

High sensitivity detection of capsaicin in red pepper oil based on reduced graphene oxide enhanced by β -cyclodextrin

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Abstract

Red pepper oil is a kind of condiment used in the food factories, and its spicy degree can be reflected by the content of capsaicin. However, the classification method of red pepper oil is too subjective to be applied in food factories. In our work, a sensitivity electrochemical sensor for detecting capsaicin was constructed based on the glassy carbon electrode (GCE) modified with β -cyclodextrin (β -CD) assisted reduced graphene oxide (rGO). The results showed that the introduction of β -CD made rGO more dispersed, increasing the electrochemical active surface area of β -CD/rGO, thus improving the charge transfer rate. Furthermore, polycyclodextrin with selective recognition ability was dispersed on the surface of rGO, providing the possibility of capsaicin enrichment on the surface of the sensor. Based on these reasons, the sensor had a lower detection limit (0.05 $\mu\text{g/mL}$), and the acceptable stability and anti-interference. Most importantly, β -CD/rGO/GCE displayed a satisfactory recovery rate (94.83% ~ 115.75%) in the detection of red pepper oil, and there was no statistical significance difference between this method and the LC-MS method.

1. Introduction

Red pepper oil, also known as chili oil, is made by hot maceration of dried chilies in cooking oil (Rui et al. 2020; Xza et al. 2020). As a flavoring oil, red pepper oil is often used to process various snack foods to give them spicy taste (Ornelas-Paz et al. 2010; Zhang et al. 2021). Depending on the intensity of spiciness, red pepper oil is usually classified as slightly spicy, medium spicy, and extra spicy. However, due to regional and individual differences, consumers have different cognition and acceptance of spicy flavor, leading to this classification method is too subjective. This makes its application in food more based on experience, which is not conducive to the standardization of food production. Therefore, it is necessary to establish a more scientific classification method for grading the spiciness of red pepper oil. In fact, the main source of spiciness in red pepper oil is capsaicin, the content of which is directly related to spiciness of food and is commonly used to evaluate spiciness of food. Therefore, the establishment of determination method for capsaicin content is of great significance to the classification of red pepper oil.

A variety of methods for the analysis of capsaicin have been reported and generally recognized, such as spectrophotometry (Perucka and Oleszek 2000), ELISA (Yang et al. 2016), high-performance liquid chromatography (Yang et al. 2016), liquid chromatography-mass spectrometry (Kozukue et al. 2005), gas chromatography (Cisneros-Pineda et al. 2006; Peña-Alvarez et al. 2008), and TLC-ESI-MS (Santos et al. 2021). Spectrophotometry is simple and inexpensive, but it lacks specificity and sensitivity, and cannot achieve the purpose of trace detection. ELISA has higher specificity, but due to the difficulty in screening specific antibodies, so it cannot be widely used in the detection of capsaicin (Yang et al. 2016).

Chromatography and mass spectrometry methods have the characteristics of high precision and good accuracy, but they need long detection period, frequent operation, expensive equipment and labor costs (Kaale et al. 2002; Peña-Alvarez et al. 2008; Wu et al. 2018).

Compared to usual tests, electrochemical analysis has significant advantages in terms of economy, timeliness, accuracy, miniaturization, and sensitivity (Sheela et al. 2011; Da Silva Antonio et al. 2019; Supchocksoonthorn et al. 2020) Up to now, there have been reports on the detection of capsaicin by electrochemical sensors based on metals and their oxides (Wang et al. 2016), carbon nanomaterials (Salimi et al. 2008; Yardm and entürk 2013), and other semiconductor substances (Yu et al. 2012; Sørstad et al. 2018). Among them, rGO is considered as a good modification material for detecting capsaicin because it not only has excellent electrical conductivity, but also can produce a large number of topological defects and continuous changes of carbon vacancies, thus enhancing the adsorption, embedding and activation properties of target molecules (Zhou et al. 2010; Zhong et al. 2019). However, rGO sheets tend to accumulate and lose the advantage of nanomaterials because of their van der Waals' forces (Wang et al. 2013; Mutyala and Mathiyarasu 2016). β -CD is an oligosaccharides consisting of seven glucose units, which can effectively disperse various nanomaterials to enhance their electrochemical detection performance. At the same time, β -CD has a toroidal structure with a hydrophobic inner cavity and a hydrophilic outer side, enabling them to selectively combine with some organic molecules (Przybyla et al. 2020). This interesting property will undoubtedly enrich the organic molecules on the electrode surface, thus improving the sensitivity of electrochemical detection (Díaz et al. 2018; Gu et al. 2021; Xu et al. 2021). Therefore, we speculate that β -CD can further enhance the detection performance of capsaicin by electrochemical sensors based on rGO.

In our work, a sensitive electrochemical sensor for detecting capsaicin was constructed by introducing β -CD into the polymerization solution of rGO. Firstly, the mixed solution of β -CD and graphene oxide (GO) was scanned by cyclic voltammetry (CV) to deposited β -CD/rGO composite material on the surface of GCE. Secondly, the electrochemical reaction mechanism of capsaicin on the sensor we prepared (β -CD/rGO/GCE) and the optimal deposition parameters of β -CD/rGO composite material were further investigated. Finally, the methodological evaluation and the application potential of β -CD/rGO/GCE for the detection of capsaicin were studied.

2. Materials And Methods

2.1. Reagents and materials

GO dispersion was purchased from Suzhou Tanfeng Graphene Technology Co. Ltd (Suzhou, China). β -CD, monopotassium phosphate, dipotassium phosphate, hydrochloric acid (HCl), sucrose, ascorbic acid, sodium glutamate, potassium chloride, potassium ferrocyanide, and potassium ferricyanide were purchased from Shanghai Sinopharm Chemical Reagents Co. Ltd (Shanghai, China). Capsaicin standard, anhydrous ethanol, and methanol were purchased from Shanghai Aladdin biochemical Technology Co. Ltd (Shanghai, China). The ultrapure water was purified with a water purification system (Sichuan Youpu Ultrapure Science and Technology Co. Ltd, Chengdu, China). Red pepper oil was obtained by Three Squirrels Inc (Wuhu, China). All electrodes were purchased from Shanghai Xianren Instrument Co. Ltd (Shanghai, China). All reagents except capsaicin used were of analytical grade.

2.2. Synthesis of β -CD/rGO/GCE

2.2.1 Preparation of β -CD/GO dispersion

β -CD dispersion was obtained by dissolving 1 mg β -CD into 3.7 mL phosphate buffers (0.1 mol/L, pH 5) and then ultrasonic treatment (40 kHz, 75 W) for 10 min. Afterward, 1.3 mL GO (2 mg/mL) dispersion was added into the mixture, which was performed by ultrasonic treatment for 10 min to obtain β -CD/GO dispersion.

2.2.2 Preparation of β -CD/rGO/GCE

The GCE was polished using 0.05 μm of alumina powder and ultrasonically washed for 10 min in ethanol and ultrapure water, respectively. To obtain β -CD/rGO/GCE, bare GCE was treated in β -CD/GO dispersion by cyclic voltammetry (CV) scanning of 25 segments between $-1.4 \sim 0.4$ V with the scan rate of 0.025 V/s. Next, β -CD/rGO/GCE was further reduced in PBS solution (0.1 mol/L, pH 5) by CV scanning of 10 segments between $-1.4 \sim 0.4$ V with the scan rate of 0.025 V/s. The preparation method of rGO/GCE was the same as that of β -CD/rGO/GCE, except that there was no β -CD in the dispersion. The preparation process of β -CD/rGO/GCE was shown in Scheme 1.

2.2.3 Characterization methods of β -CD/rGO/GCE

The characterization methods referred to previous researches with a slightly modified (Chen et al. 2021). The microscopic images of GCE, rGO/GCE, and β -CD/rGO/GCE were obtained by a SU8010 scanning electron microscope (Hitachi, Tokyo, Japan). The Raman spectra of different samples were analyzed by a Raman spectrometer (Horiba scientific, Kyoto, Japan) using 532 nm laser in the range of $500 \sim 2500 \text{ cm}^{-1}$. The charge transfer rate and electrochemically active surface area of different modified electrodes were studied in $\text{Fe}[(\text{CN})_6]^{3-/4-}$ solution (5 mmol/L) by CV method.

2.3. Application of actual sample detection

β -CD/rGO/GCE was applied to detect the content of capsaicin in red pepper oil. The preparation method of actual sample was as follows: 4 g of sample was placed into a centrifuge tube after homogeneous. Afterwards, 15 mL of methanol solution was added into the tube and ultrasonic treatment (40 kHz, 150 W) at 60°C for 15 min. To collect the supernate, the sample was centrifuged at 8000 g for 5 min. The centrifugal extraction process was repeated twice. Finally, the solution was metered to 50 mL using methanol solution, and then it was filtered using 0.22 μm filter membrane before detection. To obtain the recovery rate of our method, different concentrations (5, 10 and 20 $\mu\text{g/L}$) of capsaicin standard solution were added to the filter liquor. At the same time, the LC-MS method was compared with our method. LC-MS experiments were carried out according to Chinese national standard of GB/T 40348 – 2021 (Chen et al. 2021) by a TRIPLE QUAD 5500 LC-MS (Waters Corporation, Connecticut, USA).

2.4. Electrochemical experiments

All the electrochemical experiments were performed on a LK2010 electrochemical workstation (Tianjin Lanlike Chemical Electronics High-tech Co., Ltd, Tianjin, China) in a three-electrode system. A GCE or modified GCE as the working electrode, a platinum electrode as the counter electrode, and a saturated calomel electrode as the reference electrode. The initial parameters of CV method in capsaicin solution were as follows: the potential range was 0 ~ 1 V, the scan rate was 0.1 V/s, and the equilibrium time was 2 s. The initial parameters of CV in 5 mmol/L $[\text{Fe}(\text{CN})_6]^{3-/4-}$ solution were as follows: the potential range was - 0.2 ~ 0.6 V, and other parameters were consistent with those of in capsaicin solution. The initial parameters of differential pulse voltammetry (DPV) in capsaicin solution were that the potential range was 0.9 ~ 0.2 V, impulse amplitude was 0.05 V, pulse width was 0.2 s, pulse period was 0.5 s, and equilibrium time was 2 s.

2.5. Statistical analysis

The raw data were exported from the electrochemical workstation, then analysed by Microsoft Excel 2016 and SPSS 24.0. Imported processed data into the OriginPro2017 to draw the graphics.

3. Results And Discussion

3.1. Characterizations

3.1.1 Micromorphology Analysis

The morphological analysis of the GCE, rGO/GCE, and β -CD/rGO/GCE is shown in Fig. 1. The surface of bare GCE was smooth, while the other two electrodes showed different morphological characteristics. For rGO/GCE, it can be clearly observed that the nanosheets of rGO were tightly stacked together to form a dense film with a wrinkled texture pattern. It has been reported that these wrinkles were due to capillary effects during drying, which can not only increase the surface roughness and specific surface area of rGO, but also speed up its electron transfer rate (Zhou et al. 2022). Interestingly, β -CD/rGO/GCE presented that many tubular structures were embedded on the surface of the rGO, which may be because β -CD was electrically polymerized to form polycyclodextrins (Díaz et al. 2018). Previous studies have shown that electropolymerization of cyclodextrin can improve the ability of electrochemical sensor to recognize and detect capsaicin (Zhao et al. 2020). Therefore, we speculated that the emergence of this tubular structures provided a possibility for the synergistic effect of β -CD and rGO.

3.1.2 Analysis of Raman spectra

The Raman spectra of rGO and β -CD/rGO are shown in Fig. 1D. Both curves had obvious characteristic peaks at 1346 cm^{-1} (D band) and 1558 cm^{-1} (G band), indicating that the functional group composition of rGO was retained after the addition of β -CD. Moreover, it has been reported that the D band was related to the second-order double resonance scattering process of graphite material defects, the G band represented the E_{2g} vibration mode of the sp^2 carbon atom in the two-dimensional hexagonal lattice, and the intensity ratio of the D and G band could reflect the structure disorder degree of carbon material (Zhao

et al. 2020). Compared with rGO (the ratio was 1.38), the I_D/I_G of β -CD/rGO was markedly decreased (the ratio was 1.31), indicating that rGO was more dispersed on the electrode surface after the addition of β -CD (Ma et al. 2017). Our previous research has proved that the better the dispersion of carbon materials, the stronger the detection performance of capsaicin (Gu et al. 2021).

3.1.3 Electrode transfer capacity of different electrodes

The electrode transfer capacity of different electrodes was tested by CV scanning in 5 mmol/L $[\text{Fe}(\text{CN})_6]^{3-/4-}$ solution. It was observed from Fig. 2A that the peak current of different electrodes were significantly different, and the peak current values in descending order were β -CD/rGO/GCE, rGO/GCE, and GCE. This result showed that the β -CD/rGO/GCE had the optimum electron transfer capacity.

Meanwhile, the effect of scan rate on the redox peak current of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ solution is shown in Fig. 2B. It was found that the redox peak current value increased with the increase of scan rate, and showed a good linear relationship with the square root of scan rate, indicating that the electrochemical reaction process of $\text{Fe}^{2+}/\text{Fe}^{3+}$ was controlled by diffusion (Lyu et al. 2019). In order to further study the effect of β -CD on the electrochemical properties of rGO, the electrochemical active surface areas of different electrodes were calculated. According to Randles Sevcik equation, the electrochemical active surface area of GCE, rGO/GCE, and β -CD/rGO/GCE was 5.93, 13.28, and 20.69 mm^2 respectively, indicating that the addition of β -CD made β -CD/rGO composite have more electrochemical active sites.

3.2. Electrochemical response of capsaicin on different electrodes

The peak current of different electrodes in HCl solution containing 10 $\mu\text{g}/\text{mL}$ capsaicin is shown in Fig. 3. It could be seen that there was a weak response current on the bare GCE of capsaicin, which could be because the charge transfer rate of bare GCE was slow. After modifying the rGO, the value of peak current was significant increased, which could be due to that the electrochemical reduction process of GO can remove the oxygen-containing groups and form the wrinkled film, which significantly increased the electrochemical active surface area of rGO, promoting the electron transfer rate of electrochemical reaction (Kaleem et al. 2021; Li et al. 2021). Surprisingly, the peak current of capsaicin on β -CD/rGO/GCE was higher than that on rGO/GCE. There were two main reasons for the enhancement of electrochemical performance. One was the addition of β -CD made rGO more dispersed, which increased the electrochemical active surface area of β -CD/rGO composite, thus further improving the charge transfer rate. The other might be that polycyclodextrins can selectively combine with capsaicin into their internal cavity to form stable host-guest inclusion complexes, which effectively improved the selectivity and catalytic ability of the sensor to capsaicin.

3.3. Electrochemical reaction mechanism of capsaicin

3.3.1 CV curves analysis of capsaicin

The electrochemical redox reaction of capsaicin on β -CD/rGO/GCE was investigated by CV scanning. The voltammetric signal of capsaicin in 0.02 mol/L HCl solution was characterized by 4 cycles with a scan rate of 0.2 V/s (Fig. 4A). In the first circle of CV scanning, an irreversible redox peak appeared at 680 mV and 370 mV. However, in the second circle of CV scanning, the peak current of oxidation peak at 680 mV decreased obviously, and a new oxidation peak appeared at 440 mV. In fact, the reaction sequence of this stage was that capsaicin was oxidized at 680 mV, the product of oxidation reaction was reduced at 370 mV, and then the reduction product continued to be oxidized at 440 mV. Interestingly, with the continuation of scanning, the current value of redox peaks at 370 and 440 mV gradually increased, and the oxidation peak current of 680 mV continued to decline, indicating that the electrochemical reaction in the subsequent stage was mainly capsaicin derivatives.

3.3.2 Effect of scan rate

The β -CD/rGO/GCE was placed in capsaicin solution to study the relationship between scan rate (ν) and peak current (i_p). As shown in Fig. 4B, with the increase of scan rate, the oxidation peak potential of capsaicin moved positively, and its peak current increased gradually. After linear fitting, the peak current featured a good linear relationship with both square root of scan rate (Fig. 4C) and scan rate (Fig. 4D), indicating that the electrochemical reaction of capsaicin on the surface of β -CD/rGO/GCE was controlled by diffusion and adsorption at the same time (Rezaei and Damiri 2008; Lyu et al. 2019). Simultaneously, the oxidation peak shifted positively and the reduction peak shifted negatively by changing the scan rate, indicating that there were electrons involved in the redox reaction of capsaicin. According to Nernst equation and previous research, the number of electrons is about 2 (Supchocksoonthorn et al. 2020).

3.3.3 Effect of pH

Figure 5A displays the CV curves of capsaicin under different pH (from 1 to 8). The potential of redox peak (E_p) shifted negatively with the increase of pH, and the peak current decreased gradually, reflecting that protons were involved in the redox reaction (Chen et al. 2018). Acidic conditions were more conducive to the detection of capsaicin, owing to the side effects of reducing the concentration of capsaicin under alkaline conditions, including deprotonation and dimerization (Kachoosangi et al. 2008; Yu et al. 2012). The calibration curve between E_p and pH was E_p (V) = -0.0551 pH + 0.5425, $R^2 = 0.9918$ (Fig. 5B). In the equation, -55.1 mV/pH was very close to the theoretical slope (-59.1 mV/pH) for transferring an equal number of protons and electrons (Chen et al. 2018).

3.3.4 Electrochemical reaction pathway of capsaicin

Based on our previous study and the above results, an electrochemical reaction pathway of capsaicin was proposed. Firstly, when scanning to 680 mV, the phenolic hydroxyl group and methoxy group of capsaicin were oxidized and hydrolyzed respectively to form the o-benzoquinone structure (Lyu et al. 2019). Secondly, when scanning to 440 mV, the o-benzoquinone structure obtained two electrons and was reduced to the o-phenol structure. Finally, the electrochemical reaction in the subsequent stage was mainly that the mutual transformation of o-benzoquinone structure and o-phenol structure, and the reaction of capsaicin to o-benzoquinone gradually disappeared (Søpstad et al. 2018; Lyu et al. 2019).

3.4. Optimization of electrode modification conditions

According to the results of preliminary experiments, it was found that the performance of β -CD/rGO/GCE was mainly affected by the deposition parameters of β -CD/rGO, including the pH of the deposition solution, potential range, and sweep segment during the modification process. Thus, the optimal parameters of the above influencing factors were explored by changing the experimental conditions.

3.4.1 Influence of pH value of deposition solution

As shown in Fig. 6A, the response current of capsaicin obtained in different pH deposition solutions was significantly different (Fig. 6A). The response current increased rapidly when the pH of deposition solution increased from 3 to 5, but decreased significantly when the pH of deposition solution continued to rise. So the β -CD/rGO/GCE had the highest peak current of capsaicin when the pH value of the deposition solution was 5. It has been reported that the pH values of both too low and too high were detrimental to the deposition of GO. The too low pH could cause GO to precipitate or gelation, promoting hydrogen evolution during electrodeposition process, meanwhile too high pH could lead to rGO instability (Zhou et al. 2022). Therefore, PBS (pH = 5.0) was selected as the supporting deposition.

3.4.2 Influence of potential range

As shown in Fig. 6B, when the upper limit of potential range was 0.4 V and the lower limit of potential range increased from -1.6 V to -1.2 V, the response current of capsaicin increased first and then decreased, and reached the maximum at -1.4 V. This may be due to the fact that the lower limit was too high to carry out the electrochemical reduction reaction of GO normally, while the lower limit was too low, which could produce by-products affecting the electrochemical reaction (Wang et al. 2013; Zhou et al. 2022). However, when the lower limit of potential range was -1.4 V and the upper limit of the potential increased from 0.4 V to 0.8 V, the peak current of capsaicin fluctuated slightly, indicating that the effect of the upper limit on peak current of capsaicin was slight (Wang et al. 2013).

3.4.3 Influence of sweep segments

Additionally, in Fig. 6C, it was observed that the peak current of capsaicin increased with the raise of sweep segments, and reached the maximum in 25th segment, which may be because β -CD/rGO composite material deposited on the electrode increased with the increase of the number of sweep segments, thus increasing the active site of capsaicin (Zhou et al. 2010). However, the response current of capsaicin decreased as the number of sweep segments increased to 30, which may be because the increasing number of sweep segments led to excessive accumulation of β -CD/rGO, or because the electrochemical reaction time is too long, causing the occurrence of side reactions (Wang et al. 2013). Therefore, 25 segments were used as the optimal sweep segments.

3.5. Methodological evaluation

3.5.1 Limit of detection

The DPV respond current of capsaicin with different concentrations on the surface of β -CD/rGO/GCE was shown in Fig. 7A. In the range of 0.1 ~ 40 $\mu\text{g/mL}$, the peak current value of capsaicin increased with the increase of concentration, and showed a good linear relationship. After linear fitting, the equation was $I_p (\mu\text{A}) = 2.953 C (\mu\text{g/mL}) + 1.499$, $R^2 = 0.995$, where I_p represents peak current value of capsaicin, and C represents the concentration of capsaicin. Based on the method supplied by International Union of Pure and Applied Chemistry (IUPAC) (Xu 2021), the limit of detection (LOD) of β -CD/rGO/GCE for capsaicin was calculated to be 0.05 $\mu\text{g/mL}$.

3.5.2 Reproducibility and stability

In order to test the reproducibility of β -CD/rGO/GCE, capsaicin (30 $\mu\text{g/mL}$) was detected with the same electrode repeatedly modified for 5 times. As a result, the response current values were 85.13, 83.89, 84.50, 86.81, and 84.97 μA respectively, and the relative standard deviations (RSD) was 1.28%, indicating that β -CD/rGO/GCE had good reproducibility. To evaluate the long-term stability of β -CD/rGO/GCE, we stored them at room temperature for one week and detected 30 $\mu\text{g/mL}$ capsaicin solution. The results showed that the response current of capsaicin remained at 95% of its initial value, reflecting the good stability of the modified electrodes.

3.5.3 Anti-interference analysis

The common additives and interfering ions in foods were added into capsaicin solution in order to test the anti-interference performance of β -CD/rGO/GCE. Ascorbic acid is common endogenous interference in plant samples. Besides, sucrose and sodium glutamate are usually added to enhance the taste of food. Sucrose (0.2 mg/mL), sodium glutamate (0.2 mg/mL), ascorbic acid (0.2 mg/mL), and inorganic salt ions (0.1 mg/mL of Na^+ , Cl^- , K^+ , Ca^{2+} , SO_4^{2-} , Mg^{2+}) were added into capsaicin solution, respectively. As shown in the Fig. 7B, the peak current changes of capsaicin were not obviously under interfering substances, indicating that these interfering substances had little influence on the detection of capsaicin ($P < 0.05$).

3.6. Real sample analysis

In order to verify the accuracy and practicability of β -CD/rGO/GCE, the red pepper oil was used as the real sample (Table 1). The content of capsaicin in red pepper oil was 105.714 mg/kg by the method proposed in our paper, which was close to the LC-MS test result of 107.615 mg/kg. The recovery rates of capsaicin in red pepper oil was 94.83 ~ 115.75%, and the relative standard deviation (RSD) was less than 10.87%. Meanwhile, there was no significant difference between the results of our method and those of LC-MS through the paired t-test ($P < 0.05$). These results indicated that the method proposed in our paper had strong application potential in red oil detection.

Table 1
Standard addition recovery experiments of different capsaicin content.

Samples	Added ($\mu\text{g/mL}$)	This method			LC-MS		
		Detection ($\mu\text{g/mL}$)	Recovery rates (%)	RSD (%)	Detection ($\mu\text{g/mL}$)	Recovery rates (%)	RSD (%)
Red pepper oil	5.00	5.587	94.83	9.06	5.484	92.45	5.84
	10.00	12.420	115.75	10.87	10.641	97.80	6.51
	20.00	20.504	98.30	9.41	21.015	100.77	5.08

4. Conclusions

In this work, an electrochemical sensor for the rapid and accurate detection of capsaicin in red pepper oil was constructed based on rGO enhanced by β -CD. The excellent performance of the sensor mainly is because that the introduction of β -CD increased the electrochemical active surface area of β -CD/rGO composite, and polycyclodextrin on the surface of rGO nanosheets provided the possibility of capsaicin enrichment on the surface of the sensor. The quantitative detection range and the detection limit of our sensor for capsaicin were 0.1 ~ 40 $\mu\text{g/mL}$ and 0.05 $\mu\text{g/mL}$, respectively. Meanwhile, the sensor also had good anti-interference and repeatability. When applied to red pepper oil samples, the sensor showed an acceptable practical application potential. The recovery rates of capsaicin in red pepper oil samples was 84.86 ~ 115.23%, and the RSD was less than 10.74%. In a word, Our work is of great significance for the application of red pepper oil in food industry.

Declarations

Compliance with Ethical Standards

Conflict of interest Ning Yun declares that she has no conflict of interest. Chaoqun Lu declares that he has no conflict of interest. Tian Sun declares that she has no conflict of interest. Baocai Xu declares that he has no conflict of interest. Yunshen Song declares that he has no conflict of interest. Zibing Zong declares that he has no conflict of interest. Kangwen Chen declares that he has no conflict of interest. Ganhui Huang declares that he has no conflict of interest. Xingguang Chen declares that he has no conflict of interest. Qianhui Gu declares that he has no conflict of interest.

Ethical Approval This article does not contain any studies with human participants performed by any of the authors.

Informed Consent Not applicable.

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Data availability All data generated or analysed during this study are included in this published article and its supplementary information files.

Author Contributions Statement

Ning Yun: Conceptualization, Methodology, Formal analysis, Investigation, Writing-original draft. **Chaoqun Lu:** Conceptualization, Investigation, Writing-review and editing. **Tian Sun and Baocai Xu:** Formal analysis, Methodology, Writing-review and editing. **Yushen Song, Zibing Zong and Kangwen Chen:** Supervision and Writing-review and editing. **Ganhui Huang, Xingguang Chen and Qianhui Gu:** Project administration, Funding acquisition.

References

1. Chen XG, Chu BB, Gu QH, Liu H, Wu DH (2021) Facile fabrication of protonated g-C₃N₄/oxygen-doped g-C₃N₄ homojunction with enhanced visible photocatalytic degradation performance of deoxynivalenol. *Journal of Environmental Chemical Engineering* 9:106380
2. Cisneros-Pineda O, Torres-Tapia LW, Gutiérrez-Pacheco LC, Contreras-Martín F, González-Estrada T, Peraza-Sánchez SR (2006) Capsaicinoids quantification in chili peppers cultivated in the state of Yucatan, Mexico. *Food Chemistry* 104:1755-1760
3. Da Silva Antonio, A, L S M Wiedemann, V F da Veiga Junior (2019) Food Pungency: the Evolution of Methods for Capsaicinoid Analysis. *Food Analytical Methods* 12:1327-1345
4. Díaz D, Rodríguez L, Rojas AM, Mendoza V, González A (2018) Study of electrochemical determination of capsaicin and dihydrocapsaicin at carbon paste electrodes modified by β -cyclodextrin. *Journal of Electroanalytical Chemistry* 814:174-183
5. Gu Q, Lu C, Chen K, Chen X, Ma P, Wang Z, Xu B (2021) Electrochemical Determination of Capsaicinoids Content in Soy Sauce and Pot-Roast Meat Products Based on Glassy Carbon Electrode Modified with Beta-Cyclodextrin/Carboxylated Multi-Wall Carbon Nanotubes. *Foods* 10:1-13
6. Kaale E, Schepdael AV, Roets E, Hoogmartens J (2002) Determination of capsaicinoids in topical cream by liquid-liquid extraction and liquid chromatography. *Journal of Pharmaceutical & Biomedical Analysis* 30:1331-1337
7. Kachoosangi RT, Wildgoose GG, Compton RG (2008) Carbon nanotube-based electrochemical sensors for quantifying the 'heat' of chilli peppers: the adsorptive stripping voltammetric determination of capsaicin. *Analyst* 133:888-895
8. Kaleem S, Mehmood S, Chaudhry M, Ali A, Bhopal MF, Bhatti AS (2021) Ultrasensitive electrochemical detection of digoxin using graphite oxide and Au-NPs composites. *Sensors and Actuators A: Physical* 318:112489

9. Kozukue N, Han JS, Kozukue E, Lee SJ, Kim JA, Lee KR, Levin CE, Friedman M (2005) Analysis of eight capsaicinoids in peppers and pepper-containing foods by high-performance liquid chromatography and liquid chromatography-mass spectrometry. *Journal of Agricultural & Food Chemistry* 53:9172-9181
10. Li L, Yi L, Tan CH, Zhang TT, Xin XL, Li WH, Li JS, Lu R (2021) Study of the interaction mechanism between GO/rGO and trypsin. *Journal of Hazardous Materials Advances* 3:100011
11. Lyu W, Zhang X, Zhang Z, Chen X, Zhou Y, Chen H, Wang H, Ding M (2019) A simple and sensitive electrochemical method for the determination of capsaicinoids in chilli peppers. *Sensors and Actuators B: Chemical* 288:65-70
12. Ma M, Zhe T, Song W, Guo P, Wang J, Wang J (2017) A comparative study on the glucose sensors modified by two different β -cyclodextrin functionalized reduced graphene oxide based Au nanocomposites synthesized through developed post immobilization and in situ growth technologies. *Sensors and Actuators B: Chemical* 253:818-829
13. Mutyala S, Mathiyarasu J (2016) A reagentless non-enzymatic hydrogen peroxide sensor presented using electrochemically reduced graphene oxide modified glassy carbon electrode. *Materials Science & Engineering C* 69:398-406
14. Ornelas-Paz JD, Martinez-Burrola M, Ruiz-Cruz S, Santana-Rodriguez V, Ibarra-Junquera V, Olivas GI, Perez-Martinez JD (2010) Effect of cooking on the capsaicinoids and phenolics contents of Mexican peppers. *Food Chemistry* 119:1619-1625
15. Peña-Alvarez A, Ramírez-Maya E, Alvarado-Suárez L (2008) Analysis of capsaicin and dihydrocapsaicin in peppers and pepper sauces by solid phase microextraction–gas chromatography–mass spectrometry. *Journal of Chromatography A* 1216:2843-2847
16. Perucka I, Oleszek W (2000) Extraction and determination of capsaicinoids in fruit of hot pepper *Capsicum annuum* L. by spectrophotometry and high-performance liquid chromatography. *Food Chemistry* 71: 287-291
17. Przybyla MA, GokhanYilmaz, Remzibecer C (2020) Natural cyclodextrins and their derivatives for polymer synthesis. *Polymer Chemistry* 11:7582-7602
18. Rezaei B, Damiri S (2008) Voltammetric behavior of multi-walled carbon nanotubes modified electrode-hexacyanoferrate(II) electrocatalyst system as a sensor for determination of captopril. *Sensors & Actuators B Chemical* 134:324-331
19. Rui Z, Gca B, Bing Y, Yun WE, Mda D, Jkac D (2020) Insights into the stability of carotenoids and capsaicinoids in water-based or oil-based chili systems at different processing treatments. *Food Chemistry* 342:128308
20. Salimi A, Miranzadeh L, Hallaj R (2008) Amperometric and voltammetric detection of hydrazine using glassy carbon electrodes modified with carbon nanotubes and catechol derivatives. *Talanta* 75:147-156
21. Santos FF, Nascimento LD, Cavalcante SF, Rezende CM, Veiga-Junior VF (2021) TLC-ESI-MS as a QuEChERS approach to detection of capsaicin present in different matrices. *Natural Product*

22. Sheela T, Basavanna S, Viswanatha R, Kalachar H, Naik YA (2011) Barium Hydrogen Phosphate Modified Carbon Paste Electrode for the Simultaneous Determination of Cadmium and Lead by Differential Pulse Anodic Stripping Voltammetry. *Electroanalysis* 23:1150-1157
23. Sørpstad S, Imenes K, Johannessen EA (2018) Hybrid electrochemical sensor platform for capsaicin determination using coarsely stepped cyclic squarewave voltammetry. *Biosensors and Bioelectronics* 130:371-381
24. Supchocksoonthorn P, Thongsai N, Wei W, Gopalan P, Paoprasert P (2020) Highly sensitive and stable sensor for the detection of capsaicin using electrocatalytic carbon dots grafted onto indium tin oxide. *Sensors and Actuators B Chemical* 329:129160
25. Wang Y, Huang B, Dai W, Ye J, Xu B (2016) Sensitive determination of capsaicin on Ag/Ag₂O nanoparticles/reduced graphene oxide modified screen-printed electrode. *Journal of Electroanalytical Chemistry* 776:93-100
26. Wang YS, Yang SY, Li SM, Tien HW, Hsiao ST, Liao WH, Liu CH, Chang KH, Ma CC, Hu CC (2013) Three-dimensionally porous graphene-carbon nanotube composite-supported PtRu catalysts with an ultrahigh electrocatalytic activity for methanol oxidation. *Electrochimica Acta* 87:261-269
27. Wu T, Yuan X, Wu X, Tang Y, Lin H, Che Z, Zhou W, Li W (2018) Rapid Determination of Capsaicin and Dihydrocapsaicin in Fermented Pepper Paste by Direct Analysis in Real Time Mass Spectrometry. *Food Analytical Methods* 12:32-40
28. Chen XG, Chu BB, Xi HT, Xu JJ, Lai LZ (2018) Determination of chlorine ions in raw milk by pulsed amperometric detection in a flow injection system. *Journal of Dairy Science* 101:9647-9658
29. Xza B, Zya B, Xia B, Xma B (2020) Catalytic co-pyrolysis of microwave pretreated chili straw and polypropylene to produce hydrocarbons-rich bio-oil. *Bioresource Technology* 319:124191
30. Yang Q, Zhu J, Ma F, Li P, Zhang L, Zhang W, Ding X, Zhang Q (2016) Quantitative determination of major capsaicinoids in serum by ELISA and time-resolved fluorescent immunoassay based on monoclonal antibodies. *Biosensors & Bioelectronics* 81:229-235
31. Yardm Y, Entürk Z (2013) Electrochemical evaluation and adsorptive stripping voltammetric determination of capsaicin or dihydrocapsaicin on a disposable pencil graphite electrode. *Talanta* 112:11-19
32. Yu Y, Mo L, Wang T, Fan Y, Jie L, Chen Z, Manoj KS, Fang F, Li C, Liang J (2012) Highly sensitive determination of capsaicin using a carbon paste electrode modified with amino-functionalized mesoporous silica. *Colloids & Surfaces B Biointerfaces* 95:90-95
33. Zhang D, Sun X, Battino M, Wei X, Zou X (2021) A comparative overview on chili pepper (*capsicum* genus) and sichuan pepper (*zanthoxylum* genus): From pungent spices to pharma-foods. *Trends in Food Science & Technology* 117:148-162
34. Zhao Y, Zheng X, Wang Q, Zhe T, Bai Y, Bu T, Zhang M, Wang L (2020) Electrochemical behavior of reduced graphene oxide/cyclodextrins sensors for ultrasensitive detection of imidacloprid in brown rice. *Food Chem* 333:127495

35. Zhong F, Liu Z, Han Y, Guo Y (2019) Electrochemical Sensor for Sensitive Determination of Capsaicin Using Pd Decorated Reduced Graphene Oxide. *Electroanalysis* 31:1-8
36. Zhou A, Bai J, Hong W, Bai H (2022) Electrochemically reduced graphene oxide: Preparation, composites, and applications. *Carbon* 191:301-332
37. Zhou M, Wang YL, Zhai YM, Zhai JF, Ren W, Wang FA, Dong SJ (2010) Controlled Synthesis of Large-Area and Patterned Electrochemically Reduced Graphene Oxide Films. *Journal of Molecular Structure* 15:6116-6120

Scheme

Scheme 1 is available in Supplementary Files section.

Figures

Figure 1

SEM images of GCE (A), rGO/GCE (B), β -CD/rGO/GCE (C); Raman of different materials (D).

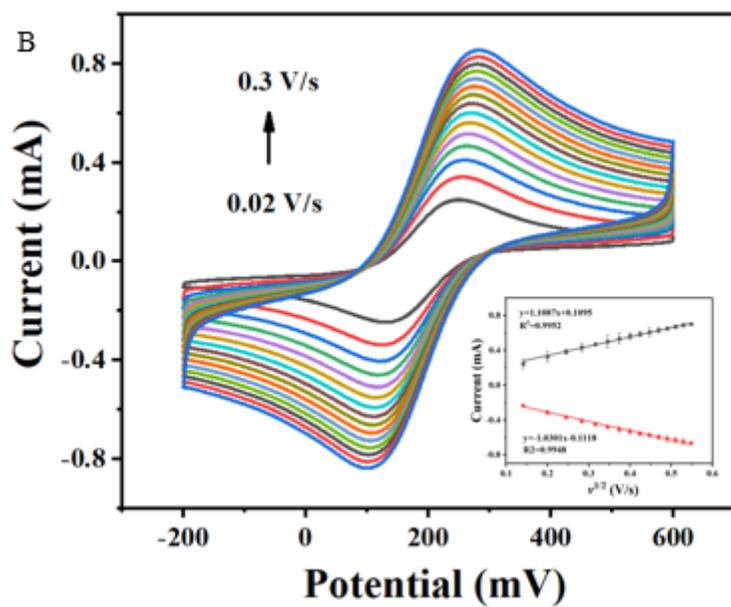
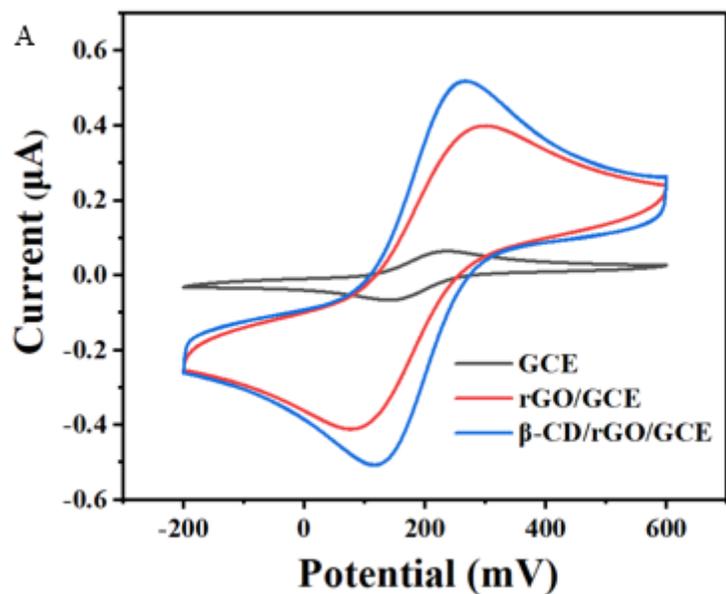


Figure 2

CV curves of 5 mmol/L $[\text{Fe}(\text{CN})_6]^{3-/4-}$ on GCE, rGO/GCE, β -CD/rGO/GCE (A); CV curves of β -CD/rGO/GCE at different scan rates (B); The illustration is the correlation between the peak current and square root of scan rate.

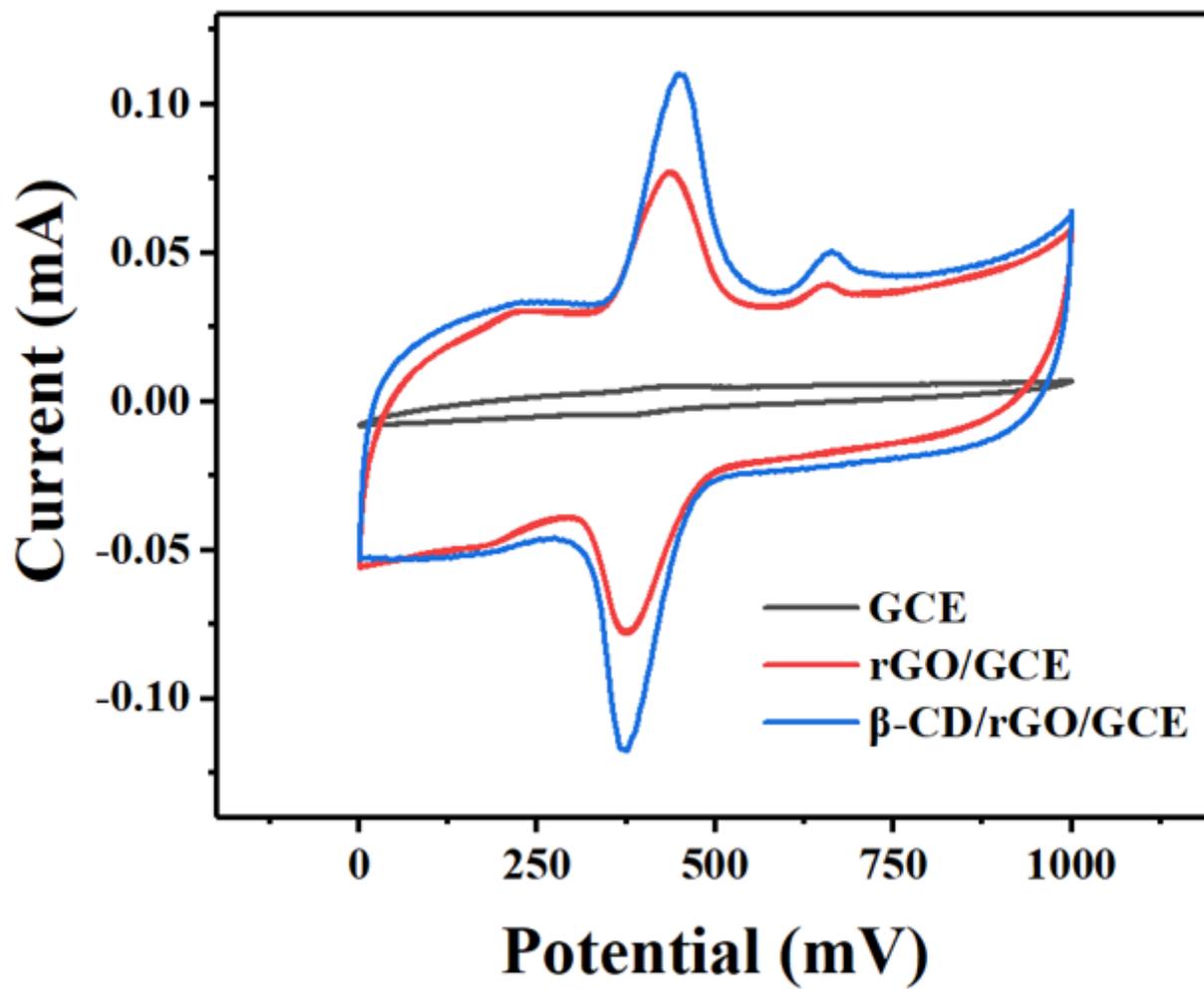


Figure 3

The CV curves of 10 µg/mL capsaicin in 0.02 mol/L HCl solution on GCE, rGO/GCE, β-CD/rGO/GCE.

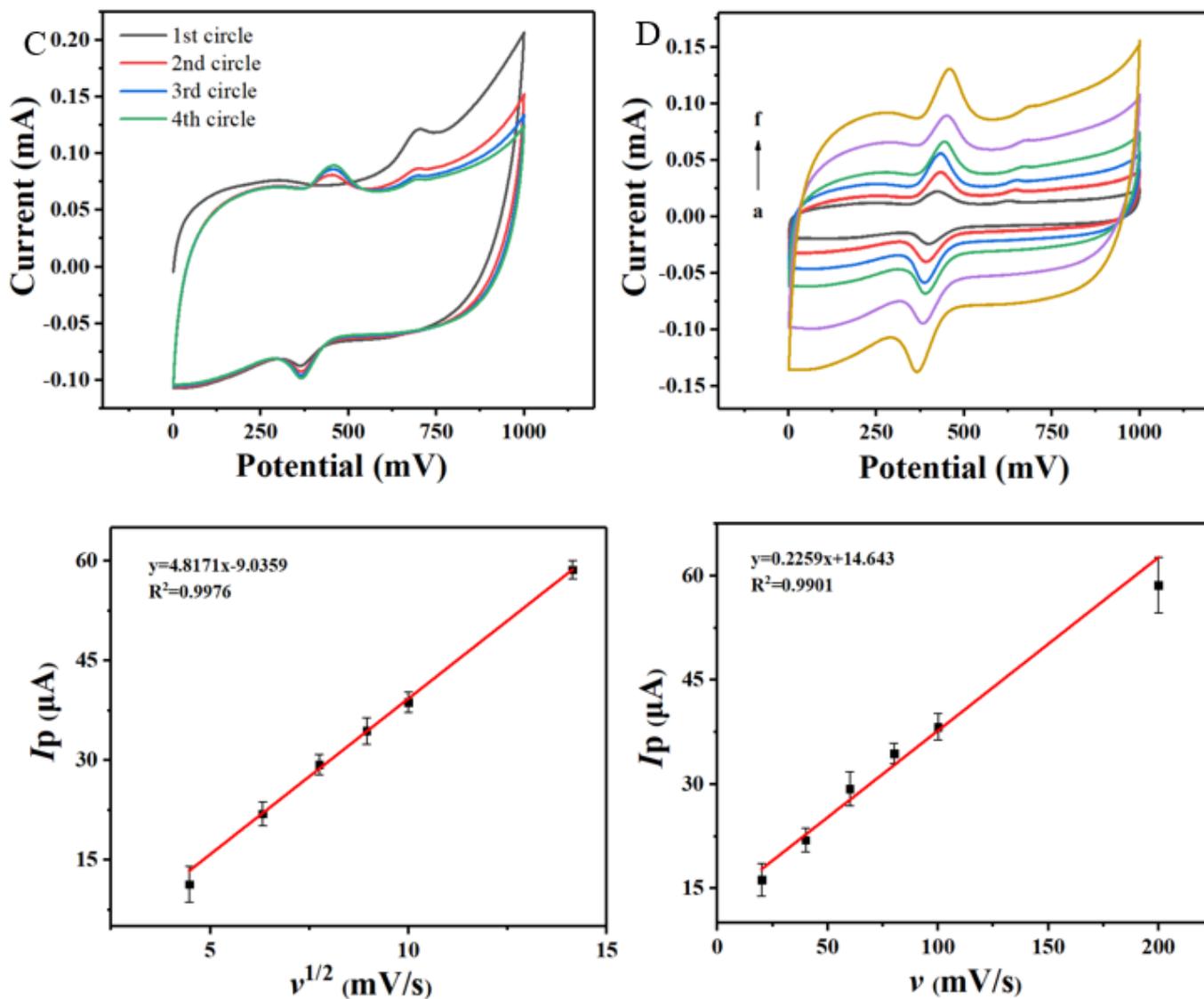


Figure 4

(A) the first circle to fourth circle of CV curves in 6 capsaicin solution on β -CD/rGO/GCE; (B) CV response of β -CD/rGO/GCE in 6 $\mu\text{g/mL}$ capsaicin containing 0.02 mol/L HCl at different scan rates from 20 to 200 mV/s (curve a-f); the linear relation of redox peak current (I_p) vs $v^{1/2}$ (C), I_p vs. v (D).

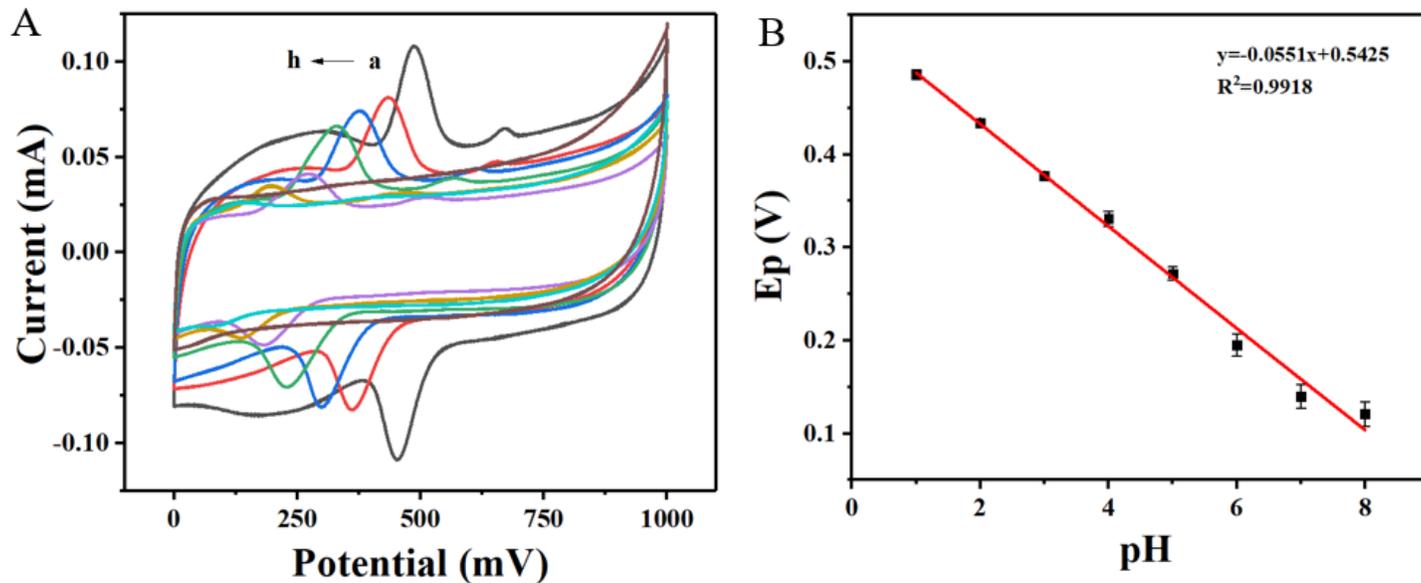


Figure 5

CV response of β -CD/rGO/GCE in 6 $\mu\text{g/mL}$ capsaicin containing different pH of PBS from 1 to 8 (curve a-h) (A) and the linear fitting of pH to oxidation peak potential (E_p) (B).

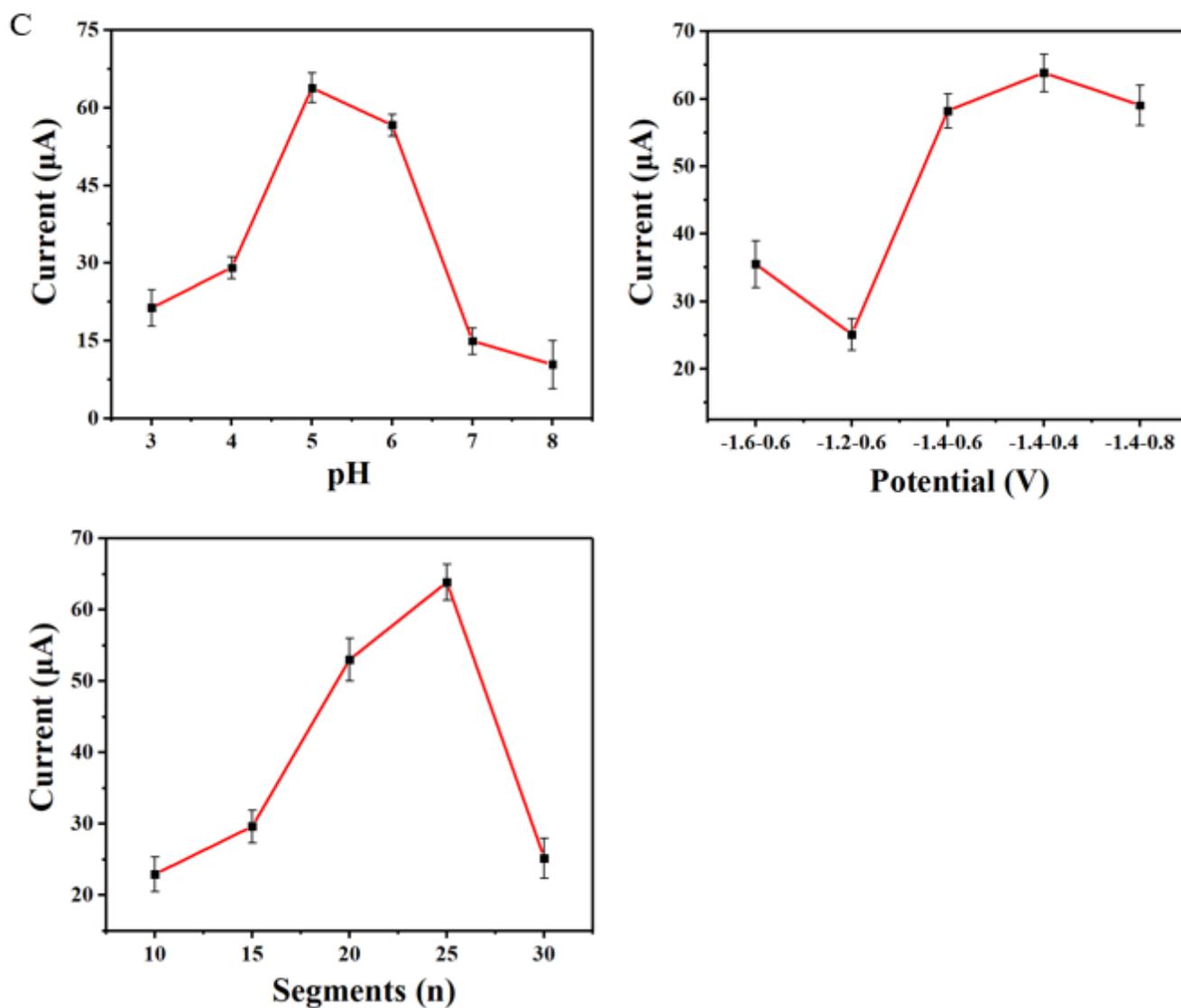


Figure 6

Optimization of deposition parameters of β -CD/rGO/GCE, including the pH (A) of the deposition solution, potential range (B), and sweep segments (C).

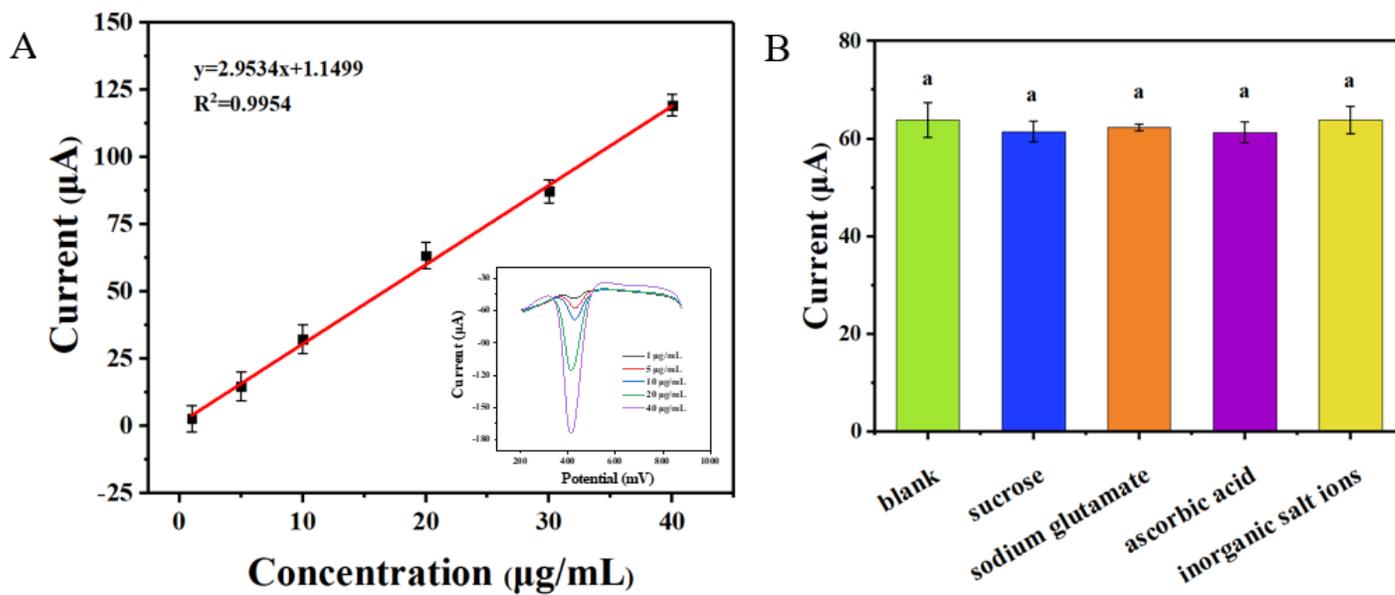


Figure 7

The standard curve of capsaicin in 0.02 mol/L HCl solution on β -CD/rGO/GCE (A); the anti-interference (B) of the method proposed in this work.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Scheme1.png](#)