

# Determination of Allura Red in the Presence of Cetylpyridinium Bromide by Square-wave Adsorptive Stripping Voltammetry on a Glassy Carbon Electrode

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A simple method to determine Allura Red (AR) in cherry gelatin, chili sauce and strawberry juice by square-wave adsorptive stripping voltammetry (SWAdV) in the presence of cetylpyridinium bromide (CPB) is reported. Using a glassy carbon electrode (GCE), Amaranth (AM), Ponceau 4R (P-4R) and AR were oxidized to very close potential values in medium acid pH 3.0 with phosphate buffer solution (PBS), making selective AR detection impossible. Under these conditions AM, P-4R and AR were oxidized at 0.80, 0.77 and 0.81 V, respectively. When small amounts of CPB were added, an AR-CPB aggregate was formed. This change displaced the oxidation almost 0.14 V in the direction of the most positive potential and increased the current almost 50%, while AM and P-4R were oxidized at almost the same potential. Under these conditions, the selective determination of AR in food was possible. Detection limit was 0.032  $\mu\text{mol L}^{-1}$ . Finally, the method was successfully applied to the determination of AR in these foods.

**Keywords** Amaranth, Ponceau 4R, Allura Red, glassy carbon electrode, cetylpyridinium bromide

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## Introduction

Features such as stability in light, changes in pH and low microbiological contamination have allowed synthetic dyes to displace natural dyes in the food industry.<sup>1</sup> Amaranth (E123, AM), Ponceau 4R (E124, P-4R) and Allura Red (E129, AR) have been used simultaneously in drinks, gelatin, ice-creams, synthetic juice, candies and chili sauce due to the different shades of red and orange colors that can be obtained by combining them. In addition, the coloring provided by synthetic dyes to foods increases visual appeal. Some health problems caused by AM, P-4R and AR are: migraines, eczema, anxiety, hyperactive behavior in children, allergies and asthma problems. In addition, rapid accumulation of these coloring agents in tissues may have mutagenic and carcinogenic potential action.<sup>1-3</sup> The chemical structure of AM, P-4R and AR contains the azole group ( $-\text{N}=\text{N}-$ ) and the aromatic rings that are the main cause of health problems.<sup>3</sup> Moreover, the maximum permissible concentrations of AM, P-4R and AR recommended by the Food Agriculture Organization (FAO) and the World Health Organization (WHO) are 0.40, 0.50 and 100.0 mg/kg, respectively.<sup>2,3</sup> However, according to Colombian legislation, the permitted levels for AM and P-4R in foodstuffs are 200 and 300 mg/kg, respectively. Also, for AR Colombia does not have a restriction. This situation suggests the need to develop new simple, sensitive and selective methodologies that allow the detection of excessive use of these dyes in foods, especially AR.

High-performance liquid chromatography with UV-Vis

detector (HPLC-DAD) has been the most widely used technique for the simultaneous determination of AM, P-4R and AR<sup>4,5</sup> with a detection limit of 0.05 mg kg<sup>-1</sup> and 0.005 mg L<sup>-1</sup>, respectively. These methodologies have proven to be sensitive and selective, but of high instrumental and operational cost. Therefore, electroanalytical techniques are very convenient for this purpose as they combine excellent sensitivity, selectivity, precision and accuracy with low instrumentation. Electrochemical oxidation of azo dyes has permitted identification with detection limits of less than 0.01  $\mu\text{mol L}^{-1}$  using selectively modified electrodes for the simultaneous detection of AM and P-4R with MnO<sub>2</sub>-modified glassy carbon electrode<sup>6</sup> and carbon nanotube-polypyrrole composite,<sup>7</sup> simultaneous detection of AR and P-4R with graphene-TiO<sub>2</sub>-glassy carbon electrode modified<sup>8</sup> and multi-walled carbon nanotubes<sup>9</sup> and simultaneous detection of AM, P-4R and AR using bismuth film electrode.<sup>10</sup> On the other hand, ionic liquids were incorporated with carbon nanotubes (CNT) on the surface of the modified electrode to improve the dispersibility of CNT and the sensitivity in the determination of AM<sup>11</sup> and CPB surfactant formed aggregate with the dye tartrazina on a hanging mercury drop electrode (HMDE). The latter allowed the determination in the presence of the dye sunset yellow.<sup>12</sup> The surfactant tends to accumulate at an electrode surface or interface, which allows through an affinity or charge interaction an increase in the concentration of the analyte at the surface of the electrode.<sup>13</sup> In addition, different charge surfactants have been used for the detection of AR by UV-Vis spectroscopy, where the interaction with this dye was more effective with a positive charge surfactant.<sup>14</sup> The determination of AR using a GCE without modification in the presence of CPB has not been reported. The use of this electrode has been reported with some modification and no CPB.

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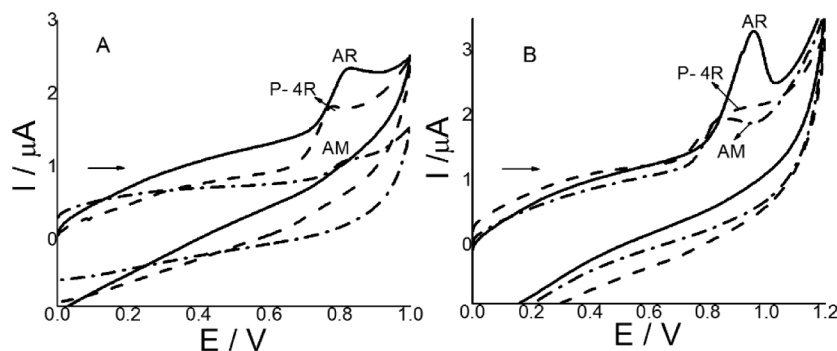


Fig. 1 Cyclic voltammograms of AM (dashed-dotted line), P-4R (dashed line) and AR (solid line)  $14.0 \mu\text{mol L}^{-1}$  without CPB (A) and with  $12.50 \mu\text{mol L}^{-1}$  CPB (B). Conditions: scan rate  $0.05 \text{ V s}^{-1}$ , pH 3.0 (PBS).

In light of the unrestricted use of AR in some countries the aim of this work was the development of a rapid, sensitive and selective methodology to detect AR using a GCE unmodified with CPB in the presence of AM and P-4R.

## Experimental

### Apparatus

Cyclic voltammograms and square wave voltammograms were obtained using a DropSens  $\mu\text{Stat 400}$  potentiostat. A GCE (CH-Instrument 3 mm), reference electrode ( $3.0 \text{ mol L}^{-1}$ ) Ag/AgCl and platinum wire electrode were used in the electrochemical cell. In the preparation of the supporting electrolytes, a pH meter Lovibond SD 50 was used.

### Chemicals

The water used in the preparation of standard solutions and sample preparation was obtained from the Purifier System Wasselab ASTM D1193. Standard solutions of  $0.60 \text{ mmol L}^{-1}$  AR, AM, 4R and  $0.06 \text{ mmol L}^{-1}$  AR were prepared from pure reagent (Sigma-Aldrich). A volume of 10 mL of these standard solutions was used throughout the study. CPB was obtained from Merck. The optimal concentration of the PBS supporting electrolyte was between  $0.05 - 0.025 \mu\text{mol L}^{-1}$   $\text{H}_3\text{PO}_4/\text{H}_2\text{PO}_4^-$ ,  $\text{CH}_3\text{COOH}/\text{CH}_3\text{COO}^-$  and  $\text{HNO}_3$  (Merck).

### Sample preparation

Food products were purchased from a local supermarket in Ibagu  (Colombia). They were previously weighed and diluted 10 times. The cherry gelatin and strawberry juice contained sugars, fumaric acid, sodium citrate, ascorbic acid, carboxymethylcellulose, citric acid, aspartame, phenylalanine, titanium dioxide, tartrazine and sunset yellow. The chili sauce contained tomato paste, pepper extract, chili, acetic acid, citric acid, carboxymethyl cellulose and sodium benzoate. All three samples contained AR.

### Cyclic and adsorptive voltammetry measurement

Prior to each measurement, the GCE was carefully polished using a soft cloth containing 0.05 and 0.3 mm alumina, washed successively with methanol and distilled water in an ultrasonic bath (40.0 Hz) for 3 min and dried at  $60^\circ\text{C}$ .

For CV, 9.5 mL of deionized water, 0.50 mL of supporting electrolyte and  $0.25 \text{ mL}$  ( $0.60 \text{ mmol L}^{-1}$ ) AM, P-4R and AR were added to the voltammetry cell. Then, after an equilibration time of 3 s cyclic voltammograms were recorded, while the

potential was scanned from 0.3 to 1.2 V with a scan rate of  $0.05 \text{ V s}^{-1}$ . Each CV measurement was repeated three times. For SWAdV, 9.5 mL of deionized water, 0.25 mL of supporting electrolyte and between  $20.0 - 100 \mu\text{L}$  ( $0.060 \text{ mmol L}^{-1}$ ) AR ( $0.11 - 0.59 \mu\text{mol L}^{-1}$  in the cell) were added in the electrochemical cell. After 3 s of equilibration time, square-wave voltammograms were recorded, while the potential was scanned from 0.2 to 1.3 V with time adsorption ( $t_{\text{ads}}$ ) of 60 s, potential adsorption ( $E_{\text{ads}}$ ) of 0.0 V, frequency ( $f$ ) of 15.0 Hz and pulse amplitude of 0.05 V. Each SWAdV measurement was repeated three times.

## Results and Discussion

### Electrochemical behavior of AM, P-4R and AR on GCE in the presence of CPB

It was found that  $14.0 \mu\text{mol L}^{-1}$  AM, P-4R and AR can be easily oxidized to pH 3.0 on a GCE. Figure 1A shows the oxidation voltammograms for AM, P-4R and AR. The results clearly show that the three dyes were oxidized between 0.78 and 0.81 V with a separation potential less than 0.03 V. Moreover, anodic peak currents were 0.38, 0.63 and  $0.45 \mu\text{A}$ , respectively. These results showed that under these conditions, the selective determination of AR in the presence of AM and P-4R is impossible. In order to determine AR in the presence of AM and P-4R, the interaction of CPB surfactant with these dyes was studied. The voltammograms of the interaction of CPB with the three dyes are shown in Fig. 1B. The effect produced by CPB on the oxidation of AM, P-4R and AR is uncertain because the CPB can act either as an extractor agent to facilitate the adsorption or interfere due to competitive adsorption.<sup>12</sup> The results showed a shift of the oxidation potential for AR from 0.81 to 0.95 V, possibly by the formation of the AR/CPB aggregate. The interaction of AR with CPB makes the oxidation require more energy. On the other hand, the current increases by almost 50% (from  $0.45$  to  $1.10 \mu\text{A}$ ) indicating that the concentration of AR increases at the surface of the electrode. Anodic peak current for AM and P-4R showed no significant changes, indicating that surfactant aggregates between AM/CPB and P-4R/CPB were lower compared to AR/CPB. Likely, the anionic charge and the smaller molecular size of AR allowed the formation of this aggregate with the cationic CPB surfactant. In addition, surfactants can change the kinetics of electron-transfer reactions.<sup>15</sup> Under these conditions, it is possible to determine AR in the presence of AM and P-4R.

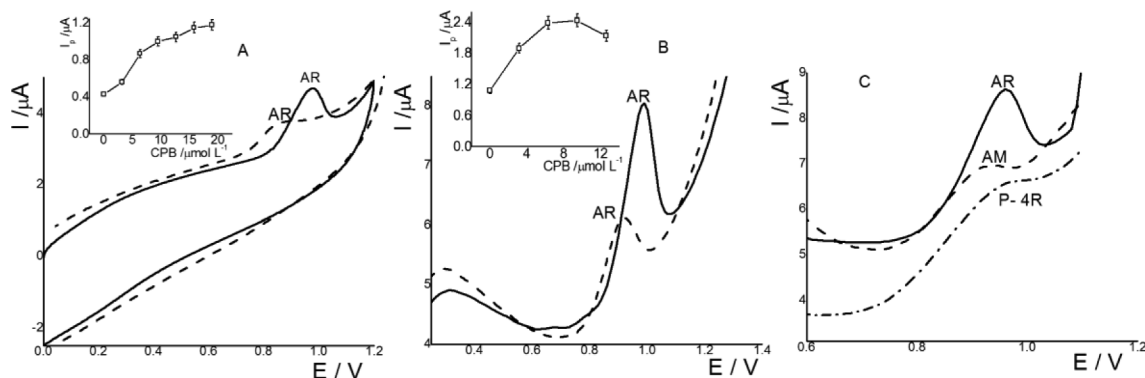


Fig. 2 (A) Cyclic voltammograms for  $14.0 \mu\text{mol L}^{-1}$  AR (dashed line) and with presence of  $18.50 \mu\text{mol L}^{-1}$  CPB (solid line). (B) Square wave voltammograms for  $2.0 \mu\text{mol L}^{-1}$  AR (dashed line) and with presence of  $12.6 \mu\text{mol L}^{-1}$  CPB (solid line). (C) Square wave voltammograms for  $2.0 \mu\text{mol L}^{-1}$  AM (dashed line), P-4R (dashed-dotted line) and AR (solid line) in the presence of  $8.5 \mu\text{mol L}^{-1}$  CPB using GCE. Conditions: scan rate  $0.05 \text{ V s}^{-1}$ , pH 3.0 (PBS),  $0.0 \text{ V } E_{\text{ads}}$  and  $60 \text{ s } t_{\text{ads}}$ .

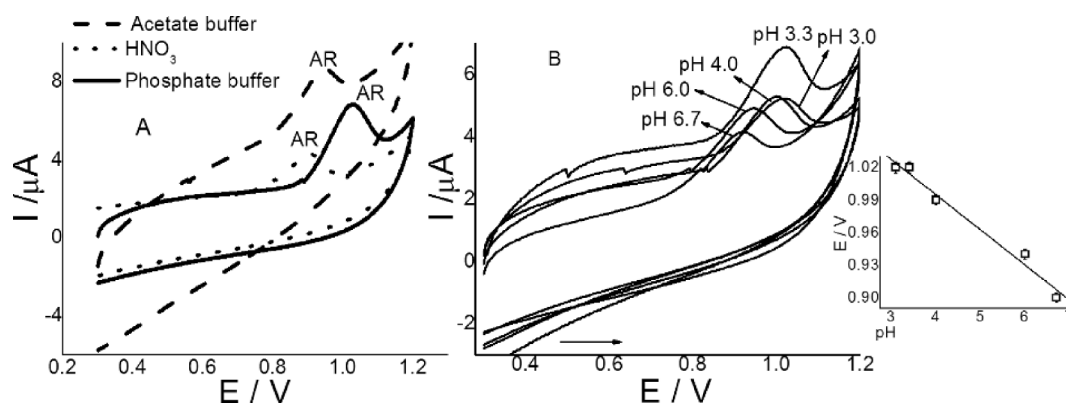


Fig. 3 (A) Cyclic voltammograms of  $14.0 \mu\text{mol L}^{-1}$  AR with  $15.6 \mu\text{mol L}^{-1}$  CPB using  $\text{HNO}_3$  (dashed line), acetate buffer (dotted line) and phosphate buffer (solid line) supporting electrolytes. (B) Cyclic voltammograms of AR at various pHs (insert  $E_{\text{pa}}$  vs. pH) using GCE; conditions as in Fig. 1.

#### Effect of the concentration of CPB

The properties of the surfactants depend mainly on the charge and concentration.<sup>13</sup> Therefore, the interaction of AR and CPB was studied as a function of the concentration between  $3.16$  and  $18.96 \mu\text{mol L}^{-1}$  CPB with  $14.0 \mu\text{mol L}^{-1}$  AR by CV (Fig. 2A) and  $2.0 \mu\text{mol L}^{-1}$  by SWAdV (Fig. 2B).

In the results, it was observed that anodic peak current increased by almost 50% for AR before the concentration of CPB began to interfere and anodic peak current for AR decreased. The optimum concentration was  $15.6 \mu\text{mol L}^{-1}$  by CV and  $8.5 \mu\text{mol L}^{-1}$  by SWAdV. In addition, the anodic peak current for AR increased using SWAdV at a lower concentration. Therefore,  $8.5 \mu\text{mol L}^{-1}$  of CPB and SWAdV were chosen for other studies. With regard to CPB concentration, in the determination of RA by UV-Vis spectroscopy, the concentration of cetyltrimethylammonium bromide (CTAB) cationic surfactant was 100 times higher.<sup>14</sup> Figure 2C shows the square wave voltammograms of  $2.0 \mu\text{mol L}^{-1}$  AM, P-4R and AR in the presence of  $8.6 \mu\text{mol L}^{-1}$  CPB. A separation between AM-AR of  $0.05 \text{ V}$  and signals for P-4R-AR were observed at almost the same potential value, but the signal for P-4R was almost 5% compared to the AR signal. These results indicate that the selective detection of AR by SWAdV is possible in the presence of AM and P-4R.

#### Effect of supporting electrolyte and pH for AR with CPB

The effect of the supporting electrolyte on the oxidation of AR was studied in order to obtain a solution with the least amount of ions in solution and to improve the conductivity in the electrochemical cell. Acetate buffer solution, PBS and  $0.001 \text{ mol L}^{-1}$  nitric acid at pH 3.0 with  $14.0 \mu\text{mol L}^{-1}$  AR and  $15.6 \mu\text{mol L}^{-1}$  CPB using a GCE were used.

Figure 3A shows the anodic peak currents for AR with  $\text{HNO}_3$  (dashed line),  $\text{CH}_3\text{COOH}/\text{CH}_3\text{COO}^-$  (dotted line) and  $\text{H}_3\text{PO}_4/\text{H}_2\text{PO}_4^-$  (solid line). The results showed that in the presence of acetate and  $\text{HNO}_3$  solutions, anodic peak currents for AR were lower compared to the phosphate solution. Moreover, the phosphate solution allowed for the oxidation of AR to more positive potentials. Therefore, a PBS supporting electrolyte was chosen for further studies. Optimum concentration of the electrolyte was  $0.05 \mu\text{mol L}^{-1}$  by CV and  $0.025 \mu\text{mol L}^{-1}$  by SWAdV. AR, like sunset yellow, has only two sulfonic acid groups and an azo group with  $\text{p}K_{\text{a}} = 9.20$ .<sup>16</sup> Therefore, the study of pH is important. The oxidation of  $14.0 \mu\text{mol L}^{-1}$  AR between pH 3.0 and 6.7 was studied using  $0.001 \text{ mol L}^{-1}$  PBS on a GCE. The voltammograms are shown in Fig. 3B and the linear relationship between  $E_{\text{pa}}$  (V) versus pH are insert in the Fig. 3B. The results showed the change from potential to less positive potential values with an increase in pH

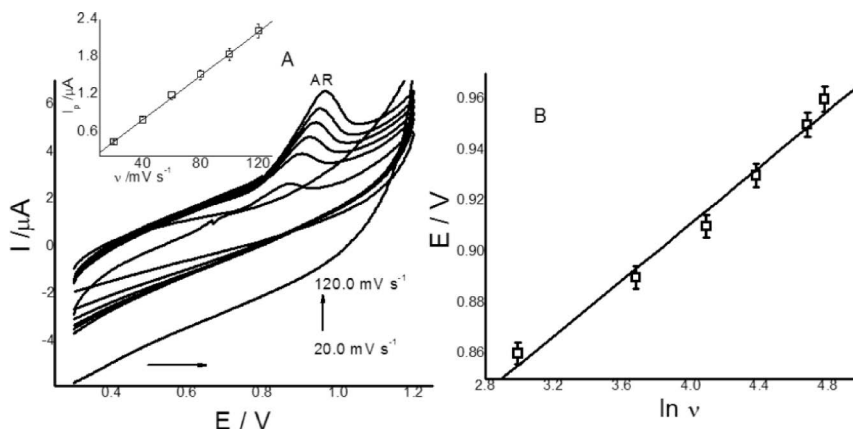


Fig. 4 (A) Scan rate voltammograms between 0.02–0.12 V s<sup>-1</sup> (insert  $I_{pa}$  vs.  $v$ ) and (B) the relationship of peaks potential vs.  $\ln v$  for AR; conditions as in Fig. 3.

value from 1.02 to 0.90 V with a regression equation of  $E$  (V) =  $-0.032\text{pH} + 1.12$  ( $R^2 = 0.989$ ) with a slope value that is almost half of the theoretical value 0.059, indicating that  $\text{H}^+ = e^-$ . The pH results are in accordance with the scan rate results previously discussed. The highest anodic peak current was observed at pH 3.3, where AR is protonated and is less hydrophilic, facilitating the adsorption. This value was chosen for further studies.

#### *The influence of scan rate ( $v$ ) on anodic peak currents for AR with CPB*

The influence of scan rate ( $v$ ) on anodic peak currents for 14.0  $\mu\text{mol L}^{-1}$  AR using a GCE and CPB was studied. Scan rate was studied between 0.02–0.12 V s<sup>-1</sup>. In Fig. 4A, the regression equation was  $I_{pa} = 0.186 + 0.017v$  ( $R^2 = 0.999$ ) for AR. These results indicate that the process is controlled by adsorption. On the other hand, the potential  $E_{pa}$  of AR increased proportionally to  $\ln v$  with the regression equation of  $E_{pa} = 0.689 + 0.055 \ln v$  ( $R^2 = 0.992$ ) as shown in Fig. 4B. It was determined that the charge-transfer coefficient generally calculated is of 0.50 for the irreversible system controlled by adsorption and  $n$  (number of electrons) obtained was of 1.0, indicating a transfer of one electron for the oxidation process of AR in a GCE. Similar results were reported using functionalized nanoparticles of graphene and nickel of poly(diallyldimethylammonium) where the value of the slope reported was 0.047  $\ln v$  and  $n = 1.09$ .<sup>3</sup>

#### *Square-wave adsorptive stripping voltammetry variables on anodic peak currents for AR*

The parameters studied for square wave stripping voltammetry were frequency (Hz), step amplitude (V) and pulse amplitude (V). Anodic peak currents for AR increased when the frequency was 15 Hz at higher frequencies the signal AR loses resolution. In real samples, it was necessary to reduce to 10 Hz. Step amplitude of 0.01 V, and pulse amplitude of 0.05 V were selected for further experiments.

#### *Calibration curve for quantifying the detection limit (DL) of AR on GCE in the presence of CPB*

Square wave adsorptive voltammograms and the net anodic currents for AR under optimized conditions, pH 3.3 (250  $\mu\text{L}$  of 0.001 mol L<sup>-1</sup> PBS), 0.0 V  $E_{ads}$  and 60 s  $t_{ads}$  were obtained and the calibration curve between 0.06 and 11.5  $\mu\text{mol L}^{-1}$  AR was plotted. A regression equation was obtained of

$I_{(\mu\text{A})} = 1.013C_{AR} + 0.141$  ( $R^2 = 0.999$ ). At higher concentrations of 12.0  $\mu\text{mol L}^{-1}$  AR, anodic peak current decreases. Possibly, the surface of the electrode was saturated. DL ( $3\sigma/b$ ) obtained was of 0.032  $\mu\text{mol L}^{-1}$ . Reproducibility as relative standard deviation was 0.50% ( $n = 7$ ) for 14.6  $\mu\text{mol L}^{-1}$  AR using two different electrodes. With respect to the detection limit, this work is compared with previous works<sup>3,8,9,17</sup> where modified GCEs have been used. Although the methods described in the earlier reports are more sensitive than the method proposed in this work, but these sensors are more complex to develop. On the other hand, the calculated detection limit in this work is low enough to achieve the detection of AR in foods.

#### *Interference study and analytical utility*

Before verifying the usefulness of the method for analysis of AR in commercial food samples, it is necessary to verify the interferences that can occur with some organic and inorganic substances that are also present in the foods. The interference of metal ions was evaluated with ICP multi-element standard solution IX (Merck) containing 100 mg L<sup>-1</sup> As, Be, Cd, Cr(VI), Hg, Ni, Pb, Se and Tl. Fortunately, no signal between 0.0 and 1.2 V was observed. With tartrazine, sunset yellow, Sudan I and Sudan II in concentrations 100 times higher, no signal was observed between 0.6 and 1.2 V. Therefore, it was determined that the developed method in this work allows for the selective detection of RA in food.

Since we did not have standard reference material with certified AR content, a series of recovery studies were carried out to verify the accuracy using food that contains AR. The samples were analyzed three times and the slope ( $m$ ), intercept ( $b$ ) and correlation ( $R^2$ ) values of each calibration curve are summarized in Table 1 and the detected amounts of AR are shown in Table 2. The results in Table 1 showed that the slope values of the three samples have a minimal variation, indicating that the matrix of the samples did not affect the versatility of the method. On the other hand,  $R^2$  values were almost at 0.99, indicating good stability. Only for the strawberry juice sample, the correlation value was less than 0.98. The results of the recovery of each obtained sample in Table 2 were satisfactory and similar to those quantified with vitreous carbon-modified electrodes.<sup>3,9</sup> The quantified values were much lower than the values allowed in Colombia.

The method lifetime was demonstrated using cyclic voltammetry after 60 cycles. The current intensity variation was 18.9% (from 0.791 to 0.641  $\mu\text{A}$ ) AR, indicating a current

Table 1 Values of the linear regression for the calibration curves of the real samples

Sample	Slope ( <i>m</i> )	Intercept ( <i>b</i> )	<i>R</i> <sup>2</sup>
Cherry gelatin			
1	0.197 ± 0.043	0.6308 ± 0.16	0.988
2	0.210 ± 0.0034	0.791 ± 0.025	0.999
3	0.122 ± 0.011	0.654 ± 0.044	0.995
Chili sauce			
1	0.193 ± 0.006	0.674 ± 0.015	0.999
2	0.188 ± 0.005	0.714 ± 0.011	0.998
3	0.201 ± 0.009	0.594 ± 0.013	0.996
Strawberry juice			
1	0.119 ± 0.034	0.845 ± 0.140	0.979
2	0.096 ± 0.064	0.850 ± 0.064	0.987
3	0.094 ± 0.0053	0.758 ± 0.0053	0.985

Table 2 Quantification and % recovery of AR in real samples

Sample	Spiked/ μmol L <sup>-1</sup>	Expected/ μmol L <sup>-1</sup>	Found/ μmol L <sup>-1</sup>	Recovery, %
Cherry gelatin	—	—	4.16 ± 0.10	—
	2.98	7.14	6.80 ± 0.10	95.5
Chili sauce	—	—	3.49 ± 0.20	—
	2.98	6.47	6.77 ± 0.20	104.2
Strawberry juice	—	—	7.96 ± 0.15	—
	2.98	10.94	11.18 ± 0.10	97.85

decrease of 0.33% for each cycle. These results indicate that the GCE with CPB can be used for a long period of time without reducing its capacity.

## Conclusions

The GCE was highly selective for AR detection in solution using a PBS supporting electrolyte at pH 3.3. The presence of CPB was very convenient to control interference caused by AM and P-4R and increase sensitivity. The detection limit was less than 0.1 μmol L<sup>-1</sup> and the matrix effects were minimal. In addition, the method can be used for more than 60 measurements with little loss of reliability. Furthermore, the proposed method decreases the time of analysis, since it is not necessary to apply

to samples pretreatment before each analysis and each analysis takes only 60 s.

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