Full Paper

Electrochemical Study of Allura Red at Glassy Carbon Electrode Modified with the Reduced Graphene Oxide and Its Determination in Food by Adsorptive Stripping Voltammetry

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Abstract- The electrochemical properties of Allura red on a glassy carbon electrode modified with the electrochemically reduced graphene oxide (ErGO/GCE) was investigated by cyclic voltammetry and differential pulse adsorptive anodic stripping voltammetry. The ErGO/GCE was fabricated of electrochemical reduced method in conditions: Graphene oxide was dispersed in water and electrochemically reduced for 15 cycles, potential scan range from 0 V to -1.5 V, scan rate 30 mV.s^{-1}. The electrochemical behaviors of Allura red on the ErGO/GCE are semi-reversible oxidation and the Allura red had adsorptive on the surface of ErGO/GCE. The optimal conditions for determination of Allura red were Britton-Robinson buffer pH = 2.5, adsorption potential 0.5 V, adsorption time 15 s, and scanning rate 12.5 mV.s^{-1}, linearity range from 10^{-7} mol.L^{-1} to 8×10^{-7} mol.L^{-1}. The limit of detection (LOD) is 2.8×10^{-8} mol.L^{-1} and limit of quantification is 9.3×10^{-8} mol.L^{-1}. The ErGO/GCE was successfully used in the determination of Allura red content in food samples with high sensitivity and accuracy, simple and low cost.

Keywords- Allura red; ErGO/GCE; Adsorptive stripping voltammetry; Electrochemically reduced graphene oxide; Food samples

1. INTRODUCTION

In order to make the visual of the final products more attractive, Allura red which is a synthetic water-soluble azo dye is usually added to candies, syrups, cake mixes, and wine. Synthetic dyes have the ability to replace natural colors thanks to their several beneficial properties, for instance, low cost, high stability to light, oxygen, and pH change. However, the synthetic color may harm people's health. Allura red contains Azo groups (–N=N) bound to aromatic rings in their molecular structures (Figure 1). The excessive intake of Allura red can be one the reasons which lead to various diseases, such as anemia, pathological lesions in the brain, liver, kidney, asthma, and even cancer [1,2]. The Food and Drug Administration establish acceptable daily intakes of 7 mg/kg/day for Allura red. Thus, detecting the content of Allura red in foods and drinks is significant for evolving food security in modern society. Therefore, a simple, fast, selective, and sensitive method for the determination of Allura red in food is necessary to develop.
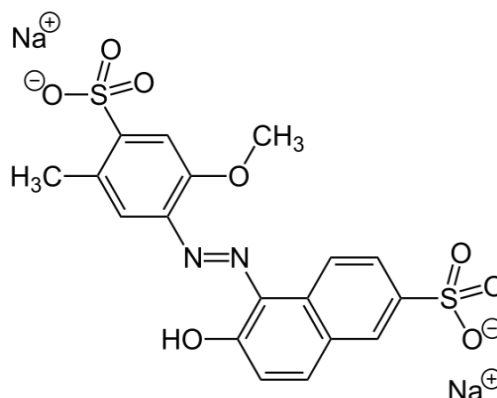

Figure 1. Chemical structure of Allura red

Currently, it is reported that there have been some analytical methods to detect Allura red such as spectrophotometry [3-5], high-performance liquid chromatography (HPLC) [6-9], electrophoresis [10-13], voltammetry [14-19]. While spectrophotometry, capillary electrophoresis, and fluorescence techniques have low sensitivity, and narrow linear ranges, chromatography can offer good selectivity and low detection limits but they often require time-consuming detection processes and complex pre-treatment steps. Moreover, these instruments are rather complicated, overpriced, and cannot be analyzed in the field. In comparison with these above methods, the adsorptive stripping voltammetry has a large number of advantages that are simple, high sensitivity, low cost, and convenience for in-situ detection. There are some chemically modified electrodes that have been known as the electrochemical detection of Allura red, for instance, multi-wall carbon nanotube (MWNT) [17,20,21], chitosan with zinc nanoparticles and multiwall carbon nanotubes modified gold electrode (CHIT/ZnONPs/MWCNTs/AuE) [22], electrochemically reduced graphene oxide-modified

screen-printed carbon electrode (ERGO-SPCE) [23], graphene oxide modified carbon electrode (GO/GCE) [23,24], cuprous oxide-electrochemically reduced graphene nanocomposite modified glassy carbon electrode (Cu2O-ErGO/GCE) [25], Titania with electro-reduced graphene oxide glassy carbon electrode (TiO2/ErGO/GCE) [26], silica with embedded cetylpyridinium chloride modified carbon paste electrode [27]. In this work, we fabricated an electrochemical sensor from reduced graphene oxide modified on the surface of a glassy carbon electrode (ErGO/GCE)

Graphene oxide (GO) has been widely used to fabricate electrochemical sensors thanks to its excellent properties, such as high surface area, excellent hydrophilicity, good biocompatibility, and low toxicity to name a few. Graphene oxide was synthesized from graphite by improved Hummer's method [28, 29]. However, graphene oxide has poor electrical conductivity so practical application is confined to fabricating electrochemical sensors. In order to improve the conductivity, GO was reduced by different methods such as chemical reduction, thermal reduction, and electrochemical reduction to form reduced graphene oxide. In this paper, ErGO/GCE was prepared by the electrochemical reduction method which was non-toxic, simple, and low-cost. Moreover, the electrochemical behavior of Allura red on ErGO/GCE was investigated in detail by cyclic voltammetry and differential pulse adsorptive anodic stripping voltammetry. Additionally, various electrochemical parameters (pH, scan rate, accumulation potential, and accumulation time) were also discussed and, the ErGO/GCE was successfully capable of Allura red detection in a soft drink as well.

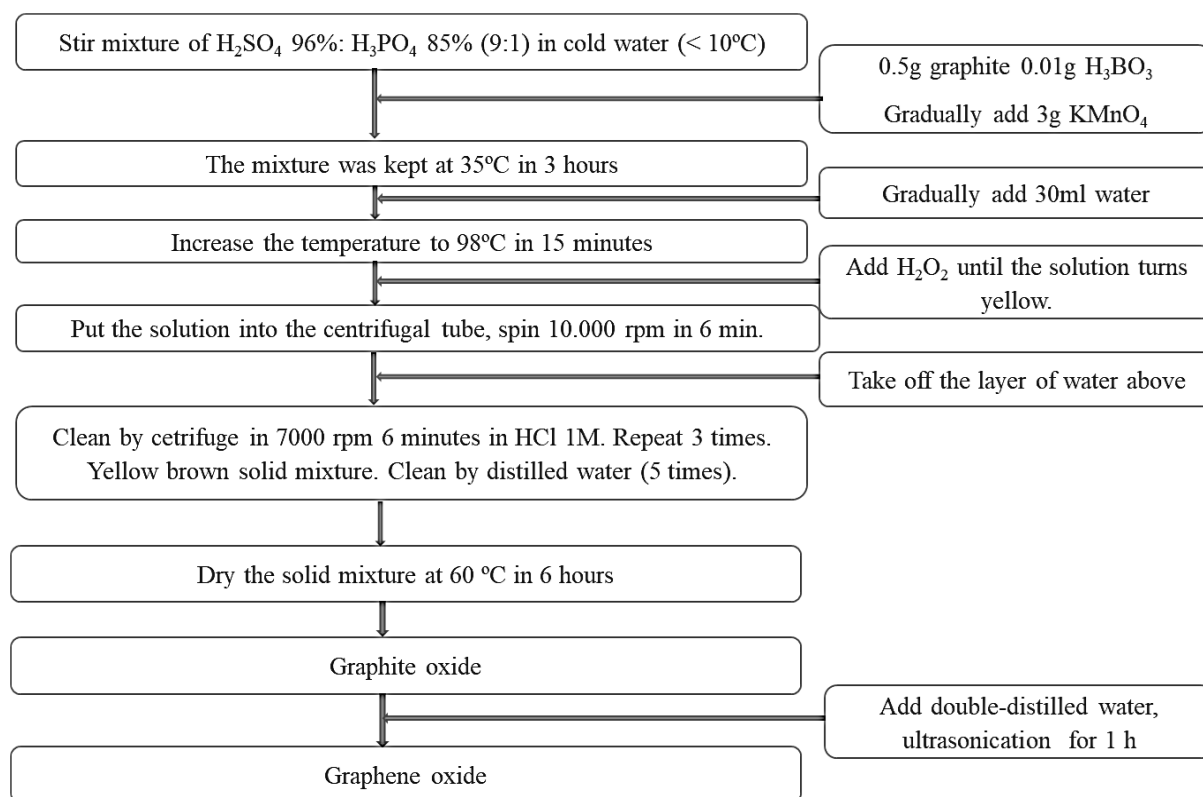## 2. EXPERIMENTAL SECTION

2.1. Reagents and instrumentation

All chemicals and reagents used in the study are analytical grades. Graphite powder, $NaNO_3$, KNO_3, H_2SO_4, H_3PO_4, $KMnO_4$, H_2O_2, NaOH, HCl, H_3BO_3, CH_3COOH, and Allura red were purchased from Sigma-Aldrich, Germany.

A stock solution of Allura red 5.10^{-3} $mol.L^{-1}$ was prepared in double distilled water. The µAutolab type III (Netherlands) was controlled by software 757 computrace VA interfaced to the electrode assembly 663 -VA Metrohm (Switzerland) was used to measure current-potential signal for determination of Allura red. Three electrodes system consists of ErGO/GC) as a working electrode, a carbon rod as a counter electrode, and a silver/silver chloride (Ag/AgCl, KCl 3 $mol.L^{-1}$) as a reference electrode.

High-performance liquid chromatography (HPLC) measurements were carried out on a Shimadzu LC-20. A system equipped with a dual wavelength absorbance detector and Lichrospher C-18 reverse phase (5µm x 250mm x 4.6mm) column was used. The mobile phase containing 90% acetate buffer (pH = 6.0) and 10 % acetonitrile with 1.0 $mL.min^{-1}$ flow rate was used.

2.2. Preparation of graphene oxide

Graphene oxide was synthesized from graphite according to the following process, Scheme 1 [1]:

Stir mixture of H_2SO_4 96%: H_3PO_4 85% (9:1) in cold water (< 10°C)

0.5g graphite 0.01g H_3BO_3
Gradually add 3g $KMnO_4$

The mixture was kept at 35°C in 3 hours

Gradually add 30ml water

Increase the temperature to 98°C in 15 minutes

Add H_2O_2 until the solution turns yellow.

Put the solution into the centrifugal tube, spin 10.000 rpm in 6 min.

Take off the layer of water above

Clean by cetrifuge in 7000 rpm 6 minutes in HCl 1M. Repeat 3 times. Yellow brown solid mixture. Clean by distilled water (5 times).

Dry the solid mixture at 60 °C in 6 hours

Graphite oxide

Add double-distilled water, ultrasonication for 1 h

Graphene oxide

Scheme 1. Graphene oxide synthesis process

2.2. Preparation of ErGO/GCE

A bare glassy carbon electrode (GCE) was polished to a shiny mirror-like surface with 0.05 µm Al_2O_3 powder. Then the GCE was alternately rinsed three times with double distilled water. The GCE electrode was immersed in 1 mg/mL graphene oxide solution and modified that electrode by cyclic voltammetry with electrochemical conditions such as a sweep rate 25 mV.s^{-1}, a range potential from 0 V to -1.5 V with 15 cycles. Then take out the working electrode, and rinse it with double-distilled water, we get a glassy carbon electrode modified reduced graphene oxide (rGO/GCE).

2.3. Analytical procedure

50.0 mL of the analytical solution containing Allura red and pH 2.5 Britton-Robinson buffer is poured into the cell. Before the voltammograms measurement, the nitrogen flow was passed through the solution to remove the entire oxygen. The concentration of Allura red in a sample was determined by differential pulse adsorptive anodic stripping voltammetry. The deposition step was carried out under an adsorptive potential of 0.5 V (*vs.* Ag/AgCl/KCl$_s$) for

15 seconds while stirring the analytical solution. After that, stop stirring the solution for 5 s, and the differential pulse stripping voltammograms were recorded in the potential range from 0.5 V to 1.1 V.

2.4. Analysis of real sample

Soft drink samples such as Sting, Red Dragon, Samurai, and Kokozo were selected from a supermarket in Ha Noi. The soft drink samples without any pretreatment were measured directly. Take V_1 mL soft drink samples into volumetric flash and add double-distilled water up to 50.0 mL (A solution). A volume of V_2 mL of A solution was poured to a 50.0 mL volumetric flask, added with 10 mL of pH 2.5 BR buffer and double distilled water to the mark. The content of Allura red in the soft drink samples was calculated by the following equation:

$$C \ (mg \ /l) = \frac{C_x \ (mol.l^{-1}) \times 25.0 \ (ml) \times 50.0 \ (ml) \times 496.42 \frac{g}{mol} \times 1000}{V_1 \ (ml) \times V_2 (ml)}$$

where C_x is the concentration of Allura red in solution sample.

2.4.1. Method evaluation

The suitable conditions were selected to determine of Allura red in soft drink and candy samples after optimizing experimental conditions and instrumental parameters. The proposed method was evaluated through linear range, recovery, limit of detection, limit of quantitation. The recovery was determined by spiking a known amount of Allura red in the soft drink samples. The recovery was calculated according to the following equation:

$$Recovery \ (\%R) = \frac{(C_f - C_i)x \ 100 \ \%}{C_{ad}}$$

where C_i is the concentration of Allura red in the sample. C_f is the concentration of Allura red in the sample spiked standard solution. C_{ad} is the concentration of standard Allura red added to the sample.

3. RESULTS AND DISCUSSION

3.1. Electrochemical behavior of Allura red at the ErGO/GCE

Figure 2 A showed the adsorptive stripping voltammograms of 5×10^{-7} mol.L^{-1} Allura red in Britton-Robinson (pH 2.5) recorded at the glassy carbon electrode-GCE (orange curve), glassy carbon electrode modified with graphene oxide-GO/GCE (blue curve) and glassy carbon electrode modified with electrochemical reduced graphene oxide-ErGO/GCE (red curve) after 1 min adsorption time. The results showed that the peak current of ErGO/GCE was higher than GO/GCE and GCE. Therefore, the ErGO/GCE was chosen for the determination of Allura red in food.
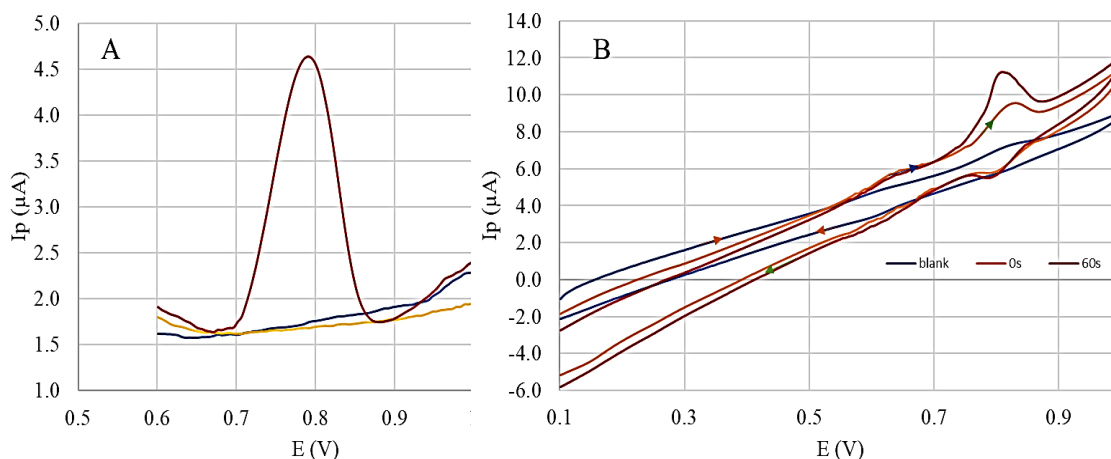
Figure 2. A) Differential pulse adsorptive stripping voltammograms of 5×10^{-7} mol.L^{-1} Allura red at different modified electrodes; B) cyclic voltammograms of blank sample and 5×10^{-6} mol.L^{-1} Allura red standard in pH 2.5 buffer recorded at the ErGO/GCE without adsorption time and after 60 s adsorption time

Figure 2B shows that on the anodic sweep from 0.1 V to 1.0 V, an oxidation peak at 0.825 V was observed (I_{ox} = 0.916 µA). On the reverse scan rate, a reduction peak appeared at 0.797 V (I_{re} = 0.425 µA). The oxidation peak current having an adsorption time was higher than the oxidation peak current without an adsorption time. These results can confirm that Allura red can be adsorbed on the surface of ErGO/GCE.

3.2. Optimization of analytical conditions

3.2.1. Effect of pH

The pH is important for the determination of Allura red by voltammetry. The pH of the electrolyte solution affected the anodic peak current, and peak potential of Allura red.

The anodic peak currents of Allura red gradually increased with pH varying from 2.0 to 2.5; then the peak current declined when the pH went upward from 3.0 to 6.0 (Figure 3A). When pH exceeded 6.5, the peak current disappeared. Therefore, pH 2.5 was selected as the optimal pH. In addition, the anodic peak of Allura red shifted toward a negative potential direction when pH increased from 2.0 to 6.0 which demonstrated the proton participation in the oxidation of Allura red. Moreover, in Figure 3B the anodic peak potential of Allura red had linear relation with pH according to the E_{pa}= -0.030 pH + 0.857 (R^2=0.9996). The slope (0.030 V/pH) was close to the theoretical value of 0.059 V/2 pH indicating an electrochemical process with an equal number of two electrons and one proton.
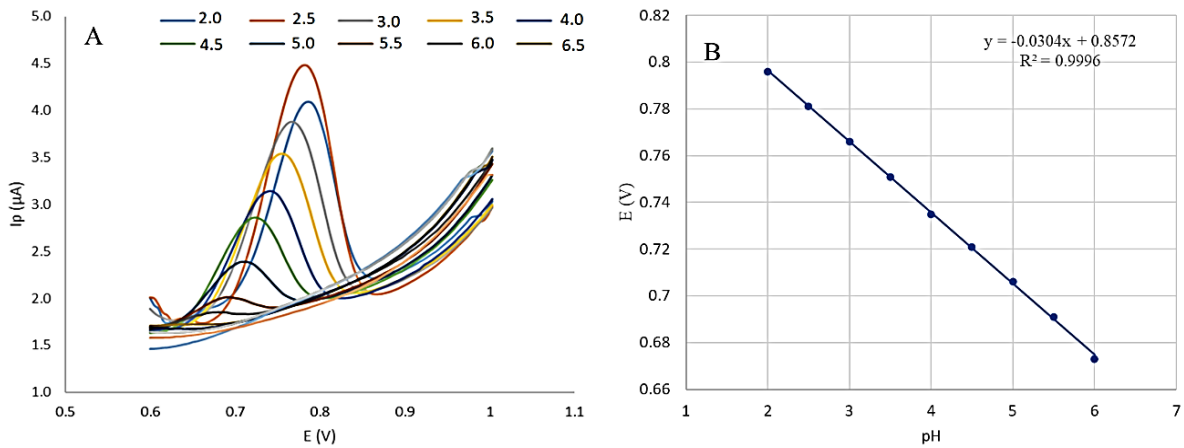
Figure 3. A) DP adsorptive stripping voltammograms of AR depending on pH from 2.0 to 6.5; B) Linear relationship between anodic peak potential and pH

3.2.2. Effect of adsorption potential and time

The adsorption potential affected to adsorptive ability of Allura red on the surface of working electrode. Therefore, the dependence of adsorption potential on the peak current was investigated. The anodic peak current of Allura red increased when the adsorption potential changed from -0.5 V to +0.5 V and reached maximum values at +0.5V, then it slightly decreased with more positive potential (Figure 4A). So, the optimal adsorption potential was + 0.5 V for determination of Allura red.
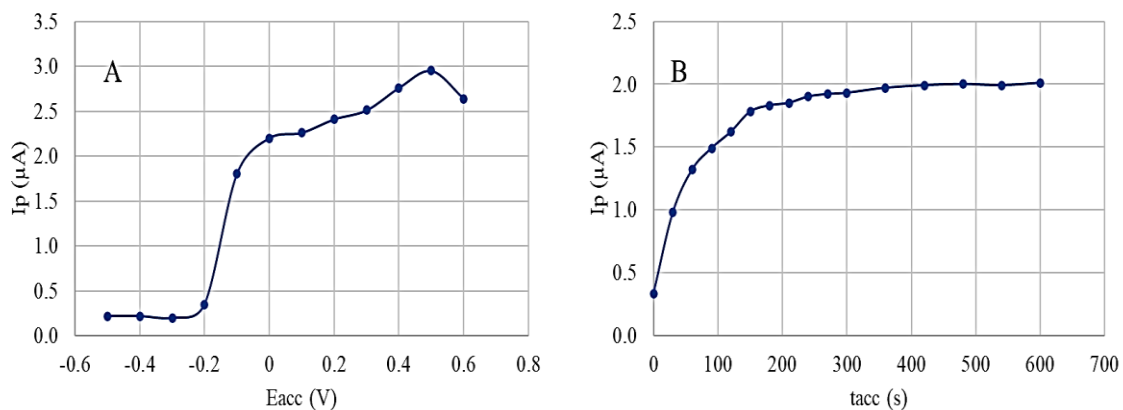

Figure 4. A) The effect of adsorption potential; B) adsorption time

The sensitivity of the adsorptive stripping voltammetry depends on adsorption time. The anodic peak current increased rapidly within a short adsorption time from 0 s to 150 s and then slowly increased when adsorption time exceeded 150 s (Figure 4B). Which indicated that the electrode surface was saturated. As a consequence, we chose 60 s with the concentration of 10^{-7} mol.L^{-1} AR to accumulate AR on the surface of ErGO/GCE.

3.2.3. Determination of Allura red

The differential pulse voltammograms (DPV) of Allura red concentrations from 1.0×10^{-7} mol.L^{-1} to 8.0×10^{-7} mol.L^{-1} with optimal conditions pH=2.5; adsorption potential +0.5 V, adsorption time 60 s, scan rate 12.5 mV.s^{-1}. The anodic peak currents of Allura red increased gradually with corresponding concentrations (Figure 5). In the concentration range from 1.0×10^{-7} mol. L^{-1} to 8.0×10^{-7} mol.L^{-1}, anodic peak current increased linearly with Allura red concentration following equation I$_p$ (μA) = 0.637 C$_x \times 10^{-7}$ (mol.L^{-1}) + 0.215 (R^2 = 0.997).
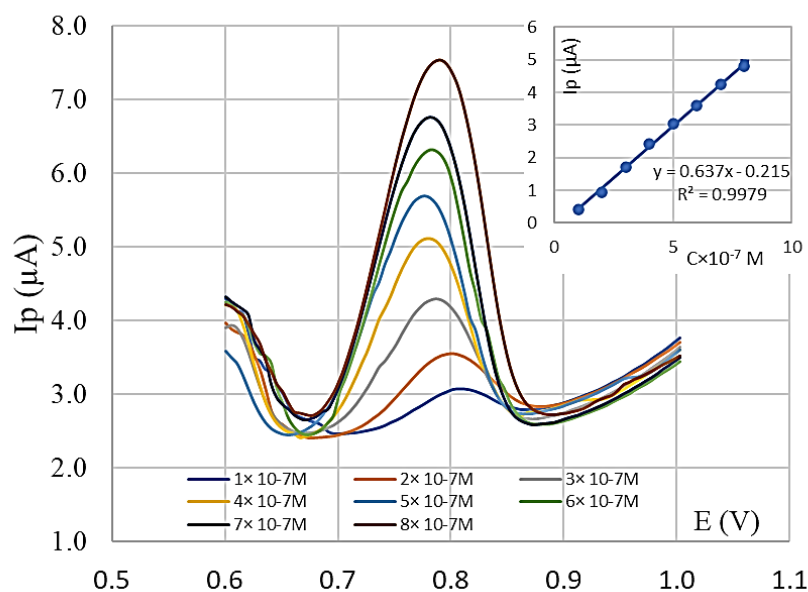

Figure 5. DPV Voltammograms of Allura red from 1.0×10^{-7} mol.L^{-1} to 8.0×10^{-7} mol.L^{-1} Experiment conditions: pH = 2.5; E$_{acc}$ = +0.5 V, t$_{acc}$ = 60 s, scan rate 12.5 mV.s^{-1}

3.2.4. Method evaluation

In order to validate a developed method for the determination of Allura red before sample analysis, detection limit (LOD), quantification limit (LOQ), and precision were evaluated. From the calibration curves, LOD, and LOQ were calculated by formula 3S$_D$/b and 10S$_D$/b, where "S$_D$" is the standard deviation of the intercept and "b" is the slope of the calibration curve [2]. The LOD and LOQ of the proposed method were 2.8×10^{-8} mol.L^{-1} and 9.3×10^{-8} mol.L^{-1}, respectively.

Ten successive measurements of a solution containing 5.0×10^{-7} mol.L^{-1} of Allura red in pH 2.5 Britton - Robinson buffer with an adsorption time of 15 s at adsorption potential +0.5 V were used to determine precision. The relative standard deviation (RSD) was 2.5% (n = 10) which was less than 3%. The results showed that the proposed analytical method had good repeatability.

To evaluate the precision of the proposed method before we applied it for the determination of Allura red in food samples, three levels of concentration of 10^{-7} mol.L^{-1}, 3×10^{-7} mol. L^{-1} and 5×10^{-7} mol. L^{-1} of Allura red was added into the soft drink sample without Allura red and was analyzed repeatedly four independent times per one level. The mean recoveries based on four replicate measurements were found to be 102.3±0.97%, 100.1±0.67 and 96.2±2.66%, respectively. The mean recovery of the method was calculated was 99.5 % and the relative standard deviation (RSD) was less than 3% showing the accuracy of the developed method.

3.3. Application of real sample

The concentrations of Allura red in the soft drink samples were determined by the adsorptive stripping voltammetry using the ErGO/GCE. The procedure for the Allura red analysis was followed as described in section 2.4. The concentration of Allura red in samples was determined by the standard addition method in order to avoid matrix effects. Standard addition voltammograms of Allura red were shown in Figure 6. The content of Allura red in samples has been shown in Table 1.
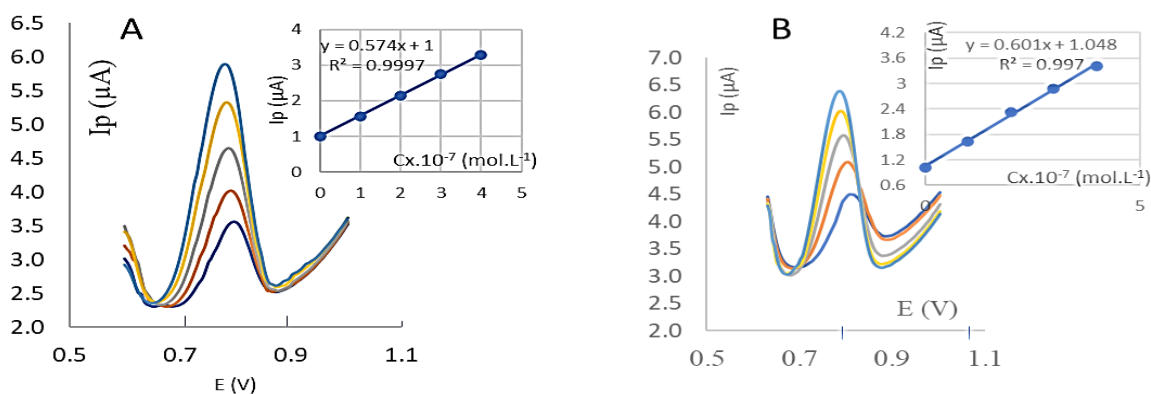

Figure 6. DPV Voltammograms of Allura red in sample: A sting sample, B samurai sample

Table 1. Contents of Allura red in soft drink (mg.L^{-1})

No	Sample	DPAdSV	HPLC	Relative error(%)
1	Sting	86.8 ± 2.4	95.1 ± 1.7	8.72
2	Red Dragon	126.5 ± 2.1	125.3 ± 1.4	0.96
3	Samurai	75.2 ± 2.0	72.5 ± 1.3	3.72
4	Kokozo	42.1 ± 2.5	44.0 ± 1.2	4.31

Table 1 showed that, the soft drink (Sting, Red dragon, Samurai, Kokozo) contained Allura red. The results were compared with the HPLC method. However, the obtained results were less than the permissible standards in food of Ministry of Health (300 mg.L^{-1}). We find that $t_{calculated}$ (0.49) is less than t_{table} (2.57) for 95% confidence and 5 degrees of freedom, so we

conclude that the results of the two methods are not significantly different. Nevertheless, the voltammetric method is simpler, faster, and requires less expensive equipment than the chromatographic method.

4. CONCLUSION

In summary, an electrochemical sensor has been developed by rGO to modify GCE for the determination of Allura red in soft drink samples. The developed method has high sensitivity, selectivity, simplicity, low cost, and very easy preparation without pretreatment. The method can be applied to the fast determination of Allura red in soft drink samples.

Declarations of interest

The authors declare no conflict of interest in this reported work.

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