Abstract: Antibiotics are widely used to kill or inhibit microorganisms, but their abuse results in various types of side effects in humans and the appearance of super bacteria with tolerance to antibiotics [1]. Excess residual antibiotics in food products have become a serious problem, and so a more reliable, accurate, and easier detection system has been required. Aptamers have strong potential as sensitive bioprobes for the development of biosensors for antibiotics detection, a specially designed aptamer for an antibiotic may be suitable for this purpose. Aptamers are synthetic RNA or DNA single-stranded oligonucleotide sequences which can be generated by an in vitro selection process called SELEX (Systematic Evolution of Ligands by Exponential enrichment) technique. Antibiotics are small molecule targets, therefore, they cannot act as an antigen independently and high specificity and affinity antibiotics antibody are difficult to prepare. Aptamers have advantages over antibodies, such as easy chemical synthesis, easy modification, and less immunogenic response, thus, which can be considered as a valid alternative to antibodies receptors to bind to the target molecules with high affinity. In this review, we concentrated on recent advances made in detection and quantification of antibiotics residues with aptamers biosensor. In this paper, the preparation of aptamers biosensor, the selection of analysis methods and the detection of real samples are introduced in detail. Future prospects toward the development of selective, sensitive aptamers biosensor systems are also discussed. Copyright © 2012 IFSA.

Keywords: Antibiotics residues, Detection, Aptamers, Biosensor.

1. Instruction

Antibiotics are widely used to kill or inhibit microorganisms, but their abuse results in various types of side effects in humans and the appearance of super bacteria with tolerance to antibiotics [1]. Therefore, it is critical to develop sufficiently sensitive methods to detect antibiotics residues for food safety and clinical diagnosis. Recently, many analytical methods, such as capillary electrophoresis [2], surface plasmon resonance [3], square-wave cathodic adsorptive stripping voltammetry [4], immunoassay [5], HPLC [6], the microbiological multi-residue system [7] and Enzyme-linked immunosorbent assay (ELISA) [8] have been reported for the detection of antibiotics. However, most of those above-mentioned methods are time-consuming, expensive and cannot be adapted to high-throughput screening and on-site detection. Thus, a low cost, less reagent consumption, sensitive and
selective technique for kanamycin detection is very desirable.

In recent years, the generation of antibodies against antibiotics has seen significant progress leading to the introduction of immunosensors for sensitive small molecules antibiotics in food samples [9-11]. Consequently, immunosensors have already gained a place in the analytical benchtop as alternative or complementary methods for antibiotics rapid detection [12-15]. They are fast, economic, and at least as sensitive as usual chromatographic techniques. However, immunosensors have not been used for analytical applications as much as expected, principally due to the instability of antibodies. Antibodies are commonly selected as the molecular recognition element but they present some limitations such as limited shelf life, thermal and chemical instability leading to denaturation of proteins and loss of binding ability. As alternatives to antibodies, aptamers (APTs) have recently attracted increasing attention due to their capability to bind a wide range of targets: nucleic acids, proteins, metal ions and other molecules with high affinity and sensitivity [16, 17]. Aptamers are peptides or oligonucleotides, which are synthesized by in vitro process with no need for animal or cell cultures [18, 19]. Aptamers exhibit many advantages as recognition elements in biosensing when compared to traditional antibodies. They are small in size, chemically stable and cost effective. More importantly, because of their simple structure, sensor layers based on aptamers can be regenerated more easily than antibody-based layers, are more resistant to denaturation and have a much longer shelf life [20, 21]. Especially for antibiotics small molecule targets, the aptamers show superior specificity to antibodies since small molecules cannot act as an antigen independently and high specificity and affinity antibody are difficult to prepare. Therefore, the search for specific aptamers for antibiotics small molecules has drawn substantial interest [22-24].

In this review, we summarize recent advances in the development of aptamer-based biosensors for antibiotics detection. We will review the key steps to construct the electrochemical aptamer biosensor including the immobilization protocols used for formation of a bio-recognition interface and the electrode modification. We also will discuss the trends and challenges associated with designing a reliable aptamer biosensor for practical applications in detail.

### 2. Selection of Aptamers

Since the SELEX (systematic evolution of ligands by exponential enrichment) technique was developed by both Gold’s group and Szostak’s group in 1990 [25], especially nanomaterials and magnetic beads are introduced to aptamer biosensor, many researchers have successfully prepared aptamer biosensors for antibiotics residues detection. To date, only a part of aptamers against antibiotics have been selected for antibodies detection applications. As shown in Table 1, the aptamer sequence of antibiotics have been selected. Therefore, for commercial applications, many aptamer sequence of antibiotics still need be selected.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Aptamer sequence 5'-3'</th>
<th>Conference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfadimethoxine</td>
<td>GAGGGCAACGAGTGTATATAGA</td>
<td>[26]</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>TGGGGTGTTAGGCTAAGCCGA</td>
<td>[26]</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>AGCGACAGGAGGTACATGAGCTAGGTGCTCCC</td>
<td>[27]</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>GCACAGGAGGUUAGCUACACUGCC</td>
<td>[28]</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>GGCGGGCGGTATAGCGG</td>
<td>[22]</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>GGATGGGTCTCAGGGGGAGGTCGGGCTGCTCGT</td>
<td>[29]</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>CGTTGGTGGTCTCCAGGGGGAGGTCGGGCTGCTCGT</td>
<td>[30]</td>
</tr>
<tr>
<td>Daunomycin</td>
<td>GGTTGTTGGGTCTTCTATGAGGGTGAAGGGTAA</td>
<td>[21]</td>
</tr>
<tr>
<td>Nenomycin B</td>
<td>GCACAGGAGGUUAGCUACACUGCC</td>
<td>[31]</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>GGCGGGCGGTATAGCGG</td>
<td>[32]</td>
</tr>
</tbody>
</table>
3. The Preparation of Electrochemical Aptamer Biosensor

The label-free aptamer biosensor supplies a simpler, and faster preparation and detection process compared with label-dependent aptamer biosensor. As a consequence, a great number of research papers have appeared over the last years describing the development of novel aptamer biosensors for detecting amounts of antibiotics residues in food samples. While an important disadvantage of this kind of electrochemical aptamer biosensor is that the signal changes due to biomolecular recognition are generally very small. Especially, antibiotics such small molecule sensing using electrochemical biosensor is more challenging. It requires us to offer highly sensitive small molecule aptamer biosensor technologies through careful consideration of sensor interface design and signal enhancement.

The oligonucleotide aptamers can easily be modified with signal moieties and can be produced at low cost. Up to now, a variety of assays have been successfully developed for aptamer based analysis of biomolecules. Nanomaterials usually can enhance the speed of electron-transfer, which lead to high background and low signal-to-noise ratio. The application of nanomaterials provides a novel approach to develop really label-free, high-sensitivity biosensors. For example, Zhou et al., have reported a simple electrochemical tetracycline (TET) aptamer biosensor with multi-walled carbon nanotubes (MWCNTs) modification [30]. The anti-TET aptamer was immobilized on the glassy carbon electrode (GCE) modified with MWCNTs. The format provides a “label-free” method to electrochemically monitor the aptamer-target interaction. Herein, MWCNTs played a role in the increase of the electroactive surface area and provided the conducting bridges for the electron-transfer of Fe(III)/Fe(II). Therefore, the proposed aptamer biosensor improved its sensitivity with a detection limit of $5 \times 10^{-9}$ M in spiked milk samples. The fabrication of aptasensor was shown in Fig. 1.

![Fig. 1. The fabrication of aptasensor modified with MWCNTs.](image)

Chandra et al., have used Au nanoparticles deposited conducting polymer to immobilized aptamer onto glassy carbon electrode [16]. At the same time, Au nanoparticles deposited conducting polymer can also enhance the speed of electron-transfer between solution and electrode.

Zhu et al., have reported a lable-free aptamer biosensor for kanamycin detection. The sensor probe is fabricated by covalently immobilizing an in vitro selected DNA aptamer for kanamycin onto gold nanoparticle comprised conducting polymer. The self-assembled poly-DPB(AuNP) nanocomposite exhibited significantly improved sensitivity.

González-Fernández et al., used amino groups to covalently immobilize tobramycin onto the surface of carboxylated magnetic microparticles [28]. They used a monovalent system for introducing the enzyme conjugate on a tagged-aptamer for specifically recognizing tobramycin. Compared with multivalent systems such as biotin-streptavidin, the sensitivity is greatly improved. The proposed tobramycin inhibition assay with monovalent labeling system is shown in Fig. 2.

![Fig. 2. The proposed tobramycin inhibition assay with monovalent labeling system.](image)

Therefore, nanomaterials can make biomolecules such as aptamer immobilize easily onto the electrode surface, and keep its bioactivity. At the same time nanomaterials can enhance the speed of electron-transfer, thus, increase the response of current of aptamer biosensor.

Microfluidic chip can develop the efficiency of antibiotics detection and realize on-site detection. Daprà et al., have reported an aptamer biosensor for kanamycin and ampicillin detection based on microfluidic chip [22]. As shown in Fig. 3 is the structure of microfluidic chip. At the same time, the polymer film is one of the immobilization methods of biomolecules on electrode surface. This entrapment process, theoretically, should occur without chemical reaction between the electrically conducting polymer (CP) films and the biomolecules, thus, this immobilization method can preserve biomolecules biological activity.

The ampicillin or kanamycin A aptamer was immobilized onto microfluidic chip with conductive bilayer tosylate doped poly(3,4-ethylenedioxythiophene) (PEDOT:Tso) and the hydroxymethyl derivative PEDOT-OH:Tso, respectively. After the immobilization of aptamer, the detection based on microfluidic chip. The ampicillin
or kanamycin A aptamer was immobilized onto microfluidic chip with conductive bilayer tosylate doped poly(3,4-ethylenedioxythiophene) (PEDOT:Tso) and the hydroxymethyl derivative PEDOT-OH:Tso, respectively. After the immobilization of aptamer, the impedance had a significant increase.

![Image](image1.png)

**Fig. 3.** The structure of Daprà et al., assembled microfluidic chip.

The use of an array of microelectrodes can overcome the disadvantage, because one of the main benefits of using a microelectrode in a sensor application is the greater sensitivity that arises from the enhanced mass-transport at these small electrodes. Hemispherical diffusion layers are formed at such electrodes and a much faster diffusion of electroactive substances occurs due to the multi-dimensional nature of this process, resulting in sigmoidal (or steady-state) cyclic voltammograms (CVs). The advantages are in the improved response time (faster response), greater sensitivity and increased response per unit electrode surface area (greater current density, increasing the signal-to-noise ratio).

Kim et al., have reported electrochemical sensing system for oxytetracycline (OTC) detection. The ssDNA aptamer was immobilized on gold interdigitated array (IDA) electrode chip by covalent chemistry [29]. As shown in Fig. 4, the aptamer is immobilized onto gold interdigitated array (IDA) electrode chip.

![Image](image2.png)

**Fig. 4.** The oxytetracycline detection progress with gold interdigitated array electrode chip.

### 4. Trends and Challenges of Aptamers Biosensor for Antibiotics Detection

The use of aptamers for the detection of small molecules still represents a challenge because of the lower affinity interaction when compared with large molecules. For small molecules, this induced fit can be very small leading to minute changes in the analytical signal. However, it has been shown that the stability of such molecules can be improved by chemical modification of the ribose ring at the 3′-position or 5′-position [33-35]. Matrix complexity is one of the greatest challenges for aptamer-based biosensor to detect antibiotics in food samples. Several components regularly present in food samples can produce interference or cross-reaction in immunology-based and aptamer-based detection systems [36, 37]. However, several aptamer biosensor platforms for detecting antibiotics have been developed. Aptamer biosensors for antibiotics detection are a promising and challenging application, with potential advantages over existing immunological biosensors [38, 39].

### Acknowledgements

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