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Electrochemical immunosensor based on MWCNT-CS as a signal amplification strategy for the detection of capsaicinoids

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We constructed an electrochemical immunosensor based on multiwalled carbon nanotube-chitosan (MWCNT-CS) for on-site detection of capsaicinoids, which are label compounds in waste oils. In this work, we fixed capsaicinoids antibodies (Abs) and ovalbumin (OVA) on the surface of the MWCNT-CS complex. The good adsorbability and conductivity of MWCNT was utilized to greatly enlarge the current response signal. OVA was embedded in the nonspecific sites of the Abs to enhance the detection sensitivity of the immunosensor. Cyclic voltammetry (CV) and differential voltammetry (DPV) were used to characterize the working principle and current response signals of the immunosensor. The parameters affecting the current response signal, such as the pH of the buffer solution, incubation time and Abs concentration, were optimized. The linear range of the immunosensor was 2–100 μg/mL, and the limit of detection (LOD) was 0.25 μg/mL. The aptasensor provides the advantages of high sensitivity, short time consumption and high stability. Overall, the results demonstrated that the developed method could meet the demands of rapid on-site assays for capsaicinoids in waste oil.

Keywords: Capsaicinoids; Immunosensor; Multiwalled carbon nanotube

1. INTRODUCTION

Capsaicinoids are alkaloids containing phenolic hydroxyl groups and are the main spicy component of peppers[1,2]. The spicy components in peppers are mainly natural capsaicinoids and dihydrocapsaicinoids, which account for more than 90% of the total capsaicinoids at present[3]. When the capsaicinoids content in fat is detected to exceed 0.5 μg/kg, it can generally be assumed that the fat is waste oil or blended with waste oil. Therefore, a method to quickly determine capsaicinoids content

of oil is necessary. In recent years, the traditional detection methods and quantification of capsaicinoids have mainly involved gas chromatography (GC)[4], spectroscopy, high-performance liquid chromatography (HPLC)[5,6], ELISA immunoassays[3], etc. However, the applicability of these methods has been restricted due to the expensive testing equipment and complicated sample processing [7–9].

Immunoassays are well known for their high sensitivity, specificity and low cost. Therefore, they have potential for rapid on-site detection of capsaicinoids in waste oil. Immunosensors are based on the interaction between antibodies (Abs) and antigens on the surface of the sensor[10]. Antibodies can be immobilized on the surface of the transducer to identify the type of antigen[11,12]. Because of the strong binding force between these biomolecules, the specificity and sensitivity of immunosensors are high, making them very desirable for many applications in different scientific fields[13–15]. In addition, immunosensors have outstanding comparative advantages compared with other biosensors[13]. At the same time, nanomaterials have great development potential in the field of biosensors, and they provide a new route for the application of immunosensors[16–18]. Currently, nanomaterials such as MWCNT, AuNPs and GO nanomaterials can not only be used as substrates to immobilize biomolecules but can also increase solid load, thereby improving catalytic efficiency and adsorption capacity [19–21]. In addition, the combination of multiwalled carbon nanotube-chitosan (MWCNT-CS) could improve the conductivity of chitosan. Moreover, MWCNT-CS modification using the good film-forming properties of chitosan could provide a good biological interface on the electrode surface and improve the ability to maintain the biological activity of antibodies. In addition, these materials could maintain the biological activity of the labelled antibody and correspond to component action, providing a good biomimetic interface for the immobilization of the Abs[22–24].

In this work, we constructed an electrochemical immunosensor using a MWCNT-CS modified electrode to improve sensitivity. The introduction of high conductivity and high stability MWCNT expanded the current response signal of the target, thereby increasing the sensitivity of detection. Cyclic voltammetry (CV) and differential voltammetry (DPV) were used to characterize the immunosensor assembly process, select conductive materials with stable effects and strong amplification ability, and determine the parameters that affected the current response signal in the experiment, including capsaicinoids antibodies. Under the optimized conditions, the current response of different concentrations of capsaicinoids to the immunosensor was established, and the limit of detection (LOD) was determined.

2. EXPERIMENTAL PROCEDURE

2.1. Materials and reagents

Glacial acetic acid (CH_3COOH), ethanol (C_2H_5OH), nitric acid (HNO_3), potassium ferricyanide ($K_3[Fe(CN)_6]$), and hydrochloric acid (HCl) were obtained from Yantai Far Eastern Fine Chemical Co., Ltd. (Shandong, China). Sodium citrate, ferric chloride ($FeCl_3$), and potassium chloride (KCl) were purchased from Tianjin HengXing Chemical Reagent Co., Ltd. (Tianjin, China). Bovine serum albumin (BSA), capsaicinoids and ovalbumin (OVA) were purchased from Sigma–Aldrich Trading Co., Ltd.

(Shanghai, China). Sodium dihydrogen phosphate dihydrate ($NaH_2PO_4·2H_2O$) and disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4·12H_2O$) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All reagents except those with an indicated content were analytical grade. The working solutions were prepared with PBS buffer (pH 7.4, containing 5 mM $[Fe(CN)_6]^{3-/4}$-and 0.1 M KCl). Aqueous solutions were prepared with ultrapure water obtained from an LS MK2 PALL-water purification system (18.2 MΩ·cm at 25℃). capsaicinoids antibodies were obtained from Wuhan Baofu Biological Engineering Co., Ltd. (Wuhan, China).

CV and DPV were carried out on a CHI 660D electrochemical workstation. The CV curve in 5.0 mM $[Fe(CN)_6]^{3-/4-}$ containing 0.1 M KCl solution (pH 7.4) was used to measure the electrode performance. The scanning rate was set at 50 mV/s, and the scanning range was -0.1–0.6V.

2.2. Design of immunosensor

The experiment used MSWNT-CS to design a high-sensitivity electrochemical immunosensor for the detection of capsaicinoids. First, MWCNT-CS were combined with a glassy carbon electrode (GCE), which we believed could provide improved conductivity due to the excellent electrical conductivity and relatively large surface area of MWCNT and the good film-forming properties of the CS; therefore, the synergy of both could improve the performance of the electrode. Then, a large amount of sealing material was bound to the nonspecific sites on the electrode where the antibody was not adsorbed. This step was performed to avoid the nonspecific binding of capsaicinoids directly to the surface of the electrode. Finally, the prepared electrode was drip-coated with capsaicinoids and placed in a 0.1 M KCl solution containing 5.0 mM $[Fe(CN)_6]^{3-/4-}$ for testing, and the concentration of capsaicinoids was quantified according to the electrical signal.

2.3 Preparation of MWCNT-CS

CS (0.1 g) was weighed, and 50 mL 1.0% (v/v) acetic acid solution was added to obtain a 0.2% CS solution. Magnetic stirring was performed for approximately 12 h to obtain a completely dissolved CS solution, and the pH of the CS solution was adjusted to 5.0. MWCNT (2 mg) were weighed, dispersed in a 4 mL 0.2% CS solution, and sonicated for 6 h. Finally, we obtained a black uniformly dispersed solution. The obtained mixture was placed in a refrigerator and stored frozen at 4 ℃.

2.4 Electrochemical testing of immunosensors

During the preparation of the immunosensor, the GCE was drip-coated with MWCNT-CS, antibodies (Abs), and OVA solution. Moreover, the assembly process of the immunosensor was characterized by cyclic voltammetry (CV). The potential range was set at -0.1–0.6 V, and the scanning rate was 0.05 V/s. After the preparation of the immunosensor, different concentrations of capsaicinoids were dripped and tested in the base solution, and the detection of capsaicinoids was characterized by

DPV. Capsaicinoids concentrations could be measured by DPV after immunization with preimmune DPV, calculated as follows:

$\Delta I=I_1-I_2$

where I_1 represents the peak current before the electrode-modified Abs specifically binds to capsaicinoids, and I_2 represents the peak current after the immune response.

3. RESULTS AND DISCUSSION

3.1 Comparison of the interface between nanocomposites and electrodes

To improve the sensitivity of the immunosensor and obtain a larger current response signal, it was necessary to modify the bare electrode with nanocomposite material to amplify the electrical signal. In the initial stage of this experiment, the MWCNT-CS and the Fe_3O_4 configuration composite were used to modify the gold electrode and the GCE, respectively, and the current amplification effect on the bare electrode was compared, as shown in Fig. 1A. Compared with the gold electrode, the GCE in the test solution of the experiment had a larger peak current, and the test results after the subsequent dripping of reagents were more obvious. Fig. 1B shows the amplification effect of the MWCNT on the current, so in this experiment, MWCNT were used to modify the GCE. It was also incated that MWCNT with excellent electrical conductivity and could be fixed stably on the GCE.

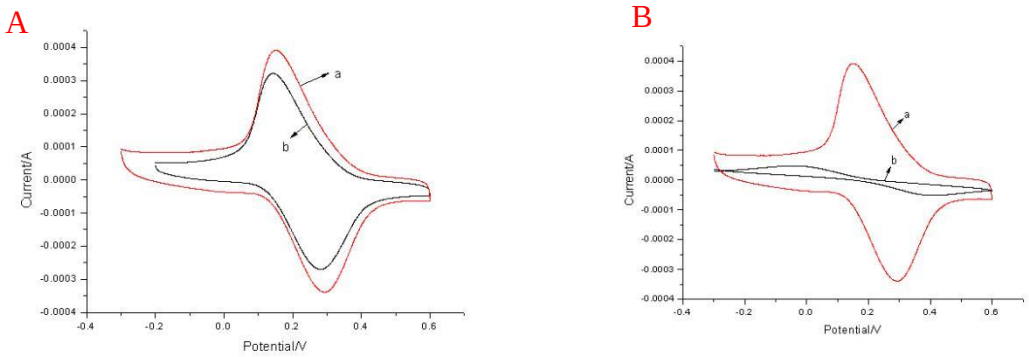

Figure 1. (A) Current response of (a) bare GCE (b) bare gold electrode; (B) CV characterization of immunosensor (a) MWCNT/GCE (b) Fe_3O_4/GCE.

3.2 Characterization of the electrochemical characteristics of the immunosensor assembly process

The CV characterization of the immunosensor assembly process is shown in Fig. 2A. When the GCE was modified with MWCNT-CS (curve b), the electric peak current Ip increased rapidly, indicating that the materials were stably fixed on the bare electrode. MWCNT coupled with the synergistic effect of chitosan caused a composite film with strong conductivity to be formed on the surface of the electrode. When the capsaicinoids Abs was dripped (curve c), the resistance of the electron transfer on the surface

increased. However, owing to the good electrical conductivity and biocompatibility of multiwalled carbon nanotubes, the peak current was still higher than that of the bare electrode. After dripping the OVA solution as a sealing material (curve d), the current value further decreased. After the complete immunosensor was dripped with a certain concentration of capsaicinoids solution (curve e), the capsaicinoids Abs specifically bound to the antigen to form an immune complex, and the current continued to decrease.

Furthermore, DPV measurements were used to evaluate the assembly process of the aptasensor, as shown in Fig. 2B. Fig. 2B shows that the information indicated by the differential voltammetry during the preparation of the electrode was consistent with the information obtained from the cyclic voltammetry curve, which further illustrated the stability of the immunosensor.

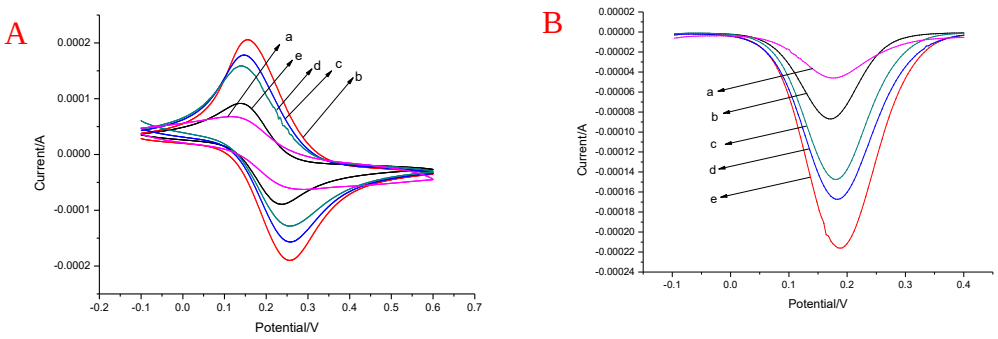

Figure 2. (A) CV characterization of the immunosensor assembly diagram (a) bare GCE (b) MWCNT/GCE (c) Abs/MWCNT/GCE (d) OVA/Abs/MWCNT/GCE (e) capsaicinoids/OVA/Abs/MWCNT/GCE; (B) DPV characterization of the immunosensor assembly diagram (a) bare GCE; (b) capsaicinoids/OVA/Abs/MWCNT/GCE; (c) OVA/Abs/MWCNT/GCE; (d) Abs/MWCNT/GCE; (e) capsaicinoids/OVA/Abs/MWCNT/GCE; (e) MWCNT/GCE;

3.3 Optimization of parameters for immunosensor performance

To establish an efficient and sensitive method for the detection of capsaicinoids in waste oil, most of the experimental conditions were optimized, including Abs concentration, incubation time, and pH value of the reaction system.

The pH value of the reaction of the immunosensor was detected. In strongly acidic or alkaline environments, Abs activity could significantly affect the effectiveness of the immunosensor performance[7]. These results are shown in Fig. 3A. There was not much change in the range of 6.8 to 7.2. The peak current change reached the maximum when the pH reached 7.2. This result indicated that the complexes were produced in high amounts and were stable in nature. However, with increasing pH, the response value of the current decreased. The results might indicate that the complex formed by the Abs and capsaicinoids was prone to decompose under alkaline conditions. As shown in Fig. 3A, when the pH was 7.2, the reduction ΔI of the modified electrode current was the largest. Therefore, pH 7.2 was selected for this experiment.

The Abs and target specific binding on the surface of the modified electrode to produce an immune response when capsaicinoids standard solution was dripped. Therefore, the concentration of Abs was also an important parameter that affected ΔI. The antibody concentration ranged from 5–60 μg/mL. The results showed that when the antibody concentration was small, the current peak value increased with increasing antibody concentration. However, once the antibody concentration rose to a certain value, further increases in antibody concentration greatly affected the current transfer impedance of the nanomaterial-modified electrode. After the electrode was finally combined with capsaicinoids, the current peak value did not change significantly, and the current peak value did not rise but fell. As shown in Fig. 3B, at Abs concentrations up to 10 μg/mL, the response signal obtained by the sensor current was the largest. Therefore, 10 μg/mL was selected as the best Abs concentration in the experiment.

The incubation time of the immunosensor had a great influence on its sensitivity. Fig. 3C shows that starting at 20 min, with the extension of the incubation time, the current peak value continued to decrease, indicating that the capsaicinoids standard solution could reach saturation after incubation with the immunosensor for 20 min. When the complex began to dissociate, the resistance to electrons began to weaken. Therefore, the optimal incubation time was determined to be 20 min.

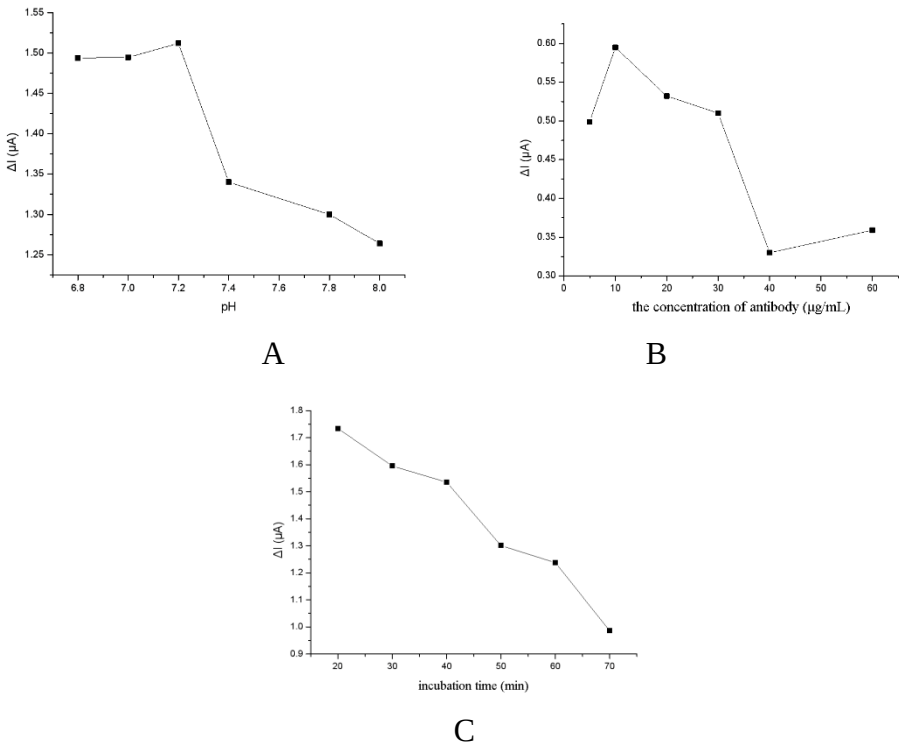

Figure 3. Optimizing the experimental conditions: (A) pH value of the reaction system, (B) concentration of antibody, and (C) incubation time

3.4 Standard curve of capsaicinoids

When capsaicinoids and specifically bound antibodies formed an immune complex, the complex hindered electron transfer on the sensor surface and reduced the response current. DPV was used to

determine the standard curve of the immunosensor for capsaicinoids. Based on the results of the above optimization experiment, we set up a standard curve to detect the concentration of capsaicinoids in various samples. The selected capsaicinoids concentration gradient was 2, 10, 20, 40, 60, 80, and 100 μg/mL. According to the test results, as shown in Fig. 4, the sensor showed a good linear relationship in the range of 2–100 μg/mL, the linear regression equation was I=0.0071C+0.1533 (R^2=0.9919), and its detection limit was 0.25 μg/mL. Compared with other methods for detecting capsaicinoids, this method had relatively high sensitivity. In addition, compared with other previously reported methods shown in Table 1, the method of thin-layer chromatography coupled with paper-based[25] could easily detect capsaicinoids. A narrow linear range made it very impractical. The methods of UPLC-MS/MS[26] and UHPLC-PDA[27] had great stability and high accuracy, however, high experiment cost and complicated operation limited their development on the field of the on-site rapid diagnosis. Large-scale instruments were limited by expensive instruments, complicated operations, and long inspection time, which made it difficult to meet the needs of on-site detections. The electrochemical method[28] had simple operation and fast response time, which could meet the needs of on-site detection. However, electrochemical method had low selectivity and accuracy.The proposed immunosensor in this work exhibited a lower detection limit and a wider linear range, which had widespread application potential for capsaicinoids detection.

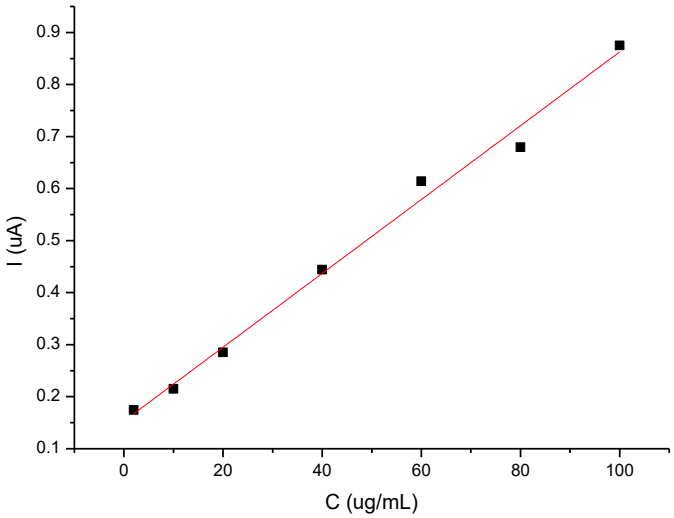

Figure 4. Standard curves of capsaicinoids.

Table 1. Comparison of the constructed electrochemical immunosensorwith other reported methods for capsaicinoids detection

Method	Liner ranges (ng/mL)	Detection limit (ng/mL)	Pros /cons	Reference
Thin-layer chromatography coupled with paper-based	5×10^4-1×10^6	5×10^4	Easily detecting, a narrow linear range.	[25]

UPLC-MS/MS	0.4-2×10^2	0.15	Stability, high experiment cost , complicated operation.	[26]
UHPLC-PDA	1×10^4-2×10^8	16.00	High accuracy, complicated operation, tedious sample pretreatment	[27]
Electrochemical	4.89×10^1-5.0×10^3	15.27	Good stability, low detection limit.	[28]
Electrochemical immunosensor	2×10^3-1×10^5	25.00	Simple operation, low cost, specifically recognize capsaicinoids	This work

3.5 The real samples

To further study the practical value of the newly built immunosensor, the recovery rates of two soybean oils were tested. The results are shown in Table 2. The recovery rates of the edible oils were calculated to be 97.1–103.40%, which proved that the immunosensor could be used for preliminary detection of capsaicinoids in waste oil samples.

Table 2. Immunosensor for actual sample detection

Sample	Detection of capsaicinoids (μg/mL)	Detection of capsaicinoids (μg/mL)	RSD (%, n=5)	Recovery (%)
	0.00	0.03	-	-
1	10.00	10.34	2.90	103.40
	50.00	51.18	3.10	102.40
2	0.00	0.04	-	-
	10.00	9.71	4.50	97.10
	50.00	49.04	3.30	98.10

4. CONCLUSION

In this experiment, MWCNT-CS was used to modify the electrode. MWCNT have unique mechanical and biological properties. These special properties were used to increase the number of antibodies that could be immobilized on bare electrodes and improve the binding area of antibodies and antigens. In this work, MWCNT-CS not only amplified the electric signal of the bare electrode but also stably bound the antibodies. By measuring the current response, the concentration of capsaicinoids in

waste oils was obtained. In order to gain an excellent result, the pH of the buffer solution, incubation time and Abs concentration were optimized. It was concluded that the relationship between current change and the capsaicinoids concentration was linear in the range of 0.2–100 μg/mL. The correlation coefficient was 0.9919, and the LOD was 0.25 μg/mL. The above test results showed that the current immunosensor prepared by this scheme had the characteristics of high sensitivity, simple preparation, fast response, good stability, and a wide linear range. The newly built immunosensor has great potential for the detection of capsaicinoids in waste oils.

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