

Research Article

Study on Determination of Sudan Red I with Graphene-modified Heat Glassy Carbon Electrode (GR/HGCE)

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Abstract: On the basis of Graphene (GR)'s unique single atomic layer structure, the author adopted drop casting method to prepare GR/HGCE, to represent the chemical behavior of Sudan Red I on GR/HGCE, the results show that the GR can effectively improve Thermal Glassy Carbon Electrode (HGCE)'s effective surface area and improve the surface properties of the electrode, the modified electrode has good catalytic activity to Sudan red I. The study established the new method with GR/HGCE for detection of Sudan Red I. This method was used to measure the content of Sudan Red I in the actual samples and the average labelling recovery is 101.4%.

Keywords: Glassy Carbon Electrode (GCE), graphene, modified electrode, Sudan red I, thermode

INTRODUCTION

Sudan Red I (chemical name: 1-phenyl azo-2-naphthol) is a kind of synthetic azo type chemical stain, scientists found that through the experiment, "Sudan Red I" can lead to rats and mice cancer (Chen *et al.*, 2011), it also reveals possibly carcinogenic properties in the study of human liver cells (Abdelghafor *et al.*, 2013), so it is prohibited being used in food production in most countries around the world. Therefore, it is very important to fast and accurately detect the Sudan Red in food (Yun *et al.*, 2014).

At present, the methods to detect the Sudan Red I mainly include Gas Chromatographic method (GC), High-Performance Liquid Chromatography (HPLC), ultraviolet spectroscopy (Grelle *et al.*, 2006), fluorescence method), capillary electrophoresis, enzyme-linked immunoassay (Cao and Ruwei, 2008), chemiluminescence method and electrical analysis method and so on. Electrochemical method has been paid to much attention because of its simple equipment, high sensitivity, low cost of analysis and being easy to automate (Brooks *et al.*, 2007). The main electrochemical method to detect Sudan Red I is modified electrode, the common modified electrodes include glassy carbon electrode modified by CNT (carbon nano tube), chitosan/graphene oxide self-assembly modified glassy carbon electrode (Chella *et al.*, 2010), 3-thiophene malonic acid modified glassy carbon electrode, nanometer WO₃ modified electrode, NiFe₂O₄@Au/GC modified electrode (Argall *et al.*, 2009), MnTMPyP/graphene oxide- multi-walled carbon

nanotubes modified electrodes, ionic liquid-oxidation graphene modified glassy carbon electrode.

Heated electrode was invented by German professor Gründler in the early 1990s (Zhang *et al.*, 2009). Main advantage of the technology is electrode heating only. The temperature of the bulk solution can remain the same. The rise of electrode temperatures can produce heat convection and enhance mass transfer and may also promote the electrode reaction (Pang and Wang, 2008), the heated electrode has been successfully applied in the electric analysis (Qu *et al.*, 2012), because of its simple heating equipment, higher detection sensitivity and lower electrode pollution effect, the technology has attracted widespread concern in the field of electrochemical analysis. But due to the limitation by heated electrode heating mode and craft (Brooks, 1986), heated working electrode area is generally smaller, influencing the effect of the detection. People now pay much attention to improve the effective area of heated electrode's plate electrode using nanometer materials especially two-dimensional plane carbon nano material, graphene modified electrode.

Graphene (GR) is a new type of two-dimensional plane of carbon nanomaterial discovered in 2004, its special single atomic layer structure determines its unique structure and excellent performance, it has good electrical conductivity, high specific surface, good mechanical stability (Wu *et al.*, 2009), chemical stability and thermal stability, so in recent years, it has been widely used in the electrochemical and electricity analysis chemistry (Gao *et al.*, 2010). Now based on

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graphene's application in the modified electrode has been reported (Gao and Xu, 2005), but it has not been applied in the modified heated electrode. Therefore, this research will use the graphene for the modification of heated electrode and apply the modified heated electrode in the detection of the dye Sudan Red I in food.

MATERIALS AND METHODS

Reagent and instrument: CHI 660B (heated glassy carbon electrode, HGCE) CHI 660B electrochemical workstation (Shanghai Chen Hua Instrument Co., Ltd.); ECT-02 electrochemical electrode temperature modulation device (Fuzhou University), three electrode system: 213 platinum electrode is counter electrode, a saturated calomel electrode is reference electrode, self-made Heated Glassy Carbon Electrode (HGCE) is as working electrode.

Sudan Red I (Germany Ehrenstorfer Company), graphene (self-made) and Phosphate Buffer Solution (PBS) are prepared according to need with Na_2HPO_4 and KH_2PO_4 . Other chemical reagents are analytically pure and experimental water is secondary distilled water.

Preparation of graphene and modified electrodes: With a reference to literature, the graphite is pre oxidized before it is oxidized to be graphite oxide, finally, by hydrazine hydrate reduction, the Graphene (GR) is made, preparation of HGCE shall refer to the reference literature (effective diameter is 3 mm).

Polish on the Chamois Towel the HGCE working face in turn with metallographic sandpaper and mixture of aluminium oxide respectively with particle size of 0.3 and 0.05 μm and water to a mirror and then wash it with ethanol, 10% NaOH solution, 1:1 HNO_3 solution and water, dry it for standby application. Place the dry HGCE in 0.5 mol/L sulfuric acid solution, scan it for 20 laps at 100 mV/sec within the scope of -1.0 V-+1.0 V (vs. SCE), until the cyclic voltammetric curve tends to be stable. Take a homemade 5 mg GR in 10 mL of N, after ultrasonic dispersion for 1 h in the N-dimethyl formamide solution, get GR suspension. Use micro syringe to take appropriate suspension to drop in the processed HGCE surface, dry it with infrared drying lamp to obtain GR/HGCE, place the dried modified electrode in PBS buffer solution (pH = 4.0) for activation.

Experimental method: Electrochemical detection adopts three electrode system, take Graphene modified Heat Glassy Carbon Electrode (GR/HGCE) as the working electrode, saturated calomel electrode as the reference electrode and the foil electrode as the counter electrode. Take PBS with pH = 4.0 as supporting electrolyte, within the scope of 0.4 to 0.9 V, do

differential pulse voltammetric scan for the Sudan Red I solution. Enrichment time: 150 sec, pulse amplitude: 50 mV, pulse width: 40 msec, scanning speed: 40 mV/sec. Every time after the scanning, put the work electrode in blank bottom liquid for circulation scanning until the peak disappears, rinse it with secondary distilled water, after the filter paper blots it up, determine it for the next time.

RESULTS AND DISCUSSION

Self-making GR and the epresentation of GR/HGCE: As shown in Fig. 1, we can see from the infrared spectrogram of GR (A) and graphite (B), the graphene has an obvious hydroxyl peak at 3450/cm (peak Fig. 1a), which is the functional group of -OH in the water, at 1630/cm there is obvious characteristic absorption peak of C = C vibration of carboxyl functional group (Fig. 1b) and the infrared spectra of graphene at 1700/cm does not represent the characteristic peak of C = O stretching vibration peak, accordingly, we can think that in the synthesis, we have thoroughly reduced the graphene oxide into grapheme.

The Scanning Electron Microscopy (SEM) of the prepared GS/HGCE in Fig. 1 is as shown in Fig. 2, the graphene on the surface of glassy carbon electrode modified by the self-made graphene has fold structure, in the disordered distribution, part of the graphene folds together, to form a multilayer structure, the existence of the graphene layer greatly increases the effective area of glassy carbon electrode.

GR/HGCE's electrochemical performance characterization: As shown in Fig. 3, the oxidation peak current of the HGCE cyclic voltammogram a of the modified GR increases obviously the oxidation current in the HGCE, it indicates that GR can promote electron transfer on the surface of the electrode, improve the electrical conductivity of the electrode and speed up the reaction rate.

In order to further verify the inference, the author has carried on the electrode EIS test for HGCE and

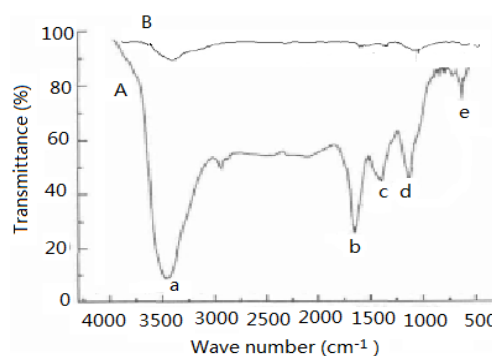


Fig. 1: The infrared spectrogram of GR and graphite

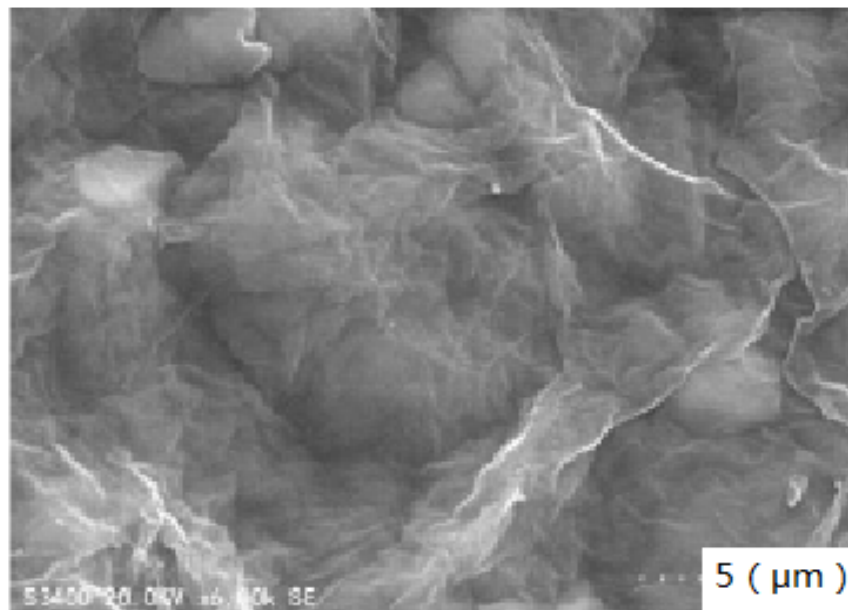


Fig. 2: SEM image of GS/HGCE

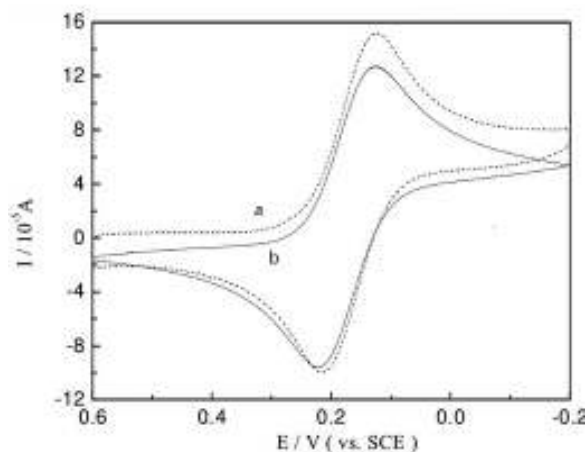


Fig. 3: Cyclic voltammograms of 1×10^{-2} mol/L $(\text{Fe}(\text{CN})_6)^{3-/4-}$ at GCE (b) and GS/HGCE (a)

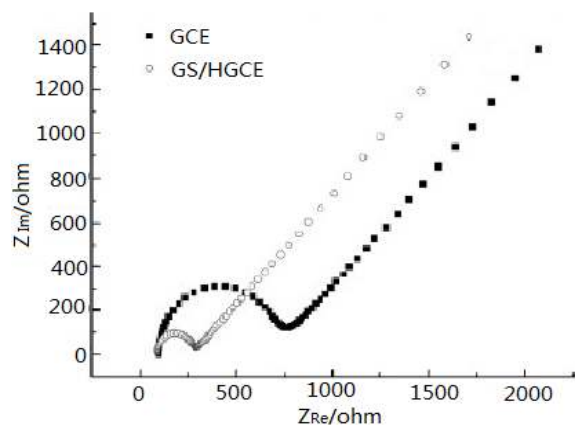


Fig. 4: Nyquist diagram of $(\text{Fe}(\text{CN})_6)^{3-/4-}$ in GCE and GR/HGCE

GR/HGCE (excitation amplitude is 5 mV, the frequency range is 1×10^{-2} - 1×10^4 Hz, the a constant potential: +200 mV). EIS is a kind of sensitive detection technology in the process of modification of the electrode surface, EIS curve is obtained from tests and the impedance value (Ret) can be obtained from the diameter of the semicircle part of the high frequency area. At room temperature, it can be seen from the Fig. 4, the impedance arc radius of the GR/HGCE is much smaller than GCE, indicating its charge transfer resistance R_{ct} is decreased obviously. The main reason why the modified electrode resistance decreased obviously is GR itself has excellent heterogeneous electron transfer ability, which can accelerate the electron transfer between the $(\text{Fe}(\text{CN})_6)^{3-/4-}$ solution and electrode surface; On the other hand, because of the existence of $-\text{C}=\text{C}-$ on the GR plane, graphene has big π conjugate system, to accelerate electrons' transfer inside the graphene base plane, so that the charge transfer resistance of GR/HGCE in the $(\text{Fe}(\text{CN})_6)^{3-/4-}$ solution decreased greatly.

Sudan red I's electrochemical behavior in GR/HGCE:

The author adopted Differential Pulse Voltammetry (DPV) to study the Sudan Red I's electrochemical behavior on the surface of HGCE (room temperature, 55°C) and the GR/HGCE (room temperature, 55°C). As shown in Fig. 5a is the DPV figure of HGCE in the blank solution, there is no oxidation peak current; Fig. 5 is Sudan Red I's DPV curve on HGCE electrode, anodic peak E_{pd} appears near to 0.647 V, the peak potential is lower than E_{pb} but the peak current rises, this is the result of GR modification; Fig. 5C is the DPV figure of HGCE at 55°C, at 0.727 V, there is an oxidation peak E_{PC} , relative to the potential of the E_{pb} peak, there is

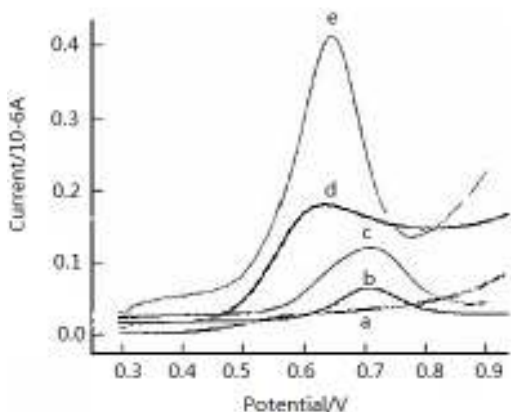


Fig. 5: DPV curves of Sudan I on HGCE, GR/HGCE in PBS (pH = 7.0)

basically no change, but the peak current rises obviously, this is due to the heating effect of the hot electrode which makes the electron transfer rate on the surface of electrode is accelerated, in turn, it increases the peak current; Fig. 5e is the DPV chart of GR/HGCE at 55°C, Epe, an oxidation peak Epe appears near 0.652 V, the GR/HGCE oxidation peak under the peak potential and the room temperature is basically identical, but the peak electricity is significantly higher than the current of oxidation peak Epb, Epc, Epd, on the electrode, there is not only the existence of GR, but also the temperature of the electrode rises, the existence of GR can make the electron transfer between the solution and the electrode surface and accelerate significantly the oxidation and at the same time, it makes the effective electrode reaction area increase, after HGCE heating up to 55°C, electrode surface has heat effect to make the electron transfer rate faster, so under the dual role of GR and heating effect, the peak current is highest.

By changing the dosage of the GR solution for the modification effect validation, the results showed that the liquid-moving machine drops 0.5 mg/mL of graphene solution on the HGCE, with the increment of modifier, electrochemical signal enhanced obviously, when volume of drop casting reached 6 μ L, the peak current is highest and the peak current reduced gradually with the increase drop casting; this indicates the coating thickness on the surface of the electrode has influenced the electron transfer, thus reduce the peak current, so this study adopts GR/HGCE working electrode prepared with 6 μ L mg/mL graphene suspension liquid.

The choice of the supporting electrolyte: Electrolytes and pH value has an important influence on the peak current of the REDOX peak of the electroactive material, this study measured the voltammetric behavior of ACOP respectively in hydrochloric acid, sulfuric acid, BR, $\text{NH}_3\text{-NH}_4\text{Cl}$, HAc-NaAc, PBS buffer solution with different concentration and pH. The

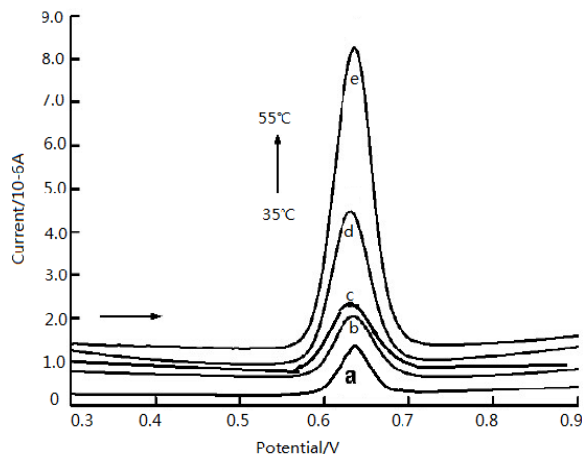


Fig. 6: The DPV curves of the Sudan I in GR/HGCE under different temperature

Pulse amplitude: 50 mV; Pulse width: 40 msec; Scan rate: 40 mV/sec

results showed that the peak current when the PBS with pH = 6.0 as bottom liquid is highest, at the same time, the peak shape is best and the background current is relatively low. Therefore, PBS with pH = 6.0 was selected as supporting electrolyte.

The influence of concentration of time: Enrichment is a kind of simple and effective method to improve electrochemical sensitivity, before the detection, Differential Pulse Voltammetry (DPV) enrichment time was investigated. Due to larger effective surface area of graphene on the electrode surface modification and also graphene contains C = C and other functional groups at the same time, GR/HGCE showed high enrichment efficiency for Sudan Red I. Within the enrichment time of 0-240 sec, Sudan Red I's oxidation peak current increases with the increase of the enrichment time obviously, when the enrichment time reached 150 sec, the increase of peak current slowed down, showing that Sudan Red I's concentration on the surface of the GR/HGCE has backed to balance. So, with a comprehensive consideration of the sensitivity and efficiency, enrichment timing was set for 150 sec.

The influence of electrode surface temperature on the detection: The rise of electrode temperature can produce heat convection to enhance mass transfer, it may also promote the electrode reaction, it can be seen from the Fig. 5 at the same time, the temperature can significantly improve the Sudan Red I's electrochemical activity on the surface of the GR/HGCE. Under the above optimal conditions, the temperature effect of electrode was investigated. As shown in Fig. 6, when the temperature of the electrode increased within the range of 35 to 55°C, the detection current signal increased gradually, but when the temperature was 60°C, inside the electrolyte it will produce bubbles to affect stability, so this study selected 55°C.

Table 1: Recovery ratios of Sudan I in samples using GR/HGCE

Sample	Sample measurement value (µg/mL)	Quantity added (µg/mL)	Labelling measured quantity (µg/mL)	Recovery rate (%)
Red duck eggs sample	0	10	10.31	103.10
	0	20	19.43	97.15
	0	30	31.72	105.73
Chili powder sample	0	10	9.97	99.70
	0	20	19.96	99.80
	0	30	30.89	102.97

Precision, reproducibility, stability, linear range and interference test: Under the selected best experimental conditions, the precision, repeatability, stability and linear range of the method was investigated. Results showed that, used GR/HGCE for 10 consecutive times, then determined the solution containing 20 µg/mL Sudan Red I, the RSD of peak current was 7.2%, showing that the electrode has good precision and reproducibility. Examined the linear range of the method by DPV, the results showed that the Sudan Red I's concentration has good linear relationship at the oxidation peak current of GR/HGCE (ipa, µA) and its concentration (C, µg/mL) within the range of 0.02-40 µg/mL, the regression equation is $ipa (\mu A) = 0.421C (\mu g/mL) + 0.205$ ($r = 0.995$, $n = 7$) and the detection limit is 6.7×10^{-3} µg/mL.

Under the selected best experimental conditions, the author investigated the interference of the common inorganic ions and organic compounds on the determination of Sudan Red I, the testing relative error is less than ±5%, 1000 times of Fe³⁺, Al³⁺, Ca²⁺, Cu²⁺, Mg²⁺, K⁺ and Zn²⁺ and 100 times of β-carotene, sucrose, glucose, mannitol, citric acid, chlorogenic acid, as well as 50 times of ascorbic acid, fructose, cysteine and tyrosine does not affect the determination.

Labelling recovery determination of Sudan red I in the actual samples: Take commercial red duck eggs and chili powder as samples, take fixed quantity of sample and place it in the buffer solution for ultrasonic 30 min before filtration, make labelling recovery determination for the filtrate. Under the condition of optimization, do labelling recovery determination for three groups of sample. The labelling recovery rate is between 97.15 and 105.73%, the average recovery is 101.4, RSD is 2.778%. The test results are shown in Table 1.

CONCLUSION

Grapheme has good electrical conductivity and higher specific surface area, adsorption ability and strong chemical stability and thermal stability, use drop casting method to prepare GR/HGCE with grapheme suspension solution, to further improve the hot electrode's electrochemical activity and improve the detection response signal enhancement; The modified heat electrodes can be analyzed in the ACOP actual sample. Using this method in the determination of

Sudan Red I's content in the actual samples, the effect is satisfying. The test method is of simple operation, high sensitivity and low detection limit, to further expand the application range of the hot electrode.

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