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Mini Review

Electroanalytical Methods for Fish Drug Determination and Control: A Review and Outlook

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Fishery drugs can kill pathogens of aquatic animals and increase the production of aquatic products. These compounds have been widely used in the past 30 years. However, in recent years, abuse and misuse of these drugs has caused great economic losses and serious consequences and has endangered human health. Accelerating the establishment of aquatic product quality inspection systems is an effective way to guarantee the quality and safety of aquatic products and public health and safety. The most important aspect is to speed up the improvement of aquatic product quality in addition to safety inspection and detection capabilities. In this review, we describe the recent development of electrochemical sensors for fishery drug detection and determination. This paper provides a reference for the aquaculture industry and academic research for selecting the most suitable detection technology for different fishery drugs.

Keywords: Fishery drug determination; Electrochemical sensor; Review; Electrode modification; Analytical determination

1. INTRODUCTION

Aquatic products refer to animals and plants produced by marine and freshwater fisheries and their processed products. Aquatic product safety is an important part of food safety [1,2]. With economic growth, aquatic products have gradually become one of the most important foods eaten daily by consumers [3,4]. At the same time, the quality and safety of aquatic products has attracted much attention. In recent years, food safety incidents and environmental pollution incidents have occurred frequently in China, which has become a major issue of increasing concern to the country, industry and consumers [5,6]. These incidents are related not only to people's health and life safety, economic

development and social stability but also to the development and credibility of food production and sale [7-12]. Therefore, how to ensure the quality and safety of aquatic products has become an urgent task. Fishery medicine and fish feed are the two main factors that could result in drug residue problems for aquatic products [13-15].

Drugs are usually classified according to pharmacology, but due to insufficient pharmacological studies of fishery drugs, they are usually classified according to the purpose of use. Fishery drugs in China can be roughly divided into six types: disinfectants, antimicrobial drugs (antibiotics, sulfonamides, furans), pesticides, metabolic improvement and strengthening agents (hormones), gene inducers, vaccines and Chinese herbal medicines [16-18]. At present, research on fishery drugs is not systematic. Fish medicine is basically composed of human medicine, veterinary medicine and pesticides or of Chinese herbal medicine supplemented by synergists, cosolvents, sustained-release agents and dispersants [8,19,20]. Many drugs have not undergone rigorous pharmacological and toxicological tests. Furthermore, there is no clear method of use and duration of discontinuation. There are no corresponding laws and regulations to guide and restrict the use of fishery drugs, and there is a lack of effective supervision and management [21]. This situation has caused a series of negative effects, destroyed the ecological balance of water systems, further exacerbated the diseases of aquatic animals and plants, and formed a vicious circle. At the same time, the drug resistance of aquatic animals and plants has increased, which has increased the difficulty of disease control [22,23]. Moreover, a serious problem is the accumulation of drugs in aquatic animals and plants, the increase in residues, which directly threatens the health of consumers [24,25]. In June 2007, the Food and Drug Administration of the United States (FDA) reported that some aquatic products imported from China had been suspended because of the discovery of antibiotics such as fluoroquinolone, chloromycetin, nitrofuran, enrofloxacin, malachite green and gentian violet. In addition, many countries and agencies worldwide have stipulated the maximum residues of veterinary drugs, such as FDA and the International Codex Alimentarius Commission (CAC), which require the maximum residues at the level of μg/kg, which put forward stricter requirements for the aquatic products export in China.

To date, the main methods that can be used to detect drug residues in aquatic products include chromatography coupled with mass spectrometry (high-performance thin-layer chromatography, gas chromatography, high-performance liquid chromatography, chromatography-mass spectrometry, etc.) [26-30], immunoassay (enzyme-linked immunoassay, fluorescence immunoassay, chemiluminescence immunoassay, colloidal gold immunoassay, immunosensor, quantum dot immunoassay, etc.) [31-35], electrochemical methods, capillary electrophoresis [36] and high-resolution mass spectrometry [37-40]. Because of the particularity of aquatic products, it is of great significance to develop new technologies and establish and improve the safety detection mechanism of aquatic products to ensure their quality and safety. Chromatography-mass spectrometry was developed relatively early and is a mature technology. It is still the main method for the determination of drug residues in food. This approach has the advantages of high sensitivity, good accuracy and good repeatability. However, chromatography coupled with mass spectrometry cannot meet the requirements for the timely, high-throughput and convenient detection of aquatic products. New immunoassay and electrochemical technologies can effectively remedy the shortcomings of instrumental analysis and can detect and analyse products in a simple, rapid and batch manner [41-44]. However, the new detection technology is still immature, and there are still

some shortcomings in the actual application process. For example, the immunoassay process is susceptible to interference and false positives or false negatives; electrochemical analysis is susceptible to interference from other substances.

In this review, we summarize the current development trends of electrochemical analysis methods towards the detection of drug residues in aquatic products. This review analyses and compares the advantages, shortcomings and development directions of the above detection technologies from the principle to the application in the detection of fishery drug residues, with the aim of demonstrating how these methods can play a significant role in the detection of fishery drugs.

2. ANTIMICROBIAL DRUG DETERMINATION

Antimicrobial drugs can inhibit or kill pathogenic bacteria and cure bacterial infectious diseases. At present, the use of antimicrobial fishery drugs faces problems such as negative effects, residuals in aquatic products and drug resistance. These drugs mainly include antibiotics, such as oxytetracycline, penicillin, doxycycline, chlortetracycline, thiamphenicol and florfenicol. Sulfonamide drugs include sulfadiazine, sulfamethylpyrimidine, sulfamethoxypyrimidine and trimethoprim. Floxacin and nitrofuran drugs include norfloxacin, ofloxacin, piperacic acid, oxaquin, naphthoic acid and furazolidone. Electrode surface modification is an effective method to enhance the performance of the common electrochemical sensor. Chemically modified electrodes are modified at the molecular level on the surface of electrodes. The surface modifiers can be molecules, atoms, compounds and polymers. The intended functions of the electrodes are chosen so that the desired reactions can be selectively carried out on the electrodes and the design of the electrode functions can be realized at the molecular level. Therefore, surface-modified electrodes have been used for the determination of antimicrobial drugs. For example, Sun and co-workers demonstrated a disposable montmorillonite and acetylene black modified microelectrode for oxytetracycline determination [45]. Feier and co-workers demonstrated a sensitive determination of cephalosporins using a bare boron-doped diamond electrode [46].

The electrochemical enzyme-linked immunosensor is a new technology developed in recent years. This method combines the chemical amplification of enzyme electrodes with the specificity of immune electrodes. It has the specificity of the immune response and the sensitivity of electrochemical analysis. The electrochemical enzyme-linked immunosensor has advantages that include relatively simple detection equipment, convenient use, a flexible method of constructing enzyme electrodes, easy integration and miniaturization of the system. The development of new electrochemical enzyme-linked immunosensors is currently an active research field. The electrochemical enzyme-linked immunosensor is a fusion technology that combines the immune reaction of an antigen and antibody with the catalytic reaction of an enzyme. The basic principle of this approach is to combine enzymes with antibodies or antigens by chemical or biological methods to form enzyme markers or to combine enzymes with antienzymes by immunological methods to form immune complexes. These enzyme markers or immune complexes still maintain their immune activity and then react with the corresponding antigens or antibodies to form enzyme-labelled or enzyme-containing immune complexes. When the enzyme on the immune complex encounters the corresponding substrates, it catalyses a hydrolysis, oxidation or

reduction reaction or forms covalent bonds, which are determined qualitatively and quantitatively by electrochemical analysis. Prado and co-workers demonstrated a β-lactamase-based biosensor for the electrochemical determination of benzylpenicillin [47]. Specifically, the initial step in chemical reduction enzyme promotes oxidation of the benzylpenicillin with concomitant electron mediator oxidation, maintaining the enzyme in its original form. Then, electrochemical regeneration of the reduced form of the mediator occurs by applying the appropriate potential, and the amount of electrons transferred in this reduction is proportional to the concentration of benzylpenicillin in the sample. Liu and co-workers demonstrated a sensitive electrochemical immunosensor based on PAMAM dendrimer-encapsulated Au for the detection of norfloxacin [48]. As shown in Figure 1, on the basis of the signal amplification of PAMAM-Au, the signal intensity was linearly related to the concentration of norfloxacin in the range of 1 μg/L to 10 mg/L.

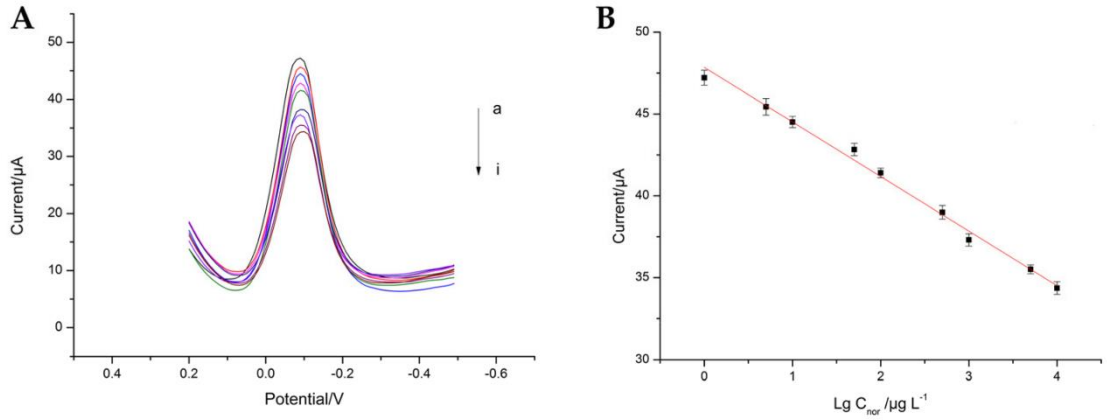

Figure 1. DPV peak current (A) and calibration curve (B) of the immunosensor at different concentrations of norfloxacin. Copyright obtained from MDPI [48].

Molecular imprinting technology (MIT) is a new and interdisciplinary molecular recognition technology. MIT is inspired by the immune function of antibodies against antigens. It refers to the process of using a specific molecule as a template to prepare a specific polymer for the template molecule. Polymers prepared by MIT have high selectivity for this particular template molecule. MIT is a reversible combination of template molecules or target molecules and functional monomers in a suitable dispersing medium by means of non-covalent or covalent interactions between molecules. After adding the crosslinking agent, the template molecule is encapsulated in an orderly manner in the precursor by external action, such as light, heat and electric field, assisted by an initiator and porogen. Finally, a series of three-dimensional holes can be obtained by eluting template molecules from the precursors. The hole has a certain "memory" and can complement the template molecule. The structure of the hole is stable and flexible. It is easy to elute template molecules, so it can be specifically recognized and recombined with template molecules (Figure 2). For example, Wang and co-workers reported an electrochemical sensor for levofloxacin based on a molecularly imprinted polypyrrole–graphene–gold nanoparticle-modified electrode [49]. Silva and co-workers reported a molecularly imprinted sensor for voltammetric detection of norfloxacin [50].

Table 1 summarizes recently developed electrochemical-based sensors for fishery antimicrobial drug determination.

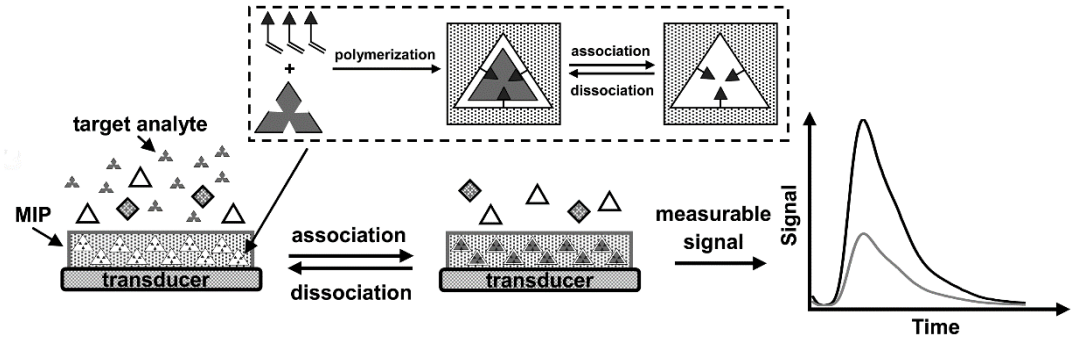

Figure 2. Schematic representations of MIP-based biomimetic sensors. Copyright obtained from MDPI [49].

Table 1. Recent developed electrochemical-based sensor for fishery antimicrobial drug determination.

Materials	Method	Target	Reference
TiN–rGO nanohybrids	Direct electrochemical determination	Chloramphenicol	[51]
Graphene	Indirect electrochemical determination	Ciprofloxacin	[52]
AuNP-coated polypyrrole	MIT	Tetracycline	[53]
aptamer-AuNPs-HRP	Aptasensor	Oxytetracycline	[54]
HRP	Electrochemical enzyme-linked immunosensor	Oxytetracycline	[55]
ART-imprinted membranes	MIT	Artemisinin	[56]
CB nanospheres modified with Cu (II)-phthalocyanine	Direct electrochemical determination	Trimethoprim	[57]
Aptamer-MNPs	Aptasensor	Oxytetracycline	[58]

3. WATER DISINFECTANT DRUG DETERMINATION

Water disinfectant drugs can regulate the physical and chemical environment of the water body. The quality of water is closely related to the occurrence of aquatic animal diseases, so the use of water quality improvement drugs is increasing annually. Common water disinfectants include halogens such as povidone iodine, sodium dichloroisocyanurate, trichloroisocyanuric acid, bromochlorohydantoin, dibromohydantoin, chlorine dioxide and bleach. Other water disinfectants include aldehydes and alcohols, such as formaldehyde solutions, glutaraldehyde and ethanol. Alkaline disinfectants include

calcium oxide and aqueous ammonia. Dye disinfectants include methyl violet, methylene blue and acridine yellow.

Several electrochemical sensors have been developed for water disinfectant drug determination. For example, Horakova and co-workers reported the electrochemical determination of methyl violet using a mercury electrode [59]. Hassan and co-workers reported an ultra-trace-level electrochemical sensor based on Nafion-stabilized ibuprofen-derived gold nanoparticles for the determination of methylene blue [60].

4. ANTIPARASITIC DRUGS DETERMINATION

Antiparasitic drugs generally have broad-spectrum insecticidal effects on Chinese worms, anchor worms, fish-gill flukes, wheelworms, third-generation worms, ring worms, tapeworms, pine algae and centipedes in water, which are external or internal parasites of aquatic animals. The inappropriate use of antiparasitic drugs in food can cause acute and chronic toxicity to the human body directly or indirectly via the environment and food chain. In addition, misuse and abuse of these drugs can cause an increase in parasite resistance and affect the development of the aquaculture industry. Antiparasitic drugs generally include methylene blue, copper sulfate, ferrous sulfate mixture, trichlorfon, deltamethrin, mebendazole and albendazole.

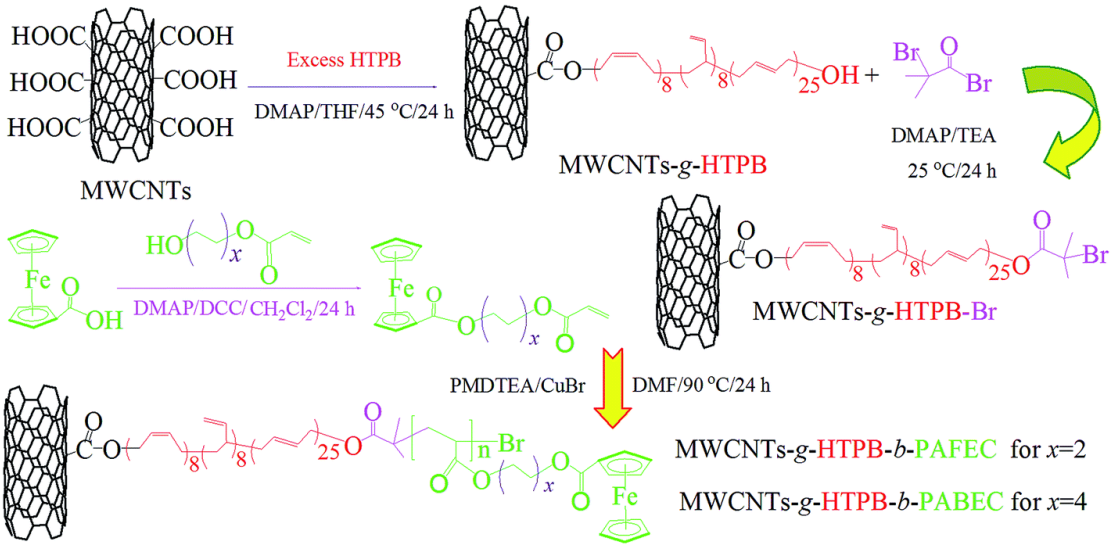

Figure 3. Synthetic strategies of MWCNT-grafted block copolymer nanohybrid composites. Copyright obtained from RSC [64].

Trichlorfon is a phosphate-type organophosphorus pesticide. The hydroxyl group in the phosphoric acid unit is replaced by an organic group to form a phosphorus-carbon bond and can also be esterified to form a phosphoric acid ester [61-63]. The daily allowable intake of organophosphorus pesticides is 0.01 mg/(kg.d), and it is easy to decompose them and decrease their toxicity under alkaline conditions, while these compounds are relatively stable in acidic and neutral solutions. Trichlorfon is a highly effective, low-toxicity and low-residual pesticide. It has good killing effects on trematodes,

nematodes, Echinococcus and Cladocera, radicals, clam hook larvae and centipedes, which endanger fish larvae and eggs. Xu et al. [64] fabricated multiwalled carbon nanotube-grafted acryloyloxy ferrocene carboxylates with different spacers (Figure 3) for electrochemical detection of trichlorfon. A similar report was reported by Anh and co-workers [65]. A poly(1,5-diaminonaphthalene)-polypyrrole nanowire bilayer was synthesized using an electrochemical method and consequently used for trichlorfon determination.

Deltamethrin is one of the most toxic pyrethroid insecticides and has contact and stomach toxicity. This chemical has a repellent effect on some pests at high concentrations. In addition, emulsifiable or wettable powders of deltamethrin are formulated as intermediate pesticides. This compound is a broad-spectrum insecticide that is effective against many pests, such as Lepidoptera, Orthoptera, Mugiptera, Hemiptera, Diptera and Coleoptera. Fruhmann and co-workers recently reported an immunoassay biosensor for deltamethrin detection based on the use of specific polyclonal antibodies, with a spacer arm placed at the cyano residue in the pyrethroid structure [66].

Table 2 summarizes recently developed electrochemical-based sensors for fishery antiparasitic drug determination.

Table 2. Recent developed electrochemical based sensor for fishery antiparasitic drug determination.

Materials	Method	Target	Reference
Graphene	Electrochemical enzyme-linked immunosensor	Trichlorfon	[67]
MWCNT@TiO_2-carboxymethyl chitosan	Direct electrochemical determination	Trichlorfon	[68]
CuO nanostructures	Direct electrochemical determination	Trichlorfon	[69]
Cu-hemin MOF grown-carbon foam	Direct electrochemical determination	Trichlorfon	[70]
Reduced graphene oxide	Direct electrochemical determination	Deltamethrin	[71]
Poly(o-anisidine)/carbon nanotubes	Direct electrochemical determination	Mebendazole	[72]
Nitrogen-doped carbon nanosheet-Fe	MIT	Mebendazole	[73]
GCE	Direct electrochemical determination	Albendazole	[74]

5. PROHIBITED DRUG DETERMINATION

Prohibited fishery drugs refer to fishery drugs with high toxicity, high persistence or carcinogenic, teratogenic and mutagenic properties. In addition, it is difficult to repair the serious damage to the water environment that fishery drugs can cause. It is strictly forbidden to spray antibiotics directly into aquaculture waters and to use newly developed drugs as the main or minor components of fishery drugs. Table 3 lists the main prohibited fishery drugs with their descriptions.

Table 3. Prohibited drugs for fishery.

Prohibited drug name	Influences	Applications
Malachite green	Carcinogenesis, teratogenesis and aquatic poisoning	Insecticidal, mainly to control melon insects; Hygromycosis control; Antibacterial
Chloramphenicol	Inhibiting bone marrow haematopoietic function, intestinal flora imbalance, immunosuppressive effect, and affecting the metabolism of other drugs in the liver	Treatment of gill rot and erythroderma in aquatic animals
Erythromycin/Tylosin	Drug resistance; increased body residues, endangering the quality and safety of aquatic products	Treatment of bacterial gill rot in aquatic animals
Nitrofurans	Haemolytic anaemia, acute hepatic necrosis, ocular damage, polyneuritis	Treatment of enteritis in fish
Sulfathiazole and sulfamidine	Acute or chronic poisoning of aquatic animals, urinary tract infection, haemolytic anaemia, ecological imbalance of normal flora, digestive disorders	Treatment of intestinal diseases in aquatic animals
Ciprofloxacin	Ciprofloxacin is used exclusively for human only.	Treatment of bacterial infections such as gill rot and erythroderma
Mercury-containing drugs	Enrichment, hepatomegaly and congestion, digestive tract inflammation, neurological symptoms	Treatment of white spot disease
Ethyl alcohol	Enrichment effect; poor tolerance and high mortality of fish; the high water content of fish leads to death.	Antibacterial effect; growth-promoting effect
Hormone drugs	Hormone residues in fish, causing serious harm to consumers; liver damage caused by high-dose use;	Promoting the synthesis of amino acids, sugars and proteins; inhibiting protein decomposition in vivo; delayed sexual

	sexual cycle arrest or disorder in fish	maturation of fish, resulting in male epigenetic reversal
Organochlorine preparations	High toxicity, slow natural degradation, long residual period, biological enrichment, carcinogenicity and damage to human functional organs	Kill fish lice and centipedes
Sodium pentachlorophenate	Damage of central nervous system, liver and kidney. Toxicity to aquatic animals	It can kill wild fish, snails, and mussels.
Acetamiprid	High toxicity, intermediate metabolites have carcinogenic effects on human body	Insecticidal action
Trypanosome arsine/potassium antimony tartrate	Highly toxic and easily accumulated in organisms	Insecticidal action
Bacilli zinc	-	It has strong inhibition and killing effects on gram-positive bacteria such as Staphylococcus and Streptococcus. It also is effective on some gram-negative bacteria, such as Chlamydia, Spirochetes and Actinomycetes.
Cyhalothrin	Fish death caused by serious impairment of normal physiological function	Insecticidal action
Avoparcin	Glycoside antibiotics; drug resistance.	Improving feed efficiency and utilization efficiency can promote growth
Phoxim	Highly toxic and highly toxic pesticide	A broad-spectrum organophosphorus soil insecticide used chiefly to control underground pests.
Carbofuran	High toxicity to humans and animals; high toxicity to the environment; long residual period	Eliminate Dactylogyrus and Gyrodactylus and hookworm
Fenbendazole	Biotoxic side effects	Improving feed efficiency and utilization efficiency; can promote growth

Malachite green is a toxic triphenylmethane chemical. It is not only a dye but also a fungicidal, bactericidal and parasitic drug. This compound can cause cancer if used excessively for a long time. Accordingly, malachite green is prohibited by the state in the field of pollution-free aquaculture. Zhu

and co-workers reported an electrochemical impedance immunosensor based on a bovine serum albumin-decorated gold nanocluster/antibody composite that was used to detect malachite green [75]. In addition, Guo et al. demonstrated a simple electrochemical method for malachite green determination based on graphene-gold nanoparticle nanohybrids [76].

Chloramphenicol is a broad-spectrum antibiotic that has bacteriostatic effects by inhibiting the synthesis of bacterial proteins. Chloramphenicol is effective for most gram-negative and gram-negative bacteria but especially strong against gram-negative bacteria. The use of chloramphenicol in aquaculture is prohibited because of its serious toxic and side effects. The determination of chloramphenicol can be achieved using MIT-based sensors. For example, Cardoso et al. designed a molecularly imprinted polymer for screen-printed electrode surface modification and subsequently used the electrode for chloramphenicol determination [77]. A similar report has been published by Yang and Zhao [78]; they prepared a MWCNT@molecularly imprinted polymer for chloramphenicol determination. Asl and co-workers fabricated an aptasensor for chloramphenicol detection based on aptamer incorporated gelatine [79]. Figure 4 shows the preparation procedure of the aptamer/gelatine-

modified screen-printed electrode for chloramphenicol detection.

Table 4 summarizes recently developed electrochemical-based sensors for fishery prohibited drug determination.

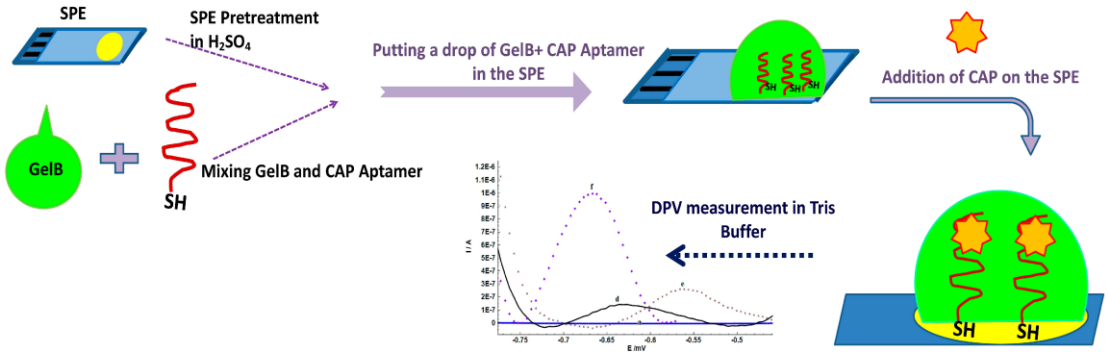

Figure 4. The preparation procedure of aptamer/gelatine-modified SPE for chloramphenicol detection. Copyright obtained from MDPI [79].

Table 4. Recent developed electrochemical based sensor for fishery prohibited drugs determination.

Materials	Method	Target	Reference
CeO_2 NPs-Nafion	Direct electrochemical determination	Malachite green	[80]
	Electrochemical Impedance Spectroscopy	Malachite green	[81]
Silica-modified electrodes	Direct electrochemical determination	Malachite green	[82]
MOF derived exfoliated porous carbon	Direct electrochemical determination	Chloramphenicol	[83]

PVA-co-PE nanofibrous membrane	Direct electrochemical determination	Chloramphenicol	[84]
Ti-Sn/γ-Al_2O_3	Direct electrochemical determination	Chloramphenicol	[85]
Boron-doped diamond electrode	Direct electrochemical determination	Erythromycin	[86]
Silver-amalgam film electrode	Direct electrochemical determination	Erythromycin	[87]

6. CONCLUSION AND OUTLOOK

In summary, electrochemical sensors can achieve high sensitivity and rapid detection of fishery drugs. However, the electrochemical enzyme sensor still has some shortcomings, which are mainly manifested in the complicated preparation process of the enzyme sensor electrode, the loss of enzyme activity when the enzyme is immobilized on the electrode surface, the limited storage time of the enzyme activity, and the rigorous requirements for the environment and sample conditions. These shortcomings of the electrochemical protease sensor will directly lead to the deviation of the electrochemical results and the poor reproducibility and stability of the sensor. Although electrochemical immunosensors have strong specificity, there are many problems for practical applications, such as the long-term development of antigens and antibodies, difficulty of antibody preparation, ease of inactivation, cross-reactions with similar compounds, and fact that they are only suitable for the detection and analysis of single fishery drugs. Therefore, electrochemical biosensors still face challenges in the detection of fishery drugs. Hopefully, to solve this problem, new bio-recognition molecules can be found that can specifically identify fishery drugs. In addition, optical biosensors using techniques such as colorimetry, fluorescence spectroscopy and chemiluminescence have advantages such as fast analysis and high sensitivity and specificity. These sensors represent an important research direction in the rapid analysis of fishery drugs. Therefore, we can combine electrochemical and optical biosensors to complement each other and realize the rapid and sensitive analysis of fishery drugs.

The development of electrochemical biosensors not only facilitates the rapid, simple and highly sensitive analysis of fishery drugs but also provides a rapid screening tool for the analysis of drug residues and reduces the risk of food safety. However, electrochemical biosensor detection technology still faces many challenges, and solutions must be developed and improved. For example, the detection limit of enzymes and immunosensors cannot meet the standard of analytical instruments, and high-performance electrodes and modified materials (such as nanocomposites) need to be developed. The selectivity of sensors is insufficient, so new sensors (such as aptamer biosensors) need to be developed to achieve the selective and specific analysis of target fishery drugs. In addition, the development of new enzyme immobilization technology and immobilization materials is necessary to reduce the loss of immobilized enzyme activity. Combined with other analytical systems, these technologies will develop

towards high-throughput and miniaturization approaches (such as array sensors). The problems listed here restrict the analytical performance of electrochemical biosensors for the detection of fishery drugs. Accordingly, these problems need to be solved urgently. They will be a topic of concern to analysts in the future.

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