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Adsorptive Stripping Voltammetric Determination of Tartrazine and Sunset Yellow in Gelatins and Soft Drink Powder in the presence of Cetylpyridinium Bromide

Marisol Gómez, Verónica Arancibia[*]*, Carlos Rojas, Edgar Nagles.*

Pontificia Universidad Católica de Chile, Facultad de Química, Vicuña Mackenna 4860, Santiago–7820436, Chile.
[*]E-mail: darancim@uc.cl

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The use of a hanging mercury drop electrode (HMDE) to determine tartrazine and sunset yellow in gelatins and soft drink powder by adsorptive stripping voltammetry in the presence of cetylpyridiniun bromide (CPB) is reported. With an HMDE it is possible to determine individually tartrazine and sunset yellow at pH values of 8.5 and 9.8, respectively. Under these conditions tartrazine was reduced at –0.60 V and sunset yellow at –0.65 V, and the linear calibration curves ranged from 6.6–300 μgL^{-1} and 3.3–160 μgL^{-1}, respectively. When CPB was added to the electrochemical cell, a tartrazine/CPB aggregate was formed, changing the reduction to 100 mV more negative potentials with a finer signal, while sunset yellow was reduced at almost the same potential (–0.55 V at pH 8.5) and the peak current decreased. Under these conditions the simultaneous determination of these dyes was possible. The limits of detection were 3.3 and 1.6 $\mu g\ L^{-1}$ for tartrazine and sunset yellow, respectively (pH 8.5, ammonium buffer, E_{ads} = –0.3 V and t_{ads} = 30 s) and the linear range was up to 100 μgL^{-1} for both. The method was validated using undyed gelatin spiked with tartrazine and sunset yellow. Finally, the method was successfully applied to the determination of these dyes in lemon-, orange- and papaya-flavored gelatin and in a peach flavored soft drink powder.

Keywords: Sunset yellow; Tartrazine; Foods dyes; Adsorptive stripping voltammetry; Soft drinks; Gelatins; Surfactants

1. INTRODUCTION

Synthetic dyes are widely used for improving the color and enhancing the visual aesthetic appeal of some foods, and this effect is maintained throughout the production process and during storage. They present high stability to light, oxygen, and pH changes, and have lower prices compared to natural dyes [1,2]. Synthetic dyes can be classified into water soluble and fat soluble, and only the former are permitted in foods. Some of the most commonly used are sunset yellow (E110), allura red

(E129), brilliant blue (E133), erythrosine (E127), tartrazine (E102), amaranth red (E123), ponceau 4R (E124), and others. They are present in soft drinks, gelatins, snacks, yoghurts, ice cream, candies, puddings, chips, pickles, honey, mustard, gum, baked goods, etc. However, these dyes must be controlled because many of them have been related to health problems. It has been reported that synthetic dyes present in foods may cause overactive behavior in children, especially if they are consumed in excess. On the other hand, some dyes in the presence of analgesics like aspirin can induce allergic or asthmatic problems. Tartrazine and sunset yellow are the second and third most widely used food dyes, added to many foods [3–5]. According to the 1994 laws of the European Union, the maximum levels of some dyes, including tartrazine and sunset yellow, should be the following: 100 μg mL^{-1} (individually or in combination) in non–alcoholic flavored drinks, 150 μg g^{-1} in desserts including flavored milk products, and 200 μg g^{-1} in candied fruits and vegetables. On the other hand, Sudan I (1–(phenylazo)–2–naphthalenol), which is an unauthorized fat-soluble dye, may be formed during the production of sunset yellow, and its presence in sunset yellow was restricted to less than 0.5 μg g^{-1} [6]. In 2011 the European Union announced that they would be reducing the maximum permitted concentration of sunset yellow (in drinks) to 20 μg mL^{-1}. Therefore, accurate and selective methods for the determination of synthetic dyes are very important. Several methods have been proposed for the analysis of synthetic dyes: Aci et al. [7] used derivative spectrometry for the determination of tartrazine, indigo carmine and, erythrosine; Bozdogan et al. [8] used first derivative spectrophotometry and partial least–squares multivariate spectrophotometric calibration for the determination of sunset yellow and Ponceau 4R; Berzas et al. [9] used the derivative spectrophotometric ratio spectrum zero crossing method for the determination of tartrazine, sunset yellow, and Ponceau 4R; Ni et al. [10] optimized chemometric analysis with spectrophotometric measures for the determination of amaranth, Ponceau 4R, sunset yellow, tartrazine, and brilliant blue; Fernández et al. [11] used a direct spectrophotometric method with multivariate curve resolution optimized by alternating least squares for the determination of amaranth, sunset yellow, and tartrazine; Dinc et al. [12], used double divisor ratio spectra derivative (graphical method), classical least squares, and principal component regression (two numerical methods) methods for the analysis of sunset yellow, tartrazine, and allura red. On the other hand, Ma [13] used high performance liquid chromatography with photodiode array detector for the determination of sunset yellow, allura red, brilliant blue, erythrosine, tartrazine, amaranth red, and ponceau 4R; and Yahya [13] applied a hybrid linear analysis method developed by Olivieri and Goicoechea [14] using chromatographic data of allura red, sunset yellow and tartrazine.

The aim of this work is to show that adsorptive stripping voltammetry with a hanging mercury drop electrode makes it possible to simultaneously determine tartrazine and sunset yellow. For many years the electrochemistry of the azo compounds has been studied with different electrodes; specifically for the determination of tartrazine Song et al. [15] used a multi walled carbon nanotube-modified pyrolytic graphite electrode; Yang et al. [16] used a glassy carbon electrode modified with acetylene black nanoparticles; for the simultaneous determination of tartrazine and sunset yellow Silva et al. [5] used a polyallylamine-modified tubular glassy carbon electrode; Zhang et al. [17] used a multi walled carbon nanotube film-modified electrode; and Ghoreishi et al. [18] used a gold

nanoparticle carbon paste electrode. However, to the best of our knowledge, the effect of cetylpirydinium bromide on the position of the signals and the peak current has not been reported.

2. EXPERIMENTAL PART

2.1. Apparatus

The voltammograms were obtained on a Metrohm model 797 VA Computrace in a three–electrode configuration. A hanging mercury drop electrode (HMDE) was used as working electrode with an Ag/AgCl/KCl 3 mol L^{-1} reference electrode, and a platinum wire auxiliary electrode. The solutions were deoxygenated with high purity nitrogen. The pH measurements were made with an Orion–430 digital pH/mV meter equipped with a combined pH glass electrode.

2.2. Reagents and solutions

Water used for sample preparation, reagent dilution, and rinsing purposes was obtained from a Milli–Q system (18.2 MΩ. Millipore, USA). All the chemicals (boric acid, acetic acid, phosphoric acid, sodium hydroxide, methanol, etc.) were analytical grade from Merck (Darmstadt, Germany). Tartrazine (Tt), sunset yellow (Sy) and cetylpyridinium bromide (CPB) were obtained from Aldrich. Stock solutions containing 10.0 mg L^{-1} of tartrazine and sunset yellow were prepared water. Britton Robinson (BR) buffer solutions were used to investigate pH in the 4.5–10.0 range. These buffers (0.4 mol L^{-1}) were prepared by mixing equal volumes of orthophosphoric acid, acetic acid, and boric acid, adjusting to the required pH with 2.0 mol L^{-1} NaOH solution. Ammonium buffers (0.4 mol L^{-1}) were prepared with ammonium acetate and adjusted to the required pH with NH_3 solution.

2.3. Sample preparation

Four kinds of powdered gelatins of the same brand were bought in a supermarket in Santiago (Chile). These gelatins contain sugars, fumaric acid, sodium cyclamate, and sodium saccharin. The lemon-flavored gelatin contained tartrazine, the orange-flavored one contained sunset yellow, while the papaya-flavored one contained both tartrazine and sunset yellow. The soft drink powder contains sugar, citric acid, fumaric acid, sodium cyclamate, maltodextrine, sodium saccharin, aspartame, titanium dioxide, tartrazine, and sunset yellow. The preparation instructions for the powdered gelatin box specified 160 g L^{-1} and for the soft drink 45 g L^{-1}. Undyed gelatin was used for validation measurements. Solutions with 1.0 g L^{-1} of gelatin or soft drink powder were prepared in water and aliquots of 0.2–2.0 mL were added to the voltammetric cell.

2.4. Procedure

9.5–mL of deionized water, 0.5 mL of Britton–Robinson buffer solution (0.4 mol L^{-1}), and 10 μL aliquots of tartrazine and/or sunset yellow solution (10.0 mg L^{-1}) were pipetted into the

voltammetric cell. The solution was purged with nitrogen (saturated with water vapor) for 5 minutes in the first cycle and for 60 s in each successive cycle. Then, after eliminating some drops (size: 8), a new mercury drop was extruded to initiate the preconcentration step for a given t_{ads} and E_{ads} at a stirring speed of 1800 rpm. After an equilibration time of 10 s, the adsorptive voltammogram was recorded, while the potential was scanned from –0.2 to –0.9 V using square wave modulation with 10 mV step amplitude, 20 mV pulse amplitude, and a frequency of 25 Hz (sweep rate 0.248 V s^{-1}). Each voltammogram was repeated three times. The calibration curves were obtained and linear regression and detection limits were calculated. To eliminate matrix effects the standard addition method was used. All data were obtained at room temperature (~25 °C).

3. RESULTS AND DISCUSSION

The electrochemical reduction of tartrazine (4,5–dihydro–5–oxo–1–(4–sulfophenyl)–4–[(sulfophenyl)azo]–1H–pyrazole–3–carboxylic acid trisodium salt) and sunset yellow (1–p–sulfophenylazo–2–naphthol–6–sulfonic acid disodium salt) was reported many years ago. At pH close to 7 the azo group is reduced as follows: R–N=N–R` + 2e− + $2H^+$ → R–NH–NH–R` [19]. With a mercury electrode these reactions take place between –0.4 and –0.8 V (pH 7.0) [20]. In order to reach the best selectivity and sensitivity, individual studies were carried out with tartrazine and sunset yellow as a function of pH, adsorptive potential (E_{ads}), and adsorptive time (t_{ads}).

3.1. Effect of operational parameters

3.1.1. Effect of pH

The influence of pH on the adsorptive peak currents of tartrazine and sunset yellow were studied in the 4.5–10.0 range (Fig. 1A). In order to keep the composition of the buffer constant when studying the effect of pH, Britton–Robinson (BR) buffers were used. The experimental conditions were: $C_{Tt} = C_{Sy} = 20.0$ μg L^{-1}; E_{ads} = –0.10 V and t_{ads} = 30 s. It was found that at pH 8.0 the peak current of tartrazine is maximum, while the peak current of sunset yellow is maximum at pH 9.8. Then, ammonium buffer solutions were used in the pH 8.0–9.0 range. Fig. 1B shows the adsorptive stripping voltammograms obtained with tartrazine solution at pH 8.0 in BR buffer (curve a) and at pH 8.5 with ammonium buffer (curve b). The peak current obtained with ammonium buffer was higher (–23.1 nA) than with BR buffer (–19.4 nA) and the potential was displaced to a more positive value (from –0.67 to –0.62 V). Further measurements were carried out individually for tartrazine at pH 8.5 (ammonium buffer) and sunset yellow at pH 9.8 (BR buffer). Tartrazine presents two strong sulfonic acid groups (pKa:2), one acetate weak acid group (pKa:5) and one azo group (pKa: 10.86). To pH 8.5 only the azo group is protonated, the charge is 2^- and the molecule is high hydrophilic. A higher pH values the net charge is increased and the tartrazine is not adsorbed on the surface electrode. On the other hand, Sunset Yellow has only two sulfonic acid groups, the azo group is deprotonated at pH 9.8 (pKa = 9.20) and the structure presents a naphthalene ring, for these reasons it is the less hydrophilic [21].

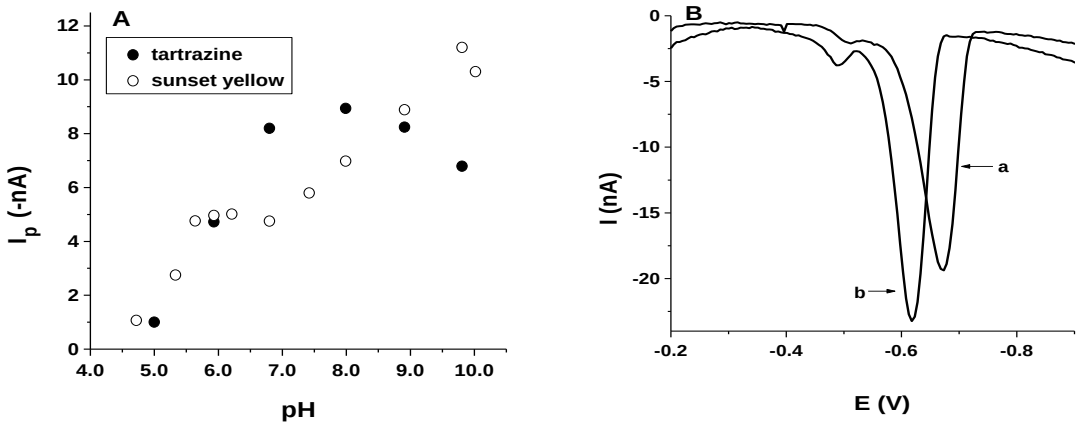

Figure 1. (A) Effect of pH on the peak current of tartrazine and sunset yellow. Conditions: $C_{Tt} = C_{Sy}$ = 20.0 μg L^{-1}; 0.02 mol L^{-1} BR buffer. (B) Tartrazine (20.0 μg L^{-1}): BR buffer (pH:8.0) (curve a); Ammonium buffer (pH:8.5) (curve b). E_{ads} = −0.10 V; t_{ads} = 30 s. Step amplitude = 10 mV; pulse amplitude = 20 mV, and frequency = 25 Hz.

3.1.2. Effect of accumulation potential (E_{ads})

Fig. 2 shows the effect of the accumulation potential on the stripping peak current of tartrazine in ammonium buffer (pH 8.5) and sunset yellow in BR buffer (pH 9.8) over the –0.1 to –0.6 V range. The experimental conditions were: C_{Tt} = 49.8 μg L^{-1}; C_{Sy} = 20.0 μg L^{-1}, and t_{ads} = 30 s. As shown in Fig. 2, the peak current of tartrazine and sunset yellow remains constant from –0.1 to –0.4 V and then tends to drop. An accumulation potential of −0.3 V gives the best sensitivity and was selected for further measurements.

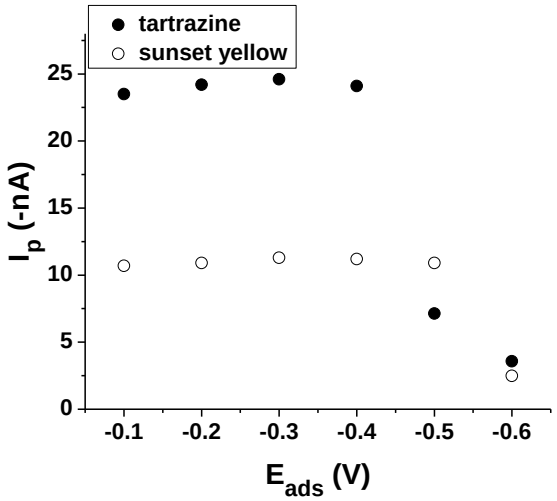

Figure 2. Effect of accumulation potential on the peak current of tartrazine and sunset yellow. Conditions: (•) C_{Tt} = 49.8 μg L^{-1}; pH 8.5 (0.02 mol L^{-1} ammonium buffer). (o) C_{Sy} = 20.0 μg L^{-1}; pH 9.8 (0.02 mol L^{-1} BR buffer). t_{ads} = 30 s. Other conditions as in Fig.1B.

3.1.3. Effect of accumulation time (t_{ads})

Figure 3 shows the effect of accumulation time on the stripping peak current of tartrazine (pH 8.5 ammonium buffer) and sunset yellow (pH 9.8 BR buffer) over the 0–350 s range. The experimental conditions were: C_{Tt} = 49.8 μg L^{-1}; C_{Sy} = 20.0 μg L^{-1}, E_{ads} = –0.3 V. Peak current increases with increasing accumulation prior to the potential scan, indicating that the synthetic dyes are readily adsorbed on the HMDE. The peak current of tartrazine increased with time up to 150 s and the peak current of sunset yellow increased with time up to 350 s, because the concentration used was lower than that of tartrazine. However, considering the speed of the measurement, t_{ads} of 30 s was used for further studies, but in the analysis of real samples higher times can be used to achieve good sensitivity.

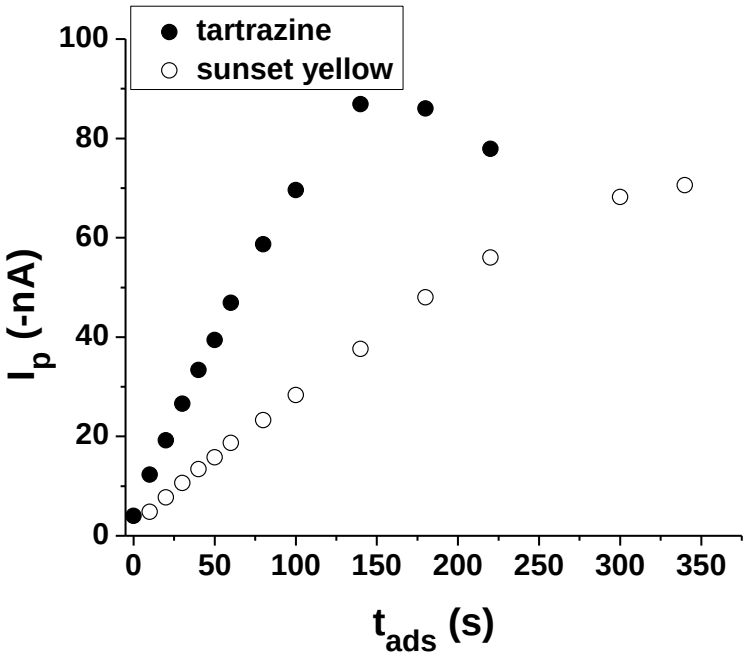

Figure 3. Effect of accumulation time on the peak current of tartrazine and sunset yellow. Conditions: (•) C_{Tt} = 49.8 μg L^{-1}; pH: 8.5 (ammonium buffer). (o) C_{Sy} = 20.0 μg L^{-1}; pH: 9.8 (BR buffer). E_{ads} = –0.3 V. Other conditions as in Fig. 1B.

3.2. Analytical parameters

The calibration graphs for the individual determination of tartrazine and sunset yellow were obtained under the optimized conditions: pH 8.5 (ammonium buffer) for tartrazine and pH 9.8 (BR buffer) for sunset yellow; E_{ads} = –0.3 V and t_{ads} = 30 s. As illustrated in Fig. 4A, for tartrazine the relation was linear until 300 μg L^{-1}, while for sunset yellow it was linear until 160 μg L^{-1} (Fig. 4B). The limit of detection (LoD) obtained with these calibration curves was 6.6 μg L^{-1} (0.012 μmol L^{-1}) for tartrazine and 2.7 μg L^{-1} (0.006 μmol L^{-1}) for sunset yellow. As t_{ads} increases the LoD gets lower.

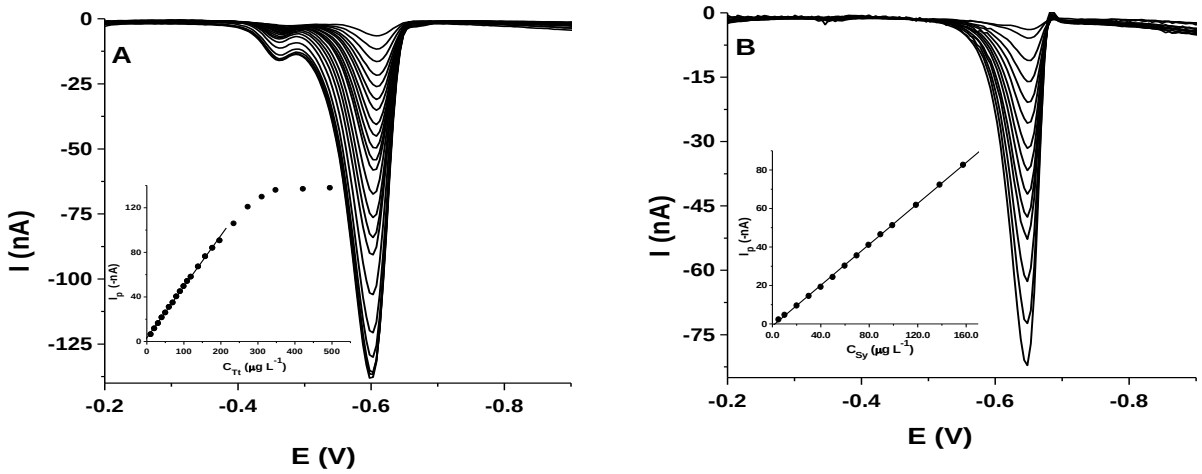

Figure 4. Adsorptive voltammograms and calibration curves for tartrazine (A) and sunset yellow (B) Conditions: (A) pH 8.5; (B) pH 9.8. E_{ads} = –0.3 V; t_{ads} = 30 s. Other conditions as in Fig. 1B.

3.3. Effect of CPB concentration

One possible way of enhancing the adsorptive process or producing changes in the peak potentials is the use of surfactants, whose beneficial effects are unpredictable, as they tend to interfere by competitive adsorption. The presence of surfactants can shift the absorbance and fluorescence properties, the solubility of the compounds and the kinetics of the electron–transfer reaction, and it can affect the reversibility of the systems, etc. Depending on the structure and concentration of the dyes and the surfactant, dye/surfactant complexes and salts, ion pairs and their aggregates, self–aggregates of the dye, dye–rich premicelles, and pure micelles of surfactants with solubilized dye monomers can be formed [22]. Electrostatic interactions and steric factors are also important in the dye–surfactant binding process.

With the purpose of determining tartrazine and sunset yellow simultaneously by changing the charge of the dyes or producing electrostatic interaction with the mercury electrode, a cationic surfactant (cetylpyridinium bromide, CPB) was added to the electrochemical cell. As illustrated in Fig. 5A, the peak current of tartrazine decreased about 27.8% in the presence of 5.6 µmol L^{-1} of CPB, and the signal was displaced from –0.62 to –0.70 V. On the other hand, the peak current of sunset yellow decreased about 66.5% and the signal remained at almost the same potential in the presence of CPB below the critical micelle concentration. The separation of the signals changed from 70 to 140 mV. Under these conditions the peak current was proportional to the concentration of the dyes over the 0.0–100.0 µg L^{-1} range, with a 3σ LoD of 3.3 (0.006 µmol L^{-1}) and 1.6 µg L^{-1} (0.003 µmol L^{-1}) for tartrazine and sunset yellow, respectively, with an accumulation time of 30 s (Fig. 5B).

With the multiwalled carbon nanotube-modified pyrolytic graphite electrode [15] a LoD of 0.93 µmol L^{-1} was found for the determination of tartrazine, LoDs of 0.188 and 0.022 µmol L^{-1}, respectively, for tartrazine and sunset yellow with the multi walled carbon nanotube film-modified electrode [17]; 0.002 and 0.030 µmol L^{-1} with the gold nanoparticle carbon paste electrode [18], and

1.8 and 3.5 μmol L^{-1}, respectively, with the polyallylamine modified tubular glassy carbon electrode [5].

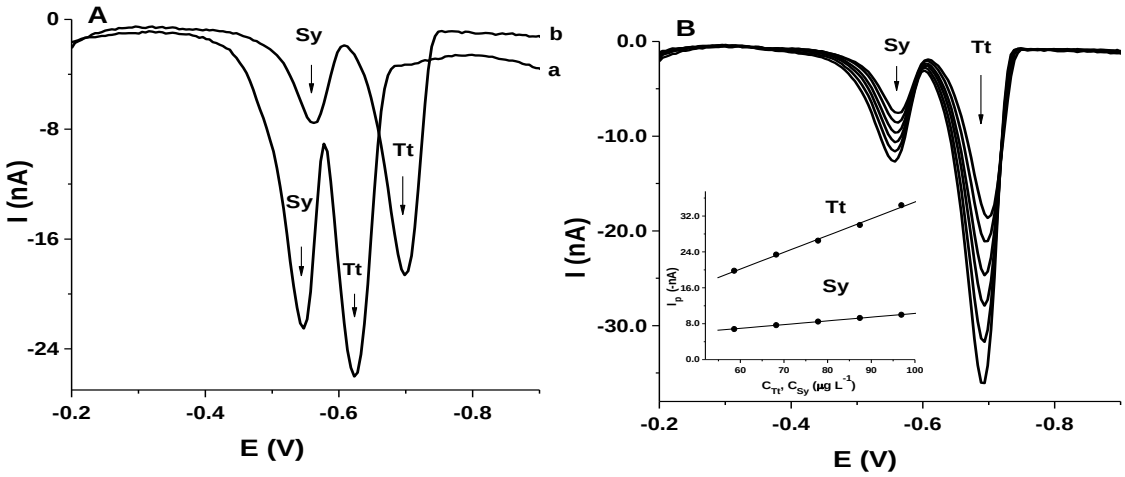

Figure 5. Adsorptive voltammograms and calibration curves of tartrazine and sunset yellow in the presence of CPB. Conditions: (A) $C_{Tt} = C_{Sy} = 48.9$ μg L^{-1}. (B) $C_{Tt} = C_{Sy}$ from 48.9 to 96.9 μg L^{-1}. pH 8.5 (0.01 mol L^{-1}. ammonium buffer); $C_{CPB} = 3.0$ μmol L^{-1}; $E_{ads} = –0.3$ V; $t_{ads} = 30$ s. Other conditions as in Fig. 1B.

3.4. Validation of the method

Undyed gelatin spiked with tartrazine and sunset yellow was used for validation measurements. An aliquot of gelatin was contaminated with tartrazine and sunset yellow solutions (2.50 mg g^{-1}) and the determination was carried out using the standard addition method, getting 2.50±0.12 mg g^{-1} (RE 0.0%) for sunset yellow and 2.44±0.11 mg g^{-1} (RE –2.4%).

3.5. Application to real samples

The proposed method was applied to the detection of tartrazine in a gelatin with lemon flavor, sunset yellow in a gelatin with orange flavor, and both dyes in gelatin with papaya flavor and in a soft drink with peach flavor. The gelatin with lemon flavor contained tartrazine 596 mg kg^{-1} (95.4 μg mL^{-1}); that with orange flavor contained sunset yellow 724 mg kg^{-1} (115.8 μg mL^{-1}); gelatin with papaya flavor contained tartrazine 65.6 mg kg^{-1} (10.5 μg mL^{-1}) and sunset yellow 12.7 mg kg^{-1} (2.0 μg mL^{-1}); while the soft drink powder contained tartrazine 654 mg kg^{-1} (29.4 μg mL^{-1}) and sunset yellow 465 mg kg^{-1} (20.9 μg mL^{-1}). The levels of tartrazine and sunset yellow in gelatins and soft drinks were below the old limit set by European law in 1994 (100 μg mL^{-1}), except for the gelatin with orange flavor. Figures 6A and 6B show some voltammograms and calibration curves obtained with these samples.

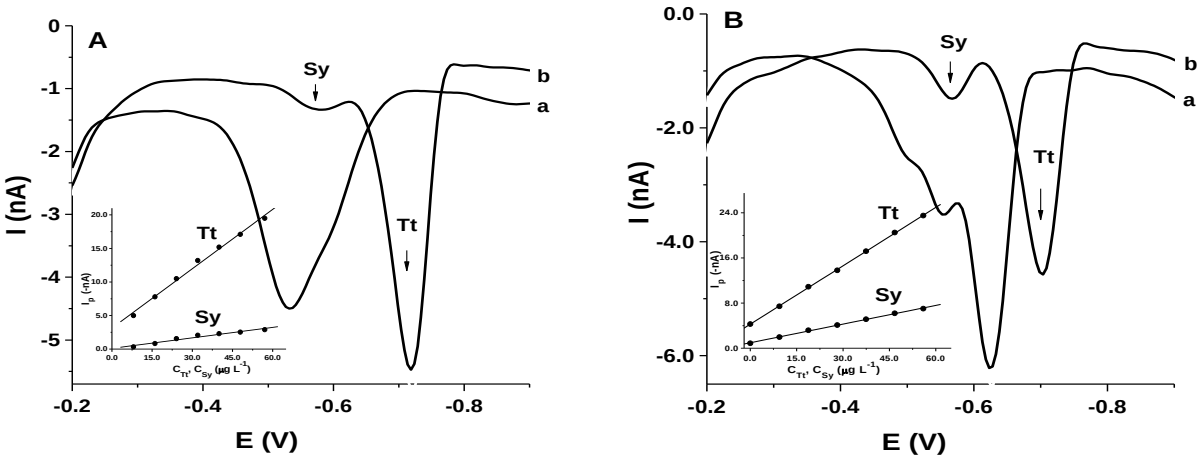

Figure 6. Adsorptive voltammograms and calibration curves of gelatin and soft drink powder in the absence and presence of CPB. Conditions: (A) Gelatin with papaya flavor; (B) Soft drink powder with peach flavor. pH 8.5 (0.01 mol L^{-1}. ammonium buffer); C_{CPB} = 3.0 μmol L^{-1}; E_{ads} = –0.3 V; t_{ads} = 30 s. Other conditions as in Fig. 1B.

4. CONCLUSIONS

A hanging mercury drop electrode was used for the simultaneous determination of tartrazine and sunset yellow in gelatins and soft drinks. In the presence of CPB the peak current of the two dyes decreased, probably because the formation of the dye-containing aggregate could change the mean diffusion coefficient value and hence the peak reduction current. However, the experimental results showed that CPB enhanced the selectivity of the method because the signals were separated from 70 to 150 mV, which was sufficient to determine each dye accurately. On the other hand, the calibration curve showed good linearity with better detection limits with 30 s of accumulation.

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References

1. S. P. Alves, D. Mares Brum, E. C. Branco de Andrade, A. D. P. Netto, *Food Chem*., 107 (2008) 489.
2. N. E. Llamas, M. Garrido, M. S. Di Nezio, B. S. Fernández, *Anal. Chim. Acta*, 655 (2009) 38.
3. J. C. Larsen, *Trends Food Sci & Technol.*, 19 (2008) S64.
4. J. J. Berzas–Nevado, J. Rodríguez–Flores, M. J. Villaseñor–Llerena, *Fresenius J. Anal. Chem.,* 357 (1997) 989.
5. M. L. S. Silva, M. B. Q. Garcia, J. L. F. C. Lima, E. Barrado, *Talanta*, 72 (2007) 282.
6. Official Journal of the European Communities. Commission Directive 1994/36/EC and 2006/33/EC.

7. B. Asci, O. A. Donmez, M. U. Ozgur, *Asian J. Chem*. 22 (2010) 5672.
8. A.Bozdogan, M. Ustun Ozgur, L. Koyuncu, *Anal. Letters*, 33 (2000) 2975.
9. J. J. Berzas, J. Rodríguez, C. Guiberteau, M. J. Villaseñor, A. Contento, *Talanta* 46 (1998) 933.
10. Y. Ni, Y. Wang, S. Kokot, *Talanta* 78 (2009) 432.
11. N. E. Llamas, M. Garrido, M. S. Di Nezio, B. S. Fernández, *Anal. Chim. Acta*, 655 (2009) 38.
12. E. Dinc, E. Baydan, M. Kanbur, F. Onur, *Talanta*, 58 (2002) 579.
13. A. Yahya, *Food Chem*., 117 (2009) 485.
14. H. C. Goicoechea, A. C. Olivieri, *TrAC Trends Anal. Chem*., 19 (2000) 599.
15. Y. Z. Song, J. M. Xu, J. S. Lv, H. Zhong, Y. Ye, J. M. Xie, *Russian J. Phys. Chem*. A, 86 (2012) 303.
16. X. Yang, H. Qin, M. Gao, H. Zhang, *J. Sci. Food Agric*. 91 (2011) 2821.
17. W. Zhang, T. Liu, X. Zheng, W. Huang, C. Wan, *Colloids and Surfaces B*: *Biointerfaces* 74 (2009) 28.
18. S. M. Ghoreishi, M. Behpour, M. Golestaneh, *Food Chem*., 132 (2012) 637.
19. I. M. Kolthoff, J. J. Lingane, Polarography, Interscience. New York, Vol I. 1952, p. 97.
20. J. Zima, J. Barek, J. C. Moreira, V. Mejstrik, A. G. Fogg, *Fresenius J. Anal. Chem*., 369 (2001) 567.
21. E. C. Vidotti, W. F. Costa, C. C. Oliveira, *Talanta*, 2006, 68 (2006) 516.
22. A. A. Shahir. S. Javadian, B. B. M. Razavizadeh, H. Gharibi, *J. Phys. Chem. B* 115 (2011) 14435.