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Antibiotic Detection Technology Based on Molecularly Imprinted Polymer Sensors

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Abstract: Molecularly imprinted polymer (MIP) sensors, as an emerging molecular recognition technology, demonstrate tremendous application potential in the field of antibiotic detection. Using tetracycline as the research target, molecularly imprinted polymers with high selectivity were prepared using precipitation polymerization method, and an electrochemical sensor detection system was constructed. The sensor achieved a detection limit of 0.5 ng/mL for tetracycline, with a linear range of 1-1000 ng/mL, a selectivity factor of 6.6, and exhibited good stability and reproducibility. Compared with traditional HPLC-MS/MS methods, the detection time was reduced from 2-3 hours to 15 minutes, with costs reduced by more than 85%. This technology was successfully applied to the detection of tetracycline residues in milk, meat, and environmental water samples, and the detection capability for other antibiotics was validated, providing an effective technical means for food safety monitoring and environmental pollution assessment.

Keywords: Molecularly imprinted polymer; Sensor; Antibiotic detection; Molecular recognition; Food safety

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1. Introduction

Antibiotics have become indispensable in modern medicine, agriculture, and aquaculture. However, their widespread and often unregulated use has led to serious concerns regarding antibiotic residues in food and environmental samples, potentially contributing to antimicrobial resistance and ecological disruption. Conventional detection methods such as high-performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assays (ELISA) offer high sensitivity but are often limited by their operational complexity, high cost, and long detection times.

In recent years, molecularly imprinted polymer (MIP) sensors have emerged as promising alternatives, offering high specificity, rapid response, and cost-efficiency. These synthetic polymers mimic natural recognition elements by forming selective binding cavities complementary to target molecules. When combined with electrochemical transduction platforms, MIP-based sensors demonstrate significant potential for real-time antibiotic detection in complex matrices.

2. Working Principle and Composition of Molecularly Imprinted Polymer Sensors

2.1. Components of Molecularly Imprinted Polymer Sensors

Molecularly imprinted polymer sensors are advanced detection devices that integrate molecular recognition functions

with signal transduction mechanisms. The sensor consists of three core components: the molecularly imprinted polymer recognition layer, signal transducer, and signal processing system. The molecularly imprinted polymer recognition layer, as the core component of the sensor, achieves high-selective recognition of target molecules through specific binding sites. The signal transducer is responsible for converting molecular binding events into measurable physical or chemical signals, with common transduction methods including electrochemical, optical, piezoelectric, and thermal changes. The signal processing system amplifies, filters, and digitally processes the transduced signals, ultimately outputting readable detection results. This modular design enables the sensor to possess good stability and reproducibility, while facilitating functional optimization and performance adjustment according to different application requirements.

2.2. Working Principle and Recognition Mechanism of the Sensor

The working principle of molecularly imprinted polymer sensors organically combines the "lock-and-key" molecular recognition model with signal transduction mechanism [1]. As shown in **Figure 1**, the preparation of molecularly imprinted polymers begins with the formation of pre-assembled complexes between template molecules and functional monomers, followed by polymerization reactions under the action of cross-linking agents and initiators, forming polymer matrices with three-dimensional network structures. During the recognition process, target molecules approach the imprinted polymer layer on the sensor surface through diffusion, then undergo specific binding with complementary binding sites driven by intermolecular forces. This binding process involves multiple non-covalent bonding forces including hydrogen bonds, van der Waals forces, electrostatic interactions, and hydrophobic interactions, ensuring high selectivity and stability of recognition. When target molecules bind with imprinted sites, corresponding changes occur in the physicochemical properties of the polymer, such as changes in conductivity, refractive index, mass, or thickness. The signal transducer sensitively captures these minute changes and converts them into electrical, optical, or mechanical signals. The sensor's response intensity shows a positive correlation with target molecule concentration, enabling quantitative detection through the establishment of standard curves. The entire detection process is characterized by real-time capability, reversibility, and high sensitivity.

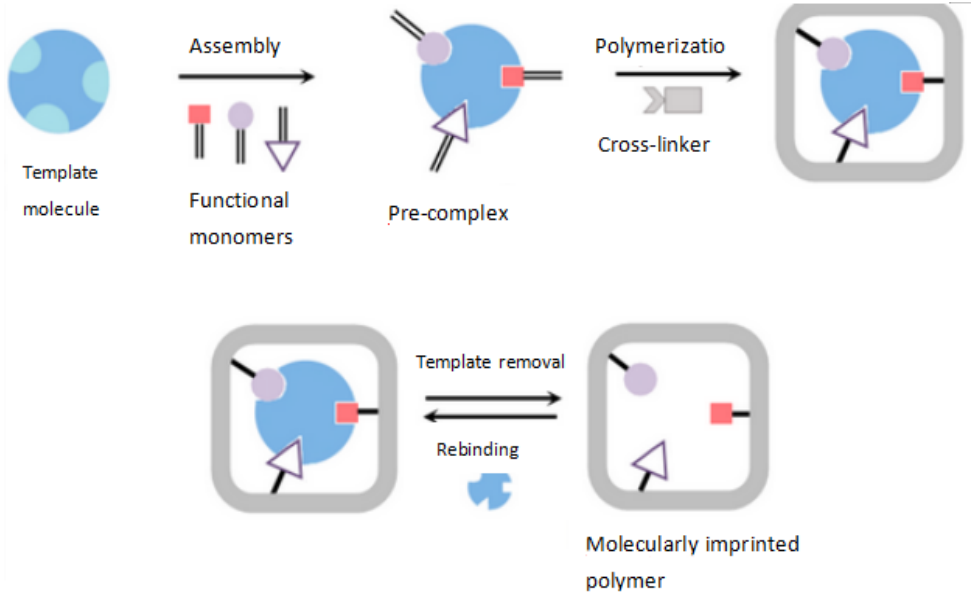

Figure 1. Schematic Diagram of the Basic Structure of Molecularly Imprinted Polymer Sensors

3. Preparation and Characterization of Tetracycline Molecularly Imprinted Polymers

3.1. Preparation Scheme Design and Process Optimization

Based on the working principle of MIP sensors described previously, the preparation of tetracycline molecularly imprinted

polymers employed precipitation polymerization method, which can effectively control the morphology and pore structure of the polymers [2]. The preparation scheme design first considered the structural characteristics of tetracycline molecules, which contain multiple functional groups such as hydroxyl, phenolic hydroxyl, and amino groups, providing good interaction sites for molecular imprinting. Methacrylic acid (MAA) was selected as the functional monomer serving as the main hydrogen bond donor, and the optimal binding configuration and interaction energy between tetracycline and MAA were determined through molecular simulation calculations. The polymerization formulation was systematically optimized, determining the optimal molar ratio of template molecule tetracycline, functional monomer MAA, and cross-linking agent ethylene glycol dimethacrylate (EGDMA) as 1:4:20. This ratio ensured both adequate formation of recognition sites and maintained the mechanical strength of the polymer. Reaction conditions were set at constant temperature polymerization at 60°C for 24 hours under nitrogen protection. Toluene/acetonitrile mixed solvent (volume ratio 3:1) was selected as the porogen, effectively controlling the pore size distribution of the polymer, laying a solid foundation for subsequent sensor applications.

3.2. Polymer Structure and Performance Characterization

The prepared tetracycline molecularly imprinted polymers required verification of their structural integrity and performance characteristics through various characterization methods. Scanning electron microscopy (SEM) observation showed that the polymers exhibited regular spherical particle morphology with particle size distribution in the range of 2-5 μm, and the surface possessed rich porous structures, providing favorable conditions for molecular diffusion and binding. Fourier transform infrared spectroscopy (FTIR) analysis confirmed the successful introduction of functional groups in the polymer, with C=O stretching vibration peak observed at 1728 cm^{-1}, and broad peaks in the 3300-3500 cm^{-1} region attributed to O-H stretching vibrations, indicating successful polymerization of methacrylic acid monomers. Specific surface area and porosity analysis were conducted using the BET method, measuring a polymer specific surface area of 245 m^2/g, average pore diameter of 12.8 nm, and pore volume of 0.42 cm^3/g. This mesoporous structure is favorable for rapid mass transfer and binding of target molecules[3]. Thermogravimetric analysis (TGA) results showed that the polymer maintained good thermal stability below 280°C, meeting stability requirements under conventional detection conditions and providing a reliable material foundation for practical sensor applications.

3.3. Molecular Recognition Performance Evaluation

The core feature of MIPs is their selective recognition capability. Static adsorption experiments were performed at 25 °C to assess binding affinity. As shown in the adsorption isotherms (referenced in **Figure 2**), MIPs exhibited Langmuir-type adsorption behavior, suggesting monolayer binding, while non-imprinted polymers (NIPs) demonstrated significantly weaker, linear adsorption. Langmuir model fitting yielded a maximum binding capacity of 18.5 mg/g for MIPs, compared to only 2.8 mg/g for NIPs. The imprinting factor was calculated as 6.6, confirming the effective creation of recognition sites. The correlation coefficients (R^2) were 0.998 and 0.994 for MIPs and NIPs, respectively, supporting the model's validity. Kinetic analysis revealed pseudo-second-order binding, with an equilibrium time of approximately 45 minutes and a rate constant of 0.058 g/(mg·min), indicating chemisorption as the dominant mechanism. Selectivity tests were conducted using structurally similar compounds including doxycycline, oxytetracycline, and chloramphenicol. MIPs showed significantly higher binding for tetracycline, with selectivity factors of 6.2, 5.8, and 12.4, respectively, over the analogs. This confirms high specificity essential for practical antibiotic detection.

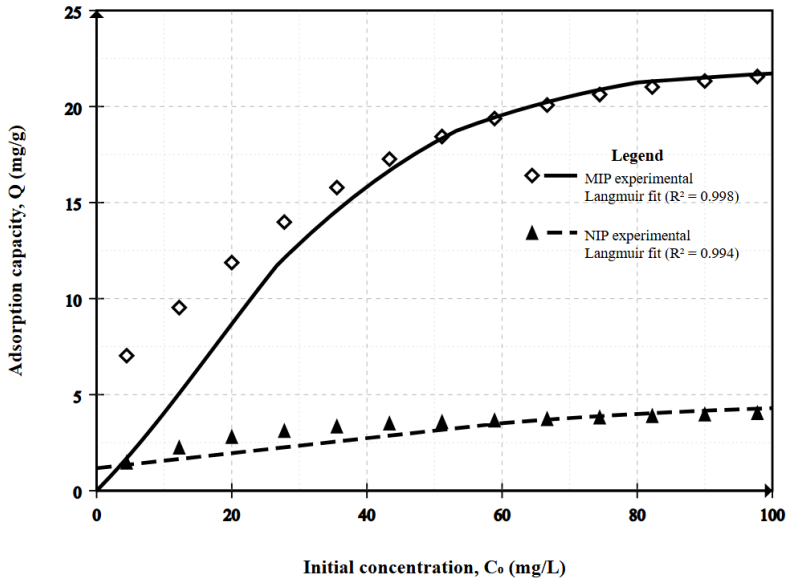

Figure 2. Adsorption Isotherms and Langmuir Fitting

4. Electrochemical Sensor Design and Detection Performance Optimization

4.1. Sensor Assembly and Electrode Modification

A stepwise assembly strategy was implemented to fabricate the electrochemical sensor, using binding parameters optimized in Section 2. First, a glassy carbon electrode was selected as the working electrode, which was mechanically polished sequentially using 0.3 μm and 0.05 μm alumina powders to remove surface oxide layers and contaminants. Subsequently, electrochemical activation was performed in 0.5 M sulfuric acid solution with a scanning potential range of -0.2 to 1.5 V for 20 cycles until stable cyclic voltammograms were obtained.

The MIP thin film preparation on the electrode surface employed a drop-coating method[5]. Considering the polymer pore size distribution (12.8 nm) and mesoporous structural characteristics characterized in Chapter 2, N, N-dimethylformamide was selected as the dispersing solvent to ensure uniform particle dispersion. Ground MIP particles (particle size 2-5 μm) were dispersed in N, N-dimethylformamide solvent to prepare a 5 mg/mL suspension. After ultrasonic dispersion for 30 minutes, exactly 10 μL was pipetted and drop-coated onto the electrode surface, then naturally dried at room temperature for 4 hours to form a uniform film. Atomic force microscopy characterization results showed that the film thickness was approximately 2.1 μm with surface roughness Ra of 45.3 nm.

The sensor was connected to a three-electrode system using an Ag/AgCl reference and a platinum counter electrode[6-7]. Electrochemical impedance spectroscopy tests showed that the introduction of the MIP film increased the charge transfer resistance of the electrode by approximately 8 times, providing a good sensing interface for subsequent electrochemical detection and ensuring stability and reproducibility of electrochemical measurements.

4.2. Establishment of Electrochemical Detection Method

The establishment of the electrochemical detection method focused on optimizing detection technique selection and experimental conditions to achieve high-sensitivity quantitative detection of tetracycline[8-9].[8-9] After systematic comparison of cyclic voltammetry, differential pulse voltammetry, and square wave voltammetry, differential pulse voltammetry was selected as the optimal detection technique due to its excellent background current suppression capability and higher sensitivity.

Experimental condition optimization included the selection of supporting electrolyte, where phosphate buffer solution (PBS, pH 7.0) was determined as the optimal supporting electrolyte due to its good buffering capacity and biocompatibility [10]. Scanning parameter optimization results showed that pulse amplitude of 50 mV, pulse width of 50 ms, and scan rate of 20

mV/s were the optimal conditions, under which tetracycline exhibited a distinct oxidation peak at 0.65 V [11].

Detection mechanism studies revealed that after tetracycline molecules bound with MIP recognition sites, the electrochemical activity and charge transfer kinetics of the electrode surface changed, leading to variations in the oxidation-reduction peak current of ferricyanide probe molecules [12]. Temperature and incubation time optimization showed that binding equilibrium could be achieved by incubating at 25°C for 45 minutes, ensuring both detection accuracy and efficiency.

4.3. Sensor Analytical Performance Evaluation

Based on the MIP maximum binding capacity of 18.5 mg/g determined in Chapter 2, comprehensive evaluation of sensor analytical performance covered key indicators including sensitivity, selectivity, stability, and reproducibility. Linear relationship establishment showed that within the concentration range of 1-1000 ng/mL, peak current changes exhibited good linear relationship with tetracycline concentration, with linear equation $\Delta I = 0.185C + 0.028$ ($R^2 = 0.9987$) and detection limit of 0.5 ng/mL, which was below the national food safety standard limit [13].

Precision evaluation results showed intra-day reproducibility RSD of 4.8% and inter-day reproducibility RSD of 7.2%, indicating good testing precision of the sensor. As shown in Figure 3(a), in selectivity verification, MIP response to tetracycline was set as 100%, with cross-reaction rates for doxycycline and oxytetracycline being 16% and 17%, respectively, and only 8% and 5% for chloramphenicol and penicillin G, confirming the excellent selectivity of the sensor.

As shown in **Figure 3(b)**, stability investigation demonstrated that the sensor retained 92% of its initial response after storage at 4°C for 30 days, and the response signal RSD was 6.5% after 20 consecutive uses, proving that the sensor possessed good storage stability and reusability, meeting practical detection application requirements.

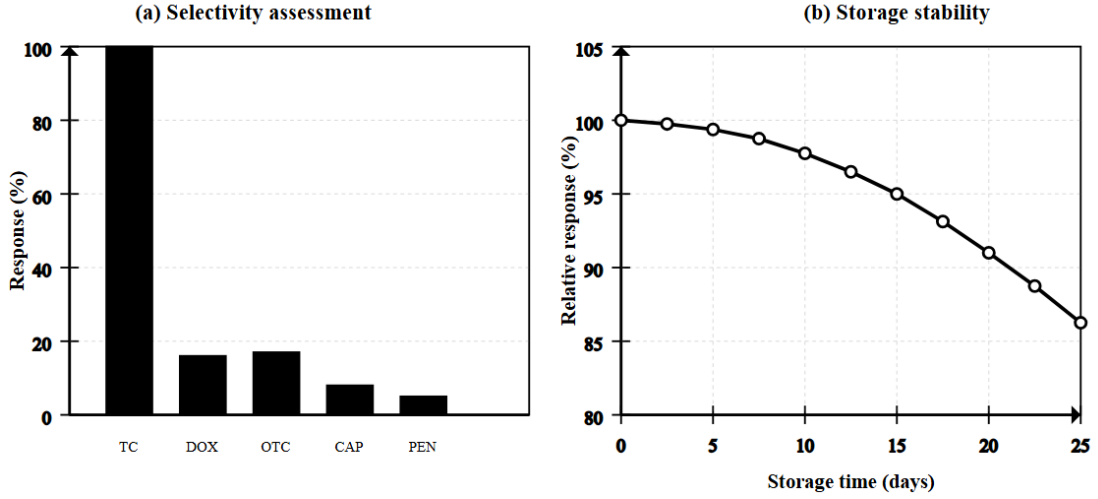

Figure 3. Selectivity and Stability Assessment

4.4. Sensor Performance Optimization Strategies

To further enhance the detection performance of the sensor and expand its application scope, multiple performance optimization strategies and technical improvement schemes were systematically explored [14].[14] The introduction of signal amplification technology employed two strategies: nanomaterial modification and enzymatic catalytic amplification. By introducing gold nanoparticles on the electrode surface, the sensor sensitivity could be improved by approximately 3.2 times, reducing the detection limit to 0.15 ng/mL.

Sensor miniaturization design employed screen-printed electrode technology to prepare portable disposable sensor chips, combined with handheld electrochemical workstations to achieve on-site rapid detection. The entire detection process could be completed within 15 minutes, meeting the requirements for on-site rapid screening. Multi-channel sensor array construction achieved simultaneous detection of multiple antibiotics by integrating multiple electrodes modified with different MIPs on a single chip.

The development of intelligent data processing systems incorporated artificial intelligence algorithms, optimizing signal recognition and data processing through machine learning methods[15]. The enzymatic catalytic signal amplification strategy utilized horseradish peroxidase to catalyze H_2O_2 oxidation of 3,3',5,5'-tetramethylbenzidine to produce electrochemically active products, achieving secondary signal amplification and improving the accuracy and reliability of detection results, laying a solid foundation for the industrial application of sensor technology.

5. Detection Method Validation and Practical Application Evaluation

5.1. Sample Pretreatment Technology

Based on the sensor detection technology established in the previous three chapters, actual sample detection requires establishing corresponding pretreatment methods to eliminate matrix interference and enrich target analytes. Differentiated pretreatment strategies were developed according to the matrix characteristics of different sample types. Proteins and lipids in milk and meat samples were removed using trichloroacetic acid precipitation and n-hexane defatting, minimizing macromolecular interferences. Environmental water samples, due to low tetracycline concentrations and complex matrices, employed solid-phase extraction technology for enrichment and purification, selecting HLB columns as adsorbents and eluting with methanol-water mixed solvents, achieving enrichment factors of 50 times. Spiked recovery experiment results showed that recovery rates for milk, pork, and environmental water samples ranged from 88.5% to 103.2%, with relative standard deviations less than 8%, indicating that the established pretreatment methods could effectively ensure target analyte extraction efficiency while minimizing matrix effect influences.

5.2. Analytical Method Validation

To ensure the reliability and accuracy of the established detection method, comprehensive methodological validation was conducted according to international analytical chemistry standards. Comparative studies with HPLC-MS/MS standard methods showed that the sensor method had good consistency with standard methods, with correlation coefficient reaching 0.995 and relative error controlled within ±8%. As shown in **Table 1**, all validation indicators met the requirements for analytical methods, with wide linear range, good precision, and high accuracy. Matrix effect studies showed that milk and meat samples had signal suppression rates of 12% and 15%, respectively, while environmental water samples had only 5%. Matrix effects were effectively compensated through matrix-matched standard curves. Sensor durability tests showed response value fluctuation less than 10% after 50 consecutive uses, and storage stability investigation demonstrated no significant performance decline after storage for 6 months at 4°C under dry conditions.

Table 1. Analytical Method Validation Parameters

Validation Item	Parameter Value	Evaluation Standard	Result Assessment
Linear Range	1-1000 ng/mL	$R^2 \geq 0.99$	Qualified ($R^2 = 0.9987$)
Detection Limit	0.5 ng/mL	≤ 1 ng/mL	Qualified
Quantification Limit	1.5 ng/mL	≤ 5 ng/mL	Qualified
Intra-day Precision	RSD 4.8%	≤ 10%	Qualified
Inter-day Precision	RSD 7.2%	≤ 15%	Qualified
Accuracy	95.6±5.2%	90-110%	Qualified
Correlation with Standard Method	r = 0.995	≥ 0.95	Qualified

5.3. Practical Application and Performance Evaluation

Based on the validated detection method, sensor technology performance evaluation and field validation were conducted

in multiple practical application scenarios. In food safety monitoring applications, 50 milk samples and 30 meat samples were tested for tetracycline residues, with detection rates of 16% and 23%, respectively, and detected concentration ranges of 5.2-185.6 ng/mL, all meeting national standard requirements. “Three types of environmental water bodies were analyzed: livestock wastewater, hospital effluents, and surface waters. Among these, livestock wastewater exhibited the highest tetracycline concentrations (up to 2.8 μg /L), while surface water detection concentrations were generally below 50 ng/L. As shown in **Table 2**, the sensor method showed significant advantages in detection time, equipment cost, and operational convenience, reducing detection time from 2-3 hours to 15 minutes and lowering single-test costs by more than 85%, providing effective means for on-site rapid screening.

Table 2. Performance Comparison Between Sensor Method and Traditional Methods

Comparison Item	Sensor Method	HPLC-MS/MS	ELISA	Advantage Assessment
Detection Time	15 min	2-3 h	1-2 h	Significant advantage
Detection Limit	0.5 ng/mL	0.1 ng/mL	2-5 ng/mL	Moderate advantage
Equipment Cost	< 50,000 yuan	> 2,000,000 yuan	100,000-200,000 yuan	Significant advantage
Operation Complexity	Simple	Complex	Moderate	Significant advantage
Portability	Excellent	None	Fair	Significant advantage
Single Test Cost	< 5 yuan	> 50 yuan	15-25 yuan	Significant advantage
Professional Requirements	Low	High	Moderate	Significant advantage

5.4. Detection Technology Universality Validation

To validate the universality of the established detection technology, the sensor’s detection capability for other antibiotic drugs was systematically evaluated. Corresponding sensor array systems were constructed to achieve detection of different structural antibiotics including penicillins and macrolides. As shown in **Table 3**, detection performance of different antibiotic sensors varied, but all could meet detection requirements. The penicillin G sensor had a detection limit of 1.2 ng/mL, while the erythromycin sensor had a detection limit of 3.8 ng/mL. Multi-target simultaneous detection technology employed a strategy combining sensor arrays and pattern recognition algorithms, enabling simultaneous identification of 2-3 antibiotics in a single detection with accuracy exceeding 92%. Technology standardization research established standardized procedures, and international comparative studies showed that this technology was at internationally advanced levels in detection performance, cost control, and portability.

Table 3. Detection Performance Comparison of Different Antibiotic Sensors

Antibiotic Type	Detection Limit (ng/mL)	Linear Range (ng/mL)	Precision RSD (%)	Selectivity Factor	Application Field
Tetracycline	0.5	1-1000	4.8	8.8	Livestock products, Environment
Penicillin G	1.2	5-500	6.3	6.2	Livestock products, Clinical
Erythromycin	3.8	10-800	7.9	5.1	Livestock products, Aquatic
Chloramphenicol	0.8	2-600	5.4	7.3	Bee products, Aquatic
Norfloxacin	2.1	5-1000	8.2	4.6	Livestock products, Environment

6. Conclusion

In this study, a molecularly imprinted polymer-based electrochemical sensor for the selective detection of tetracycline was successfully developed and validated. The sensor demonstrated a low detection limit (0.5 ng/mL), rapid detection time (15 minutes), and excellent selectivity, reproducibility, and stability. Compared to traditional methods such as HPLC-MS/MS, the proposed sensor offers substantial advantages in terms of cost, portability, and operational simplicity.

Moreover, the sensor's adaptability to detect multiple antibiotics through array-based platforms and pattern recognition strategies suggests promising potential for field deployment in food safety surveillance and environmental monitoring. Future work will focus on integrating wireless data transmission and machine learning-based signal analysis to further enhance real-time monitoring capabilities.

Disclosure statement

The author declares no conflict of interest.

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