

Short Communication

## Voltammetric Study of Sudan I on Glass Carbon Electrode and Its Interaction with DNA

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The electrochemical behavior of sudan I at glassy carbon electrode as well as its interaction with DNA were studied using voltammetric technique. The results indicated that the electrochemical oxidation of sudan I was an irreversible process and the electrode process was controlled by a adsorptive step. In the presence of DNA, the oxidation peak current of sudan I decreased and the peak potential shifted positively, which indicated that the binding of sudan I to DNA was via intercalation. The binding number is 1 and binding constant is  $1.44 \times 10^3$ .

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**Keywords:** Voltammetric study; Sudan I; Interaction

### 1. INTRODUCTION

Sudan I (1-phenylazo-2-naphthol) is a synthetic lipophilic azo chemical dye. It was widely used in solvent, oil, wax, gasoline, shoes, floor and other color enhancement [1]. World Health Organization has classified it as a category 3 carcinogen [2,3], and most countries have banned the addition of Sudan I to foods. However, in order to increase the economic benefits, some manufacturers still add them as additives to foods to improve their appearance, the foods including chili oil, ketchup, olive oil and so on [4-10].

DNA plays an important role in the reproduction, growth and development of organisms. Many molecules can interact with DNA and change their original life function and affect the gene regulation and expression. Study on the interaction between small molecules and DNA is very interesting and significant in understanding the effects of certain molecules on the replication and transcription of DNA

and the resulting mutations [11-13]. The electrochemical method is simple and promising for investigation the interaction between DNA and molecules [14-17]. Sudan I is a kind of carcinogen, it will do harm to human health when it enters human body. Studying the interaction between Sudan I and DNA is helpful to understand the pathogenesis, which is of great significance to the treatment of patients and the research of corresponding drugs.

In this study, the electrochemical behavior of sudan I on glassy carbon electrode was studied by voltammetric technique. The interaction between sudan I and DNA was also investigated. The binding number 1 and binding constant  $1.44 \times 10^3$  were obtained.

## 2. EXPERIMENTAL

### 2.1 Reagents and instrumentation

All electrochemical experiments were carried out on a CHI660B electrochemical workstation (Chenhua Instrument Company of Shanghai, China). A conventional three-electrode system including glassy carbon electrode, the saturated calomel electrode and platinum wire electrode.

The phosphate buffer solutions (PBS) were prepared by mixing the stock solutions of  $\text{NaH}_2\text{PO}_4$  and  $\text{Na}_2\text{HPO}_4$ , then adjusting the pH with  $\text{H}_3\text{PO}_4$  or  $\text{NaOH}$ . 0.01 mol/L stock solution of Sudan I (Sinopharm Chemical Reagent Co. Shanghai, China) was prepared with ethanol. 20 mg/L stock solution of calf thymus DNA (Sigma reagents Co., Ltd.) was prepared with PBS(pH=6.8). The experimental solutions were prepared with double-distilled water.

### 2.2 Experimental measurements

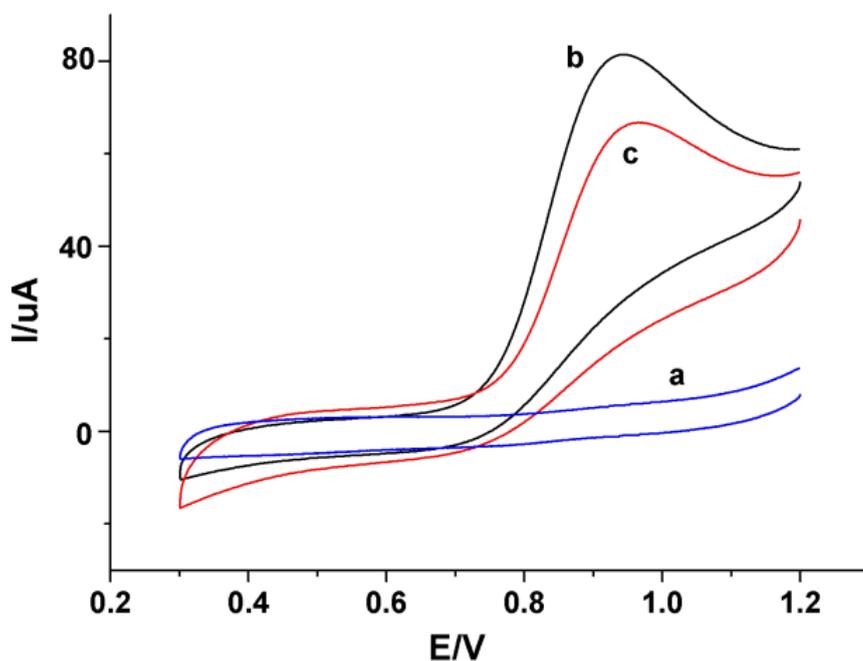
The PBS(pH=6.8) was selected as the supporting electrolyte. According to the experimental needs, the test solution was prepared with sudan I stock solution, DNA stock solution and PBS. 10 mL test solution was transferred to the three electrode system electrolyzer. The cyclic voltammograms were recorded from 0.3 to 1.2 V at scan rate of  $0.1 \text{ Vs}^{-1}$ . Before each measurement, the glassy carbon electrode(GCE) was polished with  $0.05 \mu\text{m Al}_2\text{O}_3$  slurry and then cleaned in ethanol and distilled water by ultrasonification. All the experiments were performed in the room temperature.

## 3. RESULTS AND DISCUSSION

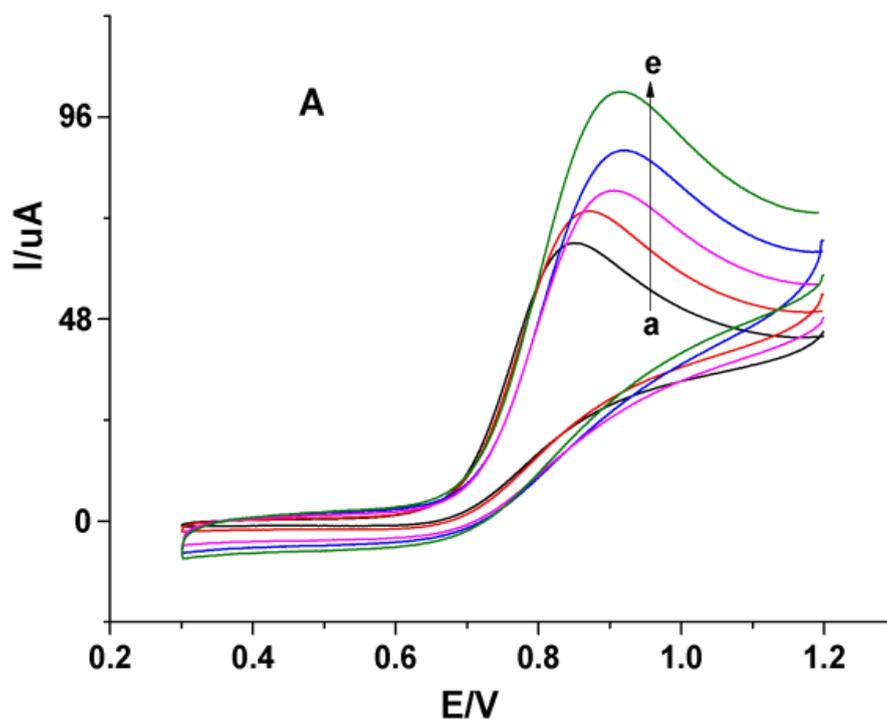
### 3.1 Cyclic voltammograms

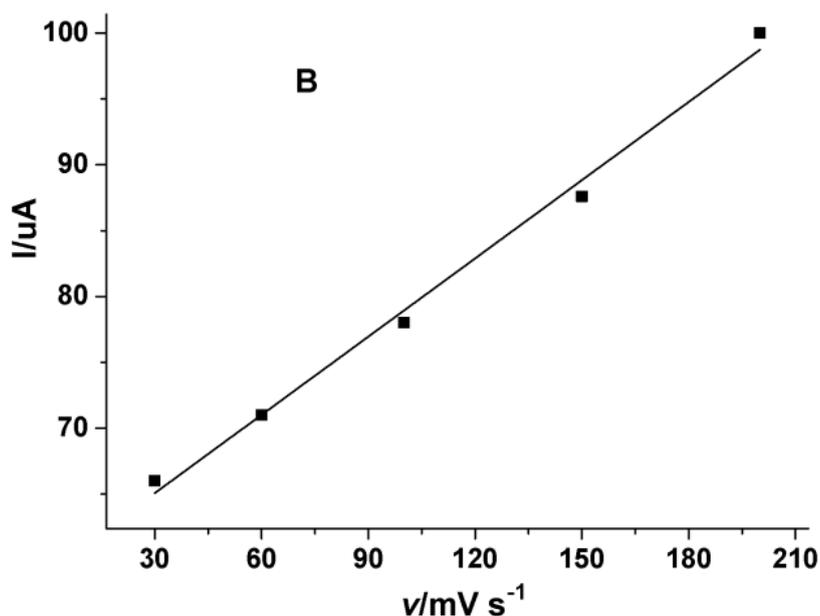
Cyclic voltammetry is commonly used in the study of the interaction between DNA and small molecules by electrochemical methods. Fig. 1 shows the cyclic voltammograms of GCE in different solutions. As can be seen from curve a, no redox peak was observed, which indicated that no reaction

was occurred in PBS. An obvious oxidation peak ( $E_{pa}$ , 0.93V) is observed in curve b, which is ascribed to the oxidation of sudan I. With the addition of DNA, an obvious oxidation peak ( $E_{pa}$ , 0.96V) was also observed in curve c, but the peak current is lower and the oxidation peak potential shift positively.



**Figure 1.** Cyclic voltammograms of GCE in different solutions. a: PBS; b:  $1.0 \times 10^{-4}$  M sudan I; c: 0.8 mg/L DNA. Scan rate:  $100 \text{ mV s}^{-1}$ .





**Figure 2.** (A) Cyclic voltammograms of sudan I at different scan rates, from a-e: 30, 60, 100, 150, 200  $\text{mV s}^{-1}$ . (B) Plot of peak current versus scan rate.

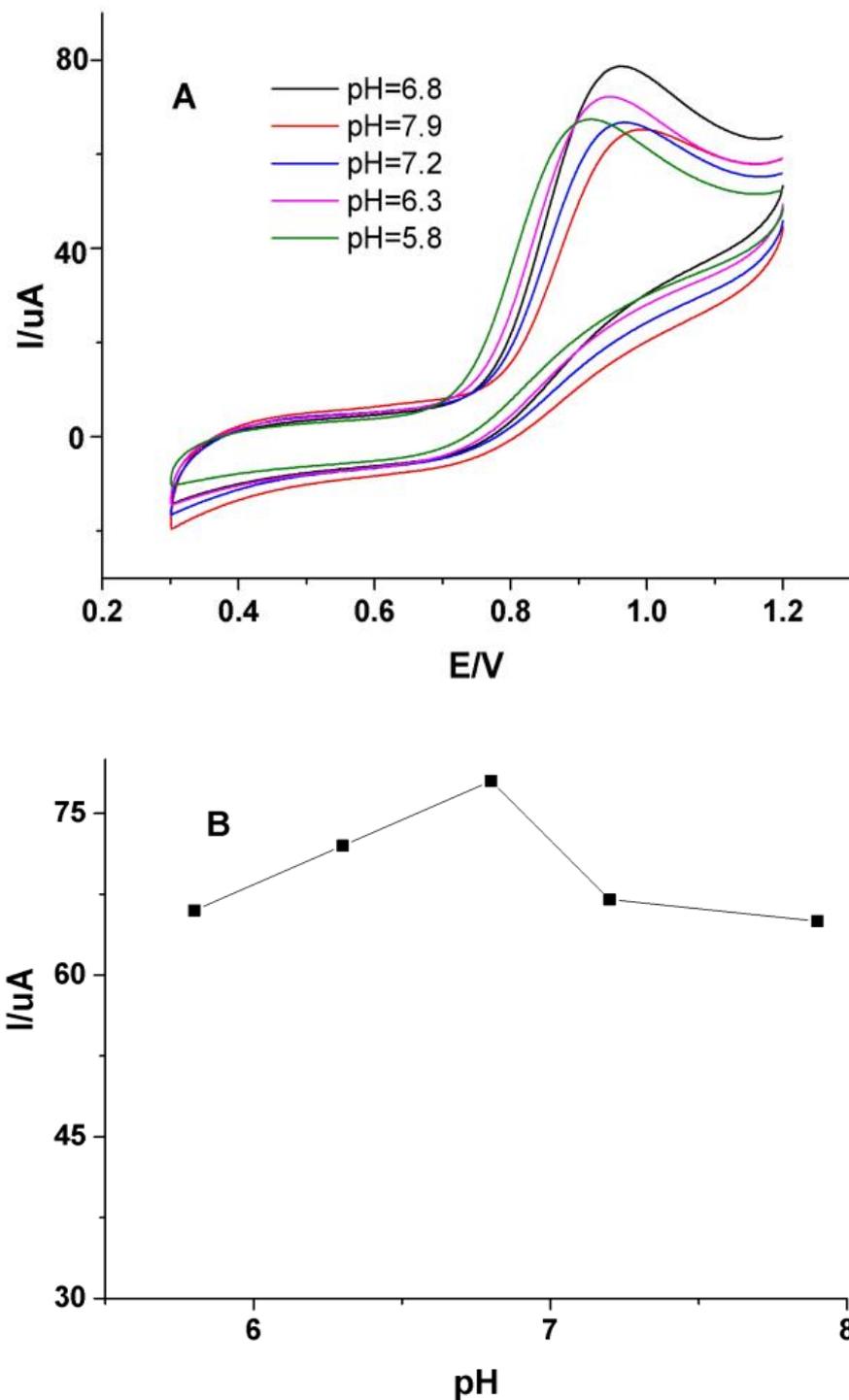
The change of peak potential and peak current of the small molecules is the main basis for judging the interaction between DNA and small molecules. Thus, the decrease in peak current and shifting in peak potential is attributed to the formation of sudan-DNA complex on the electrode surface, the result is similar to the previous reports[18].

The cyclic voltammetric behavior of  $1.0 \times 10^{-4}$  M sudan I was investigated at different scan rates from 30 to 200  $\text{mV s}^{-1}$ . As can be seen from Fig. 2A(curve a - e), the shape of the cyclic voltammogramsthe are similarly, but the potential and peak current are dependent on the scan rates. The oxidation peak current ( $I_{pa}$ ) increases with the increase of scan rate ( $v$ ), moreover, the oxidation peak potential shift positively. As shown in Fig. 2B, the oxidation peak currents ( $I_{pa}$ ) are linearly with the scan rates in the range of 30–200  $\text{mV s}^{-1}$ . The linear regression equation is  $I_{pa} (\mu\text{A}) = 59.1 + 0.20 v$  ( $\text{mV s}^{-1}$ ),  $r = 0.991$ . According to the electrochemical reaction mechanism between the peak current value and scan rate [19], the peak current and the scan rate showed a linear relationship, suggesting that the electrochemical reaction of sudan I at GCE is a surface-controlled electrode process[20].

### 3.2 Effect of solution pH

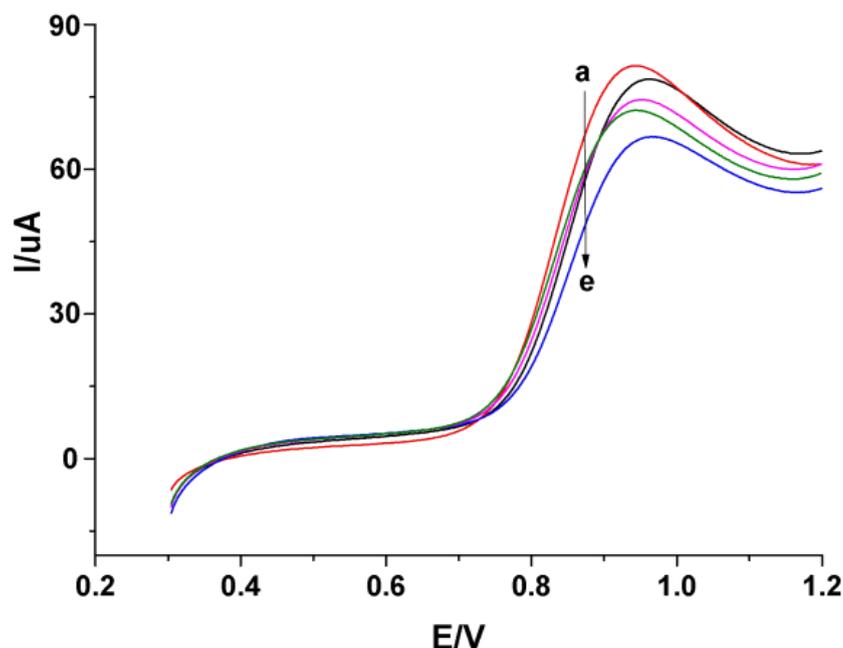
Solution pH is an important factor in studying the interaction between Sudan I and DNA. The pH value of electrolyte will affect the properties of DNA, and will also affect the electrochemical response of Sudan I at GCE[21]. The effect of solution pH was investigated. Fig. 3A shows the cyclic voltammograms of  $1.0 \times 10^{-4}$  M sudan I at different pH values. As can be seen from Fig. 3B, the shape of the cyclic voltammogramsthe are similarly, but the oxidation peak current increases firstly and then decreases, moreover, the oxidation peak potential varying with the corresponding pH, which indicates

that protons are involved in the oxidation process of Sudan I [22]. Thus, to obtain the best oxidation peak current response, a PBS solution of pH= 6.8 was chosen in the following voltammetric measurements.



**Figure 3.** (A) The cyclic voltammograms of  $1.0 \times 10^{-4}$  M sudan I at different pH values. (B) Plot of peak current versus pH values.

### 3.3 Voltammetric study on the interaction of sudan I and DNA



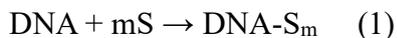
**Figure 4.** Linear scan voltammograms of  $1.0 \times 10^{-4}$  M sudan I with addition of different concentrations of DNA. a-e: 0, 0.3, 0.7, 1.2, 3.0 mg/L.

Different molecules interact with DNA by a number of modes, including electrostatic interaction, intercalation, and minor and major DNA groove binding interaction[23]. Minor groove binding makes intimate contacts with the walls of the groove, and as a result of this interaction, numerous hydrogen bonding and electrostatic interactions occur between a small molecule and DNA bases and its phosphate backbone. Major groove binding occurs via the hydrogen bonding to the DNA and can form a DNA triple helix [24]. The latter type of interaction between a small molecule and DNA can perturb the electron transfer, which will result in the perturbation and changes in electrical responses of DNA, including a considerable decrease in electrochemical signal of the DNA guanine base. This behavior can be used to estimate the binding constant between a small molecule and DNA helix. The intercalative binding is stronger than the other two binding modes because the surface of intercalative molecule is sandwiched between the aromatic, heterocyclic base pairs of DNA [25, 26].

The interaction of sudan I and DNA was studied by voltammetric technique. Fig. 4 shows the linear scan voltammograms of  $1.0 \times 10^{-4}$  M sudan I with addition of different concentrations of DNA. As can be seen from Fig. 4, with the increase of DNA concentration, the peak current of sudan I decreased and the peak potential shifted positively. Moreover, no new oxidation peak appeared. The results show that sudan I interacted with DNA formed a non electrically active compound via intercalation, this is in accordance to the previous reports[27, 28]. This phenomenon could be explained by the shielding of electroactive groups of sudan I while sudan I interacts with DNA at electrode surface.

### 3.4 Binding constant and binding number

The binding constant and binding number of DNA- sudan I compound were calculated. It is assumed that DNA and sudan I formed one kind of simple compound DNA-S<sub>m</sub>, the reaction is expressed as follows:



According to the previous report[29], the equation is:

$$\lg[\Delta I/(\Delta I_{\max} - \Delta I)] = \lg K + m \lg c_{\text{DNA}} \quad (2)$$

Where  $\Delta I$  is the change of the peak current after the addition of DNA,  $\Delta I_{\max}$  is the maximum value of change of the peak current after the addition of DNA,  $c_{\text{DNA}}$  is the concentration of DNA in the solution,  $K$  is binding constant and  $m$  is binding number. Therefore, a linear regression equation of  $\lg[\Delta I/(\Delta I_{\max} - \Delta I)] = 3.159 + 1.104 \lg c_{\text{DNA}}$ ,  $r = 0.9905$  is obtained. According to the slope and intercept of the equation, the binding number of sudan I and DNA is 1 and binding constant is  $1.44 \times 10^3$ . Mousavi et al. reported that the neutral red bind to DNA with an affinity constant of  $2.76 \times 10^4$  [30] and the binding constant of spermidine with DNA is  $1.85 \times 10^5$  [31]. The observed differences in the reported values of the binding constant might be due to the different small molecules, solution conditions and the study methods.

## 4. CONCLUSIONS

This work studied the electrochemical behavior of sudan I at glassy carbon electrode and investigated the interaction of sudan I with DNA by voltammetric technique. The results indicated that the binding of sudan I to DNA was via intercalation. The binding number 1 and binding constant  $1.44 \times 10^3$  were obtained.

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