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Simultaneous voltammetric determination of synthetic colorants in food using a cathodically pretreated boron-doped diamond electrode

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ABSTRACT

Differential pulse voltammetry (DPV) and a cathodically pretreated boron-doped diamond (BDD) electrode were used to simultaneously determine two pairs of synthetic food colorants commonly found mixed in food products: tartrazine (TT) and sunset yellow (SY) or brilliant blue (BB) and sunset yellow (SY). In the DPV measurements using the BDD electrode, the reduction peak potentials of TT and SY or BB and SY were separated by about 150 mV. The detection limit values obtained for the simultaneous determination of TT and SY or BB and SY were 62.7 nmol L^{-1} and 13.1 nmol L^{-1} or 143 nmol L^{-1} and 25.6 nmol L^{-1}, respectively. The novel proposed voltammetric method was successfully applied in the simultaneous determination of these synthetic colorants in food products, with results similar to those obtained using a HPLC method at 95% confidence level.

1. Introduction

Synthetic colorants, a very important class of food additives, are added to food products to make them more attractive, replacing their natural color that can be lost during the industrial processes, or to avoid variations in the color of the final product from different batches. These colorants are present in many common food products, such as sugar candies, sugar-coated pills, jelly beans, powdered drinks, sweets, nutrient-enhanced sports beverages, ice creams, and gelatins [1]. However, some of these substances pose potential risks to human health, especially if they are excessively consumed. For instance, as pointed out by Vidotti et al. [2], some synthetic azo-dyes can be harmful to human health and when in contact with some drugs can cause allergic and asthmatic reactions, as well as induce the development of cancer and other diseases. Consequently, the use of synthetic colorants in food products is strictly controlled by different national legislations, while acceptable daily intakes are determined and evaluated by the United Nations Food and Agricultural Organization (FAO) and the World Health Organization (WHO) [3,4].

In Brazil, the use of these synthetic food colorants is controlled by ANVISA (the National Agency of Sanitary Surveillance), which permits the use of eleven synthetic colorants [5]. Tartrazine (TT), sunset yellow (SY), and brilliant blue (BB) (Fig. 1) are commonly used, alone or mixed, in different food products and their content limits are 15.0, 10.0, and 15.0 mg/100 g, respectively [5]. Thus, the determination of synthetic colorants in foods is a necessity to ensure the fulfillment of legal requirements, as well as quality-control procedures in the food industry.

Many methods for determining TT, SY, and BB, individually or simultaneously, have been recently reported based on spectrophotometry [6–12] and liquid and gas chromatography [2,4,13–19]. Nevertheless, they are prone to many drawbacks, such as expensiveness, complicated and long procedures, and unsuitability for field use.

Some electrochemical methods for determining these food colorants by voltammetric techniques have been previously reported [1,20–28]. Song et al. [20] studied the electrochemical reduction of TT at a multiwalled carbon nanotube-modified pyrolytic graphite electrode; the validity of the proposed method was assessed by determining TT in soft drinks, when a detection limit (LOD) of 0.5 mg L^{-1} (0.9 μmol L^{-1}) was obtained. Song [21] also used a multiwalled carbon nanotube (MWCNT)-modified glassy-carbon electrode to investigate the electrochemical reduction of SY by cyclic voltammetry (CV). The proposed method was satisfactorily applied in the determination of SY in soft drinks in two concentration ranges, with a lower LOD value of 0.5 mg L^{-1} (1 μmol L^{-1}). Silva et al. [22] reported the construction of a polyallylamine modified tubular glassy-carbon electrode and its application in the electroreduction of three azo-colorants (TT, SY, and allura red) in several food samples by square-wave voltammetry (SWV). The obtained LOD values were 1.8 μmol L^{-1} for TT,

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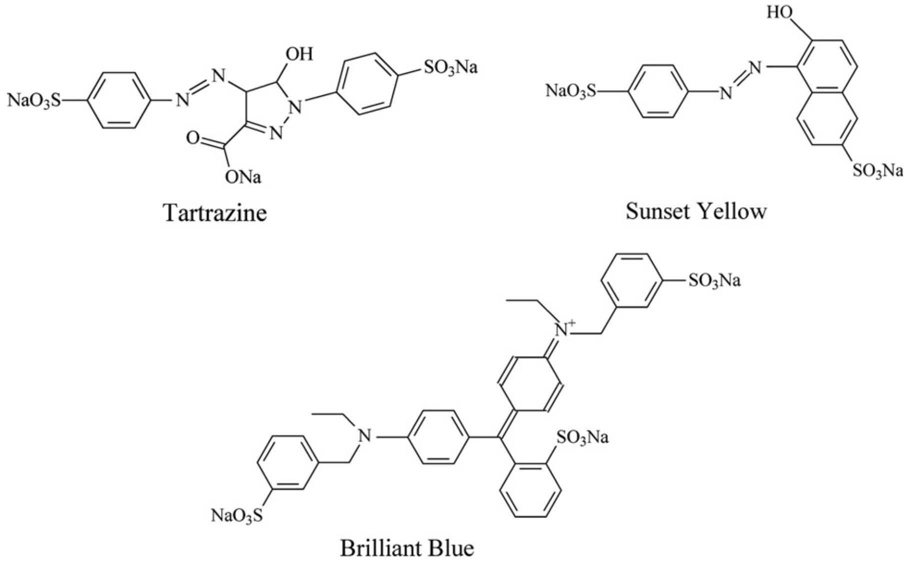

Fig. 1. Chemical structure of the tartrazine, sunset yellow, and brilliant blue molecules.

3.5 μmol L^{-1} for SY, and 1.4 μmol L^{-1} for allura red. Quite recently, Ghoreishi et al. [23] used a carbon paste electrode chemically modified with MWCNTs for the determination of BB using differential pulse voltammetry (DPV) or SWV, in the concentration range 0.05–22.0 μmol L^{-1}. In both cases two linear ranges were found for the response of the oxidation peak current with dye concentration; the lowest range was 0.05–0.8 μmol L^{-1}, for DPV (LOD=9 nmol L^{-1}), and 0.05–0.6 μmol L^{-1}, for SWV (LOD=5 nmol L^{-1}).

Moreover, few electroanalytical methods have been reported for the simultaneous determination of food colorants. Ni et al. [27] developed a method for the simultaneous determination of the amaranth, SY, TT, and ponceau 4R dyes using an adsorptive voltammetric approach at a hanging mercury drop electrode (HMDE). However, the corresponding voltammetric waves were seriously overlapped, thus hampering the simultaneous determination of the dyes without a pre-separation; then, the results were interpreted using a multivariate calibration approach. Ni and Bai [25] reported the use of a ratio derivative voltammetric method for resolving overlapping voltammograms without a pre-separation; this method was successfully applied to resolve binary mixtures of amaranth and SY. Kapor et al. [28] used a HMDE for the simultaneous voltammetric determination of TT and indigo carmine in soft drinks and candies, using their cathodic redissolution voltammetric waves. Linear calibration plots were separately obtained for both colorants in the concentration range 5.0×10^{-9}–1.0×10^{-7} mol L^{-1}, with LOD values of 7.4 nmol L^{-1}, for TT, and 0.9 nmol L^{-1}, for indigo carmine. Ghoreishi et al. [23] used DPV and a carbon paste electrode chemically modified with MWCNTs for the simultaneous determination of BB and TT in soft drinks (powdered and liquid); however, no LOD values were reported.

Boron-doped diamond (BDD) is a carbon-based electrode material that has received much attention in recent years [29–34]. To obtain BDD with metallic conductivity, a small fraction of the carbon atoms is replaced by boron atoms [37]. BDD electrodes are very attractive for many potential applications due to their interesting properties, which are significantly different from those of other conventional electrodes, e.g., glassy-carbon (GC) or platinum electrodes. The main properties of BDD electrodes are: very low and stable background current, corrosion stability in very aggressive media, extreme electrochemical stability, high response sensitivity, and a very wide working potential window, which can be larger than 3.5 V [33–36,38–41].

The properties of BDD electrodes are commonly affected by different pretreatments: plasma, electrochemical, etc. Suffredini et al. [42] reported that cathodic electrochemical pretreatments of a BDD electrode led to increased electroanalytical detection limits for chlorophenols, indicating that the analytical performance of BDD electrodes greatly depends on their surface termination, i.e., whether they are predominantly hydrogen or oxygen terminated. Recently, in our research group, this effect of cathodic pretreatments was also observed on the determination of additives in food products and drugs in pharmaceutical formulations [43–49].

In this paper we report on the coupling of voltammetric techniques and the unique properties of the BDD electrode for the development and optimization of a method for the simultaneous determination of pairs of synthetic colorants (TT and SY or BB and SY) in several food products. The proposed method is very attractive because there are no electroanalytical methods reported in the literature for the simultaneous determination of these pairs of colorants, which are frequently encountered mixed in food products.

2. Materials and methods

2.1. Apparatus

The voltammetric experiments (CV, DPV, and SWV) with a stationary BDD electrode were performed using an Autolab PGSTAT-30 (Ecochemie) potentiostat/galvanostat controlled with the GPES 4.9 software. The DP and SW voltammograms were baseline-corrected by the moving average method (peak width: 0.003) and smoothed with a Savicky and Golay algorithm using the GPES 4.9 software. A three-electrode electrochemical cell with a volume of 10 mL was used: a BDD working electrode, a Pt-wire auxiliary electrode, and an Ag/AgCl (3.0 mol L^{-1} KCl) reference electrode, to which all electrode potentials hereinafter are referred to.

The BDD film (8000 ppm) on a p-silicon wafer, obtained from the Center Suisse de Electronique et de Microtechnique SA (CSEM), Neuchatêl, Switzerland, was prepared as described elsewhere [38]. Prior to use, the BDD electrode (0.30 cm^2 exposed area) was cathodically or anodically pretreated in a 0.50 mol L^{-1} H_2SO_4 solution by applying −0.5 A cm^{-2} or 0.5 A cm^{-2} during 180 s or 60 s, respectively. For comparative purposes, a GC electrode (0.20 cm^2) was also used. Prior to use, this electrode was pretreated by sequential polishing with alumina (1 μm and

0.05 μm)/water slurries on felt pads, followed by rinsing with ultra-pure water.

The HPLC determinations of TT, SY, and BB were carried out using an LC-10 AT Shimadzu system, with a UV–Vis detector (SPD-M10-AVP) set at 430 nm, 484 nm, and 610 nm, respectively. A Shim-Pack CLC-ODS (6.0 mm × 250 mm, 5 μm) chromatographic column was used. The mobile phase consisted of an aqueous 1% (m/v) (0.13 mol L^{-1}) ammonium acetate solution, brought to pH 7.5 by the drop wise addition of a 10% (m/v) sodium hydroxide solution (mobile phase A), and a mixture of (80:20 v/v) methanol:acetonitrile (mobile phase B) at a flow rate of 1.0 mL min^{-1}, while the injection volume was 20 μL [4].

2.2. Reagents and standards

All reagents were of analytical grade: TT, SY, and BB (Sigma); H_2SO_4 (Synth). Standard aqueous 1.0 mmol L^{-1} TT, SY, and BB solutions were prepared in a 0.10 mol L^{-1} H_2SO_4 solution. All solutions were prepared using ultra-purified water (resistivity greater than 18 MΩ cm) supplied by a Milli-Q system (Millipore®).

2.3. Measurement procedures

All electrochemical measurements were carried out using a 10 mL electrochemical cell at room temperature (25 ± 1 °C). The deaeration of the supporting electrolyte was not necessary, since O_2 presence does not cause any interference in the studied experimental conditions. The instrumental parameters for SWV and DPV were optimized and their selected values are: for SWV — frequency (*f*), 30 Hz; amplitude (*a*), 40 mV; scan increment (ΔE_s), 4 mV; for DPV — scan rate (ν), 20 mV s^{-1}; *a*, 60 mV; modulation time (*t*), 5 ms. After optimizing the experimental parameters for the proposed methods, analytical curves were constructed by adding small volumes of concentrated standard solutions of the two pairs of analytes (TT and SY or BB and SY). LOD values were obtained as the concentration whose associated voltammetric response was equal to three times the average ($n=10$) voltammetric response for the blank solution [50].

2.4. Influence of the voltammetric techniques on the determination of the synthetic food colorants

The electrochemical behavior of the synthetic food colorants was investigated with different voltammetric techniques. CV was used in the preliminary studies, such as choice of the supporting electrolyte. DPV and SWV were used to investigate the simultaneous determination of the food colorants and to find the best analytical conditions.

2.5. Treatment of commercial food samples

All samples (powdered juice drinks, gelatins, and nutrient-enhanced sports drink beverages), obtained from a local supermarket, were pretreated as described in other similar works, e.g., [2,4]. The solid samples were homogenized. One-gram portions of powdered juice drinks or gelatin were accurately weighed and dissolved in 10.0 mL of a 0.10 mol L^{-1} H_2SO_4 solution. To dissolve the gelatin, the sulfuric acid solution was gently heated. Each sample solution was placed in an ultrasonic bath for 10 min for complete extraction of the colorants. This solution was then filtered through a folded paper filter. Subsequently, 500 μL aliquots of this solution were transferred to the electrochemical cell containing 9.5 mL of a 0.10 mol L^{-1} H_2SO_4 solution. In the case of the samples of sports drink beverages, 500 μL aliquots were directly transferred to the electrochemical cell containing 9.5 mL of a 0.10 mol L^{-1} H_2SO_4 solution. Voltammograms were obtained after each aliquot addition.

3. Results and discussion

3.1. Investigation of the electrochemical behavior

CV, SWV, and DPV were used to study the electrochemical behavior of TT, SY, and BB on the BDD electrode. First, CV was used to investigate the effect of the supporting electrolytes on the redox activity of these compounds: 0.10 mol L^{-1} H_2SO_4, 0.040 mol L^{-1} Britton–Robinson buffer (pH 2), and 0.10 mol L^{-1} $NaNO_3$ (adjusted to pH 2 with 0.5 mol L^{-1} HNO_3). The best results were obtained with the 0.10 mol L^{-1} H_2SO_4 solution, when peak reduction potentials of −0.35, −0.15, and −0.38 V were obtained for TT, SY, and BB, respectively. Moreover, the cyclic voltammograms obtained for all these colorants evidenced an irreversible behavior, in good agreement with data previously reported in the literature [20,21]. The effect of the CV scan rate on the reduction of TT, SY, and BB was also investigated. A plot of the peak current versus the square root of the scan rate yielded a straight line (see Supplementary Fig. SD1 in the supplementary data), indicating that the electroreduction of these colorants is a diffusion-controlled process.

Fig. 2 shows the cyclic voltammograms obtained for the three food colorants with an anodically or a cathodically pretreated BDD electrode. As can be seen, better-defined reduction waves can be observed when the cathodically pretreated electrode is used; when the anodically pretreated electrode is used, the magnitude of these waves decreases. Consequently, all subsequent experiments were carried out using a cathodically pretreated BDD electrode. Similar electrochemical-pretreatment effects were previously obtained for other compounds, such as chlorophenols [42] and food antioxidants [49]; an extensive review of the effect of cathodic pretreatments on the electroanalytical performance of BDD electrodes was recently published by Andrade et al. [51].

Next, electroanalytical procedures were developed for each colorant using SWV and DPV; thus, optimizations of the experimental parameters that affect the SWV and DPV responses were carried out (results not shown). According to the obtained results (see Table 1), the electroanalytical procedure developed using DPV yielded the best values for sensitivity. Hence, DPV ($\nu=20$ mV s^{-1}, $a=60$ mV, and $t=5$ ms) was the technique chosen for the subsequent development of an electroanalytical procedure for the simultaneous determination of TT and SY or BB and SY — see next section. However, SWV theory may be used to determine the number of electrons transferred in the redox process, using the following relationship [52]:

$$E_p = -\frac{2.3RT}{\alpha nF}\log f \tag{1}$$

where E_p is the peak potential, α the transfer coefficient, n the number of electrons involved in the redox reaction, and f the frequency; other terms have their usual meaning. The slopes obtained from the E_p *vs.* $\log f$ plots were −0.032 V for TT, −0.031 V for SY, and −0.056 V for BB; thus, by means of Eq. (1), values equal to 1.85 (TT), 1.91 (SY), and 1.05 were determined for αn. If the value of α is assumed as equal to 0.5, a value quite common for organic molecules, these results indicate that the reduction of TT and SY involves 4 electrons per molecule, whereas that of BB involves 2 electrons per molecule.

The reduction mechanism of azo-compounds has been known for some time [1,20]. The electrode process must be consistent with the mechanism that postulates a stepwise breakage of the molecule, similar to that occurring in the metabolic processes of these dyes (see Fig. 3), where R-NH_2 is sulphanilic acid and R_1-NH_2 is 5-hydroxy-1-*p*-sulphophenyl-4-(*p*-sulphophenylazo)-pyrazol-3-carboxylic acid [1]; this mechanism involves four electrons and four protons. Hence, the number of electrons involved in the redox reaction obtained by SWV theory is consistent with that in the mechanism proposed in the literature.

From the number of electrons involved in the redox reaction obtained by SWV theory and taking into account mechanisms proposed in the literature [28,53], the electrochemical reduction of BB is believed to occur involving the –C=C– bond, with the transfer of two electrons and two protons, as also shown in Fig. 3.

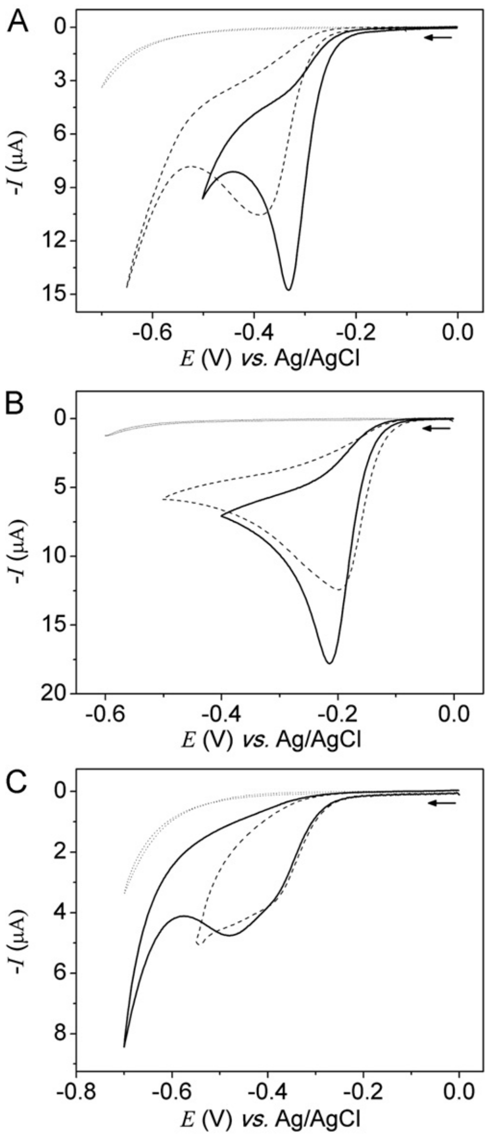

Fig. 2. Cyclic voltammograms ($\nu = 50\ \mathrm{mV\ s^{-1}}$) obtained for $0.10\ \mathrm{mol\ L^{-1}}$ H_2SO_4 (dotted line) and $1.0 \times 10^{-4}\ \mathrm{mol\ L^{-1}}$ TT (a), SY (b), and BB (c) in an aqueous $0.10\ \mathrm{mol\ L^{-1}}$ H_2SO_4 solution with an anodically (dashed lines) or a cathodically (solid line) pretreated BDD electrode.

3.2. Simultaneous determination of the food colorants

First, the DPV response of a cathodically pretreated BDD electrode was compared to that of a GC electrode in the simultaneous determination of SY and TT or SY and BB in an aqueous $0.10\ \mathrm{mol\ L^{-1}}$ H_2SO_4 solution. Fig. 4 shows the voltammetric curves obtained at the BDD and GC electrodes for $1.5 \times 10^{-5}\ \mathrm{mol\ L^{-1}}$ SY and $2.9 \times 10^{-5}\ \mathrm{mol\ L^{-1}}$ TT (Fig. 4(a)) or for $1.5 \times 10^{-5}\ \mathrm{mol\ L^{-1}}$ SY and $2.9 \times 10^{-5}\ \mathrm{mol\ L^{-1}}$ BB (Fig. 4(b)). With the cathodically pretreated BDD electrode, well-defined and separated peaks were obtained. On the other hand, the voltammograms obtained with the GC electrode did not show a good separation of peaks, especially for TT and SY; worse yet, the voltammogram obtained for SY and BB presented significantly smaller peak currents. Hence, these results allow reaffirming that further studies should be carried out only with the cathodically pretreated BDD electrode.

The DPV curves obtained with the cathodically pretreated BDD electrode (Fig. 4) presented a good peak-potential separation (about 0.15 V for both pairs of colorants), which clearly allows the simultaneous determination of these compounds. Initially, the separate determination of TT was carried out in the concentration range 5.0×10^{-7}–$6.5 \times 10^{-6}\ \mathrm{mol\ L^{-1}}$ in solutions containing SY at the fixed concentration $5.0 \times 10^{-7}\ \mathrm{mol\ L^{-1}}$ (see Fig. 5(a)). Then, the concentration of TT was fixed at $2.0 \times 10^{-6}\ \mathrm{mol\ L^{-1}}$ and that of SY was varied in the range 2.0×10^{-7}–$2.9 \times 10^{-6}\ \mathrm{mol\ L^{-1}}$ (see Fig. 5(b)). Similar procedures were carried out for BB and SY; thus the concentration of BB was varied in the range 2.0×10^{-7}–$4.8 \times 10^{-6}\ \mathrm{mol\ L^{-1}}$ in solutions containing SY at the fixed concentration $1.0 \times 10^{-6}\ \mathrm{mol\ L^{-1}}$ (see Fig. 5(b)); then, the concentration of BB was fixed at $2.0 \times 10^{-6}\ \mathrm{mol\ L^{-1}}$ and that of SY was varied in the range 2.0×10^{-7}–$2.9 \times 10^{-6}\ \mathrm{mol\ L^{-1}}$ (see Fig. 5(b)). An examination of Fig. 5(a) and (c) allows concluding that the peak reduction currents for TT and BB increase regularly as their corresponding concentrations are increased at a fixed concentration of SY (whose peak reduction currents remain fairly constant — RSD values equal to 7.9% (Fig. 5(a)) and 4.1% (Fig. 5(c)), respectively). Similarly, as shown in Fig. 5(b) and (d), the peak reduction current for SY increases regularly as its concentration is increased at a fixed concentration of TT or BB (whose corresponding peak oxidation currents remain constant — RSD values equal to 7.9% (Fig. 5(b)) or 7.2% (Fig. 5(c)), respectively).

After this preliminary study, the pairs of colorants TT and SY or BB and SY were determined by simultaneously and equally changing their concentrations in a $0.10\ \mathrm{mol\ L^{-1}}$ H_2SO_4 solution. Fig. 6 shows the DP voltammograms obtained for solutions containing the pairs of colorants TT and SY (Fig. 6(a)) or BB and SY (Fig. 6(b)). The respective analytical curves for TT and SY present a good linearity in the investigated concentration ranges (9.99×10^{-8}–$5.66 \times 10^{-6}\ \mathrm{mol\ L^{-1}}$, for TT, and 2.00×10^{-8}–$4.76 \times 10^{-6}\ \mathrm{mol\ L^{-1}}$, for SY). The corresponding analytical equations are:

$$\mathrm{TT}: -I_p/\mu\mathrm{A} = 0.0919 + 2.20 \times 10^5 (c/(\mathrm{mol\ L^{-1}}))\ (r = 0.999)$$

$$\mathrm{SY}: -I_p/\mu\mathrm{A} = -0.116 + 2.62 \times 10^5 (c/(\mathrm{mol\ L^{-1}}))\ (r = 0.994)$$

Table 1
Figures of merit for individual determinations of the SY, TT, and BB colorants using DPV and SWV.

	SY		TT		BB	
	DPV	SWV	DPV	SWV	DPV	SWV
Measured potential (V)	−0.15	−0.17	−0.30	−0.35	−0.35	−0.38
Linearity range ($\mathrm{mol\ L^{-1}}$)	4.9×10^{-8}–2.9×10^{-6}	4.9×10^{-8}–9.9×10^{-7}	3.5×10^{-7}–9.1×10^{-6}	3.5×10^{-7}–9.1×10^{-6}	4.9×10^{-7}–9.1×10^{-6}	4.9×10^{-7}–1.1×10^{-5}
Sensitivity ($\mu\mathrm{A\ mol\ L^{-1}}$)	-2.4×10^5	-7.0×10^4	-1.5×10^5	-6.6×10^4	-1.1×10^5	-2.2×10^5
Intercept (μA)	−0.023	−0.005	−0.060	0.037	0.033	0.042
Correlation coefficient, *r*	0.999	0.976	0.996	0.991	0.995	0.971

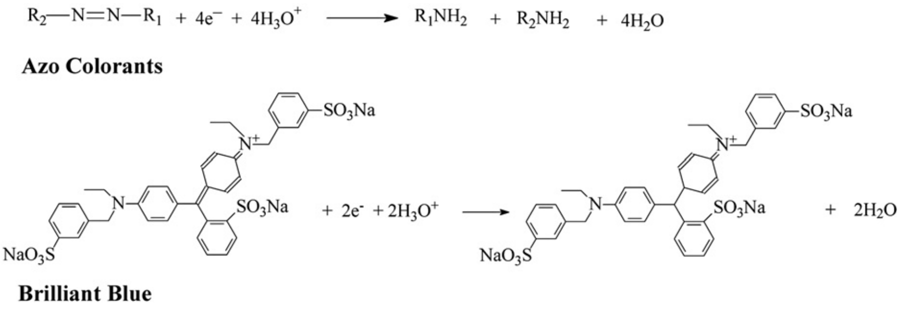

Fig. 3. Reduction mechanism for the azo-colorants and the brilliant blue colorant at the BDD electrode surface.

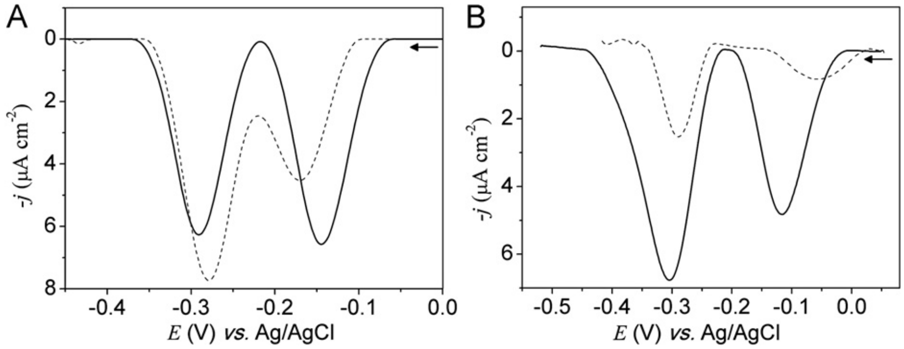

Fig. 4. Differential pulse voltammograms (with baseline correction) obtained using a mixture of 3.5×10^{-5} mol L^{-1} TT and 2.0×10^{-5} mol L^{-1} SY (a) or 3.0×10^{-5} mol L^{-1} BB and 1.5×10^{-5} mol L^{-1} SY (b), in an aqueous 0.10 mol $L^{-1}H_2SO_4$ solution, at a CG (dashed lines) or BDD (solid line) electrode. DPV parameters: $\nu=20$ mV. s^{-1}, $a=60$ mV, and $t=5$ ms.

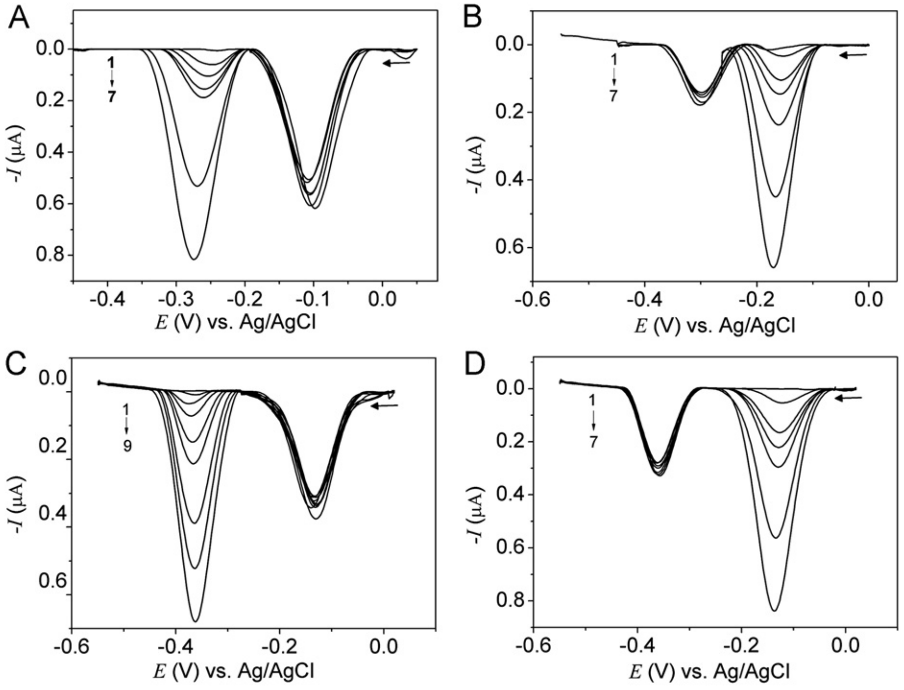

Fig. 5. (a) and (b) Differential pulse voltammograms (with baseline correction) for various concentrations of TT (a) and SY (b) at fixed concentrations of SY 1.0×10^{-5} mol L^{-1} and TT 2.0×10^{-6} mol L^{-1}, respectively, in an aqueous 0.10 mol $L^{-1}H_2SO_4$ solution. TT concentration range (2–7): 5.0×10^{-7}–6.5×10^{-6} mol L^{-1}; SY concentration range (2–7): 2.0×10^{-7}–2.9×10^{-6} mol L^{-1}. (c) and (s) Differential pulse voltammograms (with baseline correction) for various concentrations of BB (c) and SY (d) at fixed concentrations of SY (1.0×10^{-6} mol L^{-1}) and BB (2.0×10^{-6} mol L^{-1}). BB concentration range (2–9): 2.0×10^{-7}–4.8×10^{-6} mol L^{-1}; SY concentration range (2–7): 2.0×10^{-7} –2.9×10^{-6} mol L^{-1}. DPV parameters as indicated in Fig. 4.

The corresponding LOD values calculated for TT and SY were 62.7 nmol L^{-1} and 13.1 nmol L^{-1}, respectively. These detection limits are significantly lower than those obtained by other authors using modified carbon electrodes [20,22]. Nevertheless, the LOD value for TT is not lower than that obtained by Kapor et al. [28]; however, these authors determined one dye at a time, after an accumulation time, using a HDME electrode.

Similarly, BB and SY present analytical curves with a good linearity in the investigated concentration ranges (5.96×10^{-7}–9.90×10^{-6} mol L^{-1} and 9.99×10^{-8}–4.76×10^{-6} mol L^{-1}, respectively).

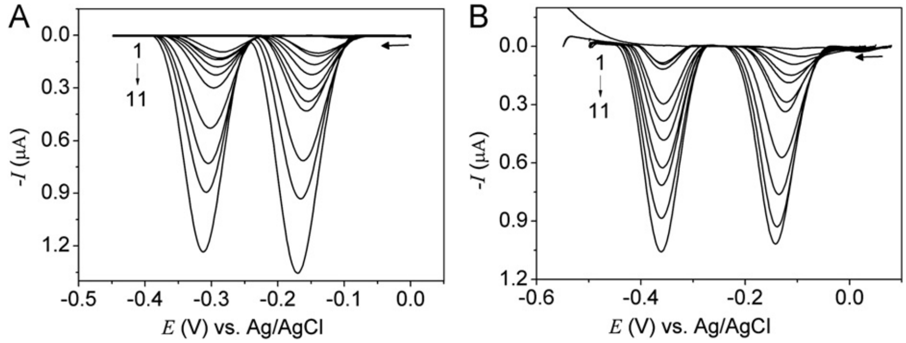

Fig. 6. (a) Differential pulse voltammograms obtained for the reduction of TT and SY in an aqueous 0.10 mol L^{-1} H_2SO_4 solution. The concentrations of both TT and SY were changed simultaneously (2–11): 9.99×10^{-8}–5.66×10^{-6} mol L^{-1}, for TT, 2.00×10^{-8}–4.76×10^{-6} mol L^{-1} for SY. (b) Differential pulse voltammograms obtained for the reduction of BB and SY contained in an aqueous 0.10 mol L^{-1} H_2SO_4 solution. The concentrations of both TT and SY were changed simultaneously (2–11): 5.96×10^{-7}–9.90×10^{-6} mol L^{-1}, for BB, 9.99×10^{-8}–4.76×10^{-6} mol L^{-1} for SY. DPV parameters as indicated in Fig. 4.

Table 2
Results obtained for the intra- and inter-day repeatability studies.

Repeatability	Intra-day (RSD %)*	Inter-day (RSD %)*	Repeatability	Intra-day (RSD %)*	Inter-day (RSD %)*
TT (mol L^{-1})			BB (mol L^{-1})		
1.0×10^{-7}	7.2	8.5	5.0×10^{-7}	5.3	6.9
9.9×10^{-7}	2.0	5.4	3.8×10^{-6}	1.5	7.8
3.8×10^{-6}	1.3	2.2	9.1×10^{-6}	1.1	8.2
SY (mol L^{-1})			SY (mol L^{-1})		
5.0×10^{-8}	5.2	6.3	1.0×10^{-7}	5.6	5.8
6.9×10^{-7}	3.4	2.3	9.9×10^{-7}	2.6	1.6
2.9×10^{-6}	5.2	4.4	4.8×10^{-6}	0.8	5.5

* $n=5$.

Table 3
Results obtained in the simultaneous determination of TT and SY in food products by HPLC or the proposed method (DPV).

Samples	TT		SY		Error 1 (%)[b]	Error 2 (%)[b]
	HPLC[a]	DPV[a]	HPLC[a]	DPV[a]		
Powdered juice (mg/100 g)						
1	55 ± 2	53 ± 2	49 ± 3	49 ± 4	−3.6	0
2	59 ± 4	61 ± 1	34 ± 2	36 ± 3	3.4	5.9
3	22 ± 3	21 ± 4	8.2 ± 0.5	8.9 ± 0.4	−4.5	8.5
4	24 ± 1	25 ± 2	28 ± 4	27 ± 1	4.2	−3.6
Gelatin (mg/100 g)						
1	14 ± 2	14 ± 1	3.1 ± 0.6	2.8 ± 0.5	0	−9.7
2	5.8 ± 0.3	5.6 ± 0.8	1.2 ± 0.4	1.3 ± 0.6	−3.4	8.3
3	14 ± 1	13 ± 1	3.1 ± 0.6	3.2 ± 0.3	−7.1	3.2
Nutrient-enhanced sports drink (mg/100 mL)						
1	1.5 ± 0.2	1.6 ± 0.5	0.35 ± 0.04	0.38 ± 0.03	6.7	8.6
2	0.61 ± 0.06	0.59 ± 0.08	1.7 ± 0.5	1.8 ± 0.4	−3.3	5.9

[a] Average of three measurements.
[b] Average errors 1 and 2 (%) = 100 × (DPV value − HPLC value)/HPLC value.

Table 4
Results obtained in the simultaneous determination of BB and SY in food products by HPLC and the proposed method (DPV).

Samples	BB		SY		Error 1 (%)[b]	Error 2 (%)[b]
	HPLC[a]	DPV[a]	HPLC[a]	DPV[a]		
Powdered juice (mg/100 g)						
1	4.3 ± 0.2	4.7 ± 0.6	8.7 ± 0.4	9.4 ± 0.5	9.3	8.0
2	32 ± 3	33 ± 1	13 ± 1	12 ± 2	3.1	−7.7
Gelatin (mg/100 g)						
1	13 ± 1	12 ± 2	2.4 ± 0.2	2.5 ± 0.4	−7.7	4.2
2	9.9 ± 0.5	10 ± 1	3.1 ± 0.4	3.4 ± 0.6	1.0	9.7

[a] Average of three measurements.
[b] Average errors 1 and 2 (%) = 100 × (DPV value − HPLC value)/HPLC value.

The corresponding analytical equations are:

$$\text{BB}: -I_p/\mu\text{A} = 0.0260 + 1.18\times10^{5}(c/(\text{mol L}^{-1}))\ (r = 0.997)$$

$$\text{SY}: -I_p/\mu\text{A} = -0.576 + 2.38\times10^{5}(c/(\text{mol L}^{-1}))\ (r = 0.995)$$

The corresponding LOD values calculated for BB and SY were 143 nmol L^{-1} and 25.6 nmol L^{-1}, respectively. Once more, the detection limit for SY is significantly lower than those obtained by other authors using modified carbon electrodes [20,22], reinforcing the attractiveness of BDD electrodes for electroanalytical purposes. In the case of BB, lower LOD values were reported in the literature [23]; however, these authors determined solely the BB dye and used a statistical approach to estimate the LOD values.

The intra- and inter-day repeatabilities were assessed by successive simultaneous measurements ($n=5$) of TT and SY or BB and SY at different concentrations, when good RSD values were obtained, as can be seen in Table 2.

Then, the selectivity of the proposed method was evaluated by the addition of possible interferents (ascorbic acid, sodium citrate, fumaric acid, sodium cyclamate, saccharin, aspartame, acesulfame-K, maltodextrin, and citric acid) to a standard solution containing TT and SY or BB and SY, at the concentration ratios (standard solution:interferent) 10:1, 1:1, and 1:10; the obtained current signals were compared with those obtained with the standard solution. The analysis of the obtained responses allowed concluding that these compounds do not significantly interfere with the here proposed method.

Finally, the proposed method was applied to simultaneously determine TT and SY or BB and SY by the standard addition method in 13 different commercial products, such as five gelatins, six powdered juice drinks, and two nutrient-enhanced sports drink beverages. The recovery experiments carried out to evaluate matrix effects after the standard-solution additions yielded a good average recovery for all food colorants (90.8–111% for SY and 87.2–112% for TT, or 94.0–114% for SY and 86.2–103% for BB), indicating that there were no important matrix interferences for the samples analyzed by the proposed DPV method. Table 3 presents the TT and SY concentrations simultaneously determined in the analyzed food products, employing the proposed DPV method and an HPLC method (two of the chromatograms obtained for samples of commercial products can be seen in Supplementary Fig. SD2 in the Supplementary data); Table 4 presents analogous results for the BB and SY concentrations. By analyzing these results obtained for the commercial food products, one can conclude that the values obtained by our novel proposed method agree quite well with those obtained by the reference HPLC method. In fact, applying the paired *t*-test to the results obtained by both methods, the resulting *t* values (2.03 for TT and 2.18 for SY, or 2.75 for BB and 2.60 for SY) are smaller than the critical one (2.31 or 3.18, $\alpha = 0.05$), indicating that there is no difference between the obtained results at a confidence level of 95%.

4. Conclusions

The obtained results allow concluding that DPV along with a cathodically pretreated BDD electrode can be used with significant benefits for the quantitative determination of TT, SY, and BB, alone or mixed as commonly found in food products. Very low detection limits were obtained in the simultaneous determination of pairs of these food colorants: TT (62.7 nmol L^{-1}) and SY (13.1 nmol L^{-1}) or BB (143 nmol L^{-1}) and SY (25.6 nmol L^{-1}). Moreover, addition and recovery studies allowed to conclude that matrix effects did not present any significant interference. Furthermore, the obtained concentrations of the colorants in commercial food products are similar to those obtained using a HPLC method. Hence, the novel proposed method may be effectively and advantageously used for the simultaneous determination of TT and SY or BB and SY in food products, since it is very simple, inexpensive, and rapid.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.04.033.

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