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Original scientific paper

MnO_2 nanorods modified screen-printed electrode for the electrochemical determination of Sudan dye in food sample

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Abstract

A novel MnO_2 nanorods modified screen-printed electrode was fabricated and used as a voltammetric sensor for Sudan determination. MnO_2 nanorods were characterized using Field emission-scanning electron microscopy (FE-SEM). Electrochemical measurements were performed using cyclic voltammetry (CV), linear sweep voltammetry (LSV), differential pulse voltammetry (DPV), and chronoammperometry (CA). The MnO_2 nanorods on the electrode surface act as an excellent catalyst for the Sudan oxidation reaction. Our modified electrode presents good electrocatalytic activity toward Sudan, a short response time of <10 s, a low detection limit of around 0.08 µM, and linear detection range from 0.25 to 300.0 µM.

Keywords

Voltammetric sensor; cyclic voltammetry; electroanalysis; Sudan red

Introduction

All food dyes can be divided into two basic, general categories: natural dyes and artificial dyes. The natural food dyes are derived from grapes, saffron, paprika, carrots, beets, and algae and are used to color a variety of foods. The artificial food dyes (AFCs), mostly derived from petroleum, contain a single or more azo functional groups (–N=N–), most frequently connecting the two aromatic parts. People associate specific colors with specific flavors, therefore, the colors of food can affect their perception of taste, especially their perception of sweets and beverages. Artificial dyes may improve on natural variations in color, may enhance colors that occur naturally, or may provide color to colorless and "fun" food, thereby making it appear more attractive and appetizing, *e.g.,* adding a red, yellow or green color to gummy sweets, which would naturally be colorless [1,2].

Sudan red I (1-phenylazo-2-naphthol), a synthetic azo dye, is considered to be a genotoxic carcinogen and classified as a category 3 carcinogen by the International Agency for Research on Cancer (IARC) (1975). Its presence is prohibited in foodstuffs for any purpose at any level worldwide. Unfortunately, a variety of foodstuffs contaminated with Sudan dyes and Para red, particularly

Sudan red I, have been detected throughout Europe and Asia [3-5]. High-performance liquid chromatography-mass spectrometry (HPLC–MS) has been proven to be an excellent method for the direct determination of Sudan reds (I, II, III, and IV). Although sensitive and specific, this method can be time-consuming and expensive. Therefore, it is necessary to develop a rapid and economical method for detecting Sudan red I [6].

The electrochemical sensors are the most developed analytical tools for detecting the analyte. Electrochemical detection has the advantages of simple operation, low cost, and easy to carry, but the specific recognition of the electrochemical method in detection is poor. The chemical modification of inert substrate electrodes offers significant advantages in the design and development of electrochemical sensors [7-26]. A further advantage of chemically modified electrodes is that they are less prone to surface fouling and oxide formation compared to inert substrate electrodes [27-39].

In this context, electrochemical sensors based on screen-printed electrodes (SPEs) have gained increasing interest as analytical tools for food analysis since SPEs provide great advantages that make these kinds of sensors have the important characteristics of ideal sensors: ease of use, low cost, and portability [40-42]. So, the screen-printed technology has significantly contributed to the transition from the traditional unwieldy electrochemical cells to miniaturized and portable electrodes that meet the needs for on-site analysis. Although a screen-printed electrode (SPE) is not as robust as a conventional electrode, such as glassy carbon or gold disk, and the surface of its working electrode is not as perfect as the one of a mirror-like polished solid electrode, the advantages of SPEs regarding cost and size led to their increasing use in the last years as transducers in sensing. The use of SPE-based sensors in the control of food spoilage as complementary analytical tools to the conventional methods allows a rapid screening at any point of the food production chain, preventing the occurrence of foodborne illnesses and the reduction of food waste [43].

The development of nanoscience and nanotechnology has allowed trials to apply different nano-materials to fabricate chemically modified electrodes [44-53]. In recent years, various nanomaterials have been used singly or in composite form to modify electrodes [54-60]. Manganese dioxide (MnO_2), as a type of transition metal oxide, has received much more attention for its role as an electrode material for sensors, batteries and supercapacitors attributed to its good electrochemical performance in the neutral electrolyte, high theoretical capacity and nontoxicity [61].

In this research work, an electrochemical sensor modified with MnO_2 nanorods (MnO_2–NRs) was designed using a screen-printed electrode for the determination of Sudan.

Experimental

Apparatus and chemicals

All the electrochemical measurements were carried out on a PGSTAT302N potentiostat/galvanostat Autolab. The measurement cell consisted of SPE (DropSens; DRP-110: Spain) containing a graphite counter electrode, a graphite working electrode, and a silver pseudo-reference electrode. Solution pH values were determined using a 713 pH meter combined with a glass electrode (Metrohm, Switzerland). Sudan and other chemicals used were analytical grade and were purchased from Merck. Orthophosphoric acid and its salts (NaH_2PO_4, Na_2HPO_4 and Na_3PO_4) were utilized to prepare the phosphate buffer solutions (PBSs), and sodium hydroxide was used for adjusting the desired pH values (pH range between 2.0 and 9.0). MnO_2 nanorods (MnO_2-NRs) were synthesized in our laboratory. So that first, 0.316 g of $KMnO_4$ was dissolved in deionized water (30 ml) under stirring. Then, 1.4 mL of HCl (3.0 M) was added into the above solution under stirring for 30 min.

The achieved solution was transferred into a Teflon Lined autoclave at 160 °C for 6 h. Next, it was cooled down at room temperature to gather the products via centrifugation, followed by washing with ethanol and deionized water several times. The product was finally dried in an oven at 60 °C for 12 h. Figure 1 shows the FE-SEM image of MnO_2-NRs.

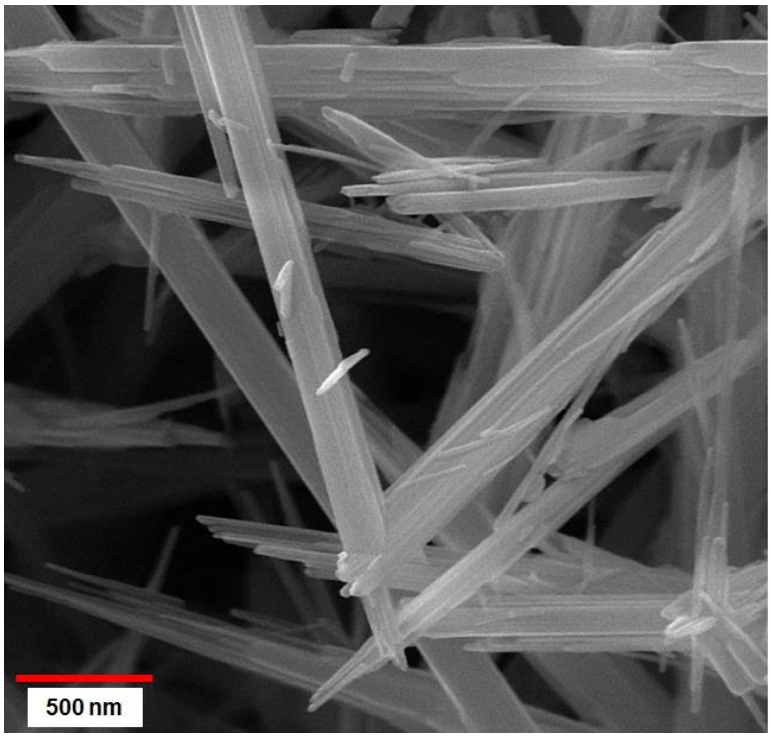
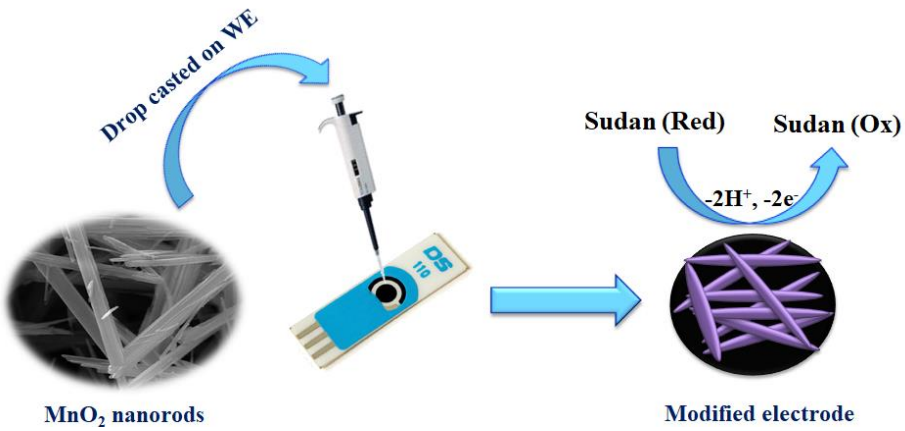


Figure 1. *FE-SEM image of MnO_2-NRs*

Preparation of MnO_2-NRs/SPE

First, 1 mg of prepared MnO_2-NRs was added into an aqueous solution (1 ml), followed by sonication for 30 min to give a homogeneous solution. Then, 4 µL of MnO_2–NRs were dispersed on the surface of SPE dropwise. Following the solvent's evaporation, the sensor's surface was washed several times with deionized water to clean free modifier molecules and subsequently air-dried. The obtained electrode was noted as MnO_2-NRs/SPE. The schematic of SPE surface modification for the oxidation of Sudan is shown in Scheme 1.

The surface areas of the MnO_2-NRs/SPE and the unmodified SPE were obtained by CV using 1 mM $K_3Fe(CN)_6$ at various scan rates. Using the Randles–Ševčik equation for MnO_2-NRs/SPE, the electrode surface was found to be 0.122 cm^2 which was about 3.9 times greater than un-modified SPE.



Scheme 1. *Schematic of SPE surface modification for the oxidation of Sudan*

Results and discussion

Electrochemical behavior of Sudan at the surface of various electrodes

The effect of the electrolyte pH on the oxidation of 70.0 µM Sudan was investigated at MnO_2-NRs/SPE using differential pulse voltammetry (DPV) measurements in the PBS in the pH range from 2.0 to 9.0. According to the results, the oxidation peak current of Sudan depends on the pH value and increases with increasing pH until it reaches the maximum at pH 7.0 and then decreases at higher pH values. The optimized pH corresponding to the higher peak current was 7.0, indicating that protons are involved in the reaction of Sudan oxidation.

The electrochemical behavior of Sudan was also investigated by CV. The cyclic voltammetry obtained using the bare SPE (trace b) and MnO_2-NR/SPE (trace a) in 0.1 M PBS (pH 7.0) in the presence of 100.0 µM Sudan is shown in Figure 2. On a bare SPE, a signal with a low oxidation current of ~3.2 µA was obtained with a peak potential of ~680 mV. In contrast, MnO_2-NRs/SPE exhibited an enhanced sharp anodic peak current (I_{pa} =11.0 µA) at a much lower overpotential E_p = 610 mV. These results confirmed that the MnO_2-NRs/SPE improved the sensitivity of the modified electrode by enhancing peak current and decreasing the overpotential of the oxidation of Sudan.

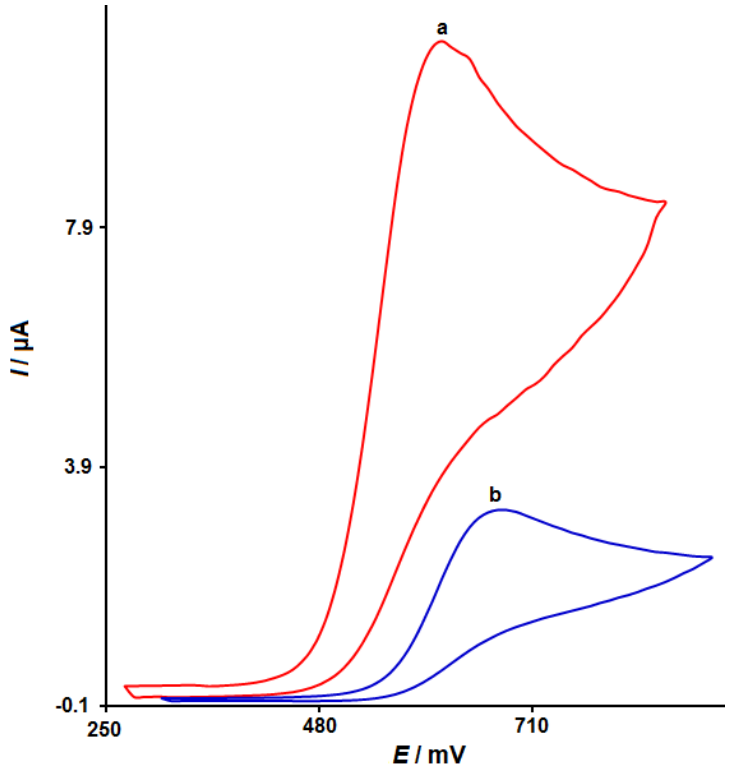


Figure 2. *Cyclic voltammograms of (a) MnO_2–NRs/SPE and (b) bare SPE in 0.1 M PBS (pH 7.0) in the presence of 100.0 µM Sudan at the scan rate 50 mVs^{-1}*

Effect of scan rate on the determination of Sudan at MnO_2–NRs/SPE

The influence of the scan rate (ν) on the peak currents (Ipa) of Sudan at MnO_2-NRs/SPE was investigated by LSV (Figure 3). Figure 4 shows the voltammetric response of 100.0 µM Sudan at MnO_2-NRs/SPE at different scan rates in the range of 5 to 400 mV/s. The oxidation peak current of Sudan increases linearly with increasing scan rate. A linear regression equation was obtained from the plot I_{pa} *vs.* $\nu^{1/2}$ (square root of scan rate) as follows; I_{pa} = 1.4523 $\nu^{1/2}$ + 0.8751 (R^2 = 0.9990) for the oxidation process, which indicates that the reaction of Sudan at MnO_2-NRs/SPE is diffusion controlled.

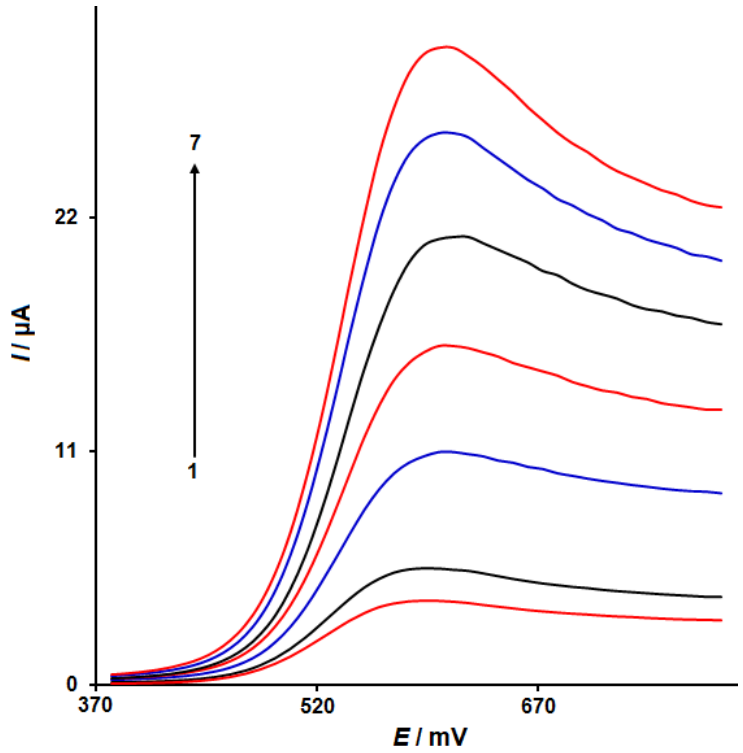


Figure 3. *Linear sweep voltammograms of MnO_2-NRs/SPE in 0.1 M PBS (pH 7.0) containing 100.0 µM Sudan at various scan rates; 1-7 correspond to 5, 10, 50, 100, 200, 300 and 400 mV s^{-1}*

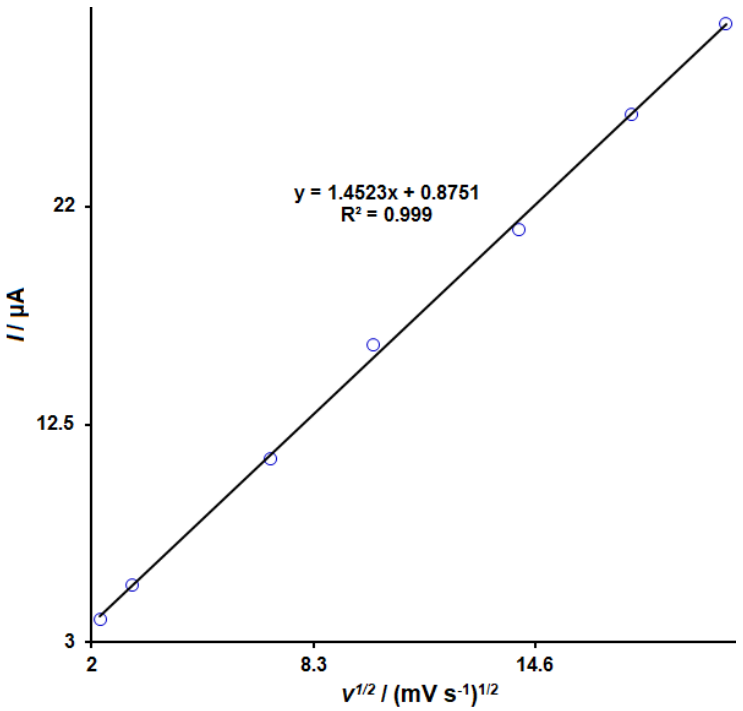


Figure 4. *Plot of anodic peak current vs. $v^{1/2}$ at different scan rates in the range of 5 to 400 mV/s*

Chronoamperometric analysis

The analysis of chronoamperometry for Sudan samples was performed using MnO_2-NRs/SPE at 0.66 V. The chronoamperometric results of different concentrations of Sudan in PBS (pH 7.0) are demonstrated in Figure 5. The Cottrell equation for the chronoamperometric analysis of electroactive moieties under mass transfer limited conditions is as in equation (1):

$$I = nFAD^{1/2}C_b\pi^{-1/2}t^{-1/2} \quad (1)$$

where D represents the diffusion coefficient ($cm^2\ s^{-1}$), and C_b is the applied bulk concentration ($mol\ cm^{-3}$). Experimental results of I *vs.* $t^{-1/2}$ were plotted in Figure 6A, with the best fits for different concentrations of Sudan. The resulting slopes corresponding to straight lines in Figure 6A were then plotted against the concentration of Sudan (Figure 6B). The mean value of D was determined to be $4.5\times10^{-5}\ cm^2/s$ according to the resulting slope and Cottrell equation.

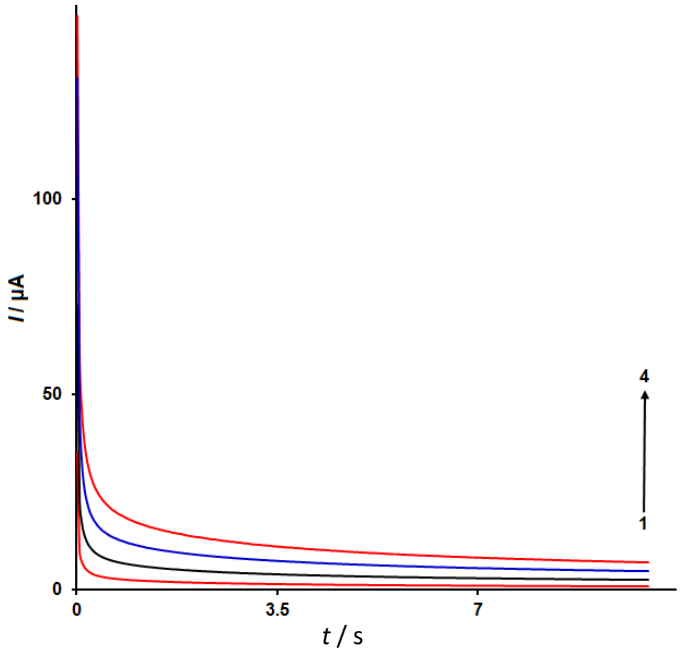


Figure 5. *Chronoamperograms obtained at MnO_2-NRs/SPE in 0.1 M PBS (pH 7.0) for different concentrations of Sudan. The 1-4 correspond to 0.1, 0.5, 1.0 and 1.5 mM of Sudan*

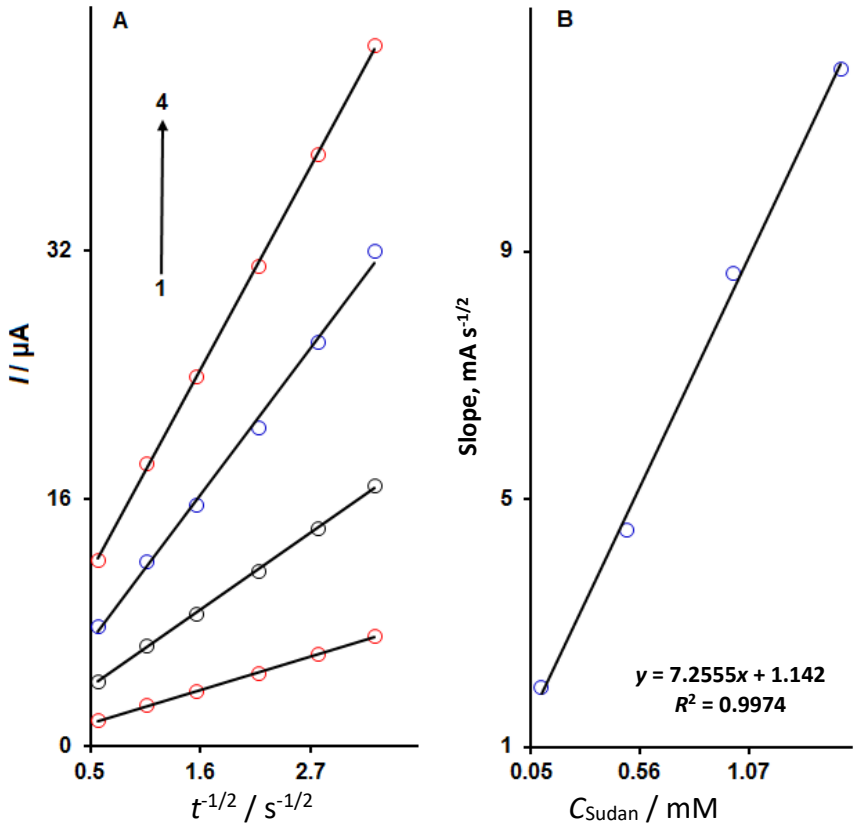


Figure 6. *(A) Plots of* I vs. $t^{-1/2}$ *obtained from chronoamperograms 1-4. (B) Plot of the slope of the straight lines against Sudan concentration (0.1-1.5 mM)*

Calibration curve

Because DPV commonly has a higher sensitivity than cyclic voltammetry, the DPV technique was applied for the quantitative detection of Sudan. Figure 7 shows the differential pulse voltammograms of Sudan at various concentrations using MnO_2-NRs/SPE (step potential of 0.01 V and pulse amplitude of 0.025 V). As seen, the oxidation peak currents of Sudan enhance gradually by increasing its concentration. The oxidation peak currents (I_{pa}) show a good linear relationship with the concentrations of Sudan ranging from 0.25 to 300.0 µM. The linear equation is $I_{pa} = 0.0952C_{Sudan} + 1.296$ ($R^2 = 0.9995$) (Figure 8). Also, the limit of detection, Cm, of Sudan was calculated using the equation (2):

$$C_m = 3S_b/m \quad (2)$$

where, m is the slope of the calibration plot (0.0952 µA/ µM) and S_b is the standard deviation of the blank response obtained from 15 replicate measurements of the blank solution. The detection limit of 0.08 µM was obtained for the determination of Sudan using this method.

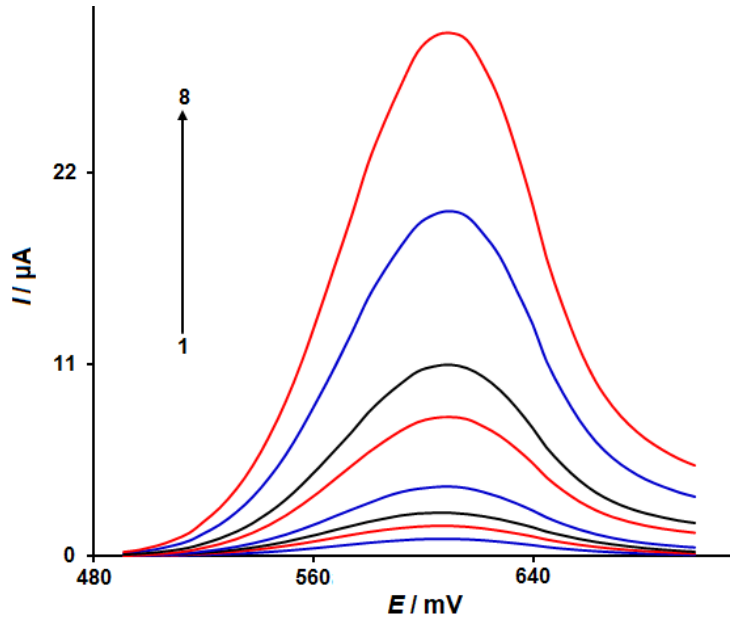


Figure 7. *DPVs of MnO_2-NRs/SPE in 0.1 M (pH 7.0) containing different concentrations of Sudan. Numbers 1-8 correspond to 0.25, 2.5, 10.0, 30.0, 70.0, 100.0, 200.0 and 300.0 µM of Sudan*

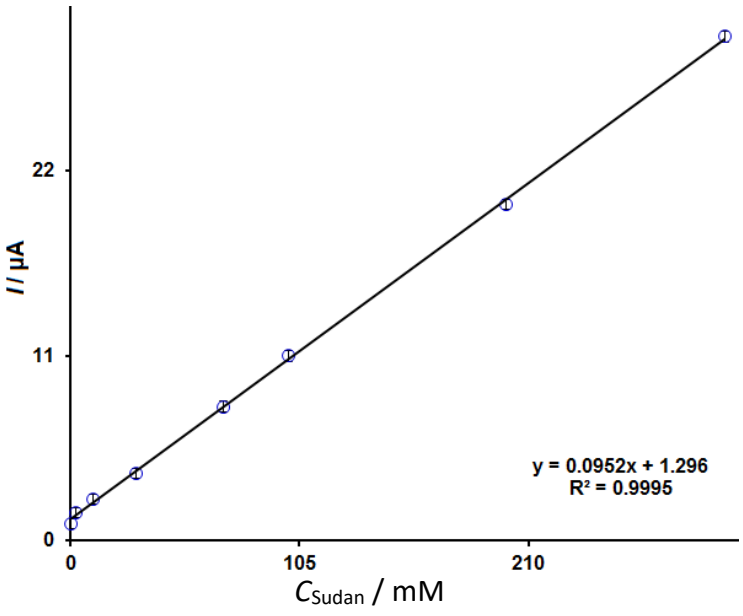


Figure 8. *Plot of the electrocatalytic peak current as a function of C_{Sudan} in the range of 0.25-300.0 µM*

Analysis of real samples

The real samples for the analysis were prepared and quantified by the DPV method. The developed sensor was applied to detect Sudan in tomato paste and ketchup sauce samples. The results are summarized in Table 1. Each measurement was repeated 3 times. The recovery and relative standard deviation (RSD) values confirmed that the MnO_2-NRs/SPE sensor has a great potential for analytical application.

Table 1. *The application of MnO_2–NR/SPE for determination of Sudan in real samples (n=3)*

Sample	Concentration, µM		Recovery, %	RSD, %
	Spiked	Found		
Tomato paste	0	-	-	-
	4.0	3.9	97.5	3.2
	6.0	6.3	105.0	1.8
Ketchup sauce	0	-	-	-
	5.0	5.1	102.0	2.2
	7.0	6.9	98.6	2.9

Conclusion

Modifications of the screen-printed electrode with MnO_2 nanorods for sensing Sudan in food samples were investigated. MnO_2-NRs/SPE electrodes were used as Sudan sensors by using CV, LSV, CA and DPV techniques. The results showed that the electrodes gave linearity of 0.25 to 300.0 µM, and a detection limit of 0.08 µM. The diffusion coefficient for Sudan using MnO_2-NRs/SPE, 4.5×10^{-5} cm^2/s was obtained. The analysis of real food samples spiked with sudan gave satisfactory results with recovery values between 97.5 and 105.0 %.

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