

Short Communication

Electrochemical sensor based Chitine modified GO for determination of sulphite in food samples

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Received: 3 June 2022 / Accepted: 18 July 2022 / Published: 7 August 2022

In this study, chitin, a polysaccharide found in wild morel mushrooms, was isolated and used to modify the electrode surface of a nanocomposite of chitin and graphene oxide (chitin/GO), an electrochemical sensor for the detection of sulfite in food samples. A Chitin/GO nanocomposite was successfully synthesized on the surface of a glassy carbon electrode (GCE) employing structural characterizations performed using XRD and SEM. Due to the synergistic electrocatalytic effect of chitin and GO, which improved the charge transfer process in the oxidation of sulfite, electrochemical characterizations using the DPV technique demonstrated that chitin/GO/GCE was a selective sulfite sensor and had comparable or even better performance than that of other reported sulfite sensors. The linear range was established as 1 to 960 μM , the detection limit as 0.021 μM , and the sensitivity as 0.02751 $\mu\text{A}/\mu\text{M}$. The practical applicability of the proposed sulfite sensor was assessed in order to determine the amount of sulfite in actual samples of vinegar and pickle water. The findings revealed that the recovery (96.00% to 98.33%) and RSD (3.17% to 4.65%) values were acceptable. The findings show that the created sulfite sensor has excellent precision, acceptable validity and excellent potential for use in actual food sample analysis.

Keywords: Polysaccharides; Chitin; Graphene Oxide; Nanocomposite; Sulfite; Food Samples; Electrochemical Sensor

1. INTRODUCTION

Sulfites are chemicals that are present in some foods naturally [1, 2]. They are used as an additive to preserve food color, lengthen shelf life, and stop the development of bacteria or fungi. Like cellophane, sulfites are also utilized in food packaging [3-5]. Sulfites are also used in raw, dry, frozen, and canned fruits and vegetables to stop enzymatic and nonenzymatic browning [6-8]. For the most

part, sulfites that are added to baked goods, sauces, snack foods, and other products are harmless [9-11]. However, sensitive individuals have been known to experience a variety of negative clinical symptoms from dermatitis, urticaria, flushing, hypotension, abdominal pain and diarrhea to potentially fatal anaphylactic and asthmatic reactions [12-14]. According to some researchers [15], sulfites and other additives may contribute to the development of colorectal cancer.

Accordingly, determining the sulfite level in food samples is very important. Distillation [16], ion exclusion chromatography [17, 18] and photometry [19, 20] are the traditional methods for determining the sulfite level in foods. These methods proved to be more sensitive and selective. Many studies have been carried out for improvement of sulfite sensing using liquid chromatography [21], mass spectrometry [22], flow injection [23], fluorometry [24], chemiluminescence [25] and electrochemical methods [26-30]. The majority of these methods, meanwhile, are either time-consuming or call for expensive equipment [31-33]. Among these other techniques, electrochemical approaches have been shown to have acceptable accuracy and selectivity for sulfite analysis in food samples [34]. However, additional studies are needed to improve the sensing capabilities of sulfite electrochemical sensors [35, 36].

The most prevalent type of carb in the diet is a polysaccharide. They are long-chain polymeric carbohydrates made up of glycosidic connections that connect monosaccharide units. Starch, cellulose, pectin, and other compounds like these are present in plants [37-40]. Plant polysaccharides are widely distributed, and as a result, the molecular weight and content of polysaccharides from various species vary. Both chitin and cellulose are structural polysaccharides made up of thousands of glucose monomers arranged in long threads. The side chains that are joined to the monosaccharide carbon rings are the only distinction between the two polysaccharides. Following cellulose in terms of the abundance of biodegradable polymers created in nature, chitin is a polysaccharide. It is a polysaccharide that has been acetylated and contains N-acetyl-d-glucosamine groups [41, 42]. It is a biopolymer with a distinctive composition that is present in the cell walls of fungi and the exoskeletons of crustaceans [43-45]. Because they may carry positive charges and are simple to bind with materials carrying negative charges, chitin nanostructures can be exploited as crucial supporting platforms for the construction of electrochemical sensors [46, 47]. Therefore, chitin, a polysaccharide, was isolated from wild morels for this investigation, and it was then used to modify the electrode surface using a nanocomposite of chitin/GO. Sulfite levels in food samples were determined using the produced nanocomposite.

2. EXPERIMENT

2.1. Extraction of chitin from wild morels

The method described by Yang et al. [48] was used to extract chitin from wild morels in the manner described below. First, wild morels were collected from China's northern Sichuan province's Aha region. The morels were then dried at room temperature and ground into a fine powder using a milling machine. 2000 mL of deionized water were used to extract 200g of morel powder over the course of five hours at 80°C. The combination was then allowed to rest for 12 hours at 4 °C. The mixture was then ultrasonically dispersed for 60 minutes after that. The mixture was then centrifuged

for 15 minutes at 1000 rpm. Separate samples of the resulting supernatant were taken and enriched using vacuum-rotary evaporation at 45°C, precipitation with ethanol (99 percent, Merck, Germany), in a volume ratio of 1:4, at 4 °C for 10 hours, and centrifugation at 1500 rpm for 12 minutes. To obtain the crude morel chitin, the precipitate was then dried for 4 hours in a vacuum oven at 80°C. The Sevag method was then used to remove the protein using the procedure described below [49]: the crude morel chitin was placed in a column (2.5 30 cm) of fast flow resin (Sephacrose, GE Healthcare, Chalfont St Giles, UK) and gradually mixed with 100 ml of deionized water and 5 ml of 0.2 M NaOH (99%), which was performed via stage gradient elution separately. In order to obtain pure chitin, the product's water-eluted fraction was finally collected, concentrated, dialyzed against water, and lyophilized.

2.2. Preparation chitin/GO/GCE

The Chitin/GO composite was prepared by ultrasonically dispersing 30 mg of GO (99%, Globalchemical Factory Co., Ltd., China) in 50 mL of deionized water for 90 minutes [50]. The resulting GO suspension was then ultrasonically mixed with 3 mg of chitin. The Chitin/GO composite was created by relaxing the obtained black suspension for 12 hours at room temperature following 30 minutes of ultrasonication. About 100 µL of the chitin/GO composite was drop cast onto the surface of the cleaned GCE in order to modify it, and it was allowed to dry at room temperature. 100 µL of the chitin was drop cast on the surface of the GCE to prepare it, and 100 L of dispersed GO was drop cast on the surface of the GCE to modify it with GO.

2.3. Characterization

The electrochemical workstation potentiostat galvanostat (TOB-CS-300, Xiamen Tob New Energy Technology Co., Ltd., China) with a three-electrode system of a reference electrode (Ag/AgCl), counter electrode (Pt wire), and working electrode was used for electrochemical characterizations using the differentiable pulse voltammetry (DPV) technique (unmodified or modified GCE). The 0.1M phosphate buffer solution (PBS) electrolyte (pH 7.3) used for all electrochemical measurements contained an equal volume of 0.1M NaH₂PO₄ (99%, Sigma-Aldrich) and 0.1M Na₂HPO₄ in a combination (99%, Merck, Germany). A D8 Discover X-ray diffractometer was used to conduct X-ray diffraction (XRD) investigations (Bruker, Karlsruhe, Germany). Modified electrode nanostructures underwent morphological evaluation using scanning electron microscopy (SEM; JEOL JSM-5200, Japan).

2.4. Preparation actual food samples

Vinegar and pickle water samples from Zhoushan, China's local markets, were diluted with 5 mL of buffer (pH 7.3) and utilized for sulfite detection by DPV using the standard addition method.

3. RESULTS AND DISCUSSION

3.1. Structural characterizations

Figure 1 shows the results of an XRD analysis of the crystal structure of chitin, GO, and chitin/GO composite powders. Chitin's XRD pattern has three diffraction peaks at 7.95° , 19.86° , and 26.87° , which are correlated to reflections of the intrinsic crystal forms (020), (110) and (120) of -chitin, respectively [51-53]. According to the XRD pattern of GO, the (001) and (100) crystalline planes of GO are ascribed to diffraction peaks at 9.82° and 41.92° , respectively [54-56]. The effective synthesis of a well-crystalline chitin/GO composite on GCE is demonstrated by the XRD pattern of the chitin/GO composite, which shows diffraction peaks of the (001) and (100) crystalline plane of GO and (020), (110) and (120) reflection of chitin [57].

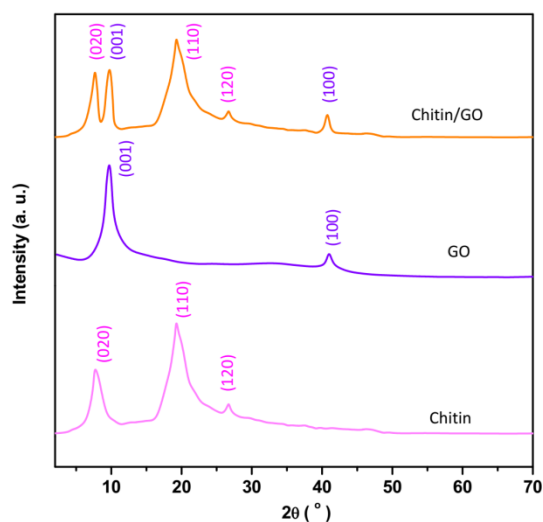


Figure 1. XRD patterns of powders of chitin, GO and chitin/GO composite.

Figure 2 displays SEM images of chitin, GO, and chitin/GO composite modified GCE. The morphology of the GO sample's SEM picture is flaky, suggesting its multilayer architecture. Individually thin, wavy, folded sheets make up GO flakes. Chitin nanofibers can be seen in the cross-section of GO nanosheets in the SEM image of the chitin/GO composite modified GCE [58]. This could lead to an improvement in the mechanical strength of the chitin/GO composite sponges, which also increases porosity and roughness to improve analyte absorption and electrolyte diffusion ability throughout the sponges [59-62].

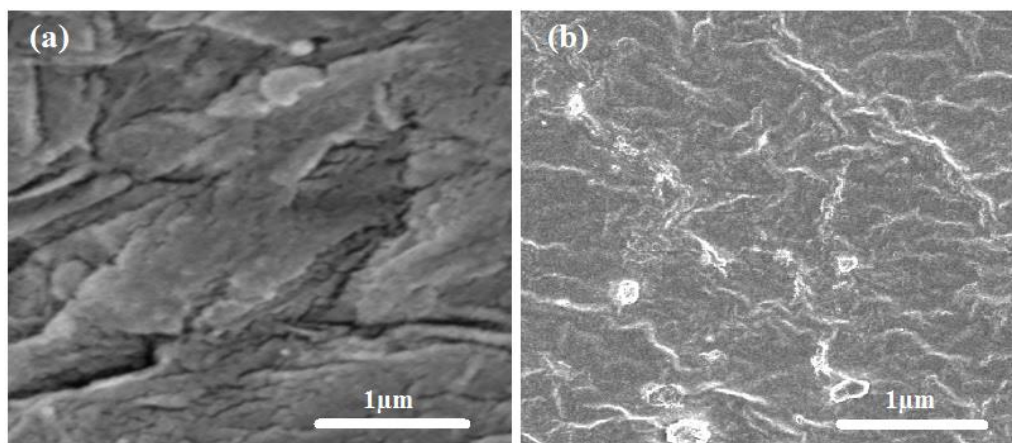


Figure 2. SEM images of GO and chitin/GO composite modified GCE.

3.2. Electrochemical studies

Figure 3 shows the DPV responses of GCE, chitin/GCE, GO/GCE, and chitin/GO/GCE in 0.1 M PBS (pH 7.3) without and with 50 M sulfite solution in the potential window from 0.1 V to 0.7 V at a scan rate of 15 mV/s. According to electrochemical DPV responses in the absence of sulfite solution, there is no clear peak for any electrode. For an electrolyte containing a 50 M sulfite solution, the GCE electrode displays a very weak peak at 0.501 V, while chitin/GCE, GO/GCE, and chitin/GO/GCE exhibit anodic peaks at 0.362 V, 0.355 V, and 0.348V, respectively. These results indicate that the sulfite is being oxidized via electro-oxidation, which involves a one-electron transfer process followed by a chemical step of second order via the following reactions [63-65]:



Where $\text{H}_2\text{S}_2\text{O}_6$ (dithionic acid) can be decomposed through a disproportionation step, dilute solutions of the acid are known to be stable with the occurrence of decomposition on concentration only above 50 °C [63, 66, 67]. The peak current of chitin/GO/GCE is evidently greater than that of chitin/GCE, GO/GCE, and chitin/GO/GCE peak and is noticed at a lower potential of 0.62 V than that of the other electrodes, as shown by the DPV curves.

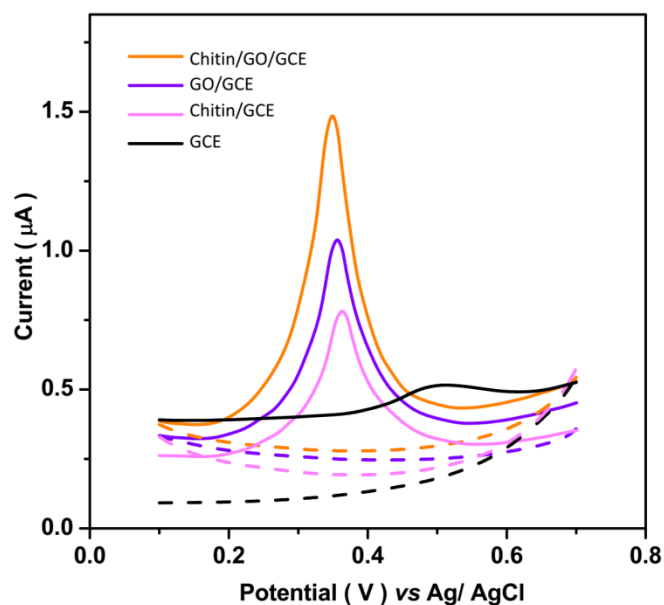


Figure 3. DPV responses of GCE, chitin/GCE, GO/GCE and chitin/GO/GCE at the potential window from 0.01 V to 0.7 V with a scan rate of 15 mV/s in 0.1 M PBS (pH 7.3) without (dashed line) and with 50 μM sulfite solution (solid line).

It shows that the charge transfer process in the oxidation of sulfite is improved by the synergistic electrocatalytic impact of chitin and GO. The chitin nanostructures show certain exceptional physical qualities, including the capacity to form films, nontoxicity, biocompatibility, and greater mechanical strength [68-72]. In comparison to other materials, carbon-based nanocomposites with chitin hold more active sites because of the high nitrogen content of chitin [73-75]. The biorenewable nitrogen present in chitin can be embedded into a variety of N-functional groups relevant to the unique face-to-face contact with GO, which is favorable for the enhancement of the electrocatalytic performance. The incorporated GO nanosheets form conductively interconnected networks to enhance the charge transfer in chitin based electrodes [73, 76]. The large electrochemically active surface areas of the chitin/GO nanocomposite effectively improve the transport of electrons between the electrode and the sulfite, resulting in a more quick and sensitive current response [68, 77]. As a result, the Chitin/GO nanocomposite modified GCE was used in the following electrochemical investigations for the measurement of sulfite.

Figure 4 shows the DPV responses and corresponding calibration plot of chitin/GO/GCE in the potential window of 0.01 V to 0.7 V with a scan rate of 15 mV/s after successively adding 60 μM sulfite solution into 0.1 M PBS (pH 7.3). With each addition of 60 μM sulfite solution in the range of 1 to 960 M, it has been discovered that the DPV peak current of chitin/GO/GCE increases linearly. With a correlation value of 0.99911, it is seen that the electrocatalytic peak current (I_P) and the sulfite concentration (C) have a linear connection as follows [78-81]:

$$I_P (\mu\text{A}) = 0.02751 C (\mu\text{A}/\mu\text{M}) + 0.19921 \quad (3)$$

The suggested sulfite sensor is shown in Table 1 for comparison with other sulfite electrochemical sensors published in the literature. Based on the aforementioned linear relationship, the detection limit ($S/N=3$) can be computed to be 0.021 μM and the sensitivity can be determined to

be 0.02751 $\mu\text{A}/\mu\text{M}$. The presence of GO with a high number of active edge planes and inert basal planes that are entrapped with chitin nanostructures in chitin/GO nanocomposite is said to give the proposed sulfite sensor using chitin/GO/GCE a comparable or even better performance than that of other reported sulfite sensors. This enhances the electrochemical properties and electron transfer ability [82-84].

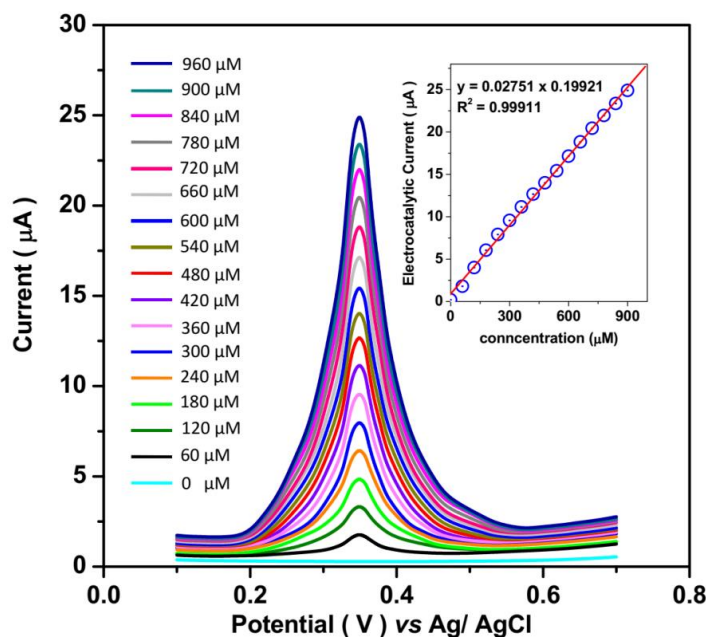


Figure 4. DPV responses and corresponded calibration plot of chitin/GO/GCE after successive adding 60 μM sulfite solution into 0.1 M PBS (pH 7.3) at the potential window from 0.1 V to 0.7 V with a scan rate of 15 mV/s.

Table 1. Performance of sulfite proposed sensor in present study and other reported sensor in literatures.

Electrode	Technique	LOD (μM)	Linear range (μM)	Ref.
Chitin/GO/GCE	DPV	0.021	1 to 960	Present study
PTZ-IL/MWCNT/GCE	Amperometry	9.3	30–1177	[85]
CTAB/Chit /CNT/GCE	DPV	9.6	30–800	[86]
p-ADPA-4-ATP-Au/GCE	CV	1.5	5–160	[87]
IL/graphene/CPE	DPV	0.02	0.05–250	[88]
Prussian Blue//GCE	CV	80	0–4000	[89]
Au/silsesquioxane/CPE	SWV	6.98	20.1–358.5	[90]
La ³⁺ -doped Co ₃ O ₄ / screen printed electrode	DPV	0.090	0.7–1000	[91]

PTZ-IL: Phenothiazine imidazolium ionic liquid with hexafluorophosphate counter anion; CTAB/Chit/CNT/GCE: cetyltrimethylammonium bromide/chitosan/carbon nanotube/ glassy carbon electrode; p-ADPA-4-ATP-Au/GCE: poly(4-aminodiphenylamine)-4 aminothiophenol/ Au composite/glassy carbon electrode; CV: cyclic voltammetry; IL/graphene/CPE: ionic liquids/graphene/carbon paste electrode; SWV: Square wave voltammetry;

The interference potential of common chemicals in food samples on the detection of sulfite was investigated in order to investigate the selectivity and interference influence of the proposed sulfite sensor in the current study. The results of the DPV studies of the electrocatalytic currents of chitin/GO/GCE at 0.348 V in 0.1 M PBS (pH 7.3) under the addition of 10µM sulfite and 50 µM of interfering compounds are summarized in Table 2. It can be inferred that the electrocatalytic currents of the chitin/GO/GCE responded exceptionally well to the addition of sulfite and weakly to the addition of the invented sulfite sensor have a suitable selectivity for sulfite determination in food samples.

Table 2. The results DPV studies of electrocatalytic currents of chitin/GO/GCE at 0.348 V in 0.1 M PBS (pH 7.3) under addition 10 µM sulfite and 50 µM of interfering compounds.

Compound	Added (µM)	Electrocatalytic current (µA) at 0.348 V	RSD
Sulfite	10	0.2755	±0.0041
Fructose	50	0.0631	±0.0028
Glucose	50	0.0290	±0.0015
Ascorbic acid	50	0.0424	±0.0011
Folic acid	50	0.0704	±0.0013
2-aminophenol	50	0.0320	±0.0014
Zn ²⁺	50	0.0213	±0.0019
Cu ²⁺	50	0.0201	±0.0012
NH ₄ ⁺	50	0.0251	±0.0014
NO ₃ ⁻	50	0.0577	±0.0021
Mg ²⁺	50	0.0446	±0.0020
K ⁺	50	0.0420	±0.0019
Fe ³⁺	50	0.0389	±0.0010
SO ₄ ²⁻	50	0.0265	±0.0012
Cl ⁻	50	0.0223	±0.0018
Ce ²⁺	50	0.0329	±0.0010

Table 2. The analytical findings of determination of sulfite in the prepared real samples

Sample	spiked (µM)	DPV			Total sulfite assay kit		
		detected (µM)	Recovery (%)	RSD (%)	detected (µM)	Recovery (%)	RSD (%)
vinegar	0.00	0.00	--	3.48	0.00	--	3.33
	1.00	0.97	97.00	4.20	0.98	98.00	4.04
	2.00	1.96	98.00	3.25	1.97	98.50	3.58
	3.00	2.91	97.00	4.04	2.97	99.00	4.11
pickle water	0.00	0.00	--	3.22	0.00	--	3.28
	1.00	0.96	96.00	4.65	0.95	95.00	4.14
	2.00	1.94	97.00	3.17	1.96	98.00	3.58
	3.00	2.95	98.33	4.10	2.94	98.00	4.09

The determination of sulfite in actual samples of vinegar and pickle water was carried out using DPV at a potential window of 0.1 V to 0.7 V with a scan rate of 15 mV/s, and total sulfite assay kit (Enzymatic, Neogen, USA) was used to determine sulfite in prepared real samples before and after the addition of sulfite. This was done in order to assess the practical applicability of the developed sulfite sensor. The usual addition approach was used to acquire the analytical studies. The results are described in Table 3, which also demonstrates the excellent agreement between the two studies and the satisfactory recovery (96.00% to 98.33%) and RSD (3.17% to 4.65%) values. The findings demonstrate that the created sulfite sensor has respectable validity, accuracy, and considerable potential in the practical evaluation of food samples.

4. CONCLUSION

In this study, chitin, a polysaccharide, was taken from wild morel mushrooms and used to chemically synthesize a modified GCE called a chitin/GO nanocomposite. The effective production of a well-crystalline chitin/GO nanocomposite on the GCE surface was demonstrated by structural characterization. Due to the synergistic electrocatalytic effect of chitin and GO, which improved the charge transfer process in the oxidation of sulfite, electrochemical characterizations demonstrated that chitin/GO/GCE was a selective sulfite sensor and had comparable or even better performance than that of other reported sulfite sensors. The linear range was established as 1 to 960 μM , the detection limit as 0.021 μM , and the sensitivity as 0.02751 $\mu\text{A}/\mu\text{M}$. In order to determine the amount of sulfite in actual samples of vinegar and pickle water, the practical applicability of the proposed sulfite sensor was assessed. The findings revealed that the recovery (96.00% to 98.33%) and RSD (3.17% to 4.65%) values were acceptable. The findings show that the created sulfite sensor has excellent precision, acceptable validity and excellent potential for use in actual food sample analysis.

ACKNOWLEDGEMENT

This work was sponsored in part by the 2021 Doctoral Startup Fund Project, Jilin Agricultural Science and Technology University (JASTU [2021] No. 5012).

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