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Electrochemical Behavior of Sudan I and Sudan II at Graphene Modified Electrodes

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The electrochemical behavior of Sudan I and Sudan II was investigated by using graphene modified glassy carbon electrode with cyclic voltammetry and differential pulse voltammetry. Both azo dyes demonstrated multiple well-defined peaks in their voltammogramms and the signal intensity was better than the voltammogramms conducted by naked glassy carbon electrode. The optimization for the method was performed based upon the pH value and graphene amount. By applying the optimal condition, Sudan I could be linearly detected in the wide concentration range between 1 and 5 mg/L and Sudan II was capable in the range between 0.5 and 7.5 mg/L. The detection limit was 0.1 mg/L for Sudan I and 0.3 mg/L for Sudan II. This method provides potential application for detecting Sudan I and Sudan II in food matrix.

Keywords: Azo dyes, Electrochemistry, Cyclic voltammatry, Differential pulses voltammatry, Graphene modified electrodes.

INTRODUCTION

Food colour additives reinforce the colour and make the food more appealing for eating[1]. However, some illegal dyes were also used in food products and caused the food safety issues. Azo dyes are one of the most common colour dyes that have been using in various industries due to their simple synthetic scheme and stable colour constancies. These dyes are categorized as class 3 carcinogens by the International Agency for Research on Cancer (IARC) and therefore were prohibited as addictive in food products[2]. However, the fraudulent use of azo dyes in food still occurred occasionally. Sudan dyes were recently found in chili powder, hot sauces, ketchup in some countries[3,4]. In 2005, Sudan I was also found in China in some roasted chicken wings, chicken burgers and some other commercial products, *e.g.* piccalilli, chili sauce, *etc*., which led to a great panic to consumers. At this circumstance, developing analytical methods for detecting this group of compounds becomes crucial. Nowadays, HPLC and LC/MS/MS are generally applied to classify and quantify the amount of azo dyes in food matrix[1,5]. But these methods usually take long time and laborious for sample extraction, therefore more robust and convenient method for screening analysis of azo dyes in food needs to be established. The electrochemical (EC) technique provides a rapid detection and a handy installation and has been widely used for developing analytical method for chemical contaminants detection. Several studies regarding Sudan I analysis using electrochemical methods have been done[6,7]. Moreover, the modified electrodes have been proved to give better sensitivity and selectivity than un-modified electrodes and several approaches regarding electrodes modification for Sudan I detection have been pursued. For example, montmorillonite calcium was used to improve the sensitivity of Sudan I detection, the limit of detection (LOD) reached 0.02 mg/L with 2 min accumulation time[8]. As well, ionic liquid-multiwalled carbon nanotube was used for Sudan I detection[3]. On the contrary, the analysis of other azo dyes such as Sudan II, which are also commonly detected in food has not been reported and the electrochemical behavior of this azo compound has not been elucidated.

Graphene is a material newly discovered by researchers in England[9]. Graphene is made of 2-dimentional carbon atom layers. This material provides a decent conductivity and has been widely used in electronic mechanics, such as transducers and gas sensors. Graphene has also been used for electrochemical electrode modification. It not only has good conductivity[10], but also has decent stability, it would not change forms in corrosive acid or any strong base, which could also make as inert material that provide protection against those reagents, therefore very suitable for electrochemical electrodes modification. Moreover, the electrochemical sensitivity can be significantly improved after electrodes modification by graphene[11]. Taking these advantages into consideration, it is meaningful to investigate the electrochemical behavior of Sudan dyes after graphene application.

The aim of the present study was to analyze Sudan I and II using electrochemical with graphene modified electrodes. Graphene was suspended on glassy carbon electrodes (GCE) for voltammerty scanning. The validation of the method and the potential usage of graphene were also evaluated.

EXPERIMENTAL

The aqueous solution of graphene was synthesized by arc-discharging method[12]. Sudan I and Sudan II were purchased from Sigma Aldrich (Beijing, China). The chemical structure of Sudan I and II were shown in Fig. 1. Acetonitrile with HPLC grade (Fisher, USA) was used to dissolve Sudan I and Sudan II. The concentration of all those stock solutions was made in 0.5 mg/mL. The 0.1 M phosphate buffer solution was made by dissolving disodium hydrogen phosphate and sodium dihydrogen phosphate (Sinopharm Chemical Reagent Co., Ltd, China) into deionized water. The serial pH values were fixed by 10 M of sodium hydroxide or phosphoric acid.

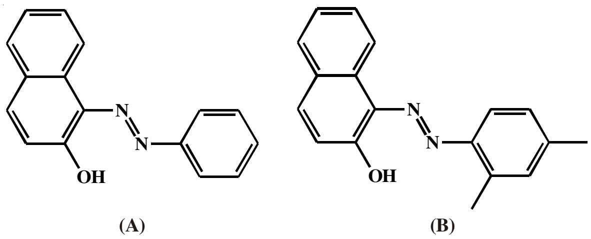

Fig. 1. chemical structures of Sudan I (A) and Sudan II (B)

CHI 650 d electrochemical workstation (Chen Hua Instruments, Shanghai, China) was used for sample analysis and data processing. In this study, cyclic voltammetry (CV) and difference pulse voltammetry (DPV) were performed to analyze the electrochemical behavior of different azo dyes. Three-electrode system was used in this experiment for sample detection. This system was composed by a graphene modified GCE, an Ag/AgCl reference electrode and a platinum wire counter electrode. Additionally, the magnetic stirrer was used in the reaction vial during analysis, in case the analyte could maintain homogeneity throughout the experiment.

Working electrodes preparation: The glassy carbon working electrodes were polished with 1, 0.3 and 0.05 μm of alumina slurry, respectively, followed by flushing with deionized water and then blows away any water residue with rubber air pump. A volume of 10 μL of graphene-water suspension solution was applied onto glassy carbon electrode contact surface and dried under the room temperature.

Optimization of working condition: The pH value of the buffer was investigated in this experiment. Previous results showed that Sudan I was more favorable for the reaction under acidic condition[13], the method was conducted by setting the amount of graphene and the amount of the analytes as constant, the pH value of the buffer was gradually oriented from 3.2 to 7.7, with the increment of 0.5. The optimal condition was determined based on the cyclic voltammetric and differential pulse voltammetric performance for each sample.

The amount of the graphene that applied on the GCE was also important. In present experiment, the amount of graphene was differently applied on each individual GCE (0.1, 0.25, 0.5, 0.75, 1 and 3 μg) by setting pH value and amount of the analytes as constant. Determination of the optimizing condition was based on the performance of each modified GCE.

Cyclic voltammetric studies: The cyclic voltammogram of the blank buffer solution was recorded at the beginning to ensure that there was no extra peak interference and then 0.1 mM of Sudan I or Sudan II was pipetted respectively. The starting potential of -0.4 V was used, with turning potential of 1.2 V and back to -0.4 V eventually. The effect created from the scan rate was investigated over the range 0.1 to 0.9 V/s.

Differential pulse voltammetry (DPV): Differential pulse voltammetry was performed with the blank buffer solution at first, followed by the same solution containing 0.1 mM of Sudan I or Sudan II. The starting potential was determined at -0.4 V and the final potential was set at 1.2 V. The increment potential was 4 mV, amplitude was 0.05 V, sampling width was 0.0167, pulse width was 0.2 s and pulse period was 0.5 s.

RESULTS AND DISCUSSION

Cyclic voltammetric behavior of Sudan I and II at grahene modified glassy carbon electrode: The investigation was started with the comparison of cyclic voltammetries of azo dyes under bare GCE and with the graphene modified GCE. In case to confirm that the voltammetric signal was directly conducted by azo dyes, we tested the blank buffer solution by applying graphene modified GCE. The result showed that buffer solution did not conduct any signal that would interfere with our analysis. The electrochemical behavior of Sudan I showed that while GCE without graphene modification, there was an obvious oxidation peak appeared around 0.9 V with peak current height was 1.5 μA (Fig. 2). However, there was no reduction peak observed in the voltammogram and red precipitate appeared in the vial within 4 min after the solution was electrified. This phenomenon indicates that the reaction occurred in the vial was irreversible, which directly affected the differential pulse voltammetry result that the quantification for the reaction can not be done accurately. The repeatability was very poor as well. Similar observation was also reported that naked GCE did not work well for detecting Sudan I in the buffer solution[7]. Whereas with the support of graphene modified electrode, the redox couple becomes more observable from voltammogram (Fig. 2). Two oxidation peaks were observed around 0.2 V and 0.8 V, with current peak height 0.1 μA and 0.5 μA, respectively. The reduction peak appeared around 0.8 V in the negative-going scan with current peak height 0.3 μA. In this case, the electron transfer in the reaction was balanced and the quality of the reaction solution could sustain longer period than the same condition with bare GCE (3 min for bare GCE *vs.* 10 min for graphene modified GCE). This observation is also consistent with the previous report that graphene could provide more conductivity and enhance the voltametric results[14]. In spite of the CV performance, DPV for Sudan I was also improved after the graphene modified GCE was applied into the system and becomes more observable (Fig. 3). An obvious and sharp peak appeared at 0.77 V, with current about 1.4 μA.

Similar to Sudan I, with the support of graphene suspension covered on the electrode, the intensity of the Sudan II

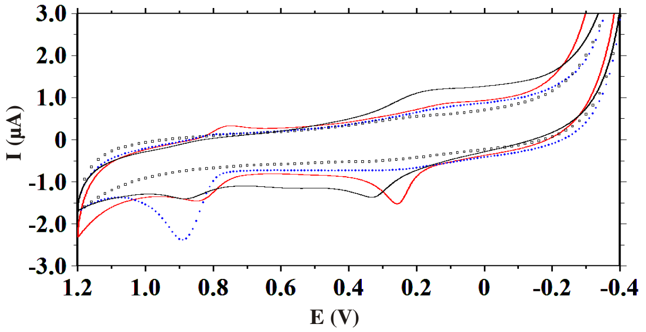

Fig. 2. Cyclic voltammetry of Sudan I in pH 4.7 and Sudan II in pH 3.7 phosphate buffer. Blue dotted line indicates the Sudan I bare electrode. Red solid line indicates the Sudan I graphene modified GCE. Brown square line indicates the Sudan II bare electrode. Green solid line indicates the Sudan II graphene modified GCE

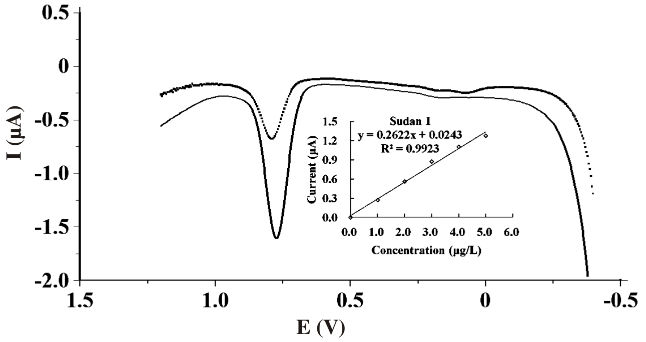

Fig. 3. Differential pulse voltammetry of graphene modified electrode in pH 4.7 phosphate buffer containing 5 mg/L Sudan I. Solid line denotes the presence and dotted line indicates the absence of graphene. Inset: calibration curve for DPV of Sudan I

electrochemical signal was tremendously enhanced and selectivity was also improved. In the CV results for Sudan II with concentration at 5 mg/L, two oxidation peaks appeared at potential 0.33 V and 0.88 V, with peak height 0.4 µA and 0.3 µA, respectively. One reduction peak appeared around 0.17 V in the negative-going scan (Fig. 2). On the other hand, the voltammogram that conducted by the bare electrode did not provide any peak signal. Result from DPV scan indicated that two peaks appeared at 0.81 V and 0.24 V, with current 0.12 µA and 0.1 µA, respectively (Fig. 4), on the voltammogram conducted by graphene modified GCE. On the contrary, the peak signal conducted from bare electrode has lower current than the signal conducted from modified GCE. Due to the improved conductivity from graphene, the whole reaction system has been exceptionally activated and current signal was successfully enhanced. Previous study showed that graphene could significantly improved signals in DNA sensors[7], our study also suggests that graphene modified GCE can also significantly improve the electrochemical behavior of Sudan II. Yang *et al.*[15] also reported that the naked GCE could not induct the reaction vial at the same condition. Additionally, from the CV result conducted by graphene modified GCE, the observable redox couple has been established and the reaction becomes reversible and the reactant consumed slower, which the quality of the reaction solution could be maintained. By applying graphene modified GCE, the electrochemical behavior for both azo dyes were enhanced and their CV results represent in similar pattern. However, their DPV performances were significantly different. Two oxidation peaks appeared in Sudan II when using DPV scanning, whereas only one oxidation peak was observed for Sudan I throughout each voltammogram. By analyzing their voltammogramms, we speculate that the structure difference is the origin that led their difference on electrochemical behavior. For Sudan I and II, the only difference comes from the benzyl groups on the side of the azo linkage, that Sudan I is benzyl group and Sudan II is a dimethyl substituted benzyl group. The dimethyl substitution significantly changed the Sudan II electrochemical behavior conducted in the DPV. Such discrepancy indicates that the substituent groups on the aromatic ring have great effects on the electrochemical behavior of the compounds. By comparing the structure of both azo dyes, we believes that graphene modified GCE could be used for detections on more compounds if the compound has electron-rich benzene ring or other aryl groups with high electron density.

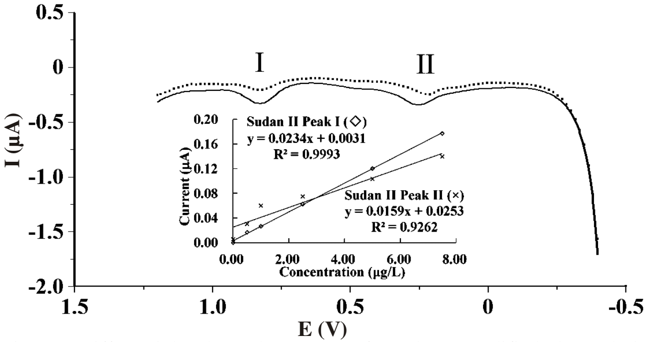

Fig. 4. Differential pulse voltammetry of graphene modified electrode in pH 3.7 phosphate buffer containing 5 mg/L Sudan II. Solid line denotes the presence and dotted line indicates the absence of graphene. Inset: calibration curve for DPV of Sudan II peak I and peak II

Effect of graphene amount to the electrochemical behavior of Sudan I and II: The optimization of the graphene amount was investigated in this experiment. Following the similar procedure reported previously[15-17], we used differential pulse voltammetery to quantify the responding current at each condition and by tabulating the value of the amount of graphene and the responding current, we optimized the value for each type of azo dyes. The results showed that even though graphene modified electrode conducts decent results, the enhancement of responding current in voltammogramms was not completely followed with the increment of graphene that applied on GCE.

For Sudan I electrochemical behavior study, certain amount of graphene was applied from 0.1 to 3 µg, with a step increment. The results show that by increasing the amount of graphene that applied on electrode, the strength of responding current from DPV result was also increased. The current value reached to maximum while 0.5 µg graphene was suspended on surface of GCE and began to shrink down when more graphene was used. Therefore, 0.5 µg of graphene was determined as the optimum value for Sudan I. The amount of 10 µg of graphene was also tested on GCE surface, but there was no peak signal observed. Since there was signal conducted while bare electrode applied into the system, it appears that the applied 10 µg of graphene was too thick that the contact between electrode and solution was completely isolated. Similar to Sudan I, the optimal electrochemical signal for Sudan II was obtained while 0.75 μg of graphene was suspended on GCE. When more graphene was applied, the responding current began to decrease.

pH effect : It is also reported that pH value of buffer solution was a crucial factor influencing electrochemical behavior in electrochemical study[13,18]. The optimization of pH value was built up according to the similar ideas indicated from reference[19], which compared the results from DPV scans for both azo dyes and all measurements were repeated for five times to test the reproducibility. In our experiments, the variation of pH value in buffer in relation to the voltammogram of analytes was also evaluated by DPV. The evaluated range was from 3.2 to 7.7, by step increment of 0.5. Results showed that after the pH value reached to certain point, the DPV signals for both azo dyes were inclined to decrease. The current of Sudan I reached maximum when pH value was 4.7 in buffer and for Sudan II, the maximum current signal appeared at the pH value 3.7 (Fig. 5). Moreover, the DPV result for both azo dyes also indicated that the potential for the peaks approached towards lower value while higher pH buffer was applied (Insets in Fig. 5). Similar observation was also reported that the higher pH for buffer, the lower reaction potential for the peak[4,16]. This could be caused by energy conservation that the more compensation of negative charges from buffer solution, the less voltage to initiate the peak occurred.

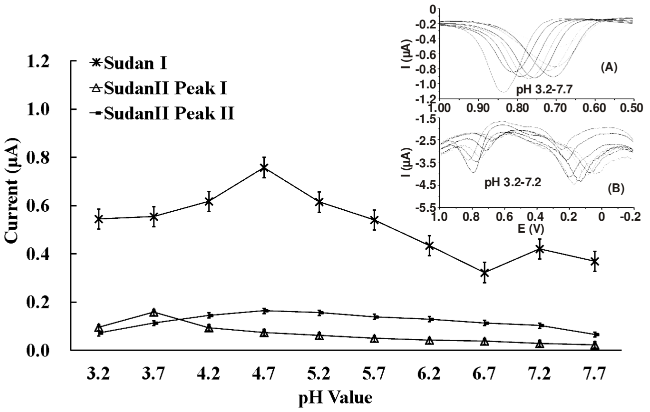

Fig. 5. Effect of pH on responding current in differential pulse voltammetry for azo dyes. The error bars were measured from three individual measurements for each sample. Insets: the effect of pH on peak potential in differential pulse voltammetry for Sudan I (A) and Sudan II (B)

Linearity and detection limit: By applying the optimized condition, DPV for both azo dyes were performed for method validation. Data was presented as the mean value of four parallel samples at one concentration and the coefficient variation was less than 10 % for both compounds. Linear calibration curve was obtained for Sudan I within the range from 1 to 5 mg/L and the correlation coefficient (R^2) was 0.9923. The regression equation was $I_p = 26.224\ C + 2.4343$. The method gives high sensitivity with the limit of detection (LOD) at 0.1 mg/L when evaluated with the signal to noise ratio at 3 (S/N = 3).

For Sudan II, the calibration curves were linear within the range between 0.5-7.5 mg/L. For the peak at 0.82 V, the regression equation was $I_p = 2.3368\ c\ (mg/L) + 0.3061$ and a corresponding R^2 value of 0.9993. And for the peak at 0.25 V, the regression equation was $I_p = 1.59\ c\ (mg/L) + 2.5278$ and a corresponding R^2 value of 0.9262. Detection limit for Sudan II was estimated to be 0.3 mg/L (S/N = 3).

Conclusion

The electrochemical behavior of Sudan I and Sudan II on graphene modified GCE was studied. We found that graphene modified electrode could obviously improve electrochemical behavior for both azo dyes. The effect of pH and graphene amount on volumetric behavior for Sudan dyes was also evaluated. With the exploration completed above, the graphene modified GCE provides adequate results and potential usage for detecting Sudan I and II in food products.

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