Detection of Sunset Yellow by Adsorption Voltammetry at Glassy Carbon Electrode Modified with Chitosan

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The simple modification of the surface of a glassy carbon electrode with chitosan (CS) allowed the development of a electroanalytical method useful for the detection and quantification of sunset yellow (SY-E110) by square wave adsorption voltammetry (SWAdV). The electrode surface was characterized with electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV). The results showed that the presence of CS on the surface of the electrode, allowed the oxidation of the SY to 1.01 V in a process controlled by adsorption. Under optimal conditions (pH 2.5, t_{ADS} 60s, E_{ADS} 0.10 V) the anodic peak current was proportional to the concentration of SY between 0.25 to 3.25 μmol L^{-1}, with a limit of detection of 0.098 μmol L^{-1}. The relative standard deviation (RSD) for a solution with 0.080 mmol L^{-1} of E110 was 2.5% for five different electrodes (n=5). The method was validated with unflavored gelatin samples spiked with known amounts of SY. The potential interferences of other synthetic dyes were studied and finally the method was tested with real samples of foods containing SY and other synthetic dyes. The results were equally acceptable compared to previous reports.

1. INTRODUCTION

The monitoring of synthetic dyes in foods of mass consumption is of great importance, especially in recent years due to the increase in the excessive use of these dyes to improve the visual aspect of food [1]. The main problem that causes people who consume excessive foods treated with these dyes, such as sunset yellow, is related to the health problems they can cause, such as: hyperactive behavior in children, allergies and asthmatic problems and in extreme cases, development of various types of cancer, especially in the liver [2]. The chemical structure from sunset yellow contains the

azole group (-N = N-) and aromatic rings, the main cause of the problems that cause health [3, 4]. The European Food Safety Authority (EFSA), considers SY Admissible Daily Intake (ADI) of 1.0 mg / kg [5].

The use of electrochemical sensors coupled with stripping techniques has been the most used in recent years for the quantification and monitoring of dyes. This is due to its sensitivity, selectivity and low operational cost. These techniques consist of two stages. A stage of accumulation of the analyte on the modified electrode or sensor and another of stripping with a sensitive technique such as square wave voltammetry (SWV) and differential pulse (DPV). The analyte can accumulate by electro-deposit (ASV) or by adsorption (AdV) on the surface of the electrode [6]. On the other hand, the development of new electrochemical sensors with a great variety of new materials and substrates has increased, such as: ionic liquids [7,8], carbon nanotubes and surfactants [2], graphene oxide [9] and nano gold particles [10]. This has allowed to quantify synthetic dyes in different matrices with great sensitivity (under $\mu mol\ L^{-1}$) and selectivity.

The modified electrode or sensing surface can be considered as the innovation of this investigation and special attention should be devoted to its manufacture and maintenance. These surfaces must be stable, sensitive, selective and easy to manufacture. In addition, they should not have redox reactions at values of similar analyte potential. The majority of synthetic dyes are electro-active at positive potential values [11]. This feature reduces the variety of substrate material used for sensor development. The substrates of carbon paste and glassy carbon modified with nano-materials are the most used. On the other hand, chitosan is a natural polymer of low cost and friendly to the environment. These properties have allowed in recent years to be used as a dispersing agent adsorbent of biological, organic and organometallic molecules in the modification of electrodes used in the detection of dopamine [12-14], ascorbic acid[12,13], uric acid[13], ponceau 4R [15] and morphine [14]. In addition, chitosan has been used in the detection of SY by reduction using a glassy carbon modified electrode with bismuth film. In this report it is observed how the presence of chitosan increases the cathodic peak current for SY improving the sensitivity. [16]

In this work, together with optimizing the methodology to detect SY using a glassy carbon electrode modified with CS, its usefulness in the analysis of samples of foods containing SY in the presence of other dyes such as tartrazine (TZ) and amaranth (AM) will be evaluated. On the other hand, the detection of SY using a simple modification of glassy carbon with CS has not been reported.

2. EXPERIMENTAL

2.1 Reagents

Water type A used for the entire study was obtained from the Wasselab ASTM D1193 system. The standard solutions of synthetic dyes: sunset yellow (SY), tartrazine (TZ), Allure red (AR), amaranth (AM) and sudan (SD) were prepared from pure reagent obtained from Sigma-Aldrich. The electrolyte solution was prepared from phosphoric acid (Merck) and adjusting the required pH with 2.0 mol L^{-1} NaOH solution and diluting 10 times with water to a concentration 0.01 mol L^{-1}.

2.2 Instrument and electrodes

Cyclic and adsorption voltammograms were obtained with a μStat 400 DropSens potentiostat and the Nyquist electrochemical impedance graph was obtained with a Versa STAT 3 from Princeton Applied Research. The electrochemical cell was composed of a system of three electrodes; Glassy carbon electrode (GCE), reference electrode of Ag / AgCl / KCl 3 mol L^{-1} and a platinum wire as an auxiliary electrode were obtained from Chi-Instruments. Stirring at 500 rpm was used. In the electrolyte preparation, an Orion 430 pH meter with a glass membrane electrode was used.

2.3 Preparation of the modified glassy carbon electrode with chitosan.

Glassy carbon electrode (GCE) was polished before each experiment with 0.3 and 0.05 μm of alumina powder and rinsed with water type A. Next, was place in a container with ethanol and is taken to an ultrasound bath for 5 minutes to remove adsorbed particles. Subsequently, 8.5 mg of low molecular weight chitosan (Sigma-Aldrich) was dispersed in 1.0 mL of acetic acid (1%). 40.0 μL of freshly prepared chitosan solution was deposited directly on the surface of the glassy carbon and maintained at 70°C for 10 minutes to remove excess solvent[12]. The preparation of the electrode and the oxidation of the SY have been summarized in figure 1.
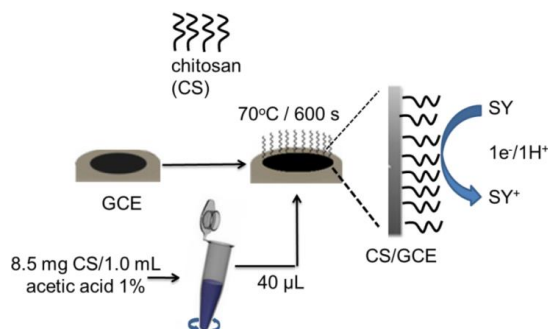

Figure 1. Preparation of the modified electrode with chitosan and SY oxidation.

2.4 development of measures

In the electrochemical cell, 9.5 mL of type A water and 0.50 mL of 0.01 M L^{-1} phosphate electrolyte were added. Background current was scanned. Subsequently, the respective voltammograms for SY was scanned between 0.12 and 1.65 μmol L^{-1} for AdSV and between 1.0 and 0.10 mmol L^{-1} for VC.

2.5 Sample preparation

The food samples (cherry flavored gelatin, powdered juice and orange flavored soft drink) were obtained from a supermarket in the city of Ibague. The solid samples, between 0.20 and 0.10 g were

weighed and dissolved in 10.0 mL of water type A. The liquid samples were analyzed directly using between 0.10 and 0.50 mL of sample.

3. RESULTS AND DISCUSSION

3.1 Characteristics of the surface modified with chitosan

In order to identify the conductive properties of the modified glassy carbon electrode with chitosan (CS/GCE), the changes of the redox potentials and electrochemical impedance to a solution of $FeCN_6^{3-/4-}$ 10.0 mmol L^{-1} in KCl 10.0 mmol L^{-1} were studied. The results are shown in Figure 2. The $FeCN_6^{3-/4-}$ redox system was less quasi-reversible with the modified chitosan electrode (Figure 2A red curve) with a lower ΔE of 100.0 mV, changing from 0.410 mV with the electrode without CS at 0.31 mV with CS. In addition, the anodic and cathodic peak currents increased by almost 5.0% compared to the glassy carbon without CS (Figure 2A black curve). These results indicate that the redox system for $FeCN_6^{3-/4-}$ requires less energy and the transfer of charge is facilitated. The Nyquist graph for the electrode modified with CS and without CS is shown in Figure 2B. The results showed a very small semi-circle for the glassy carbon electrode modified with CS at high frequencies (Fig. 2B red curve) related to the double capacitive layer, indicating less resistance to current flow in a faradic system. The glassy carbon without CS developed a large circumference circle (Fig. 2B black curve) that affect the faradic processes. Similar results were reported using glassy carbon with more complex modifications [9]. The results of cyclic voltammetry and electrochemical impedance showed have a relationship where CS on the glassy carbon surface improves the activity of the surface.
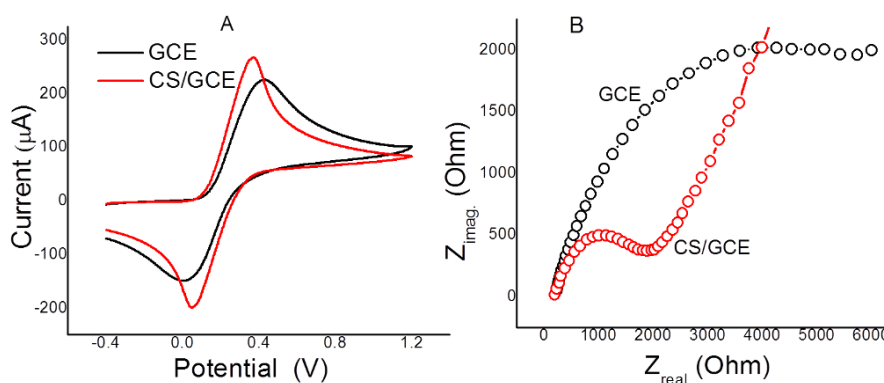

Figure 2. cyclic Voltammograms (A) and Nyquist graph (B) for $FeCN_6^{3-/4-}$ using GCE (black curves) and CS/GCE (red curves).

3.2. Electrochemical property of sunset yellow on a chitosan-modified glassy carbon electrode (CS/GCE)

The oxidation of SY occurs by the deprotonation of H^+ ions from the phenol group of the aromatic double ring in the chemical structure of SY[17] In this case, using glassy carbon without CS

(Figure 3A) no significant anodic current signal was observed with SY 14.6 µmol L^{-1}. When SY was added to the electrochemical cell containing a modified glassy carbon electrode with CS (Figure 3B red curve) an anodic peak current for SY at 1.01 V was clearly observed with a current intensity of 4.5 µA. This result indicates that the modified surface presented activity to the oxidation of the SY. Possibly CS acts as an extractor agent concentrating SY on the surface of the electrode. This accumulation has been previously justified by means of interactions; it can include hydrogen bridges, hydrophobic interactions and electrostatic interactions [9]. With the use of more complex electrodes in their modification, such as copper complexes [3], carbon nanotubes with graphene [9] and TiO$_2$ with graphene [17] anodic peak current for SY was observed at less positive potential values, indicating that the catalytic effect of the CS is lower. These results allow choosing a modified glassy carbon electrode with CS for subsequent studies.
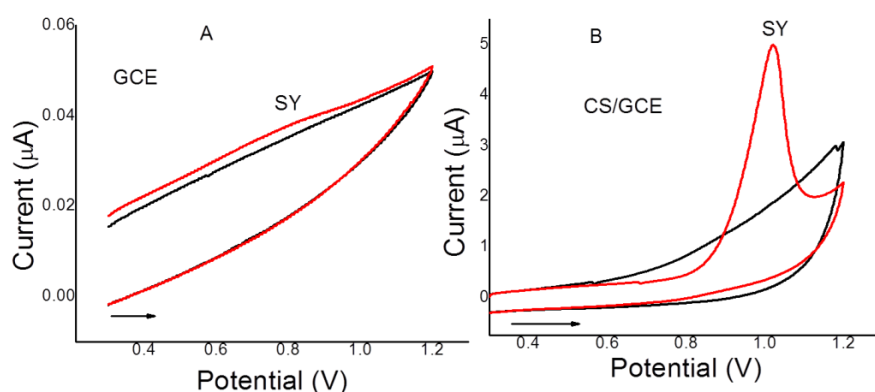
3.3. Effect of pH value

Figure 3. Cyclic voltammograms for SY 14.6 µmol L^{-1} with GCE without CS (A) y GCE with CS (B) without SY (black curves) and with SY (red curves). pH 2.5, scan rate 100.0 mV s^{-1}.

In order to increase the anodic current intensity, the effect of the pH of the solution used as an electrolyte, in this case phosphoric acid was studied. The voltammograms were developed with SY 14.6 µmol L^{-1} varying the pH value between 2.0 and 6.0 in the electrochemical cell. Figure 4A shows the results. It was clearly observed that at less acid pH values, the potential was shifted to less positive values. Indicating that protons (H$^+$) are involved in the oxidation of SY, confirming the previous reports [17, 9]. Between the pH values of 2.5 and 3.7, the highest anodic current intensity was observed. In Figure 4B it was clearly observed that the pH value of the highest anodic current intensity was 2.5. This value was chosen as optimal in further studies.

3.4. Effect of scan rate (U)

In order to know which process controls the transport of SY to the surface of the glassy carbon electrode modified with CS, the effect of the scan rate on anodic peak current for SY 14.6 µmol L^{-1}

was studied. Figure 5 shows the voltammograms of SY when the scan rate (U) was varied upwards from 10.0 to 80.0 mV s^{-1}.
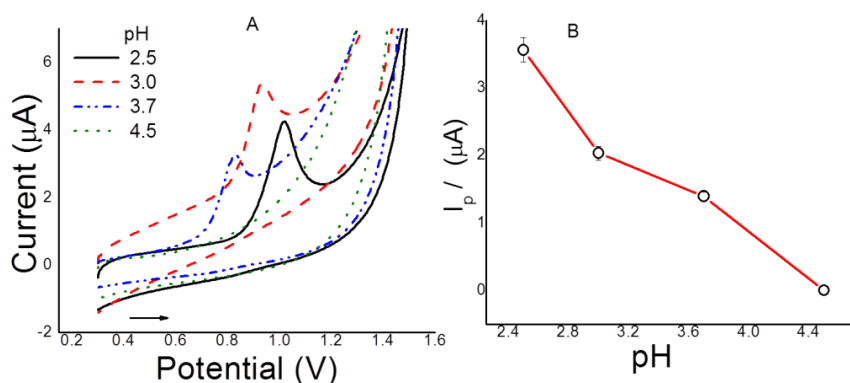

Figure 4. Cyclic voltammograms for SY 14.6 µmol L^{-1} at pH 2.5, 3.0, 3.7 y 4.5 with CS/GCE (A) and linear relationship for anodic peak current and pH (B). Scan rate 100.0 mV s^{-1}.

The results showed that anodic peak current increased proportionally, indicating a mass transport controlled by adsorption (figure 5A). On the other hand, the linear relationship between the potential and lnU (figure 5B) gave a correlation of (r^2) 0.994 with a regression equation of $E_p = 0.7734 + 0.0228 \ln U$. This slope value is almost half the theoretical value for the Nernst equation of 0.059, indicating that the number of oxidized protons equals the number of electrons in a ratio of 1:1. These results are similar to those reported by Qiu et al., Where they reported the oxidation of a single proton in a process controlled by adsorption using carbon nanotubes and graphene [9].
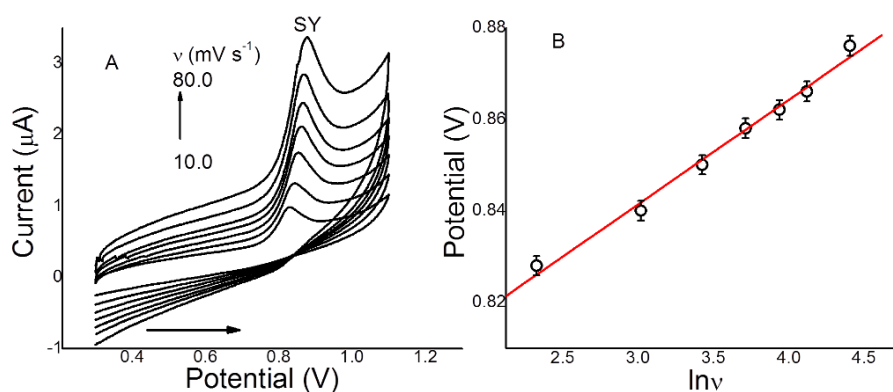

Figure 5. Cyclic Voltammograms for SY 14.6 µmol L^{-1} scan rate (υ) 10.0 at 80.0 mV s^{-1} (A) linear relationship between potential and lnU (B) with CS/GCE. pH 2.5.

3.5 Effect of time and potential of accumulation (t$_{ADS}$/ E$_{ADS}$).

In order to use the shortest possible time of each analysis without affecting the sensitivity, the time and accumulation potential for SY were studied on a modified glassy carbon electrode with CS

using square wave modulation of 15 Hz in the scanning stage. The study was developed between-0.2 and 0.2 V and between 10 and 90 s. In time greater, the intensity of current is constant, possibly it is product of the saturation of the surface of the electrode. 60 s and 0.1 V were chosen as optimal and used in all subsequent studies.

3.6. Detection limit, reproducibility and stability

Figures 6A-B shows the adsorption voltammograms as a function of SY concentration and the linear relationship for anodic peak current for SY concentration. The concentration of SY was increasing from 0.25 to 3.25 µmol L^{-1}. The experimental conditions were: pH 2.5 (500 µL phosphoric acid 0.01 mol L^{-1}); 0.1 V for 60 s. The calibration curve for SY was linear up to 3.25 mol L^{-1}. At higher concentrations, the anodic peak current remained constant, possibly due to the saturation of the electrode surface. Quantification limit ($10\sigma/b$) and the detection limit ($3\sigma/b$) where b is the slope and σ the standard error of the curve [18] were 0.80 and 0.098 µmol L^{-1} respectively. The precision expressed as relative standard deviation (R.S.D.) was 2.5% using SY 1.0 µmol L^{-1}. The stability of the sensor was 3.0% calculated from the relative standard deviation (R.S.D.) using five different electrodes with SY 1.0 µmol L^{-1}. The results were equally acceptable compared to the data summarized in Table 1 where electrodes with more complex modifications were used with an average reproducibility of 2.5%.
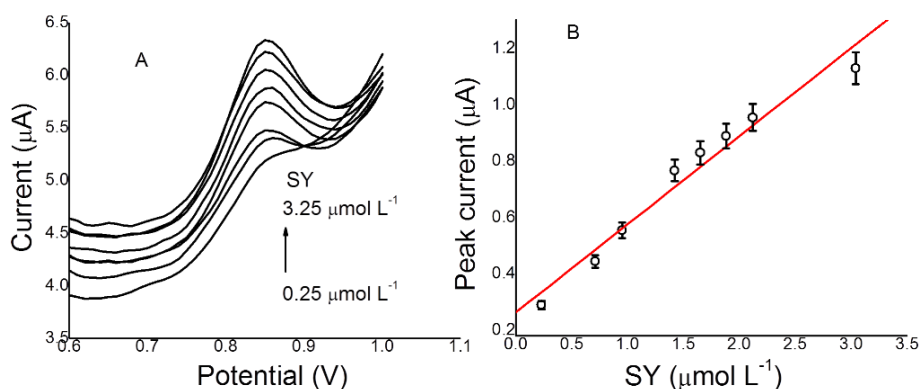

Figure 6. Adsorptive voltammograms (A) and calibration curve (B) for SY between 0.25 y 3.25 µmol L^{-1}; pH: 2.5, at 0.10 V and 90s with CS/GCE.

3.7 Validation and interference analysis

The absence of certified standards forced the development of a validation with samples of commercial unflavored gelatin with a 12.0% of protein content spiked with known amounts of SY. The results are summarized in table 2. The results clearly show a relative error lower than 20.0%, which is acceptable, considering that the quantities analyzed are below 10.0 µmol L^{-1}. In addition it was observed in figure 7A that the sample matrix changed the oxidation of SY from 1.01 V to a less positive potential of 0.85 V. On the other hand, the values of the slopes of the calibration curves of

both samples were between 0.13 and 0.19. This small change indicates that the stability of the sensor was not affected by the sample matrix. Figure 7 shows the adsorption voltammograms and the calibration curve for sample 1 of table 2.

Table 1. Modified electrodes reported for SY.

Electrode	Modification	DL (nM)	pH	Application	Ref.
CPE	Cu-BTC	0.050	8.0	Soft drink	[3]
GCE	G_O/MWCNTs	25.0	5.0	Orange juice	[9]
CPE	nAu	30.0	4.0	Orange juice	[10]
CPE	G_o/TiO$_2$	6.0	2.0	candy, ice cream, gelatin, orage juice	[17]
GCE	Bi-CHIT	10.0 μM	7.0	Candy	[16]
GCE	CS	98.0	2.5	Gelatin, orange juice, powdered juice	This work

GCE: glassy carbon electrode; CPE: carbon paste electrode; Cu-BTC: Cu- benzenetricarboxylate; G_O/MWCNTs: graphene-multi walled carbón nanotubes; nAu: gold nano particles; G_o/TiO$_2$: graphene-titanium oxide; Bi-CHIT/GC: glassy carbon-chitosan bismuth film.
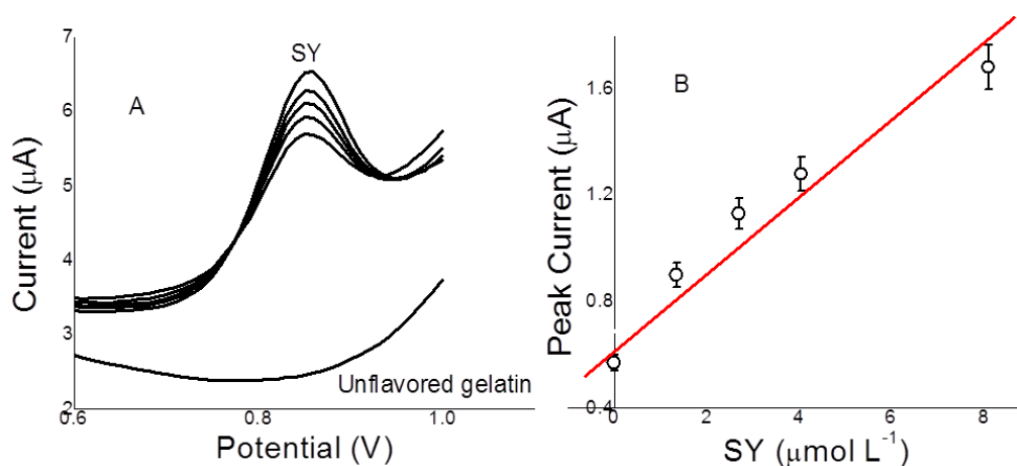

Figure 7. Adsorptive Voltammograms (A) and calibration curve (B) for unflavored gelatin (sample 1, tabla 1) with CS/GCE. Same conditions as in figure 6

Interference study was developed by analyzing the electroactive activity of potential substances that may be present simultaneously with SY in food samples, such as; tartrazine (TZ), amaranth (AM), ponceau 4R (P-4R) and sudan (SD) which are substances used as dyes that have a chemical structure very similar to SY. Some metal ions were also studied using the standard ICP multi-element standard solution IX (Merck) containing As, Be, Cd, Cr (VI), Hg, Ni, Pb, Se and Tl 100 mg L^{-1}. The results showed that the presence of these metal ions does not affect the SY signal in concentrations 500.0 times higher. In Figure 8 it is clearly observed that only TZ has a considerable signal at similar

concentrations of SY but appears at a more positive potential of 1.21 V. Only AM and PR-4 were oxidized at a very close potential of 1.0 V but anodic peak currents were only 2.0% compared to SY anodic peak current. Sudan does not cause any kind of interference. These results indicate that glassy carbon modified with CS allows obtaining a selective surface for detection of SY.

Table 2. Results for unflavored gelatin samples spiked with known amounts of SY (n = 3)

Sample	added (μmol L^{-1})	Found (μmol L^{-1})	% Relative Error
*1	1.36	1.60±0.01	17.6
2	5.63	6.73±0.05	19.5

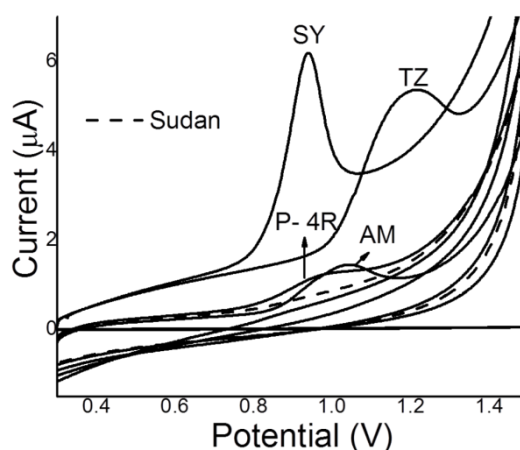*Adsorptive voltammograms and calibration curve show in the figure 7

Figure 8. Cyclic Voltammograms for SY, TZ, AM, SD y P-4R (14.6 μmol L^{-1}) with CS/GCE. pH 2.5, scan rate 100.0 mV s^{-1}.

3.8 Usefulness with real samples

In order to verify the usefulness of the sensor, samples from different matrix were analyzed such as orange marmalade, orange juice powder and orange juice containing SY and other dyes using the standard addition method. The results are summarized in Table 3. The results obtained have been similar to previous reports for SY using techniques such as high efficiency liquid chromatography (HPLC) and electroanalytical techniques, with values of 21.2 and 30.0 mg L^{-1} in orange juice [1,10]. The results obtained are below 10.0 mg/100 mL, which is the maximum amount allowed in some countries of South America [1]. On the other hand, the lifetime of the sensor was studied observing the change in the anodic peak currents after 50 cycles using CV. The results are shown in figure 9. It was clearly observed that the current decreased from 0.75 to 0.55 μA (27.6%) indicating a decrease of 0.50% of the anodic peak current for each cycle. These results indicate that the sensor can be used for a long period of time with a small activity loss.

Table 3. Results of SY detected in real food samples (n=3)

Sample	found (μmol L^{-1})	% RSD
orange marmalade	63.89±0.05	1.50
Tropical marmalade	75.03±0.07	2.20
orange juice powder	169.50±0.1	3.10
Tropical juice powder	101.70±0.09	2.9
Orange soft drink	39.96±0.05	1.3
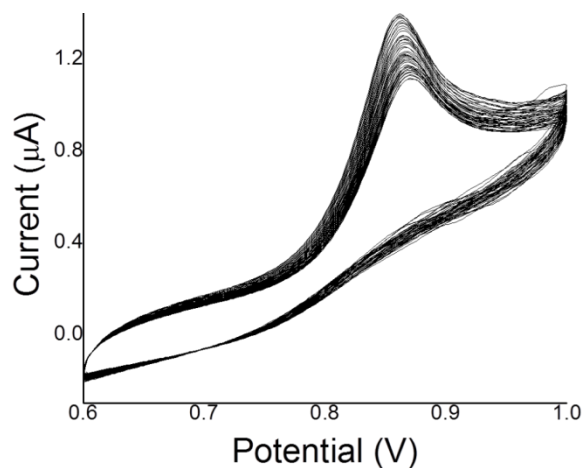
Figure 9. Lifetime for CS/GCE sensors performed with SY in PBS 0.01 mmol L^{-1} electrolyte. Scan rate of 50 mVs^{-1}.

4. CONCLUSIONS

Glassy carbon surface modified with chitosan was very convenient for the detection of SY in the presence of other dyes. The main characteristics of the census surface are related to the easy modification, sensitivity, stability. The analysis time was short because no more complete treatment of the samples was necessary before each analysis. On the other hand, the error presented is below 20% with samples spiked of less concentration than 10.0 μmol L^{-1}.

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