV	phi29 DNA polymerase and nicking endonuclease Nt.AlwI	milk and water	10–200 nM	5.13 nM	[64]
Tobramycin	EIS	SnOx@TiO_2@mC	human serum and human urine	0.01–5 ng mL^{-1}	6.7 pg mL^{-1}	[65]
Kanamycin	DPV	SA-AuNPs/OMC-CS	milk	0.1–1000 nM	0.03569 nM	[66]
Streptomycin	EIS	PdNPs/CNT/Chi	milk	0.10–1500 nM	18 pM	[67]
Chloramphenicol	DPV	Si-Fe/NOMC	eye drop	1.0–500 µM	0.03 µM	[68]
Ampicillin	EIS	POP	milk	1.0×10^{-5}–5.0 ng mL^{-1}	1.33×10^{-6}	[69]
Ciprofloxacin	DPV	3D Au-PAMAM/rGO	raw milk	1 µM–1.0 nM	1.0 nM	[70]
Kanamycin	DPV	GR-TH/HNP-PtCu	pork meat and chicken	5×10^{-7}–5×10^{-2} µg mL^{-1}	0.42 pg mL^{-1}	[71]

Table 1. *Cont.*

Antibiotic	Technique	Modification	Sample	Detection Range	Limit of Detection (LOD)	Ref.
Tetracycline	DPV	MBCPE/Fe_3O_4NPs/OA	drug, milk, honey, and blood serum	1.0×10^{-10}–1.0×10^{-7} M	2.9×10^{-11} M	[72]
Tetracycline	DPV	C-WO_3@AuNPs	water, milk, honey, and black tea	0.1–100 nM	4.8×10^{-2} nM	[73]
Enrofloxacin	SWV	AuPt@h-CeO_2/MoS_2	water and milk	5.0×10^{-6}–1.0×10^{-2} ng mL^{-1}	1.02×10^{-7} ng mL^{-1}	[74]
Ampicillin	DPV	T7 exonuclease	milk	0.02–40 nM	4.0 pM	[75]
Chloramphenicol (CAP) and oxytetracycline (OTC)	SWV	NMOF	milk	10^{-4}–50 nM	CAP 0.033 pM OTC 0.048 pM	[76]

Gold nanoparticles (AuNPs) on the surface of Cu_2O nanomaterials (Cu_2O@Au); linear sweep voltammetry (LSV); multi-wall carbon nanotubes (MWCNTs); metal −organic frameworks (MOFs); g-C_3N_4 on the surface of TiO_2 microspheres and gold nanoparticles (TiO_2-g-C_3N_4@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/TiO_2) nanocomposite; carbon nanorods (PCNR) formed by porous carbon nanosphere and multifunctional graphene composite (GR–Fe_3O_4–AuNPs); nanosheets of MoS_2 (MoS_2 nanosheets) and a composite composed of MoS_2 nanosheets, Au nanoparticles (AuNPs), and hemin (HE) (denoted as MoS_2-Au-HE); minated zirconium-based MOFs (UiO-66-NH_2), Au and Pd nanoparticles (AuPd NPs@UiO-66-NH_2/$CoSe_2$); ionic liquid (IL)-ferroferric oxide (Fe_3O_4); polyaniline@copper-1,3,5-benzenetricarboxylic acid (PAN@Cu-BTC); mesoporous carbon nanospheres embedded with SnOx (x = 0, 1, or 2) and TiO_2 nanocrystals (SnOx@TiO_2@mC); mesoporous carbon–chitosan (OMC-CS)/gold nanoparticles–streptavidin (AuNPs-SA); palladium nanoparticles decorated on chitosan–carbon nanotube (PdNPs/CNT/Chi); 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(amidoamine) dendrimer (3D Au-PAMAM/rGO); thionine-functionalized graphene (GR-TH) and hierarchical nanoporous (HNP) (GR-TH/HNP-PtCu); magnetic bar carbon paste electrode (MBCPE) with Fe_3O_4 magnetic nanoparticles and oleic acid (OA), (MBCPE/Fe_3O_4NPs/OA); tungsten trioxide-modified with multi-walled carbon nanotubes (MWCNTs) and gold nanoparticles (C-WO_3@AuNPs); composite of Au- and Pt-coated hollow cerium oxide (AuPt@h-CeO_2/MoS_2). 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-NH_2-AGATGGGGGTTGAGGCTAAGCCGA-3; STR aptamer (STP), 5-NH_2-GGGGTCTGGTGTTCTGCTTTGTTCTGTCGGGTCGT3; complementary single strand of KAP (cKAP), 3-NH_2-TCTACCCCCAACTCCGATTCGGCT-5; complementary single strand of STP (cSTP), 3-NH_2-CCCCAGACCACAAGACGAAACAAGACAGCCCAGCA-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.

Antibiotic	Sequences	Ref.
Penicillin	5′-NH_2-CTG AATTGGATCTCTCTTCTTGAGCGATCTCCACA-3′	[91]
Streptomycin	5′-NH_2-GGGGTCTGGTGTTCTGCTTTGTTCTGTCGGGTCGT-3′	[92]
Oxytetracycline	5′-SH-CGACGCACAGTCGCTGGTGCGTACCTGGTTGCCGTTGTG	[93]
Kanamycin	5′-Bio-ACCGCGGGGUUGCGGACCGGGAGCUCCAGC-NH_2-3′	[47]
Tobramycin	5′-Bio-GGCACGAGGUUUAGCUACACUCGUGCC-NH_2-3′	[47]
Streptomycin	(SH-cDNA): 5′-ACGACCCGACAGAACAAAGCAGAACACCAGACCCC-SH-3′ Amino-modified STR aptamer (NH_2-Apt): 5′-NH_2-GGGGTCTGGTGTTCTGCTTTGTTCTGTCGGGTCGT-3′	[94]

Other approaches that modify aptamers have been conducted to conjugate aptamers with active targeting drugs or nanoparticles. The process consists of inserting a functionality at the 3′ and 5′ ends of the sequence to interact with the coupling co-participant on the surface of the nanomaterial [89].

As reported in the development of aptameric electrochemical biosensors, it is important to evaluate the influence of biorecognizer immobilization on analytical performance [95]. Effective strategies are also relevant in aptamer design [96]. Some of these strategies are discussed below.

3.2. Configurations of Electrochemical Aptasensors

Several configurations can be used in the process of obtaining an electrochemical aptameric 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].

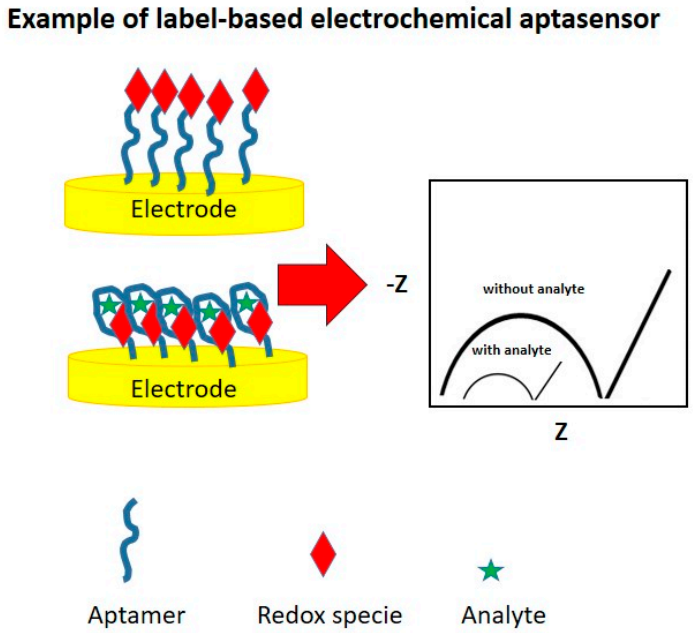

Figure 5. Illustration of label-based electrochemical aptasensor.

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].

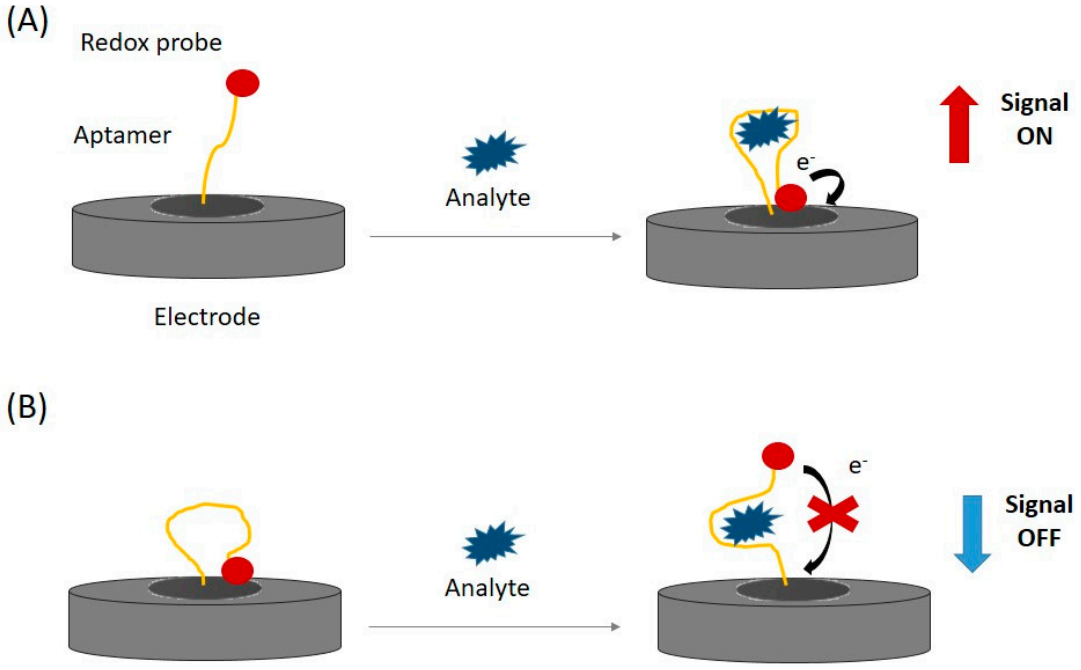

Figure 6. Design strategy based on "signal on/off" that occurs when aptamer interacts with an analyte. (**A**) Electrochemical aptasensor with off to on and (**B**) on to off (adapted from ref. [7]).

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 $[Ru(NH_3)_6]^{3+}$ 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

1. Fang, P.; Qu, H.; Mao, Y.; Zheng, L. Aptamers for mycotoxin recognition in food: Recent advances and future considerations. *Adv. Agrochem* **2023**, *2*, 213–220. [CrossRef]
2. Uğurlu, O.; Man, E.; Gök, O.; Ülker, G.; Soytürk, H.; Özyurt, C.; Evran, S. A review of aptamer-conjugated nanomaterials for analytical sample preparation: Classification according to the utilized nanomaterials. *Anal. Chim. Acta* **2023**, 342001. [CrossRef]
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]

4. Wang, L.; Peng, X.; Fu, H.; Huang, C.; Li, Y.; Liu, Z. Recent advances in the development of electrochemical aptasensors for detection of heavy metals in food. *Biosens. Bioelectron.* **2020**, *147*, 111777. [CrossRef] [PubMed]
5. Torres-Vázquez, B.; de Lucas, A.M.; García-Crespo, C.; García-Martín, J.A.; Fragoso, A.; Fernández-Algar, M.; Perales, C.; Domingo, E.; Moreno, M.; Briones, C. In vitro Selection of High Affinity DNA and RNA Aptamers that Detect Hepatitis C Virus Core Protein of Genotypes 1 to 4 and Inhibit Virus Production in Cell Culture. *J. Mol. Biol.* **2022**, *434*, 167501. [CrossRef] [PubMed]
6. Khan, S.; Hussain, A.; Fahimi, H.; Aliakbari, F.; Bloukh, S.H.; Edis, Z.; Babadaei, M.M.N.; Izadi, Z.; Varnamkhasti, B.S.; Jahanshahi, F.; et al. A review on the therapeutic applications of aptamers and aptamer-conjugated nanoparticles in cancer, inflammatory and viral diseases. *Arab. J. Chem.* **2022**, *15*, 103626. [CrossRef]
7. Nur, Y.; Gaffar, S.; Hartati, Y.W.; Subroto, T. Applications of electrochemical biosensor of aptamers-based (APTASENSOR) for the detection of leukemia biomarker. *Sens. Bio-Sens. Res.* **2021**, *32*, 100416. [CrossRef]
8. Yu, H.; Alkhamis, O.; Canoura, J.; Liu, Y.; Xiao, Y. Advances and Challenges in Small-Molecule DNA Aptamer Isolation. *Charact. Sens. Dev. Angew. Chem. Int. Ed. Engl.* **2021**, *60*, 16800–16823. [CrossRef] [PubMed]
9. Alkhamis, O.; Canoura, J.; Yu, H.; Liu, Y.; Xiao, Y. Innovative engineering and sensing strategies for aptamer-based small-molecule detection. *TrAC Trends Anal. Chem.* **2019**, *121*, 115699. [CrossRef]
10. Lin, X.; Sun, X.; Luo, S.; Liu, B.; Yang, C. Development of DNA-based signal amplification and microfluidic technology for protein assay: A review. *TrAC Trends Anal. Chem.* **2016**, *80*, 132–148. [CrossRef]
11. Xie, Y.; Huang, Y.; Li, J.; Wu, J. A trigger-based aggregation of aptamer-functionalized gold nanoparticles for colorimetry An example on detection of Escherichia coli O157:H7. *Sens. Actuators B Chem.* **2021**, *339*, 129865. [CrossRef]
12. Yu, H.; Zhao, Q. Competitive fluorescence assay for Cd^{2+} based on aptamer structure-switching. *Microchem. J.* **2023**, *194*, 109348. [CrossRef]
13. Yang, J.; Liu, H.; Huang, Y.; Li, L.; Zhu, X.; Ding, Y. One-step hydrothermal synthesis of near-infrared emission carbon quantum dots as fluorescence aptamer sensor for cortisol sensing and imaging. *Talanta* **2023**, *260*, 124637. [CrossRef] [PubMed]
14. Lee, S.H.; Back, H.; Joo, H.J.; Lim, D.-S.; Lee, J.E.; Lee, H.J. Simultaneous detection method for two cardiac disease protein biomarkers on a single chip modified with mixed aptamers using surface plasmon resonance. *Talanta* **2024**, *267*, 125232. [CrossRef] [PubMed]
15. Gupta, R.; Prakash, N.; Paul, D.; Mukherji, S. Anti-nucleolin aptamer mediated specific detection of cancer cells by Localized Surface Plasmon Resonance-based U-bent optical fiber. *Biosens. Bioelectron. X* **2023**, *13*, 100318. [CrossRef]
16. Zhao, S.; Huang, J.; Li, D.; Yang, L. Aptamer-based chemiluminescent optical fiber immunosensor with enhanced signal amplification for ultrasensitive detection of tumor biomarkers. *Biosens. Bioelectron.* **2022**, *214*, 114505. [CrossRef] [PubMed]
17. Meng, X.; Li, J.; Wu, Y.; Cao, X.; Zhang, Z. Rational design of hairpin aptamer using intrinsic disorder mechanism to enhance sensitivity of aptamer folding-based electrochemical sensor for tobramycin. *Sens. Actuators B Chem.* **2023**, *394*, 134354. [CrossRef]
18. Kim, E.R.; Dang, T.T.-T.; Lee, S.J.; Nguyen, T.T.-Q.; Park, J.-W.; Gu, M.B. A highly sensitive sandwich-type electrochemical aptasensor using a pair of novel truncated aptamers for the detection of vaspin. *Chem. Eng. J.* **2023**, *477*, 147002. [CrossRef]
19. Khan, R.; Deshpande, A.S.; Proteasa, G.; Andreescu, S. Aptamer-based electrochemical biosensor with S protein binding affinity for COVID-19 detection: Integrating computational design with experimental validation of S protein binding affinity. *Sens. Actuators B Chem.* **2024**, *399*, 134775. [CrossRef]
20. Zhang, L.; Guo, W.; Lv, C.; Liu, X.; Yang, M.; Guo, M.; Fu, Q. Electrochemical biosensors represent promising detection tools in medical field. *Adv. Sens. Energy Mater.* **2023**, *2*, 100081. [CrossRef]
21. Zhong, S.; Chen, L.; Shi, X.; Chen, G.; Sun, D.; Zhang, L. Recent advances in electrochemical aptasensors for detecting cardiac biomarkers: A review. *Microchem. J.* **2023**, *193*, 109063. [CrossRef]
22. Azzouz, A.; Hejji, L.; Kumar, V.; Kim, K.-H. Nanomaterials-based aptasensors: An efficient detection tool for heavy-metal and metalloid ions in environmental and biological samples. *Environ. Res.* **2023**, *238 Pt 1*, 117170. [CrossRef] [PubMed]
23. Ikebukuro, K.; Kiyohara, C.; Sode, K. Electrochemical detection of protein using a double aptamer sandwich. *Anal. Lett.* **2004**, *37*, 2901–2909. [CrossRef]
24. Zhang, Y.; Chen, S.; Ma, J.; Zhou, X.; Sun, X.; Jing, H.; Lin, M.; Zhou, C. Enzyme-catalyzed electrochemical aptasensor for ultrasensitive detection of soluble PD-L1 in breast cancer based on decorated covalent organic frameworks and carbon nanotubes. *Anal. Chim. Acta* **2023**, *1282*, 341927. [CrossRef] [PubMed]
25. Li, G.; Li, H.; Li, X.; Huang, H.; Bian, H.; Liang, J.; Zhou, Z. A label-free electrochemical aptasensor for low-density lipoprotein detection using MoS2-Au-Fc nanosheets as a high-performance redox indicator. *Microchem. J.* **2023**, *193*, 109068. [CrossRef]
26. Chen, K.; Zhao, H.; Wang, Z.; Zhou, F.; Lan, M. Sandwich-type electrochemical aptasensor based on polydopamine-Au-metal ions as signal label and coralloid Au-conductive carbon architecture-modified electrode for the simultaneous detection of cardiac troponin I and myoglobin. *Sens. Actuators B Chem.* **2023**, *390*, 134044. [CrossRef]
27. Chen, Y.; Gu, Y.; Yi, X.; Huang, H.; Li, Y.; Yang, B.; Guo, S.; Bai, L. Conductive nano-gold self-assembled MXene@hemin with high catalytic activity achieved by strong metal-support interactions: A powerful nanozyme for development of electrochemical aptasensor in tuberculosis diagnosis. *Chem. Eng. J.* **2023**, *466*, 143112. [CrossRef]
28. Li, L.; Ma, R.; Wang, W.; Zhang, L.; Li, J.; Eltzov, E.; Wang, S.; Mao, X. Group-targeting aptamers and aptasensors for simultaneous identification of multiple targets in foods. *TrAC Trends Anal. Chem.* **2023**, *166*, 117169. [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]

30. Kang, M.; Yao, Y.; Yuan, B.; Zhang, S.; Oderinde, O.; Zhang, Z. A sensitive bimetallic copper/bismuth metal-organic frameworks-based aptasensors for zearalenone detection in foodstuffs. *Food Chem.* **2024**, *437 Pt 1*, 137827. [CrossRef]
31. Lai, Z.; Mahdavi, B.; Baghayeri, M. Label-free and sensitive determination of toxic Cd(II) in environmental waters using a Fe3O4-PEI-Au based electrochemical aptasensor. *Alex. Eng. J.* **2023**, *83*, 251–256. [CrossRef]
32. Zhang, Z.; Karimi-Maleh, H. In situ synthesis of label-free electrochemical aptasensor-based sandwich-like AuNPs/PPy/Ti3C2Tx for ultrasensitive detection of lead ions as hazardous pollutants in environmental fluids. *Chemosphere* **2023**, *324*, 138302. [CrossRef] [PubMed]
33. Yalagandula, B.P.; Mohanty, S.; Goswami, P.P.; Singh, S.G. Optimizations towards a nearly invariable WO_3-functionalized electrochemical aptasensor for ultra-trace identification of arsenic in lake water. *Sens. Actuators B Chem.* **2024**, *398*, 134730. [CrossRef]
34. Khosropour, H.; Keramat, M.; Laiwattanapaisal, W. A dual action electrochemical molecularly imprinted aptasensor for ultra-trace detection of carbendazim. *Biosens. Bioelectron.* **2024**, *243*, 115754. [CrossRef] [PubMed]
35. Zein, M.I.H.L.; Hardianto, A.; Irkham, I.; Zakiyyah, S.N.; Devi, M.J.; Manan, N.S.A.; Ibrahim, A.U.; Hartati, Y.W. Recent development of electrochemical and optical aptasensors for detection of antibiotics in food monitoring applications. *J. Food Compos. Anal.* **2023**, *124*, 105644. [CrossRef]
36. Joshi, A.; Kim, K.-H. Recent advances in nanomaterial-based electrochemical detection of antibiotics: Challenges and future perspectives. *Biosens. Bioelectron.* **2020**, *153*, 112046. [CrossRef] [PubMed]
37. Hui, X.; Fang, W.; Wang, G.; Liu, H.; Dai, X. Waste recycling of antibiotic mycelial residue: The feasible harmless treatment and source control of antibiotic resistance. *J. Clean. Prod.* **2023**, *401*, 136786. [CrossRef]
38. Ding, R.; Chen, Y.; Wang, Q.; Wu, Z.; Zhang, X.; Li, B.; Lin, L. Recent advances in quantum dots-based biosensors for antibiotics detection. *J. Pharm. Anal.* **2022**, *12*, 355–364. [CrossRef]
39. Yu, C.; Pang, H.; Wang, J.-H.; Chi, Z.-Y.; Zhang, Q.; Kong, F.-T.; Xu, Y.-P.; Li, S.-Y.; Che, J. Occurrence of antibiotics in waters, removal by microalgae-based systems, and their toxicological effects: A review. *Sci. Total Environ.* **2022**, *813*, 151891. [CrossRef]
40. Singh, H.; Thakur, B.; Bhardwaj, S.K.; Khatri, M.; Kim, K.-H.; Bhardwaj, N. Nanomaterial-based fluorescent biosensors for the detection of antibiotics in foodstuffs: A review. *Food Chem.* **2023**, *426*, 136657. [CrossRef]
41. Wang, Q.; Zhao, W.-M. Optical methods of antibiotic residues detections: A comprehensive review. *Sens. Actuators B Chem.* **2018**, *269*, 238–256. [CrossRef]
42. Fu, L.; Mao, S.; Chen, F.; Zhao, S.; Su, W.; Lai, G.; Yu, A.; Lin, C.-T. Graphene-based electrochemical sensors for antibiotic detection in water, food and soil: A scientometric analysis in CiteSpace (2011–2021). *Chemosphere* **2022**, *297*, 134127. [CrossRef] [PubMed]
43. Hu, S.; Wei, Y.; Wang, J.; Yu, Y. A photo-renewable ZIF-8 photo electrochemical sensor for the sensitive detection of sulfamethoxazole antibiotic. *Anal. Chim. Acta* **2021**, *1178*, 338793. [CrossRef] [PubMed]
44. Sgobbi, L.F.; Razzino, C.A.; Machado, S.A.S. A disposable electrochemical sensor for simultaneous detection of sulfamethoxazole and trimethoprim antibiotics in urine based on multiwalled nanotubes decorated with Prussian blue nanocubes modified screen-printed electrode. *Electrochim. Acta* **2016**, *191*, 1010–1017. [CrossRef]
45. Elfiky, M.; Salahuddin, N.; Hassanein, A.; Matsuda, A.; Hattori, T. Detection of antibiotic Ofloxacin drug in urine using electrochemical sensor based on synergistic effect of different morphological carbon materials. *Microchem. J.* **2019**, *146*, 170–177. [CrossRef]
46. Bai, Z.; Chen, Y.; Li, F.; Zhou, Y.; Yin, H.; Ai, S. Electrochemical aptasensor for sulfadimethoxine detection based on the triggered cleavage activity of nuclease P1 by aptamer-target complex. *Talanta* **2019**, *204*, 409–414. [CrossRef]
47. Li, F.; Wu, Y.; Chen, D.; Guo, Y.; Wang, X.; Sun, X. Sensitive dual-labeled electrochemical aptasensor for simultaneous detection of multi-antibiotics in milk. *Int. J. Hydrogen Energy* **2021**, *46*, 23301–23309. [CrossRef]
48. Guan, J.; He, K.; Gunasekaran, S. Selection of ssDNA aptamer using GO-SELEX and development of DNA nanostructure-based electrochemical aptasensor for penicillin. *Biosens. Bioelectron. X* **2022**, *12*, 100220. [CrossRef]
49. Naseri, M.; Niazi, A.; Bagherzadeh, K.; Konoz, E.; Samadikhah, H.R. Modified electrochemical aptasensor for ultrasensitive detection of tetracycline: In silico and in vitro studies. *Food Chem.* **2023**, *421*, 136195. [CrossRef]
50. Wei, X.; Yin, M.; Zhang, L.; Sun, Y.; Luo, Y.; Xu, D. Octahedral Cu2O nanomaterials as electrochemical aptasensor for sensitive detection of tetracycline in milk. *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* **2024**, *304*, 123361. [CrossRef]
51. Vanani, S.M.; Izadi, Z.; Hemmati, R.; Saffar, B. Fabrication of an ultrasensitive aptasensor for precise electrochemical detection of the trace amounts of streptomycin in milk. *Colloids Surf. B Biointerfaces* **2021**, *206*, 111964. [CrossRef] [PubMed]
52. Yang, Y.; Yan, W.; Guo, Y.; Wang, X.; Zhang, F.; Yu, L.; Guo, C.; Fang, G. Sensitive and selective electrochemical aptasensor via diazonium-coupling reaction for label-free determination of oxytetracycline in milk samples. *Sens. Actuators Rep.* **2020**, *2*, 100009. [CrossRef]
53. Li, F.; Zhu, J.; Liu, Y.; Lil, Z.; Kang, H.; Li, R. Magnetic bead-based electrochemical aptasensor doped with multi-wall carbon nanotubes for the detection of ampicillin in milk. *Int. J. Electrochem. Sci.* **2020**, *15*, 7520–7530. [CrossRef]
54. Song, J.; Huang, M.; Jiang, N.; Zheng, S.; Mu, T.; Meng, L.; Liu, Y.; Liu, J.; Chen, G. Ultrasensitive detection of amoxicillin by TiO_2-g-C_3N_4@AuNPs impedimetric aptasensor: Fabrication, optimization, and mechanism. *J. Hazard. Mater.* **2020**, *391*, 122024. [CrossRef] [PubMed]

55. Wang, T.; Yin, H.; Zhang, Y.; Wang, L.; Du, Y.; Zhuge, Y.; Ai, S. Electrochemical aptasensor for ampicillin detection based on the protective effect of aptamer-antibiotic conjugate towards DpnII and Exo III digestion. *Talanta* **2019**, *197*, 42–48. [CrossRef] [PubMed]
56. Liu, X.; Hu, M.; Wang, M.; Song, Y.; Zhou, N.; He, L.; Zhang, Z. Novel nanoarchitecture of Co-MOF-on-TPN-COF hybrid: Ultralowly sensitive bioplatform of electrochemical aptasensor toward ampicillin. *Biosens. Bioelectron.* **2019**, *123*, 59–68. [CrossRef] [PubMed]
57. Huang, Y.; Yan, X.; Zhao, L.; Qi, X.; Wang, S.; Liang, X. An aptamer cocktail-based electrochemical aptasensor for direct capture and rapid detection of tetracycline in honey. *Microchem. J.* **2019**, *150*, 104179. [CrossRef]
58. Zhu, Y.; Li, X.; Wu, M.; Shi, M.; Tian, Q.; Fu, L.; Tsai, H.-S.; Xie, W.-F.; Lai, G.; Wang, G.; et al. A novel electrochemical aptasensor based on eco-friendly synthesized titanium dioxide nanosheets and polyethyleneimine grafted reduced graphene oxide for ultrasensitive and selective detection of ciprofloxacin. *Anal. Chim. Acta* **2023**, *1275*, 341607. [CrossRef]
59. Yin, J.; Guo, W.; Qin, X.; Zhao, J.; Pei, M.; Ding, F. A sensitive electrochemical aptasensor for highly specific detection of streptomycin based on the porous carbon nanorods and multifunctional graphene nanocomposites for signal amplification. *Sens. Actuators B Chem.* **2017**, *241*, 151–159. [CrossRef]
60. Song, H.-Y.; Kang, T.-F.; Lu, L.-P.; Cheng, S.-Y. Highly sensitive aptasensor based on synergetic catalysis activity of MoS2-Au-HE composite using cDNA-Au-GOD for signal amplification. *Talanta* **2017**, *164*, 27–33. [CrossRef]
61. Li, S.; He, B.; Liang, Y.; Wang, J.; Jiao, Q.; Liu, Y.; Guo, R.; Wei, M.; Jin, H. Sensitive electrochemical aptasensor for determination of sulfaquinoxaline based on AuPd NPs@UiO-66-NH2/CoSe2 and RecJf exonuclease-assisted signal amplification. *Anal. Chim. Acta* **2021**, *1182*, 338948. [CrossRef] [PubMed]
62. Zhao, G.; Xu, Q.; Zhang, Q.; Guo, Y.; Sun, X.; Wang, X.Y. Study on Aptasensors Modified by Ionic Liquid-Fe_3O_4 Based on Microarray Electrodes for Tetracycline Detection. *Int. J. Electrochem. Sci.* **2016**, *11*, 1699–1706. [CrossRef]
63. Zhan, F.; Zhao, Y.; Dai, X.; Zeng, J.; Wang, Q. Electrochemically synthesized polyanine@Cu-BTC MOF as a bifunctional matrix for aptasensing of tetracycline in aquatic products. *Microchem. J.* **2024**, *196*, 109512. [CrossRef]
64. Nie, J.; Yuan, L.; Jin, K.; Han, X.; Tian, Y.; Zhou, N. Electrochemical detection of tobramycin based on enzymes-assisted dual signal amplification by using a novel truncated aptamer with high affinity. *Biosens. Bioelectron.* **2018**, *122*, 254–262. [CrossRef] [PubMed]
65. Wang, M.; Hu, B.; Yang, C.; Zhang, Z.; He, L.; Fang, S.; Qu, X.; Zhang, Q. Electrochemical biosensing based on protein-directed carbon nanospheres embedded with SnO_x and TiO_2 nanocrystals for sensitive detection of tobramycin. *Biosens. Bioelectron.* **2018**, *99*, 176–185. [CrossRef]
66. Li, F.; Wang, X.; Sun, X.; Guo, Y.; Zhao, W. A dual-signal amplification strategy for kanamycin based on ordered mesoporous carbon-chitosan/gold nanoparticles-streptavidin and ferrocene labelled DNA. *Anal. Chim. Acta* **2018**, *1033*, 185–192. [CrossRef]
67. Aghajari, R.; Azadbakht, A. Amplified detection of streptomycin using aptamer-conjugated palladium nanoparticles decorated on chitosan-carbon nanotube. *Anal. Biochem.* **2018**, *547*, 57–65. [CrossRef]
68. Yalikun, N.; Mamat, X.; Li, Y.; Hu, X.; Wågberg, T.; Dong, Y.; Hu, G. Synthesis of an iron-nitrogen co-doped ordered mesoporous carbon-silicon nanocomposite as an enhanced electrochemical sensor for sensitive and selective determination of chloramphenicol. *Colloids Surf. B Biointerfaces* **2018**, *172*, 98–104. [CrossRef]
69. Yuan, R.; Yan, Z.; Shaga, A.; He, H. Design and fabrication of an electrochemical sensing platform based on a porous organic polymer for ultrasensitive ampicillin detection. *Sens. Actuators B Chem.* **2021**, *327*, 128949. [CrossRef]
70. Mahmoudpour, M.; Kholafazad-kordasht, H.; Dolatabadi, J.E.N.; Hasanzadeh, M.; Rad, A.H.; Torbati, M. Sensitive aptasensing of ciprofloxacin residues in raw milk samples using reduced graphene oxide and nanogold-functionalized poly(amidoamine) dendrimer: An innovative apta-platform towards electroanalysis of antibiotics. *Anal. Chim. Acta* **2021**, *1174*, 338736. [CrossRef]
71. Qin, X.; Yin, Y.; Yu, H.; Guo, W.; Pei, M. A novel signal amplification strategy of an electrochemical aptasensor for kanamycin, based on thionine functionalized graphene and hierarchical nanoporous PtCu. *Biosens. Bioelectron.* **2016**, *77*, 752–758. [CrossRef] [PubMed]
72. Jahanbani, S.; Benvidi, A. Comparison of two fabricated aptasensors based on modified carbon paste/oleic acid and magnetic bar carbon paste/Fe_3O_4@oleic acid nanoparticle electrodes for tetracycline detection. *Biosens. Bioelectron.* **2016**, *85*, 553–562. [CrossRef] [PubMed]
73. Song, J.; Lin, X.; Jiang, N.; Huang, M. Carbon-doped WO_3 electrochemical aptasensor based on Box-Behnken strategy for highly-sensitive detection of tetracycline. *Food Chem.* **2022**, *367*, 130564. [CrossRef] [PubMed]
74. Zhang, B.; Lv, L.; Ma, X.; Xie, L.; Lin, M.; Chen, H.; He, B. Au@ZnNi-MOF labeled electrochemical aptasensor for detection of enrofloxacin based on AuPt@h-CeO_2/MoS_2 and DNAzyme-driven DNA walker triple amplification signal strategy. *Biosens. Bioelectron.* **2022**, *210*, 114296. [CrossRef] [PubMed]
75. Wang, X.; Dong, S.; Gai, P.; Duan, R.; Li, F. Highly sensitive homogeneous electrochemical aptasensor for antibiotic residues detection based on dual recycling amplification strategy. *Biosens. Bioelectron.* **2016**, *82*, 49–54. [CrossRef] [PubMed]
76. Chen, M.; Gan, N.; Li, T.; Wang, Y.; Xu, Q.; Chen, Y. An electrochemical aptasensor for multiplex antibiotics detection using Y-shaped DNA-based metal ions encoded probes with NMOF substrate and CSRP target-triggered amplification strategy. *Anal. Chim. Acta* **2017**, *968*, 30–39. [CrossRef] [PubMed]
77. Dong, X.; Yan, X.; Li, M.; Liu, H.; Li, J.; Wang, L.; Wang, K.; Lu, X.; Wang, S.; He, B. Ultrasensitive detection of chloramphenicol using electrochemical aptamer sensor: A mini review. *Electrochem. Commun.* **2020**, *120*, 106835. [CrossRef]

78. Liu, Y.; Deng, Y.; Li, S.; Chow, F.W.-N.; Liu, M.; He, N. Monitoring and detection of antibiotic residues in animal derived foods: Solutions using aptamers. *Trends Food Sci. Technol.* **2022**, *125*, 200–235. [CrossRef]
79. Xie, J.; Zhang, L.; Liu, Z.; Ling, G.; Zhang, P. Application of electrochemical sensors based on nanomaterials modifiers in the determination of antipsychotics. *Colloids Surf. B Biointerfaces* **2022**, *214*, 112442. [CrossRef]
80. Kaya, S.I.; Yıldırım, S.; Cetinkaya, A.; Erkmen, C.; Uslu, B.; Ozkan, S.A. Nanomaterial-based electroanalytical sensors for the selected prohibited anabolic agents, hormones and metabolic modulators and their sensitive assays. *TrAC Trends Anal. Chem.* **2021**, *145*, 116457. [CrossRef]
81. Parihar, A.; Choudhary, N.K.; Sharma, P.; Khan, R. Carbon nanomaterials-based electrochemical aptasensor for point-of-care diagnostics of cancer biomarkers. *Mater. Today Chem.* **2023**, *30*, 101499. [CrossRef]
82. Gañán, J.; Martínez-García, G.; Morante-Zarcero, S.; Pérez-Quintanilla, D.; Sierra, I. Nanomaterials-modified electrochemical sensors for sensitive determination of alkaloids: Recent trends in the application to biological, pharmaceutical and agri-food samples. *Microchem. J.* **2023**, *184 Pt A*, 108136. [CrossRef]
83. Hui, Y.; Yang, D.; Wang, W.; Liu, Y.; He, C.; Wang, B. Truncated affinity-improved aptamer for selective and sensitive detection of streptomycin in dairy products with label-free electrochemical aptasensor. *J. Food Compos. Anal.* **2023**, *121*, 105422. [CrossRef]
84. Gorgani, L.; Mohammadi, M.; Darzi, G.N.; Raoof, J.B. Electrochemical aptasensor based on bimetallic CuZr-MOF for ultrasensitive detection of miR-21. *Sens. Actuators B Chem.* **2023**, *378*, 133194. [CrossRef]
85. Tığ, G.A.; Pekyardımcı, Ş. An electrochemical sandwich-type aptasensor for determination of lipocalin-2 based on graphene oxide/polymer composite and gold nanoparticles. *Talanta* **2020**, *210*, 120666.
86. Peng, H.; Hui, Y.; Pu, M.; Yang, D.; Zhao, A.; Wang, W.; Wu, S.; Wang, B. Electrochemical aptasensor based on PEI-Fe-MOF/Au@Ag NPs nanocomposites for streptomycin detection in dairy products. *J. Food Compos. Anal.* **2021**, *116*, 105091. [CrossRef]
87. Uhuo, O.V.; Waryo, T.T.; Douman, S.F.; Januarie, K.C.; Nwambaekwe, K.C.; Ndipingwi, M.M.; Ekwere, P.; Iwuoha, E.I. Bioanalytical methods encompassing label-free and labeled tuberculosis aptasensors: A review. *Anal. Chim. Acta* **2022**, *1234*, 340326. [CrossRef] [PubMed]
88. Zhong, T.; Li, S.; Li, X.; JiYe, Y.; Mo, Y.; Chen, L.; Zhang, Z.; Wu, H.; Li, M.; Luo, Q. A label-free electrochemical aptasensor based on AuNPs-loaded zeolitic imidazolate framework-8 for sensitive determination of aflatoxin B1. *Food Chem.* **2022**, *384*, 132495. [CrossRef]
89. Odeh, F.; Nsairat, H.; Alshaer, W.; Ismail, M.A.; Esawi, E.; Qaqish, B.; Bawab, A.A.; Ismail, S.I. Aptamers Chemistry: Chemical Modifications and Conjugation Strategies. *Molecules* **2019**, *25*, 3. [CrossRef]
90. Li, F.; Wang, X.; Sun, X.; Guo, Y. Multiplex electrochemical aptasensor for detecting multiple antibiotics residues based on carbon fiber and mesoporous carbon-gold nanoparticles. *Sens. Actuators B Chem.* **2018**, *265*, 217–226. [CrossRef]
91. He, H.; Wang, S.-Q.; Han, Z.-Y.; Tian, X.-H.; Zhang, W.-W.; Li, C.-P.; Du, M. Construction of electrochemical aptasensors with Ag(I) metal−organic frameworks toward high-efficient detection of ultra-trace penicillin. *Appl. Surf. Sci.* **2020**, *531*, 147342. [CrossRef]
92. Hui, Y.; Yang, D.; Wei, L.; Pu, M.; Mao, Y.; Chen, X.; Wang, B. Rapid and label-free electrochemical aptasensor based on a palladium nanoparticles/titanium carbide/polyethyleneimine functionalized nitrogen-doped carbon nanotubes composite for amplified detection of streptomycin. *Food Chem.* **2024**, *432*, 137271. [CrossRef] [PubMed]
93. Liu, S.; Wang, Y.; Xu, W.; Leng, X.; Wang, H.; Guo, Y.; Huang, J. A novel sandwich-type electrochemical aptasensor based on GR-3D Au and aptamer-AuNPs-HRP for sensitive detection of oxytetracycline. *Biosens. Bioelectron.* **2017**, *88*, 181–187. [CrossRef] [PubMed]
94. Meng, X.Z.; Gu, H.W.; Yi, H.C.; He, Y.Q.; Chen, Y.; Sun, W.Y. Sensitive detection of streptomycin in milk using a hybrid signal enhancement strategy of MOF-based bio-bar code and target recycling. *Anal. Chim. Acta* **2020**, *1125*, 1–7. [CrossRef] [PubMed]
95. Hianik, T.; Ostatná, V.; Sonlajtnerova, M.; Grman, I. Influence of ionic strength, pH and aptamer configuration for binding affinity to thrombin. *Bioelectrochem* **2007**, *70*, 127–133. [CrossRef] [PubMed]
96. Kadam, U.S.; Hong, J.C. Advances in aptameric biosensors designed to detect toxic contaminants from food, water, human fluids, and the environment. *Trends Environ. Anal. Chem.* **2022**, *36*, e00184. [CrossRef]
97. Meirinho, S.G.; Dias, L.G.; Peres, A.M.; Rodrigues, L.R. Voltammetric aptasensors for protein disease biomarkers detection: A review. *Biotechnol. Adv.* **2016**, *34*, 941–953. [CrossRef] [PubMed]
98. Grabowska, I.; Zapotoczny, S.; Chlopicki, S. Multiplex electrochemical aptasensors for detection of endothelial dysfunction: Ready for prime time? *TrAC Trends Anal. Chem.* **2023**, *169*, 117372. [CrossRef]
99. Li, F.; Guo, Y.; Wang, X.; Sun, X. Multiplexed aptasensor based on metal ions labels for simultaneous detection of multiple antibiotic residues in milk. *Biosens. Bioelectron.* **2018**, *115*, 7–13. [CrossRef]
100. Wang, Y.; Gong, C.; Zhu, Y.; Wang, Q.; Geng, L. Signal-on electrochemical aptasensor for sensitive detection of sulfamethazine based on carbon quantum dots/tungsten disulfide nanocomposites. *Electrochim. Acta* **2021**, *393*, 139054. [CrossRef]