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Mini Review

Recent Development and Progress of Electrochemical Sensors for Antibiotic Detection

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Antibiotics can effectively inhibit the infection of pathogenic microorganisms and the growth and reproduction of bacteria, so they are used to treat various human and animal diseases. Antibiotics are widely used in agriculture, animal husbandry and aquaculture. In addition to disease prevention and treatment, antibiotics are often added in feed to promote the growth and development of animals. Although the use of antibiotics has increased the production of agriculture, animal husbandry and aquaculture, thus bringing huge economic benefits, the resulting residual problems cannot be ignored. In this review, the research status of various kinds of electrochemical sensors used in the detection of antibiotics in water is reviewed, and the preparation, performance, mechanism, advantages and disadvantages of modified electrodes for electrochemical detection methods are categorically analyzed.

Keywords: Electrochemical sensor; Antibiotic; DNA sensor; Electrode modification; Electroanalysis

1. INTRODUCTION

Antibiotics are a kind of medicine that is often used by people. It has been one of the greatest medical discoveries by human beings. It is a kind of secondary metabolite produced by microorganisms (including bacteria, fungi, actinomycetes) or higher animals and plants in the process of their life activities, which imparts disease resistance or participates in other activities that can interfere with the development of other living cells [1–4]. Existing antibiotics mainly include cephalosporins, penicillins, chloramphenicols, aminoglycosides, tetracyclines, macrolides and lincomamide. With the progress of science and technology and the continuous development of pharmaceutical chemistry and organic chemistry, new antibiotics are still being synthesized and applied [5–11]. On the one hand, the use of antibiotics affects the normal life activities of animals and plants and significantly improves the color, growth cycle and morphology of animals. On the other hand, the overuse of antibiotics also results in low nutrition and a poor sense of taste [12–16]. For human life activities, antibiotics not only cure

diseases, alleviate pain and save the lives of patients but also have adverse consequences caused by improper use. The long-term intake of food containing antibiotic residues can lead to the accumulation of antibiotics in the body, which can lead to tissue and organ diseases, and even to cancer [17–20].

Antibiotics in the food chain come from a wide range of sources: To promote the rapid growth of animals and plants or achieve the goal of disease treatment, some breeders have added excessive antibiotics to feed [21–24]. Thus, the antibiotics in the food chain come from the original animals and plants. To promote the growth of animals and control diseases, breeders have improperly overused antibiotics, such as sedatives, clenbuterol and other illegal drugs [25–30]. In terms of industrial production, to prolong the shelf life of food, prevent mildew and improve the taste of food, some manufacturers have illegally used antibiotics in food processing [31–36]. In terms of packaging, some of the additives in food packaging materials will migrate to the food in the process of packaging and transportation, thus polluting the food.

With the inappropriate use of antibiotics in agriculture, animal husbandry, fishery and other food-related industries, only a small amount of antibiotics are ingested by living tissues to be metabolized and discharged from the body; thus, a large portion of the antibiotics remain in livestock and food-related crops [37–41]. Especially for livestock, antibiotics remaining in animal bodies gather in high-nutrition organisms through a process of biomagnification in a food chain; biomagnification has caused a serious health threat to human beings who eat high-nutrition organisms as food and has seriously interfered with the physiological functions and normal life activities of human beings. In addition to naturally occurring antibiotics, scientists have also made many different kinds of antibiotics through in vitro synthesis procedures [42–47]. With the continuous synthesis and application of antibiotics, the abuse of antibiotics has become increasingly serious. The inappropriate use of antibiotics in the breeding of livestock, on the one hand, can clearly promote the growth of animals; additionally, antibiotics can effectively prevent bacterial infection and control diseases, thus greatly improving the economic benefits of animal husbandry. On the other hand, due to an improper use of antibiotics, pathogenic bacteria have developed a strong resistance, which has induced the production of superbacteria. Residual antibiotics are transmitted to the human body through biomagnification in the food chain, which destroys the normal flora in a human body, reduces the ability of the immune system in a human body, affects normal life activities in a human body, and even causes drug poisoning in serious cases [48–51]. For example, as the most widely used antibiotics in animal husbandry, tetracycline antibiotics are difficult to fully absorb and can be inactivated by the gastrointestinal tract of animals after entering the organism; thus, most of them are discharged in their original form. Although some antibiotics are transformed by organisms, their activity is often reduced, but some antibiotics may be transformed into more toxic metabolites and discharged out of the body; these toxic metabolites may enter surface water and pollute the ecological environment after periods of rainfall [52–54]. At present, technology cannot completely remove residual antibiotics in the environment, and there are few studies on the impact of disinfection technology on antibiotics. Antibiotics remaining in drinking water are low in content, but harmful, and they seriously affect human physiological functions, the immune system and normal life activities [55–57].

At present, the main methods for the detection of antibiotics in food are instrumental analyses, microbiological methods, immunoassays and sensor methods. Instrumental analyses are the most commonly used physical and chemical analysis methods for the detection of antibiotics, mainly

including chromatography (thin layer chromatography, high-performance liquid chromatography, ion chromatography, supercritical chromatography and meteorological chromatography), spectroscopy (spectrophotometry and fluorescence spectrometry) and chromatography-mass spectrometry [58–69]. Instrument analyses and detection methods are simple and fast, but they require expensive detection equipment, and their detection procedures are complex and expensive. A microbiological method is a simple and direct way to detect antibiotics. It is based on the inhibition of antibiotics on microbial metabolism to achieve the quantitative detection of antibiotics. However, the detection time is long, and the resulting error is large. Immunoassays provide the analysis and determination of antibiotics by the specific recognition of antigens and antibodies, mainly including enzyme-linked immunosorbent assays (ELISAs), fluorescence immunoassays (FIAs), enzyme immunoassays (EIAs) and radioimmunoassays (RIAs). A sensor is a kind of analytical device that can sense the measured information and convert it into electrical signal, optical signal or acoustic signal according to certain rules. Compared with conventional analysis technology, sensor analysis technology has advantages of a simple operation, fast speed and low detection cost.

2. BIOSENSOR TECHNOLOGY AND ELECTROCHEMICAL BIOSENSOR

Biosensor technology is an interdisciplinary subject developed between life science and information science. It is formed by the interdisciplinary combination of biology, medicine, optics, electrochemistry and thermology. It has the advantages of low detection cost, fast analysis speed, high sensitivity, and strong specificity. Biosensor technology can realize continuous real-time monitoring in complex systems, so it can be widely used in biological and clinical medicine, agriculture, animal husbandry, materials and chemistry, military and environmental protection [70,71]. Biosensors can be divided into tissue sensing technology, enzyme sensing technology, immune sensing technology, microbial sensing technology and DNA sensing technology [72–74].

As an important biosensor, electrochemical biosensors use bioactive materials (such as proteins, antibodies, cells, nucleic acids, enzymes, tissues, etc.) as the sensitive elements, an electrode and electrochemical workstation as the conversion device; electrical signals relating to the concentration of the target substance are outputted as detection signals to realize the quantitative analysis and detection of the target analyte [75–77]. Electrochemical biosensors combine the advantages of the high selectivity of biometric systems and the high sensitivity of electrochemical conversion devices and have been successfully applied in various fields of analysis and detection. Electrochemical biosensors have the following characteristics: strong specificity, fast response, low sample consumption, repeatable use, and small size; additionally, they are low cost, easy to carry and can achieve continuous on-line monitoring. According to the differences in sensitive elements, electrochemical biosensors can be divided into DNA sensors, enzyme sensors, immune sensors and cell sensors [78–80].

Electrochemical DNA sensing technology generally refers to the immobilization of a single strand DNA (probe) on the electrode surface by chemical adsorption or covalent binding and then the combination of electrically active substances as hybridization indicators. Its working principle is usually based on an aptamer as the recognition element. When the aptamer and the target molecules generate

specific recognition, the biological information of the aptamer target that is formed is transformed into an electrochemical signal related to the target concentration by the electrochemical workstation. In a certain range of analyte concentrations, the obtained electrochemical signal has a linear relationship with the analyte concentration to realize the quantitative detection of the target analyte and achieve the purpose of analysis and detection.

A commonly used electrochemical aptamer sensor is composed of an electrode with a fixed aptamer and an electrochemically active indicator. Its specific working principle is as follows: under certain conditions, the nucleic acid aptamer is modified on the electrode surface after pretreatment. Then, the target is modified on the electrode surface and incubated at a certain temperature for a period of time [81–83]. The specific recognition of the target molecule and aptamer can induce a conformational change in the aptamer on the electrode surface. Then, the electrochemical response of the electroactive indicator on the electrode surface is measured to realize the recognition and concentration measurement of the target molecule substance [84–86]. The electrochemical aptamer sensor transforms the biological signal of the interaction between the aptamer and target molecule into an electrochemical signal by AC voltammetry, differential pulse voltammetry, square wave voltammetry, cyclic voltammetry, electrochemical impedance spectroscopy and stripping voltammetry. Electrochemical aptamer sensors have the advantages of high sensitivity and selectivity, high automation and low price, so they are considered to be the most promising analytical and testing methods.

3. ELECTROCHEMICAL MODIFIED SENSORS FOR ANTIBIOTICS DETECTION

The main functions of nanomaterials in electrochemical biosensors are as follows: (1) They accelerate the electron transfer rate, for instance, carbon nanotubes with good conductivity, which can be connected between biomolecules and electrodes, not only accelerate the electron transfer rate between biomolecules and electrodes but also increase the reversibility of the redox reaction on the electrode surface [87–89]. (2) In a catalytic reaction nanomaterials have the characteristics of small particle size, large specific surface area, high surface energy, and high catalytic efficiency [90,91]. (3) For the immobilization of biomolecules, nanomaterials have a large specific surface area, high surface free energy, an abundance of surface functional groups and good biocompatibility, which can immobilize a large number of biomolecules on the electrode surface and maintain a good biological configuration and activity [92,93]. (4) For labeling biomolecules, the use of nanomaterials as biomarkers can greatly improve the performance of biomarkers and significantly improve the sensitivity of existing analytical methods [94,95]. (5) As a reaction control switch on the electrode surface magnetic nanomaterials can be used because of their special magnetism and good physical and chemical properties, to control the electrocatalytic process and select the materials to be tested [96–98]. Table 1 summarizes recently developed electrochemically modified sensors for antibiotic determination.

Table 1. Recently developed electrochemically modified sensors for antibiotic determination.

Materials	Target	Reference
AuNPs/GQD-SH	Streptomycin	[99]
Molecularly imprinted polymer modified carbon nanotube-gold nanoparticles	Tetracycline	[100]
Biotin-avidin-conjugated metal sulfide nanoclusters	Tetracycline and chloramphenicol	[101]
CdS QDs	Chloramphenicol	[102]
Nanoporous gold	Kanamycin	[103]
CP/AuNPs	Kanamycin	[104]
GR-Fe_3O_4 MNPs, PEDOT-AuNPs	Penicillin	[105]
Fe_3O_4 MNPs	Tetracycline	[106]
AuNPs	Tetracycline	[107]
Ag@Fe_3O_4, TH-GS	Kanamycin	[108]
GR-TH/HNP-PtCu	Kanamycin	[109]
3DCNTs@CuNPs@MIP	Chloramphenicol	[110]
PPy_3C/ERGO	Streptomycin	[111]
GO/ZnO	Chloramphenicol	[112]
CO_3O_4@rGO	Chloramphenicol	[113]
Cl-RGO	Chloramphenicol	[114]
PoAP/GQD	Levofloxacin	[115]
MIP/Ag@AuNps/Ils	Ceftizoxime	[116]
Nanodiamonds	Pyrazinamide	[117]
Aptamer/Fe_3O_4@mC	Oxytetracycline	[118]
Carbon black within a dihexadecylphosphate film	Amoxicillin and nimesulide	[119]
Iron-nitrogen co-doped ordered mesoporous carbon-silicon	Chloramphenicol	[120]
Fe_3O_4/IL	Tetracycline	[121]
$CoFe_2O_4$@CdSe core-shell nanoparticles	Rifampicin	[122]
MIL-101(Cr)/XC-72	Chloramphenicol	[123]

4. ELECTROCHEMICAL BIOSENSORS FOR ANTIBIOTICS DETECTION

Electrochemical detection with biosensor technology is mainly divided into direct detection and indirect detection. Direct detection is based on the direct electron transfer between the nucleic acid molecule itself and the electrode [124,125]. It is based on the electrochemical activity of some components of DNA (ribose and base) under certain potential conditions. Indirect detection methods are based on redox media to carry out electronic transfer and, with the help of indicators with electrochemical activity and DNA selective recognition, combine to achieve quantitative detection. Because the application of direct detection is limited by the condition of DNA itself, the most widely used indirect detection is based on electroactive substances [126–128]. The specific electrochemical measurement methods mainly include chronopotentiometry, voltammetry and an electrochemical impedance method. Electrochemical DNA biosensors that have the advantages of rapid and sensitive

detection, low cost, low energy consumption and easy miniaturization have attracted increasing attention from researchers and have very good development prospects [129]. Indirect detection can be divided into the following situations: (1) An unmarked hybridization indicator is a kind of electroactive substance that can interact with DNA molecules. Therefore, the concentration of the target molecule can be determined indirectly by measuring the redox signal of the indicator [130–132]. Generally, the interaction mode of indicator molecules and DNA can be divided into electrostatic binding, surface binding and insertion binding. Electrostatic binding is the combination of indicator molecules and negatively charged DNA nucleic acid skeletons through electrostatic interactions. The surface binding is that the indicator molecule binds with the DNA base through hydrophobic interactions on the channel surface. Insertion binding is when an indicator molecule is inserted between base pairs of nucleic acid molecules through van der Waals forces, oxygen bonds and stacking. The commonly used hybrid indicators are mainly anthracycline antibiotics, dyes, metal complexes and ferrocene derivatives [133,134]. (2) Electroactive substance labeling is mainly to mark the electroactive substance at the end of a nucleic acid chain or between two adjacent bases of a nucleic acid chain and then carry out a specific hybridization reaction with the DNA on the electrode surface through the principle of complementary base pairing; thus the molecules undergo self-assembly with electrochemical activity on the electrode surface, thus indirectly identifying and realizing the target by measuring the electrical signal of the assembled molecules for the quantitative detection of standard molecules [135,136]. Commonly used electroactive substances are ferrocene and methylene blue. For the first time, Fan et al. [137] labeled a beacon molecule with a hairpin structure on an electrode surface; this structure was modified with a ferrocene molecule at one end of the probe molecule and labeled with a sulfhydryl group on the other end. First, the hairpin probe is decorated on the surface of a gold electrode by self-assembly decoration. When there is no detected target molecule, there is no specific recognition hybridization reaction between the target molecule and the nucleic acid molecule. The hairpin probe modified with ferrocene molecules is in a stable hairpin configuration, and the ferrocene molecule is close to the surface of the gold electrode, which is conducive to electron transfer between the electrode and the electrolyte [138,139]. Therefore, it is beneficial to obtain a high electrochemical signal; however, when there are target molecules, the specific hybridization between the target and the aptamer makes the hairpin structure open, forming a rigid double chain structure, and the ferrocene molecules are far away from the electrode surface; thus, the insulated double chain structure blocks the transmission of electrons and the electrochemical signal decreases. This distance-based change leads to a change in the electron transfer efficiency, which can easily and quickly reflect the concentration of the object to be measured [140,141]. (3) A labeling oxidoreductase usually uses a strong binding force between biotin and avidin to combine a single strand DNA labeled with biotin with an enzyme modified with avidin or directly marks the oxidoreductase at the end of a single strand DNA [142]. After hybridization of a DNA chain labeled with enzyme and the capture probe fixed on the electrode surface, the strong catalytic function of the labeled enzyme can catalyze the redox reaction in the electrolyte under a certain potential and indirectly realize the quantitative analysis and detection of the target molecule by measuring the current intensity of the redox reaction [143–145]. This method successfully converts the hybridization reaction into a detectable electrical signal and realizes electrochemical detection. It is a relatively simple and

sensitive detection method at present. Table 2 summarizes recently developed electrochemical biosensors for antibiotic determination.

Table 2. Recently developed electrochemical biosensors for antibiotic determination.

Materials	Target	Reference
Protein G-modified screen-printed dual carbon electrodes	sulfonamide and tetracycline	[146]
RNA aptamer	Aminoglycoside	[147]
Target-induced and T7 exonuclease-assisted dual recycling signal amplification	Ampicillin	[148]
Aptamer-metal ions-NMOF	Kanamycin and chloramphenicol	[149]
Magnetic hollow porous nanotracers coupling exonuclease	Chloramphenicol and oxytetracycline	[150]
Metal ions doped MOFs	Oxytetracycline and kanamycin	[151]
Y-shaped DNA-based metal ions encoded probes with NMOF	chloramphenicol and oxytetracycline	[152]
Biotinylated ssDNA aptamer	Tetracycline	[153]
Penicillinase	Penicillin G	[154]
Aptamer-MWCNTs	Tetracycline	[155]
Fe_3O_4 MNPs-AuNPs-aptamer	Chloramphenicol	[156]
GR-Fe_3O_4-AuNPs-aptamer	Streptomycin	[157]
GO-aptamer	Kanamycin	[158]
N-GQDs-aptamer	Chloramphenicol	[159]
Horseradish peroxidase-functionalized gold nanoprobe	Kanamycin	[160]
Au(L-cysteine)-Pt(penicillinase) nanowire	Penicillin	[161]
nano zirconium- NMOF-aptamer	Kanamycin and chloramphenicol	[162]
Aptamer/SnOx@TiO_2@mC	Tobramycin	[163]
CdS-KAP+PbS-STP/cKAP+cSTP/OMC-AuNPs/CNF	Kanamycin and streptomycin	[164]
Ce-MOF@COF-aptamer	Oxytetracycline	[165]

4. CONCLUSION

Antibiotics, as metabolites of microorganisms, can kill or inhibit the growth of microorganisms. In recent years, it has been widely used in the treatment of non-viral diseases, especially in animal husbandry and agriculture. The excessive and improper use of antibiotics has led to an increase in its residue in food year after year. Long-term consumption of food containing antibiotic residues will lead

to the accumulation of antibiotics in a body, making a human body resistant to antibiotics and inducing the production of superbacteria. In recent years, the problem of antibiotic residues in food has attracted great attention from all over the world, and many countries have also formulated a series of indicators for the largest antibiotic residues in food. However, the existing traditional methods of antibiotic detection are limited by various internal and external conditions. Therefore, it is urgent to develop a highly sensitive and selective method for the detection of antibiotic residues in food. In this paper, a biosensor for the detection of antibiotic residues in food was constructed by electrochemical sensing and the conformational changes of aptamers. The constructed sensor has a low detection limit and wide linear range, good selectivity and stability, and good recovery rate in the detection of real samples.

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