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Mini Review

Recent Progress and Novel Perspectives of Electrochemical Sensor for Cephalosporins Detection

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Cephalosporin drugs belong to the class of β-class lactone antibiotics. Cephalosporins have been widely used in clinical practice due to their advantages, such as a wide antibacterial spectrum, strong antibacterial effect, high clinical efficacy and few adverse reactions. They are used to treat bacterial infections, respiratory problems and more. The accumulation or storage of antibiotics and their metabolites in animal tissues and organs can occur due to non-compliance with off-label regulations or incorrect use of cephalosporins. If the residue is not well controlled, it will pose a serious threat to human health. Methods for the analysis of cephalosporin residues have been widely reported, including high-performance liquid chromatography, high-performance capillary electrophoresis, immunoassays and microbiological assays. In this review, we briefly introduce various analytical methods for the detection of cephalosporin. Then, we describe the electrochemical analysis technique used to detect the concentration of cephalosporin.

Keywords: Electrochemical sensor; Water environment, Cephalosporins, Electrode modification. Analytical chemistry.

1. INTRODUCTION

According to their chemical structure and clinical use, antibiotics are mainly divided into six categories: β-lactam, aminoglycosides, macrolides, lincomycin, tetracycline and chloramphenicol [1–6]. Among them, β-lactam antibiotics mainly include penicillin and cephalosporins [7–10]. Among these, cephalosporins are still the largest antibiotics in clinical application [11–14]. Due to the differences in the production processes of various antibiotics, cephalosporin antibiotic wastewater has the characteristics of complex components, high concentrations of colloidal solids, high biological inhibition, high ammonia nitrogen and high salinity [15–21]. Figure 1 shows the chemical structure of

the cephalosporin antibiotics. Cephalosporins can be divided into first-, second-, third- and fourth-generation agents. Figure 2 shows the detailed structures and names of these cephalosporins.

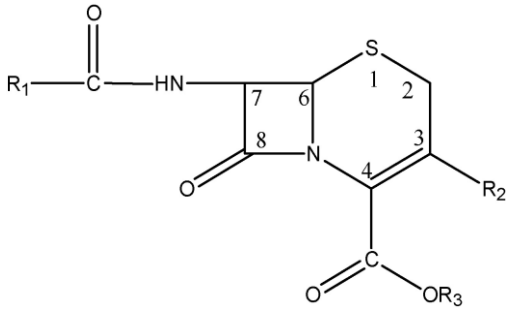

Figure 1. General chemical structure of cephalosporin antibiotics.

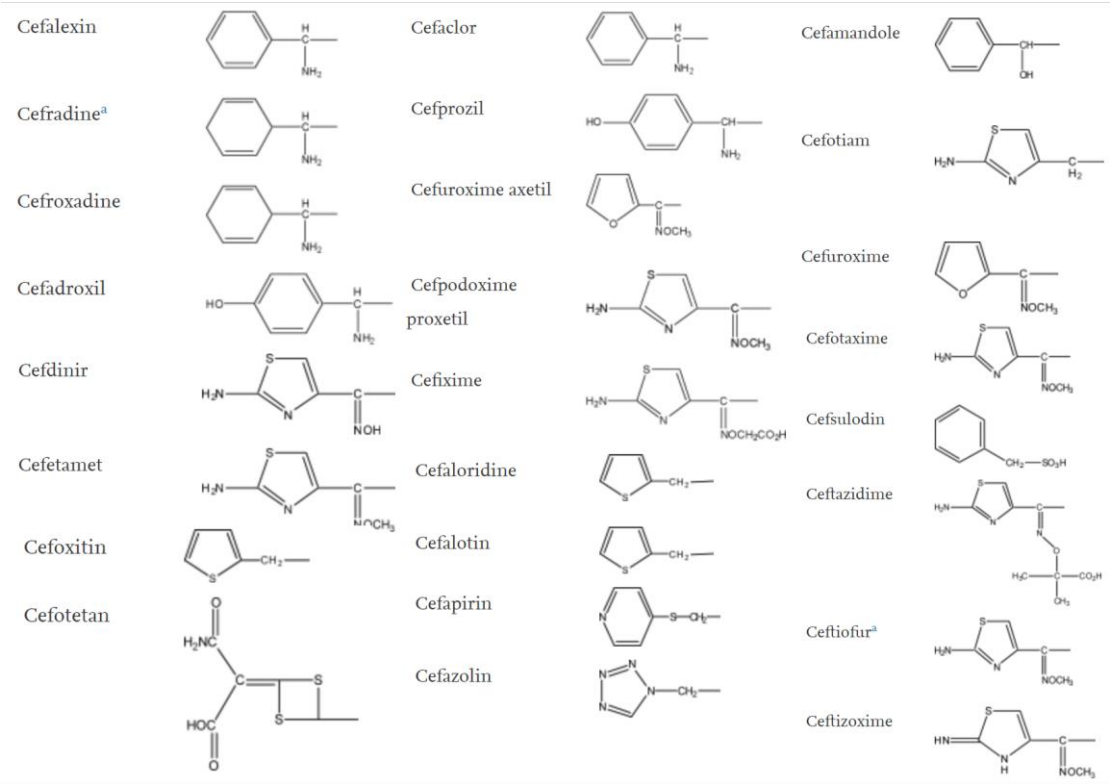

Figure 2. List of chemical structures of the cephalosporin antibiotics

With the integration of the global economy and the internationalization of food trade, antibiotic residues have become a worldwide challenge and an important global public health problem [22–26]. Excessive intake of antibiotics can directly cause acute and chronic toxic effects on the human body, increase bacterial resistance, affect intestinal flora disorders, and cause chromosomal aberrations and genetic mutations [27–32]. This intake can occur indirectly through the food chain and the environment, adversely affecting human health. Therefore, the determination of antibiotic residues, especially cephalosporins, has attracted considerable attention in academic fields as well as industry [33–37]. In this review, we first introduce analytical determination methods for industry determination, including microbiological analysis, immunoassay, liquid chromatography, liquid chromatography-mass

spectrometry, capillary electrophoresis and electrochemical sensors. Then, we summarize the recent progress of fabricating different types of electrochemical sensors for cephalosporin detection.

2. ANALYTICAL METHODS FOR CEPHALOSPORINS DETECTION

Currently, the detection methods of cephalosporin antibiotic residues can be approximately divided into microbial analysis, immunoassay and instrumental analysis [38–43].

The microbial detection method is mainly based on the antibiotics on the specific microbial physiological function, reproduction and metabolism of the inhibition, qualitative and quantitative detection of antibiotic residue [44–47]. Li et al. [48] used the common BSDA method (paper method of bacillus thermophilus), TTC method (triphenyltetrazolium chloride method) and test-tube diffusion method to detect 6 kinds of β-lactam antibiotic residues in milk, including cefapirin. The maximum residue limit in the EU standard is taken as the standard, and the detection limit of the BSDA method can reach 0.50 times that of MRLs, but the detection time is long, and the operation is tedious. The maximum residue limit of the TTC method for 6 antibiotics in the experiment was 2 ~ 6 times, which did not meet the standard. The detection limit of the tube diffusion method is 0.5 ~ 1.5 times that of MRLs, and this method has certain advantages over the other two methods in terms of the detection time and operation difficulty. However, due to the complexity of sample composition, the specificity of microbial assays is not strong in practical applications. This method is susceptible to interference by other antibiotic residues and other substances with antibacterial effects, and false positive results appear. Therefore, it is difficult to achieve standardized quantitative testing

The immune analysis method based on the antigen-antibody identification core response analysis method involves a separate kind of physical and chemical analysis technology to meet the selectivity and sensitivity, suitable for the separation of the trace components in complex matrix detection, but the antibody preparation technology is complex and cannot readily achieve a high throughput. The technology currently used for drug residue analysis of the immune analysis method mainly includes the radiation immunity analysis method and enzyme-linked immunoassays [49–56]. The radiation immunity test in the application of antibiotic residue analysis contains the CHARM I and CHARM II methods. CHARM I is dedicated to milk, such as the β-lactam class antibiotic residue detection method, and the CHARM II method can also be used for other foods for the rapid detection of antibiotic residues. Enzyme-linked immunoassay (ELISA) involves the adsorption of known antigens or antibodies on the surface of the solid phase carrier so that enzyme-labelled antigens and antibodies react on the solid phase surface [57–60]. The colour reaction of the enzyme with the substrate can be used for the quantitative determination of the substance to be measured. Jiang et al. [61] established a rapid method for the detection of β-lactam antibiotic residues in dairy products using ELISA.

In the application of the enzyme-labelled receptor (ELR) assay, the researchers developed an ELR for the detection of cefixime and penicillin G in milk up to a limit of 20 μg/kg [62–65]. IDEXX has developed Parallux laboratory instruments based on the principle of immune receptors and has realized the use of this method to detect a variety of antibiotics and other substances that interfere with fermentation. This method has been approved by the United States FDA for the detection of cephalosporin residues. Other immunoassays used in the detection of antibiotic residues include

colloidal gold immunochromatography. This method can simultaneously detect cephalosporin antibiotics and streptomycin. In addition, fluorescence immunoassay and immunoaffinity chromatography are also used in the detection of antibiotics [66–70].

In recent years, instrumental analysis technology has been widely used in the detection of antibiotic residues in food of animal origin due to its convenience, rapidity and accuracy, with advantages of high sensitivity, a low detection limit, a low quantitative limit and good reproducibility. Currently, instrumental analysis and detection methods for cephalosporin veterinary drug residues developed mainly include high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), ultra-high-performance liquid chromatography (UPLC) and capillary electrophoresis (CE).

Liquid chromatography (HPLC) is one of the oldest methods for detecting cephalosporin residues in food of animal origin. This method has the advantages of a high separation efficiency, a high sensitivity and a wide application range. The matrix components of the samples are relatively complex, and the application of chromatography can separate the substances to be tested and the interfering components in the complex components through the selection of mobile phase and fixed direction and analyse and identify the components to be tested by a spectroscopic or mass spectrometer detector [71–74].

Traditional HPLC technology is often equipped with ultraviolet or fluorescence optical detectors. Cephalosporin antibiotics have good ultraviolet absorption, so the DAD detector is often used in the analysis of residues of this kind of antibiotic in food. Sørensen et al. [75] used this technology to establish the detection method for 4 kinds of cephalosporin residues in milk. The sample was centrifuged and degreased, acetonitrile precipitated the protein, and then the supernatant liquid nitrogen was taken and blown dry to concentrate to 2 mL. Samples were purified using a tC18 SPE solid phase extraction column, and a gradient elution programme of the methanol-acetonitrile aqueous solution was set. The detection limits of four cephalosporin antibiotics were 7 ~ 11 μg/kg, and the linearity and recovery rate were good, in the range of 20 ~ 200 μg/kg.

Ultra-high-performance liquid chromatography (UPLC) was developed on the basis of an HPLC system to make the chromatographic separation of a higher resolution of a separation technology [76–82]. Compared with traditional HPLC technology, UPLC has a smaller delay volume and higher detector sensitivity, so it can obtain the most effective separation of samples in the shortest time. Wang et al. [83] used UPLC to set up a fast analysis at the wavelength of 268 nm for screening ten antibiotic residues, including cefoperazone in milk, with detection limits of 0.003 ~ 0.022 μg/g and quantitative limits of 0.01 ~ 0.08 μg/g.

In liquid chromatography, the optical detector is unable to analyse and identify the structure of the sample. LC-MS technology, which combines the high separation efficiency of liquid chromatography and the high sensitivity of mass spectrometry detectors, has gradually become one of the most widely used techniques in the detection of antibiotic residues in recent years [84–89]. In the identification of cephalosporin, Liu et al. [90] successfully isolated and identified cephalosporin antibiotic residues in milk by using a hydrophilic interaction chromatography column and LC/MS detection technology. Compared with the capabilities of the traditional inverted HILIC hydrophilic column, the analysis speed and separation efficiency are significantly improved.

UPLC-MS technology combines the advantages of UPLC with a short separation time, high efficiency, high sensitivity and selectivity of a mass spectrometry detector and has become a promising analytical technology in the field of antibiotic detection. However, this technology also has some limitations, such as the high cost of instrument parts and consumables and the need to be equipped with independent chromatographic columns. The detection method provided by this technology is inferior to that provided by HPLC and other technologies, and the detection method often needs to be explored and developed in residual detection.

Capillary electrophoresis (CE) is also called high-performance capillary electrophoresis. It has the characteristics of a simple instrument, fast analysis speed, less consumption of samples and reagents and economic and environmental protection. In recent years, the method has been applied in the field of food science by researchers, and a series of residue detection techniques have been developed. Ultraviolet detectors and electrochemical detectors are the two most commonly used detectors in capillary electrophoresis technology. In recent years, there have been reports on the detection of biochemical substances by CE and mass spectrometry detectors. However, due to the small diameter of the capillary tube used in this method, the optical path becomes shorter and the sensitivity is lower, the separation reproducibility is lower than that of HPLC technology, and there are also certain limitations in the field of detection of antibiotic residues in food.

Electrochemical detection is a method to analyse the electrochemical properties and changing behaviour in solution or other media. This method is divided into potential analysis, voltammetry and polarography, electrolysis and coulomb analysis. The method has a high sensitivity, good selectivity, a wide linear range and other advantages. In addition, the study of the electrode process can explore the mechanism of action of drugs. In the next section, we summarize the recent progress and prospective design of electrochemical sensors for cephalosporin detection.

3. ELECTROCHEMICAL SENSORS FOR CEPHALOSPORINS DETECTION

Cephalosporins usually have electrochemical activity, so characteristic redox reactions can occur at the electrode. Based on this characteristic, different researchers have reported determination methods based on electrochemical analysis.

Cephalosporin can be directly measured by ion selective electrodes. Dumkiewicz et al. [91] proposed a pseudo-liquid membrane-phase ion-selective electrode, which can be used for the efficient determination of cefuroxime. The selective detection of cefuroxime by different ion exchangers and dielectric solvents was studied [92]. Commercial electrodes have direct responses to some cephalosporins. For example, cefalexin monohydrate can be oxidized on the surface of a glassy carbon electrode. Although cefaclor has no obvious redox peak at the mercury electrode, the content can still be determined by polarography and cathodic stripping voltammetry (CSV) [93,94].

Differential pulse polarography (DPP) can be used to measure the content of cefepime on a mercury electrode [95]. Another study investigated the electrochemical behaviour of cefoperazone and ceftriaxone on the surface of a carbon paste electrode by differential pulse polarography and cyclic voltammetry. The results showed that the two cephalosporins showed obvious electrochemical oxidation

peaks at the potential of +1.05 V [96]. CSV can directly determine the content of ceftazidime in R-B buffer solution [97]. In another study, researchers activated glassy carbon electrodes and carbon paste electrodes to study the electrooxidation reaction of cefotaxime in a buffer solution with different pH values [98]. The results showed that 0.2 M PBS was the most favourable medium for the detection of cefotaxime.

Cefalexin has no obvious redox peak on commercial electrodes. However, the degradation products of cefalexin have obvious reduction peaks [99]. Therefore, ASV can be used to detect the degradation products of cefalexin and reflect the concentration of cefalexin itself. In another study, the concentration of cefazolin sodium was measured by DC polarography, cyclic voltammetry, the control potential coulomb method and SWASV [100].

Different voltammetry techniques have been used to study the electrochemical behaviour of cefuroxime and cefoperidol in B-R buffer solutions [101]. Cefloctin has a very obvious reduction peak in the electrochemical scanning process [102]. Therefore, cefloctin can be used for ASV detection at the mercury electrode. The same technique was used to detect cefoperazone levels [94]. Ojani and co-workers demonstrated a sensitive electrochemical sensor based on a poly(o-anisidine)/SDS/Ni modified electrode for the determination of cephalosporins [103]. In this study, POA/CPE was prepared by electropolymerization of anisidine on a carbon paste electrode. Adding SDS in the preparation process can improve the growth rate of the polymer. Then, Ni/POA/CPE was prepared by electro-aggregation of Ni(II) on the POA/CPE surface. Charge transfer on the Ni/POA/CPE surface can be used to catalyse the oxidation of cephalosporins. The kinetic parameters of cephalosporin in electrochemical oxidation were studied. The rate constant K obtained by the time-amperometric method shows that Ni/POA/CPE can optimize the kinetic limit of cephalosporin oxidation, reduce the overpotential and improve the detection sensitivity. Under the optimized conditions, the method can be used to determine the content of cephalosporin in aqueous solution.

A bare boron-doped diamond electrode has been used for the determination of seven cephalosporins [104]. The electrochemical behaviour of the seven cephalosporins was studied by bare BDDE. Because the nuclear structure of cephalosporins has an anodic oxidation peak, a fast and efficient method for the determination of cefalexin has been established. In this study, the parameters were optimized. The influence of other cephalosporins on the detection of cefalexin was also studied. The proposed electrochemical detection method has a very low detection limit and can be used for the detection of environmental samples. Finally, the method was applied to flow analysis, and the continuous detection of cefalexin at different concentrations was realized. Another report studied the electrochemical behaviours of cephalosporins at carbon paste electrodes modified with CoSalophen [105]. Cephalosporins exhibit significant oxidation on CoSalophen-modified carbon paste electrodes. The surface modification of the electrode can greatly improve the selectivity and sensitivity of the sensor. Because the CoSalophen-modified carbon paste electrode has a specific recognition function for sulfur compounds, dimers can be formed during the oxidation of sulfur-containing cephalosporins. After optimization, this method has been applied to the detection of cefazolin in serum, and a very good result has been obtained.

The combination of carbon materials and precious metal materials can effectively improve the sensing performance of commercial electrodes. Shahrokhian et al. [106] proposed immobilizing

platinum nanoparticles on graphene nanosheets and carbon nanospheres and then modifying the surface of glassy carbon electrodes. The modified electrodes were characterized by a series of methods. The electrochemical study showed that the modified electrode had a specific response to cefepime and could be used for the detection of cefepime. This improvement is due to the synergistic effect of bonding carbon nanomaterials and noble metal nanomaterials.

Shahrokhian et al. [107] synthesized platinum nanoparticle-multi-walled carbon nanotube nanocomposites and used them for the surface modification of glassy carbon electrodes. The modified electrode was used to determine the content of ceftriaxone in the presence of lidocaine. The modified electrode was characterized, and the electrochemical behaviour of ceftriaxone on the modified electrode was studied. Under the optimum conditions, the modified electrode can detect ceftriaxone linearly in the range of 0.01-10 μM. The detection limit was 9.01 nM. The results also confirm that this method can be used for the clinical detection of ceftriaxone.

Molecular imprinting is another way to detect cephalosporins. Yang et al. [108] synthesized an advanced biosensor for the determination of cefotaxime by molecular imprinting technique. $HAuCl_4$ was directly reduced by sodium citrate in [BMIM] [BF4] aqueous solution. Porous platinum nanoparticles can be well embedded in it, forming a suspension and coated on the hydroxylated graphene oxide-modified glassy carbon electrode. Then, using cefotaxime as the template and o-phenylenediamine as the monomer, the molecularly imprinted pattern was prepared on the modified electrode. The parameters were optimized by experiments. The molecularly imprinted biosensor had a good response to cefotaxime and could be linearly detected between 3.9 nM and 8.9 μM. The detection limit was 0.1 nM. The electrochemical biosensor could detect cefotaxime in real samples. A similar work was reported by Yola et al. [109]. In this study, 2-aminoethyl mercaptan-multi-walled carbon nanotubes were used to modify glassy carbon electrodes and as a sensitive imprinted electrochemical biosensor for the determination of cefixime. XPS and FTIR were used to characterize the electrode modifiers. Molecularly imprinted films were formed by pyrrole using cyclic voltammetry. The bioelectrochemical sensor had the characteristics of strong specificity and high sensitivity. The biosensor could detect 0.1 nM-10 nM cefixime with a linear response. The detection limit was 22 pM. The biosensor had excellent stability and reproducibility. In addition, the biosensor could detect the content of cefixime in human serum. A similar work was also reported by Karimian et al. [110].

4. CONCLUSION

Electrochemical analysis is a quick way to detect cephalosporin. However, commonly used commercial electrodes are subject to electrocatalytic performance limits, and the poor specificity is not applicable to direct quantification. As a result, electrode surface modification is the main aspect of successful electrochemical detection of cephanmycin. Carbon nanomaterials are widely used for the surface modification of electrodes. Combined with carbon nanomaterials and precious metal nanoparticles, this approach can provide very effective sensitivity to biosensors for cephonin. On the

other hand, the preparation of molecular imprinted biosensors with molecular recognition can greatly increase the specificity of the electrode.

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