Original scientific paper

Synthesis of vanadium oxide nanoplates for electrochemical detection of amaranth in food samples

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Abstract

Amaranth dye is an organic compound largely used in the food and beverage industries with potential toxicity effects on humans. In this paper, a new electrochemical sensor used for the determination of amaranth in foods was reported, where a kind of V_2O_5 nanoplates (V_2O_5-NPs) was employed as electrode modifying materials. The V_2O_5 nanoplates modified electrode enhanced its electrochemical signal obviously in the determination of amaranth in foods and exhibited a wider linear response ranging from 0.1-270.0 µM with a low detection limit of 0.04 ± 0.001 µM ($3S_b/m$). This work offers a new route in developing new electrochemical sensors for the determination of colorant additives and other hazardous components in foods.

Introduction

The rapid development of the food industry leads to an increasing number of specific products with a certain shape, colour, taste, smell, texture, *etc.* Thus, different food additives such as presservatives, sweeteners, thickeners, and colouring agents are used to improve the organoleptic properties of the food. Dyes play a special role here, as the food quality and taste are often associated with its colour. Colorants have been used throughout history, from the ancient ages to the present days. In ancient times, natural colorants were used to make cave paintings and some of these can still be found in the Altamira cave (Spain) and Lascaux cave (France). Dyes are classified into natural and synthetic. Natural dyes are extracted from plant and animal sources by physical methods. With the great development of science and technology, synthetic dyes are the foundation of various industries, including food, textile, pharmaceuticals, paper, leather, and cosmetics owing to their versatile colours, easy preparation, and low costs. Synthetic dyes can be classified by their chemical structures. The groups of atoms deciding the dye colours are called

chromophores and auxochromes. The chromophores include azo (-N=N-), carbonyl (-C=O), nitro (-NO$_2$), methine (-CH =) and quinoid groups [1,2].

Azo dyes form the largest group of artificial food dyes. Synthetic azo dyes such as amaranth, tartrazine, sunset yellow, brilliant blue etc., are used in most food items to make them more attractive. When compared with many natural dyes, these offer harmful effects on the human body, and many of them are suspected carcinogens too. The proposed study is the determination of water-soluble synthetic food colourant amaranth, (trisodium(4E)-3-oxo-4-[(4-sulfonato-1-naphthyl) hydrazono] naphthalene-2,7-disulfonate), using voltammetric techniques. Amaranth has been extensively used in food, textiles, pharmaceuticals, etc. due to its attractive dark red to purple colour .Therefore, it is very important and indispensable to develop a sensitive, rapid analytical method for the determination of amaranth in diverse food products [3-6].

There have been numerous investigations to achieve techniques for effective and green detection of azo dyes [7-9]. The electrochemical methods are showing great attention in studying analytes redox behaviour, and a high interest in electrochemical methods giving a fast response, low cost, high sensitivity, and selectivity exists [10-15]. Voltammetry is a technique utilized for the analysis of compounds that undergo oxidation or reduction. In the voltammetry methods normally using electrodes such as glassy carbon electrodes (GCE), carbon paste electrodes (CPE), screen printed electrodes (SPE), along with some modifiers [16-22]. Different modifiers will show different electrochemical activities [23-28]. SPEs are designed to analyse low sample volumes and include three electrodes (working, reference and counter electrode) in the same device. SPEs are mass produced at low cost and are thus disposable. Considering such remarkable advantages, SPEs have received widespread acceptance in fields of analytical chemistry, including food analysis [29].

In recent years, interests have focused on the use of nanosized materials in various fields [30-38]. Nanomaterials are applied in the fabrication of electrochemical sensors due to an increased surface area of the electrodes, facile electron transfers and decreased surface fouling [39-44]. The vanadium oxide (V$_2$O$_5$), as n-type semi-conducting metal oxide, has impressive features like optical bandgap energy (2.3 eV), thermo-electric potentials, appreciable chemical, and thermal stability. Accordingly, the V$_2$O$_5$ has been used for different purposes like electrochemical sensors and lithium-ion battery [45-47].

Therefore, the current work aimed to employ the stripping voltammetric technique using a screen-printed electrode modified with V$_2$O$_5$ nanoplates for sensitive, selctive and accurate detection of amaranth in real specimens.

Experimental

Chemicals and apparatus

The electrochemical measurements were performed with an Autolab potentiostat/galvanostat (PGSTAT 302N; Eco Chemie: The Netherlands). Moreover, General Purpose Electrochemical System (GPES) software has been used to control the experimental condition. Notably, the SPE (DropSens; DRP-110: Spain) involved 3 major sections of the graphite counter electrode, a graphite working electrode, as well as a silver pseudo-reference electrode. Finally, we employed a Metrohm 710 pH-meter to measure the pH.

All reagents had an analytical grade. These products have been purchased from Merck (Darmstadt; Germany).

Orthophosphoric acid was utilized to freshly prepare all needed phosphate buffer solutions (PBSs), and sodium hydroxide was responsible for adjusting the desired pH values (the pH range between 2.0 and 9.0).

V_2O_5 nanoplates synthesis

According to a typical production protocol, the ammonium metavanadate (0.5 g, AMV, from Sigma-Aldrich) was dispersed in deionized water (50 mL) while magnetic stirring. The solution pH value was dropwise adjusted to about ~2 using nitric acid while stirring continuously at 323 K. The evaporation of water during stirring resulted in an orange-coloured gel of vanadium complex that was then dissolved in methanol (20 mL) in a vial and subsequently allowed to age at an ambient temperature for 24 hours. The change in the gel colour from light to dark orange occurred when it was left in methanol. Afterward, the resultant product was washed several times with deionized water prior to the next testing.

Preparing the electrode

The V_2O_5 nanoplate stock solution in the aqueous solution (1 mL) was prepared through the dispersion of V_2O_5 nanoplates (1 mg) under ultra-sonication for half an hour, whereas 5 µl aliquot of V_2O_5 nanoplates was cast on carbon working electrode, followed by the solvent evaporation at the room temperature. The surface areas of V_2O_5-NP/SPE and the unmodified SPE were obtained by cyclic voltammetry (CV) using 1 mM $K_3Fe(CN)_6$ at various scan rates. Using the Randles-Sevcik equation for V_2O_5-NP/SPE, the electrode surface was found to be 0.096 cm^2 which was about 3.1 times greater than unmodified SPE. The brief preparation process of the V_2O_5-NP/SPE for the determination of amaranth is shown in Scheme 1.
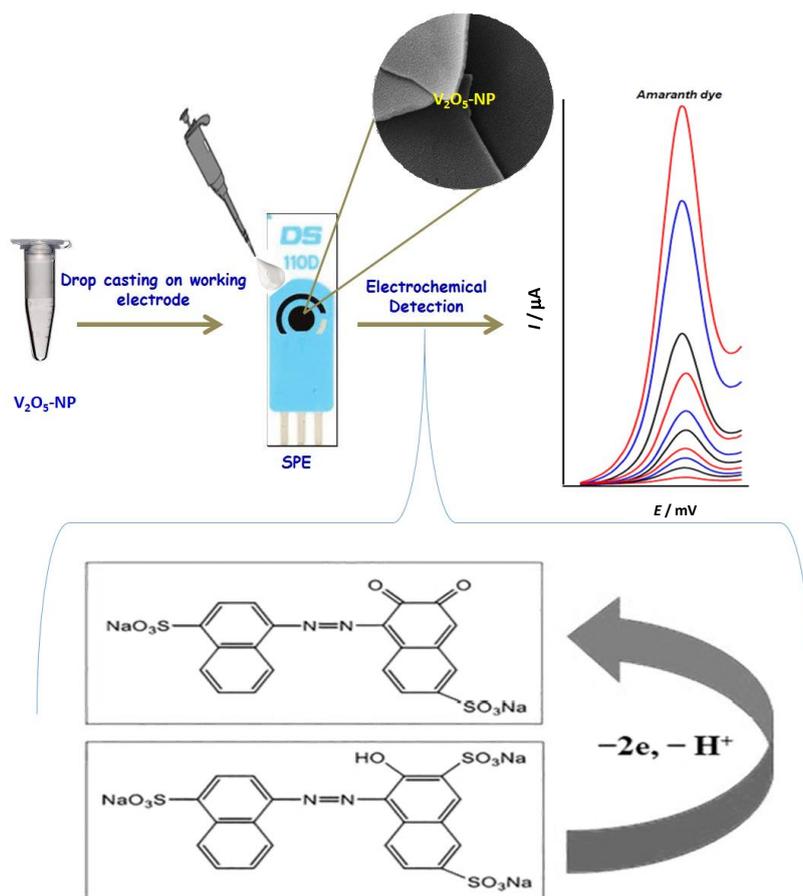
Scheme 1. *The schematic preparation of V_2O_5-NP/SPE for determination of amaranth*

Result and discussion

Structure and morphology

The morphology and structure of the prepared sample were then investigated by field-emission scanning electron microscopy (FE-SEM) (Figure 1). It is observed that V_2O_5 nanoparticles with plate-like morphology have grown well. The thicknesses of the V_2O_5 nanoplates are around 26 nm.
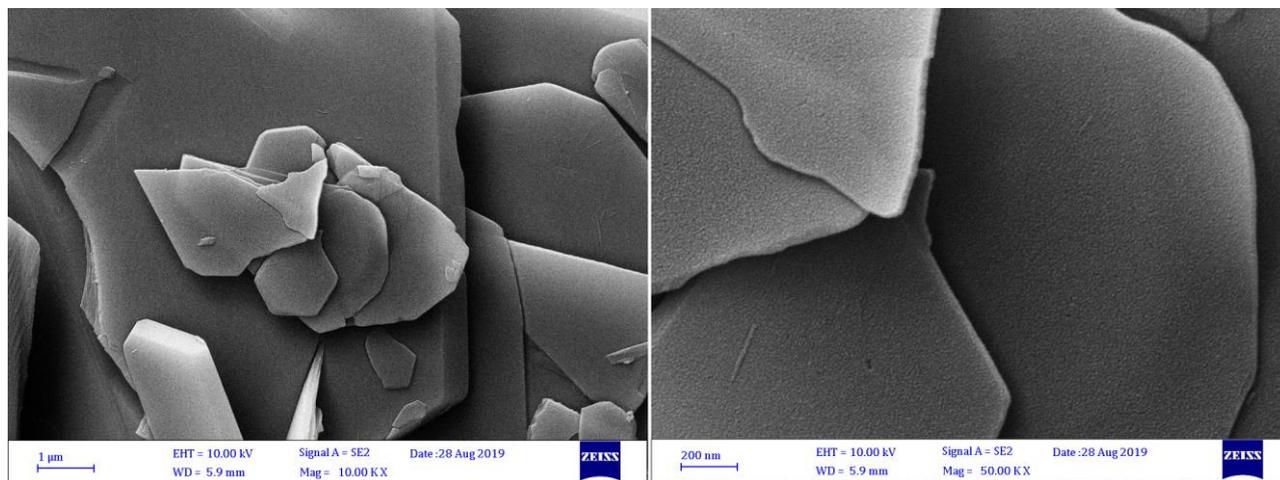
Figure 1. *The FE-SEM images of V_2O_5 nanoplates at different magnifications*

The elemental analysis from the energy dispersive X-ray spectroscopy (EDX) measurement is presented in Figure 2. The EDS spectrum shows peaks corresponding to V (73.6 wt.%) and O (26.4 wt.%) elements, thereby confirming the successful formation of the V_2O_5 nanoplates without any impurities. Also, to investigate the distribution of elements in the V_2O_5 nanoplates, the elemental mapping images of V_2O_5 are shown in Figure 3.

Element	Content, wt.%	σ
V	73.6	1.3
O	16.4	1.3
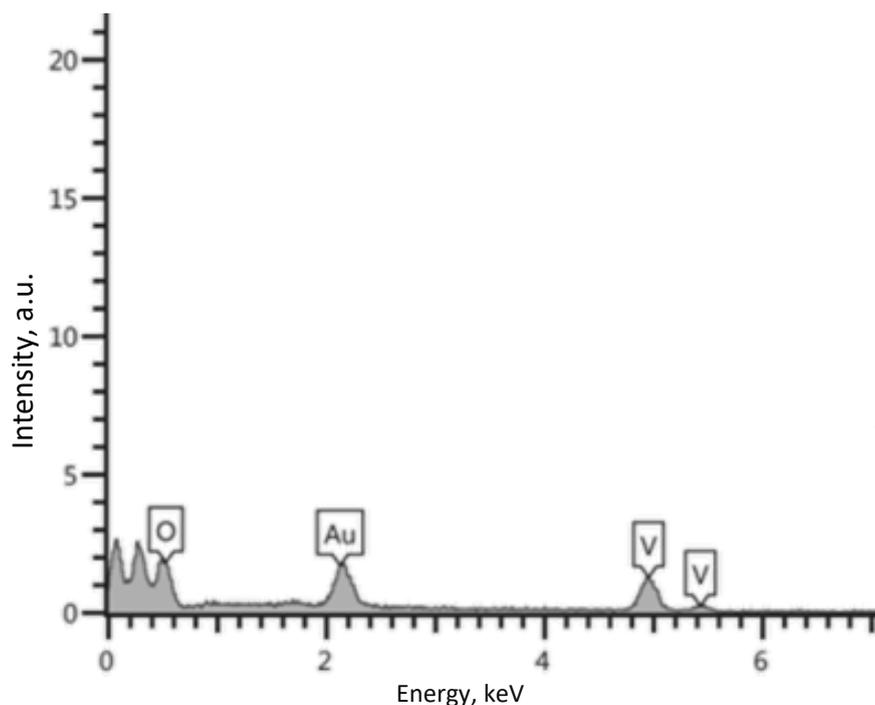
Figure 2. *The EDX spectrum of V_2O_5 nanoplates*
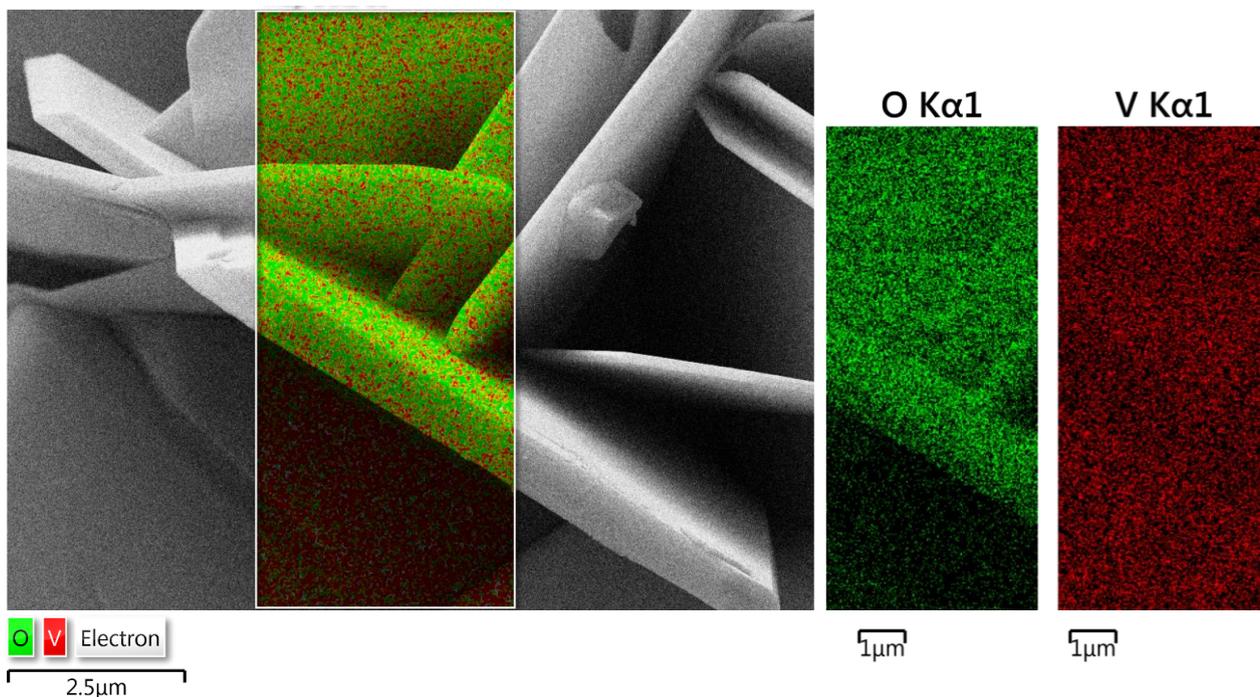
Figure 3. *The elemental mapping images of V$_2$O$_5$ nanoplates*

Electrochemical response of amaranth at different electrodes

The effect pH value of electrolyte solution was investigated by differential pulse voltammograms (DPV) in 0.1 M phosphate buffer solution (PBS) at the pH range from 2.0 to 9.0 containing 40.0 µM amaranth on the V$_2$O$_5$-NPs/SPE surface. The oxidation peaks current of amaranth reached a maximum value at pH 7.0, and therefore PBS with pH 7.0 was chosen as the optimum pH to detect amaranth.

Figure 4 displays the linear sweep voltammograms (LSV) of the amaranth at unmodified SPE (curve b) and V$_2$O$_5$-NPs/SPE (curve a), with the same concentration of 200.0 µM in 0.1 M PBS (pH 7.0).
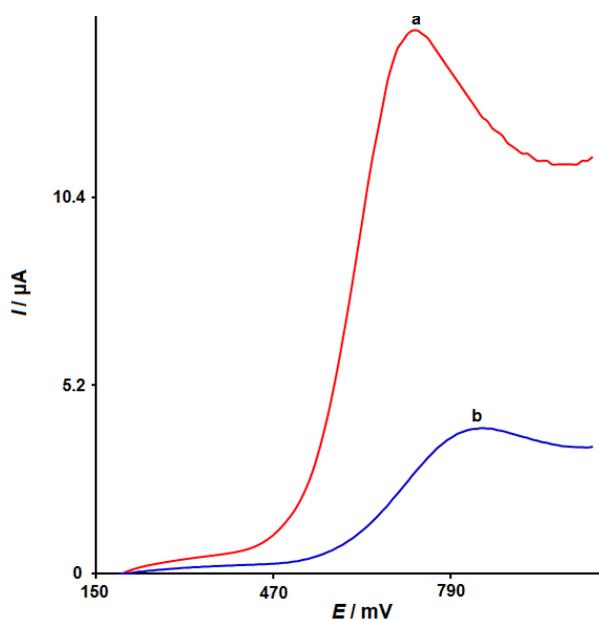
Figure 4. *Linear sweep voltammograms curves of unmodified SPE (curve b) and V$_2$O$_5$-NP/SPE (curve a) in 0.1 M PBS containing 80.0 µM amaranth; scan rate: 50 mV s^{-1}*

The anodic peak potential for the oxidation of amaranth at V$_2$O$_5$-NPs/SPE (curve a) is about 740 mV compared with 790 mV, for that on the unmodified SPE (curve b). Similarly, when the oxidation of

amaranth at the V_2O_5-NPs/SPE (curve a) and unmodified SPE (curve b) are compared, an extensive enhancement of the anodic peak current at V_2O_5-NPs/SPE, relative to the value obtained at the unmodified SPE (curve b), is observed. In other words, the results clearly indicate that the V_2O_5-NPs improve amaranth oxidation.

Effect of scan rate on the determination of amaranth at V_2O_5-NPs/SPE

The influence of the scan rate (v) on the peak currents (Ipa) of amaranth at V_2O_5-NPs/SPE was investigated by LSV. Figure 5 shows the voltammetric response of 80.0 µM amaranth at V_2O_5-NPs/SPE at different scan rates in the range of 10 to 300 mV s^{-1}. The oxidation peak current of amaranth increases linearly with increasing scan rate. Linear regression equation was obtained from the plot I_{pa} and *vs.* $v^{1/2}$ (square root of scan rate) as follows; $I_{pa} = 1.4242\,v^{1/2} - 1.7969$ ($R^2 = 0.9991$) for the oxidation process, which indicates that the reaction of amaranth at V_2O_5-NPs/SPE is diffusion controlled.
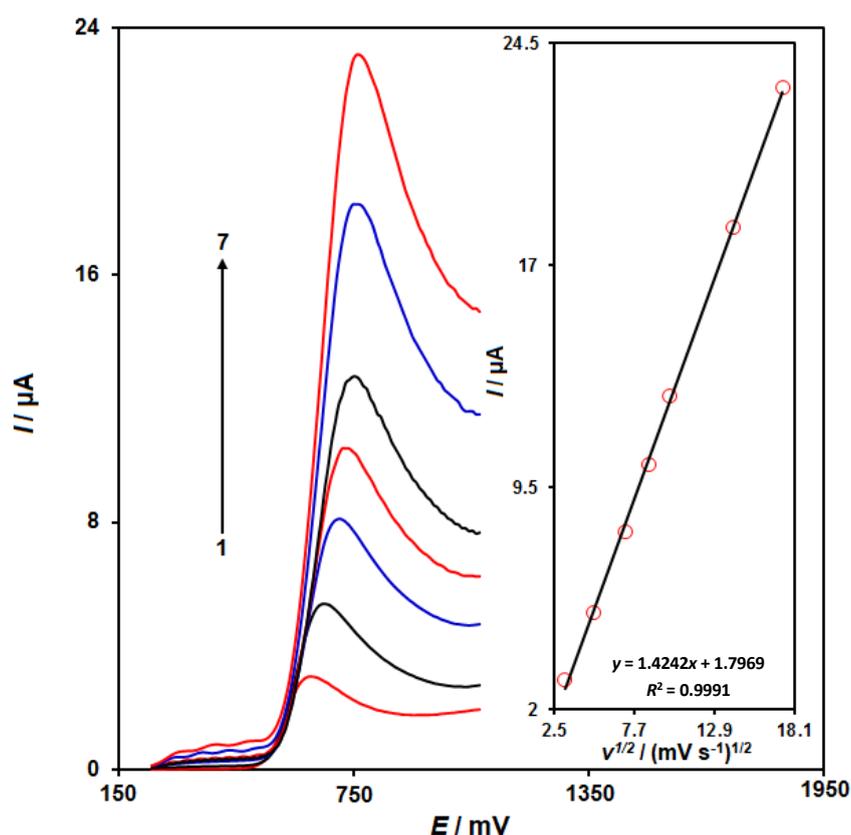
Figure 5. *Linear sweep voltammograms of V_2O_5-NP/SPE in 0.1 M PBS (pH 7.0) containing 80.0 µM amaranth at various scan rates; 1-7 correspond to 10, 25, 50, 75, 100, 200 and 300 mV s^{-1}, respectively. Inset: variation of anodic peak current vs. $v^{1/2}$*

Chronoamperometric analysis

Chronoamperometric measurements of amaranth at V_2O_5-NPs/SPE were carried out by setting the working electrode potential at 0.78 V for the various concentrations of amaranth in 0.1 M PBS (pH 7.0) (Figure 6). For an electroactive material (amaranth in this case) with a diffusion coefficient of *D*, the current observed for the electrochemical reaction at the mass transport limited condition is described by the Cottrell equation. Experimental plots of *I* vs. $t^{-1/2}$ were employed, with the best fits for different concentrations of amaranth (Figure 6A). The slopes of the resulting straight lines were then plotted *vs.* amaranth concentration (Figure 6B). From the resulting slope and Cottrell equation, the mean value of the *D* was found to be 3.3×10^{-6} cm^2 s^{-1}.
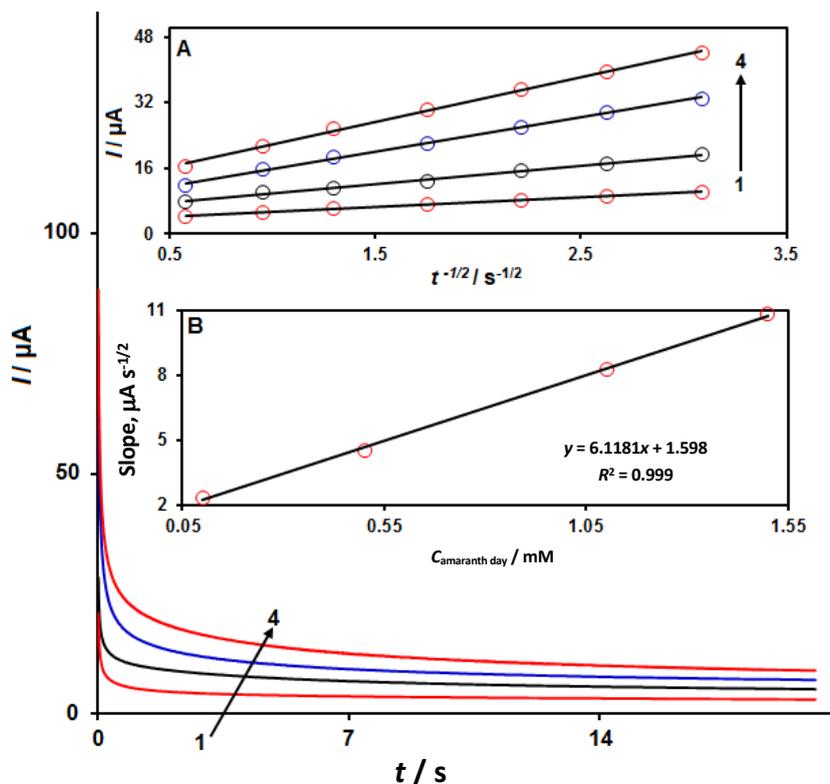
Figure 6. *Chronoamperograms obtained at V_2O_5-NP/SPE in 0.1 M PBS at pH of 7.0 for different concentrations of amaranth (1-4 refer to: 0.1, 0.5, 1.1 and 1.5 mM). A - The I plot versus $t^{-1/2}$ observed by chronoamperograms 1-4; B - The slope plot of the straight line vs. concentration of amaranth*

Calibration curve

Because DPV commonly has a higher sensitivity than CV, the DPV technique was applied for the quantitative detection of amaranth. Figure 7 shows the differential pulse voltammograms of amaranth at various concentrations using V_2O_5-NPs/SPE (Step potential = 0.01 V and pulse amplitude = 0.025 V). As seen, the oxidation peak currents of amaranth enhance gradually by increasing its concentration.
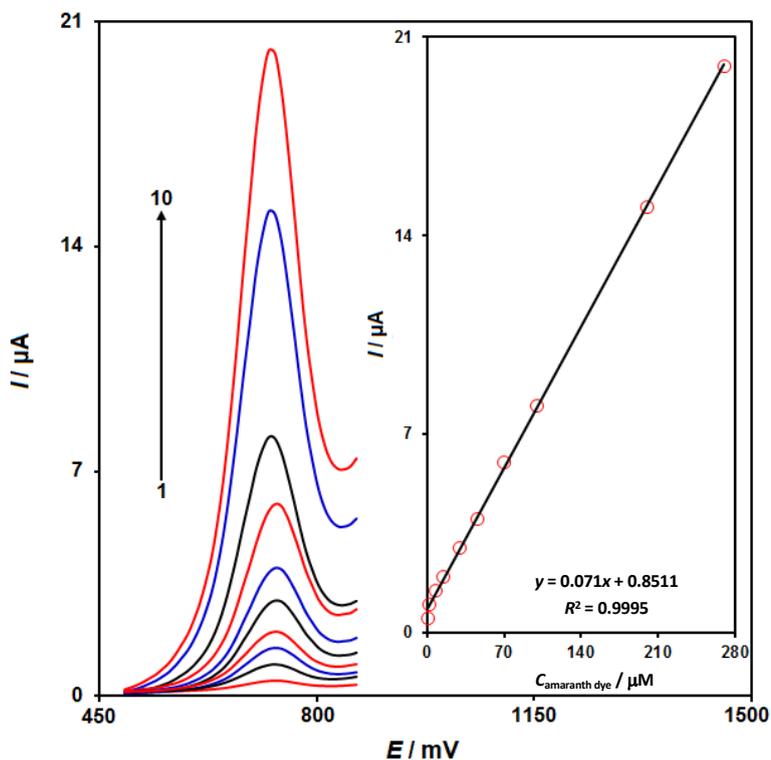
Figure 7. *DPVs of V_2O_5-NP/SPE in 0.1 M (pH 7.0) containing different concentrations of amaranth. Numbers 1–10 correspond to to 0.1, 2.0, 7.0, 15.0, 30.0, 45.0, 70.0, 100.0, 200.0 and 270.0 μM of amaranth. Inset: plot of the electrocatalytic peak current as a function of amaranth concentration in the range of 0.1-270.0 μM*

The oxidation peak currents (I_{pa}) show a good linear relationship with the concentrations of amaranth ranging from 0.1 M to 270.0 μM. The linear equation is $I_{pa} = 0.071c_{amaranth} + 0.8511$ ($R^2 = 0.9995$) (Figure 7 (inset)). Also, the detection limit, c_m, of amaranth was obtained using equation (1)

$$c_m = 3S_b / m \tag{1}$$

In the equation, m is the slope of the calibration plot (0.071 μA. μM^{-1}) and S_b is the standard deviation of the blank response which is obtained from 20 replicate measurements of the blank solution. The limit of detection (LOD) was estimated to be 0.04±0.001 μM. In addition, Table 1 shows that the V$_2$O$_5$-NPs/SPE can compete with other sensors for the determination of amaranth.

Table 1. *Linear range and LOD obtained at the V$_2$O$_5$-NP/SPE for the determination of amaranth compared with other sensors.*

Electrochemical sensor	Method	Linear range, μM	LOD, μM	Ref.
Multiwalled carbon nanotube/Gold electrodes	Differential pulse voltammetry	1.0–10.0	0.068	[48]
Graphene/TiO$_2$-Ag based composites/Gold electrodes	Linear sweep voltammetry	0.3-100.0	0.1	[49]
Ni–Mo-MOF/Screen printed graphite electrodes	Differential pulse voltammetry	0.15–500.0	0.05	[50]
Co$_3$O$_4$-CeO$_2$/Graphene nanocomposite modified electrode	Differential pulse voltammetry	2.0–96.0	0.1591	[51]
V$_2$O$_5$ nanoplate/ Screen printed electrodes	Differential pulse voltammetry	0.1-270.0	0.04	This work

V$_2$O$_5$-NPs/SPE repeatability and stability

The V$_2$O$_5$-NPs/SPE stability was tested by keeping the new sensor in the PBS at the pH value of 7.0 for 15 days, and then the cyclic voltammograms (CVs) were consequently recorded in the solution consisting of amaranth (20.0 μM) for the comparison with the CVs recorded before submersion. The peak amaranth oxidation was not altered, whereas the current was reduced in the signals by 2.8 % compared to the initial responses. This means satisfactory stability of V$_2$O$_5$-NPs/SPE and antifouling activity of the modified SPE for the amaranth oxidation. Additionally, the resultant products for the modified SPE were measured by the CV in the absence and presence of amaranth. At last, the CVs were recorded in the presence of amaranth u after 15 potential cycles at 50 mV s^{-1}, such that the currents were decreased up to >2.6 % but the peak potential did not alter.

Analytical application

The determination of amaranth in real samples such as apple Juice and water samples was performed using V$_2$O$_5$-NPs/SPE sensor. The concentration values of amaranth were calculated by the standard addition method. The results are summarized in Table 2, the recovery is between 96.4 and 104.3 %, and the relative standard deviations (RSDs) are all less than or equal to 3.4 %. The experimental results confirmed that the V$_2$O$_5$-NPs/SPE sensor has a great potential for analytical application.

Table 2. *Determining amaranth in real samples by using V$_2$O$_5$-NP/SPE (n=5).*

Sample	c / μM		Recovery, %	RSD, %
	Spiked	Found		
Apple juice	4.5	4.6	102.2	2.7
	5.5	5.3	96.4	3.4
	6.5	6.4	98.5	1.8
	7.5	7.6	101.3	2.2

Sample	c / μM		Recovery, %	RSD, %
	Spiked	Found		
Tap water	5.0	4.9	98.0	3.2
	6.0	6.1	101.7	2.1
	7.0	7.3	104.3	3.0
	8.0	7.9	98.7	1.9

Conclusions

The voltammetric behavior of synthetic food colorant, amaranth, was studied using a V_2O_5-NP/SPE in 0.1 M PBS of pH 7.0. A well-defined oxidation peak was obtained for amaranth at 0.730 V with the modified electrode. The diffusion-controlled oxidation of amaranth at the modified electrode can be attributed to the electrocatalytic nature of V_2O_5 nanoplate since the bare electrode has not produced an electrochemical signal under the same experimental conditions. The oxidation peak current varies linearly with a concentration in the range from 0.1-270.0 μM with a limit of detection at 0.04±0.001 μM. The diffusion coefficient for amaranth using V_2O_5-NP/SPE, 3.3×10^{-6} cm^2 s^{-1} was obtained. The sensor was successfully employed for the determination of amaranth in different samples.

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