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Application of Improved Electrochemical Sensor in Food Safety Inspection

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This paper analyzes the detection mechanism of electrochemical sensor by integrating nanotechnology with electrochemical analysis, and achieves excellent detection results after applying the proposed method to detect the clenbuterol (CB) and chloramphenicol (CAP) in food. Specifically, the modified electrode and Cu-Au marked CB antibody are prepared in this research and the detection method of electrochemical sensor is optimized. The results show that the proposed method realizes the rapid detection of CB in food. The detection range falls between 0.1 and 500ng/ml and the detection limit reaches 0.05ng/ml. The proposed method is also adopted for the treatment of samples of actual pig liver. According to the detection results, the recovery rate is between 94.25% and 101.14%. For the detection of CAP, the electrochemical sensor boasts advantages like wide detection range and low detection limit, and the results obtained by the sensor shows no significant difference from those obtained by the traditional high-performance liquid chromatography. Thus, the proposed detection method is proved to be accurate and fast.

1. Introduction

Food safety has a direct bearing on people's health and social stability. In recent years, our health is under serious threats from environmental pollution, misuse of pesticide, and excessive use of food additives. In this background, it is of great practical significance to implement food safety inspection. Therefore, rapid and accurate food safety inspection methods have become a focal point of current research (Galera et al., 2006; Ashrafi and Vytřas, 2012; Gupta et al., 2014; Chang et al, 2010; Clarke, 2016; Van and Delva, 2016; Mannebeck et al., 2016; Borchiellini et al., 2017).

The existing food safety inspection methods include gas chromatography, liquid chromatography, mass spectrometry, etc. However, these methods do not support real-time inspection of food owing to high cost, long detection cycle and other defects. Featuring high sensitivity, short cycle and accurate results, the electrochemical detection method has become a popular new inspection method in recent years. The electrochemical detection method mainly detects the heavy metals (Inam and Somer, 2000; Afkhami et al, 2013), pesticide residues (Yang et al, 2012; Anandhakumar et al., 2014; Grennan et al, 2003; Oliveira et al, 2012), antibiotics (Thavarungkul et al, 2007; Jin et al, 2013; Gonçalves et al, 2014) and other prohibited additives in food (Zhang et al., 2003; Et al, 2012; Moraes et al, 2013; Najafi et al., 2014).

Clenbuterol (CB) and chloramphenicol (CAP) are two cheap and widely used food additives. However, they have been banned in China from being used as auxin or food additives. In this paper, nanotechnology and electrochemical analysis are combined to analyze the detection mechanism of electrochemical sensor, and are applied in detecting the CB and CAP in food. The research findings provide a theoretical reference for relevant studies on food safety inspection.

2. Detection and analysis of CB by electrochemical sensor

2.1 Test materials and instruments

The reagents include: CB reagent, bovine serum albumin (BSA), Nafion solution, $CuSO_4.5H_2O$, $HAuCl_4.4H_2O$, $NaBH_4$, HBr, $HClO_4$, NaOH (pH adjusting solution), and Na_2HPO_4 (buffer solution). All reagents are not purified.

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Preparation of clenbuterol compound: A dispersion liquid was prepared by adding carboxyl radicals to the phosphate buffer solution (PBS). Then, the mixture of MEDC and MNHS was added, and the resulting solution was stirred and centrifuged. The supernatant was separated and allowed to stand for 24h. The phosphate buffer solution was added again, and the resulting solution was stirred to form the dispersion liquid for further use.
Preparation of pig liver samples: A number of fresh pig livers were purchased and divided into three equal parts. The three parts were added with 10μg/g, 20μg/g and 50μg/g CB reagents, respectively, and allowed to stand for a period of time. After that, 10g of each part was weighed and taken to prepare the sample extracts of pig liver.
Test instruments: The test uses UV-Vis spectrometer, transmission microscope, and ELISA reader. The electrochemical system has three electrodes: modified electrode, reference electrode, and counter electrode.

2.2 Test results and analysis

Because the CB antigen is electrochemically inert, its concentration has to be measured indirectly by a marker in the electrochemical immunoassay. In this paper, the core-shell Cu-Au composite nanoparticles are prepared as the marker for CB. The content of CB is determined indirectly by measuring the concentration of dissolved Cu^{2+} with the modified electrode.
Figure 1 shows the UV-Vis spectra of Cu, Au and Cu-Au nanoparticles. As shown in the figure, the characteristic absorption peaks of Cu and Au nanoparticles appear at 575nm and 530nm, respectively, while the characteristic absorption peak of Cu-Au nanoparticles appear near 542nm. The absorption peak difference between Cu-Au nanoparticles and Cu nanoparticles signifies that Cu-Au bimetallic particles have been formed and marked on CB antibody.

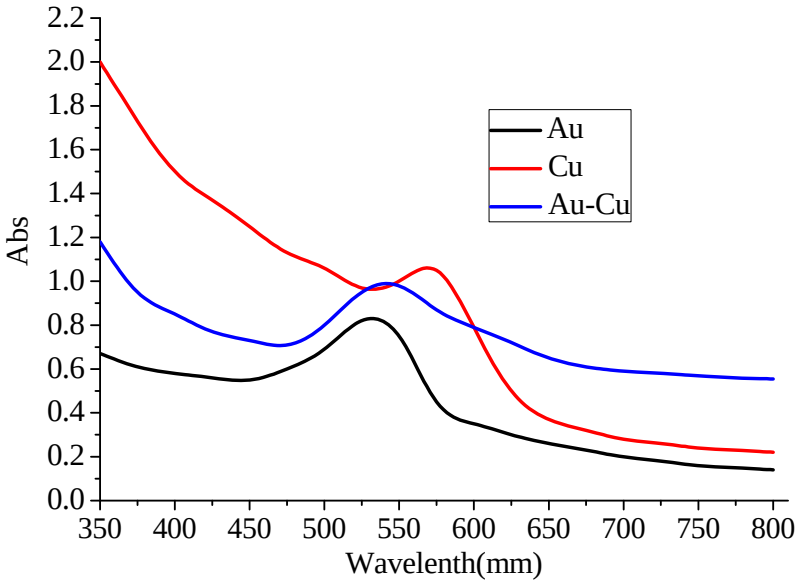

Figure 1: UV-vis spectra of Au, Cu and Cu-Au NPs

The temperature and time of incubation have an important effect on the release of Cu^{2+} into the solution. Figure 2 depicts how the concentration of released Cu^{2+} changes with incubation temperature and time. It can be seen that the released Cu^{2+} concentration first increases and then decreases as the temperature keeps rising, and the concentration reaches the maximum at 36°C. The incubation time curve shows a tendency to gradually stabilize after the initial rapid increase. When the time surpasses 60min, the increase of Cu^{2+} concentration is very limited. These trends are attributable to the fact that the antibody and antigen in the solution are subjected to damages and irreversible reaction at an excessively high temperature, and the reaction time is extended due to low molecular activity at an excessively low temperature. Therefore, the incubation temperature should be 36 °C, and the incubation time should be 60min.
In order to improve the detection accuracy of Cu^{2+} in solution, the parameters like working electrode and deposition time are optimized, and $HClO_4$ is selected as electrolyte solution. Figure 3 shows the effect of deposition potential and deposition time on the ASV signal. As can be seen from the figure, the released Cu^{2+} concentration falls gradually with the rise of potential. Thus, -0.5V is taken as the deposition potential of the

test. In terms of deposition time, 300s is set as the deposition time of Cu^{2+} because of the slight increase of released Cu^{2+} concentration after that moment.

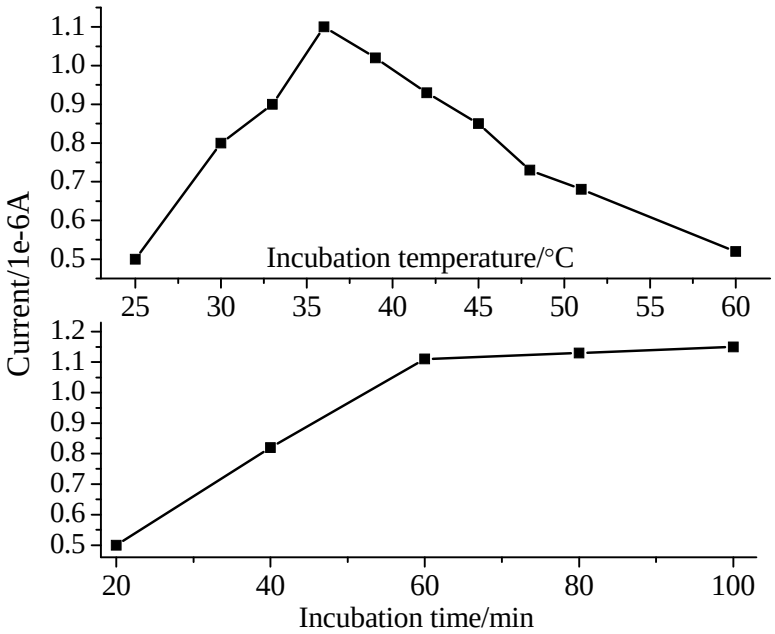

Figure 2: The relationship between Cu^{2+} release and incubation temperature and time

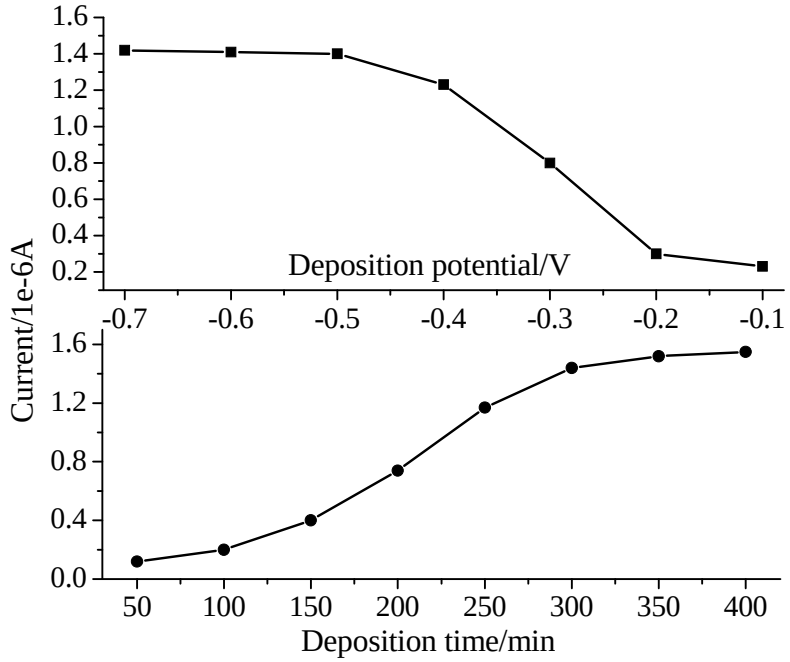

Figure 3: Effect of deposition potential and deposition time on the ASV signal

The three solutions containing different concentrations of CB (0ng/ml, 5ng/ml and 30ng/ml) are tested after the above optimization. Figure 4 presents the released Cu^{2+} concentrations at different current intensities. When the electrodes are at proper positions, the released Cu^{2+} concentration is relatively low at high concentration of CB.

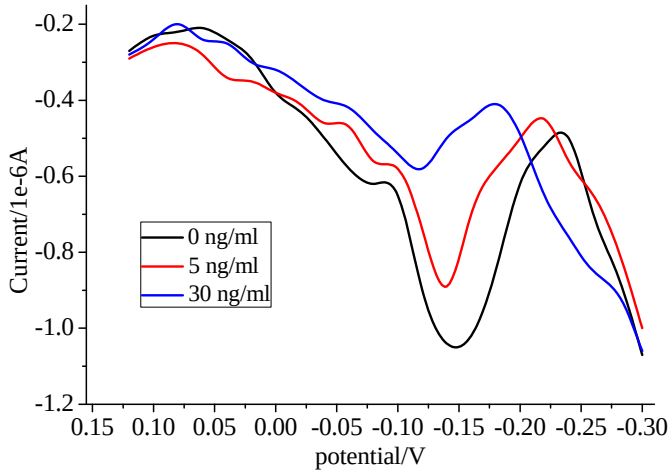

Figure 4: The effect of current on Cu^{2+} release from Cu-Au at 0, 5 and 30ng/ml CB

After preparing the solutions of pig liver samples, the author adds 15μg sample extract into 150ml PBS solution, and measures the CB content by electrochemical sensor and ELISA. The detected results of the three samples A, B and C are shown in Table 1. According to the data in the table, the two methods differ slightly in terms of recovery rate, signifying the effectiveness of the proposed detection method.

Table 1: Determination of CB in pig liver samples

Sample	Spiked (μg/g)	Immunosensor			ELISA		
		Detection (μg/g)	Recovery (%)	RSD (%)	Detection (μg/g)	Recovery (%)	RSD (%)
A	0	0	94.25	6.5	0	95.15	7.2
	20	18.85			19.03		
B	0	0	101.14	5.6	0	98.76	5.8
	50	50.57			49.38		
C	0	0	100.72	4.1	0	96.29	4.5
	100	100.72			96.29		

3. Detection and analysis of CAP by electrochemical sensor

In this section, CAP in food is detected by the electrochemical sensor method. Being one of the most widely used antibiotics, CAP has strong toxic and side effects on the human body. Thus, it is very meaningful to study the detection of CAP.

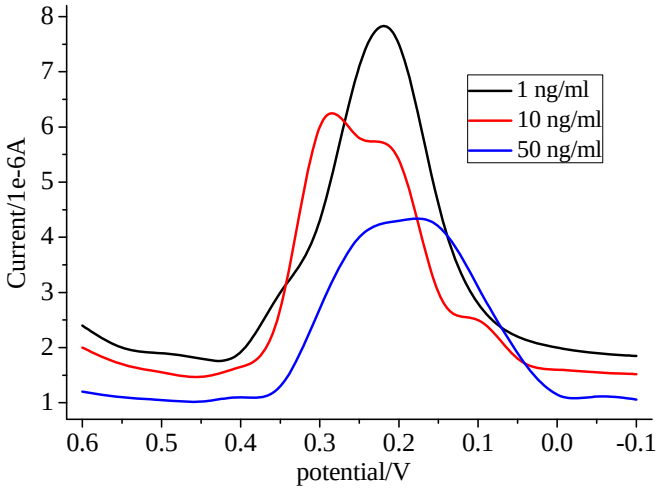

Figure 5: DPV of the immunosensor at different CAP concentrations: 1, 10 and 50ng/ml

The test materials include CAP, BSA, $HAuCl_4.H_2O$, chitosan and PBS. Beef, pork and fish are selected as test samples, each of which is injected with 5μg/g, 20μg/g and 50μg/g spiked samples, respectively.
The optimized conditions mentioned in the previous section are adopted in this test of CAP. Figure 5 shows the detected results of the three concentrations of CAP by electrochemical sensor. It can be inferred from the figure that the CAP concentration gradually grows with the reduction of current. The trend is the result of the suppressed electron transport for the growing presence of CAP on the surface of the sensor narrows down the effective area of the electrodes.
Similarly, the method is applied to detect the CAP content in the actual samples. Table 2 lists the detected CAP contents of beef, fish and pork. It can be seen that the proposed method recovers 84-98% of spiked samples, and there is no significant difference from the results obtained by the traditional high-performance liquid chromatography. Thus, the proposed method is proved to be effective.

Table 2: Recoveries of CAP by the Immunosensor and HPLC-UV

Sample	Spiked (μg/g)	Immunosensor		HPLC-UV	
		Detection (μg/g)	Recovery (%)	Detection (μg/g)	Recovery (%)
Beef	5	4.38	87.6	4.02	80.4
	20	18.15	90.75	17.33	86.65
	50	46.29	92.58	45.75	91.5
Fish	5	4.59	91.8	4.22	84.4
	20	17.97	89.85	18.47	92.35
	50	42.19	84.38	44.61	89.22
Pork	5	4.92	98.4	4.72	94.4
	20	17.27	86.35	18.83	94.15
	50	43.83	87.66	46.27	92.54

4. Conclusion

This paper analyzes the detection mechanism of electrochemical sensor by integrating nanotechnology with electrochemical analysis, and achieves excellent detection results after applying the proposed method to detect the CB and CAP in food. The conclusions are as follows:
(1) The author prepares modified electrode and Cu-Au marked CB antibody, optimizes the detection method of electrochemical sensor, and eventually achieves the rapid detection of CB in food. The detection range falls between 0.1 and 500ng/ml and the detection limit reaches 0.05ng/ml. The detected results on the samples of actual pig liver indicates that the recovery rate is between 94.25% and 101.14%.
(2) For the detection of CAP, the electrochemical sensor features wide detection range and low detection limit, and the results obtained by the sensor shows no significant difference from those obtained by the traditional high-performance liquid chromatography. The results validate the accuracy and speed of the proposed detection method.

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