Talanta 99 (2012) 883–889

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Flow injection simultaneous determination of synthetic colorants in food using multiple pulse amperometric detection with a boron-doped diamond electrode

Roberta A. Medeiros, Bruna C. Lourencao, Romeu C. Rocha-Filho, Orlando Fatibello-Filho *

Departamento de Química, Universidade Federal de São Carlos, C.P. 676, 13560-970 São Carlos-SP, Brazil

ARTICLE INFO

Article history:
Received 24 May 2012
Received in revised form
17 July 2012
Accepted 18 July 2012
Available online 23 July 2012

Keywords:
Tartrazine
Sunset yellow
Brilliant blue
Food samples
MPA
Cathodically pretreated BDD electrode.

ABSTRACT

A single-line flow injection system and multiple pulse amperometric detection using a boron-doped diamond electrode were employed to develop and optimize a simple, low-cost, and rapid method for the simultaneous determination of two pairs of food colorants: tartrazine and sunset yellow (TT–SY) or brilliant blue and SY (BB–SY). A dual-potential waveform was used: $E_{det.1} = -150$ mV (400 ms duration) and $E_{det.2} = -450$ mV (100 ms duration) vs. Ag/AgCl (3.0 mol L^{-1} KCl). Polarization at $E_{det.1}$ or $E_{det.2}$ causes reduction of SY or the respective pair of colorants, TT–SY or BB–SY; hence, with proper current correction, both colorants in each pair can be determined. The obtained linear response ranges (detection limits) were 5.0–60.0 (2.5) and 1.0–50.0 (0.80) μmol L^{-1}, for TT and SY, or 5.0–60.0 (3.5) and 1.0–50.0 (0.85) μmol L^{-1}, for BB and SY, respectively. Investigation of possible interferents (other food colorants or additives) showed no significant interference with the methods here proposed, which were then used to simultaneously determine the pairs of colorants in industrialized food samples, with results that showed good agreement with those obtained using a comparative HPLC method.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Synthetic colorants constitute a category of food additives commonly added to industrialized foods to restore their natural colors, lost during processing, or to avoid variations in the color of the final product in different batches, and to obtain products that are aesthetically and psychologically more attractive. They are present in common foods, such as sugar candies, sugar-coated pills, jelly beans, powdered drinks, sweets, nutrient-enhanced sports drink beverages, ice creams, and gelatins [1,2]. However, the use of these colorants is strictly controlled by the legislation of different countries, because some of these substances pose potential risks to human health, especially if they are excessively consumed. Some synthetic azo-colorants, for example, can be noxious to human health and cause allergic and asthmatic reactions, or induce the development of cancer and other diseases [3].

Tartrazine (TT), sunset yellow (SY), and brilliant blue (BB)—see Fig. 1—are three of eleven synthetic colorants commonly added to food by Brazilian industries. The use of these colorants is controlled by ANVISA (the Brazilian National Agency of Sanitary Surveillance), which establishes the permitted limits (per 100 g of food product): 15.0, 10.0, and 15.0 mg, for TT, SY, and BB, respectively [4]. These colorants are commonly used in different foods, either alone or as mixtures. Thus, the determination of synthetic colorants in food products is necessary to ensure the fulfillment of legal requirements as well as the quality control procedures of the food industry.

Several methods, such as spectrophotometry [5–8], liquid and gas chromatography [3,9–12], and capillary electrophoresis [13–15], have been used for the single or simultaneous determination of TT, SY, and BB. On the other hand, electrochemical techniques offer analytical options that are promising alternatives to classical approaches, due to characteristics such as their relatively low operational cost and rapid and sensitive detection procedures that are suitable for faster analyses [16]. Thus, some voltammetric methods have already been reported in the literature for the determination of different food colorants, singly or simultaneously [2,17–27]. Specifically in our laboratory and very recently, differential pulse voltammetry and a cathodically pretreated boron-doped diamond (BDD) electrode were used to simultaneously determine two pairs of these food colorants (TT–SY or BB–SY), when the reduction peak potentials of TT and SY or BB and SY were separated by about 150 mV and the detection limits obtained for their simultaneous determination were 62.7 nmol L^{-1} and 13.1 nmol L^{-1} or 143 nmol L^{-1} and 25.6 nmol L^{-1}, respectively [27]. Notwithstanding, as far as we know, the simultaneous determination of TT, SY, and BB in industrialized foods using flow injection analysis (FIA) and amperometric detection, without a previous HPLC separation procedure, has never been reported.

* Corresponding author. Tel.: +55 16 33518098; fax: +55 16 33518350.
E-mail address: bello@ufscar.br (O. Fatibello-Filho).

0039-9140/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2012.07.051

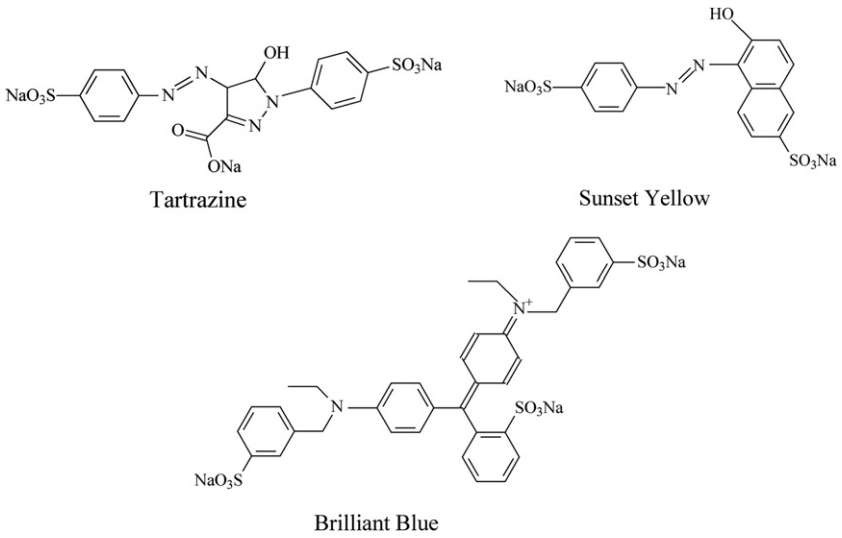

Fig. 1. Chemical structure of the three food colorants whose determination is reported here.

FIA, a very popular technique in analytical applications introduced by Ruzicka and Hansen in 1975, is simple and easy to use. It has some important advantages, such as the possibility of being automated, smaller sample and reagent consumption (with reduction of waste generation), and high sampling rates [28,29]. FIA systems with electrochemical detection are set up based on a single-line configuration, because no reagent is required to develop a chromogenic product, they are easy to handle, and show excellent sensitivity; thus, such systems have been widely used for the determination of various electroactive species at very low concentrations [28,30,31].

FIA methods with amperometric detection are usually reported in the literature for a single analyte determination; however, a few of these flow procedures are for the simultaneous determination of different analytes [32–34]. Usually, HPLC is employed for the separation of the analytes previously to their quantification, or two or more sensors are used with the application of a different constant potential at each sensor, whose resulting signals are analyzed with a multivariate calibration method. Alternatively, multiple pulse amperometry (MPA) and a FIA system have been used for the simultaneous determination of different analytes [35–39]. The use of MPA, which involves the application of an appropriate waveform of potential pulses on a single working electrode, allows the resolution of the analyte mixture with no need of separation of the analytes by a chromatographic column, any chemical pretreatment of the sample or electrode modification, or even the use of chemometric techniques for data analysis [35].

Recently we reported a FIA–MPA method for the simultaneous determination of the antioxidants butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) using a cathodically pretreated BDD electrode [37]. A dual-potential waveform was employed: $E_{det.1}=850$ mV and $E_{det.2}=1150$ mV vs. Ag/AgCl (3.0 mol L^{-1} KCl), each for the duration of 200 ms. The use of $E_{det.1}$ or $E_{det.2}$ caused the oxidation of BHA or BHA and BHT, respectively; hence, current subtraction was used to determine the concentration of both species.

In the last decade and a half, BDD films emerged as excellent electrode materials for several electrochemical applications, especially electroanalytical ones. BDD is a carbon based material that has numerous unique properties, such as extremely wide potential window, stable voltammetric and amperometric background current, long-term stability, low adsorption, and low sensitivity to dissolved oxygen [40–42]. However, the electrochemical activity of BDD electrodes for a given analyte commonly depends on their predominant surface termination (hydrogen or oxygen) [43–48], which can be changed by a proper electrochemical pretreatment (cathodic or anodic pretreatment, respectively) [49,50].

Thus, the purpose of this paper is to report on the development and optimization of a simple, low-cost, and rapid method for the simultaneous determination of two pairs of synthetic food colorants (TT–SY or BB–SY) in several food products, without a previous separation step, by coupling FIA and MPA with a BDD electrode. This method is based on a single-line flow injection system and an appropriate waveform of potential pulses that is applied to a cathodically pretreated (predominantly hydrogen terminated) BDD electrode.

2. Materials and methods

2.1. Apparatus

The BDD electrode (8000 ppm) and the electrochemical flow cell used in the experiments are described elsewhere [37,45]. Prior to the flow experiments, the BDD electrode surface (0.30 cm^2) was cleaned with isopropanol and rinsed with ultrapure water; then, it was sequentially pretreated anodically and cathodically in 0.5 mol L^{-1} H_2SO_4 by applying 0.5 A cm^{-2} for 60 s and -0.5 A cm^{-2} for 180 s, respectively; thus, the BDD surface was made predominantly hydrogen terminated [46,49,50].

In the flow experiments, a stainless steel tube was used as counter electrode, along with a miniaturized Ag/AgCl (3.0 mol L^{-1} KCl) reference electrode, to which hereinafter all working electrode potentials are referred. The voltammetric and amperometric measurements were carried out using an AUTOLAB PGSTAT-30 (Eco Chemie) potentiostat/galvanostat controlled with the GPES 4.0 software.

The HPLC determinations of SY, TT, and BB were carried out using an LC-10 AT Shimadzu system, with an UV–vis detector (SPD-M10-AVP) set at 430, 484, and 610 nm, respectively. A shimpack CLC-ODS (6.0 mm × 250 mm, 5 μm) chromatographic column was used. The mobile phase consisted of an aqueous 0.13 mol L^{-1} ammonium acetate solution, set to pH 7.5 by dropwise addition of a 1.7 mol L^{-1} 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 [3].

2.2. Reagents, supporting electrolyte, carrier solution, and standards

All reagents were of analytical grade: SY, TT, and BB from Sigma-Aldrich; H_2SO_4 from Synth. Standard 1.0 mmol L^{-1} SY, TT, and BB solutions were prepared with a 0.1 mol L^{-1} H_2SO_4 aqueous 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

Cyclic and hydrodynamic voltammograms were obtained for each colorant before the MPA–FIA determinations. The electrode potential applied in these determinations was selected taking into account the limiting current range in the hydrodynamic voltammograms. After optimizing the experimental parameters for the proposed methods, analytical curves were constructed by injection of volumes of the standard solutions of the analytes (TT – SY or BB – SY) at different concentrations. The limit of detection (*LOD*) was obtained as the concentration whose associated amperometric response was equal to three times the average voltammetric response for the blank solution (n=10) [51].

2.4. Treatment of food samples

All food samples were obtained from a local supermarket: powdered juice drinks and gelatins, and nutrient-enhanced sports drink beverages. After homogenization, accurately weighed 1.0 g portions of the powdered juice drinks or gelatins were dissolved in 10.0 mL of a 0.1 mol L^{-1} H_2SO_4 solution; to dissolve the gelatin, the H_2SO_4 solution was gently heated. The sample solutions were placed in an ultrasonic bath for 10 min for the complete extraction of the colorants. These solutions were filtered through a folded filter paper. Previous to injection into the electrochemical flow cell, a 500 μL aliquot was further diluted to 10.0 mL with the supporting electrolyte (0.1 mol L^{-1} H_2SO_4). A similar dilution was carried out for the samples of the sports drink beverages.

3. Results and discussion

3.1. Investigation of the electrochemical behavior of SY, TT, and BB, and flow injection parameters

Cyclic voltammograms obtained for the three dyes with the cathodically pretreated BDD electrode in 0.10 mol L^{-1} H_2SO_4 are shown in Fig. 2. The SY, TT, and BB dyes exhibit well-defined irreversible reduction peaks, at around −214 mV, −333 mV, and −380 mV, respectively. The use of 0.10 mol L^{-1} H_2SO_4 led to the best results for these colorants when compared with other supporting electrolytes, such as 0.040 mol L^{-1} Britton–Robinson buffer (pH 2) or 0.10 mol L^{-1} KNO_3 (pH 2, adjusted with a 0.50 mol L^{-1} HNO_3 solution); furthermore, the effect of the H_2SO_4 concentration (0.01 to 0.5 mol L^{-1}) was also investigated, when 0.1 mol L^{-1} yielded the highest current intensities. According to the literature, the electrochemical reduction of the azo-colorants (TT and SY) occurs by a four-electron process, whereas that of BB occurs by a two-electron process [2,17].

After this previous study, hydrodynamic voltammograms were obtained by chronoamperometry, to investigate the electrochemical response of the cathodically pretreated BDD electrode under dynamic conditions and optimize its operating potentials for the FIA–MPA measurements. For these experiments, increasingly negative potentials were applied in steps, and the resulting steady-state currents plotted as a function of the applied potential (see Fig. 3); from these plots one can apprehend that: (a) for SY, the cathodic current starts to increase at about 0.0 mV and reaches a maximum value in the potential range −200 to −350 mV; (b) for TT, the cathodic current starts to increase at about −200 mV and reaches a maximum value in the potential range −450 to −600 mV; (c) for BB, the cathodic current starts to increase at about −200 mV and, similarly to TT, approaches a maximum value in the potential range −450 to −550 mV. Hence, to maximize the electric current response for these analytes in the flow system, an electrode potential equal or more negative than −200 mV should be applied for the electrochemical reduction of SY, whereas a value equal or more negative than −450 mV should be applied for the reduction of TT and BB.

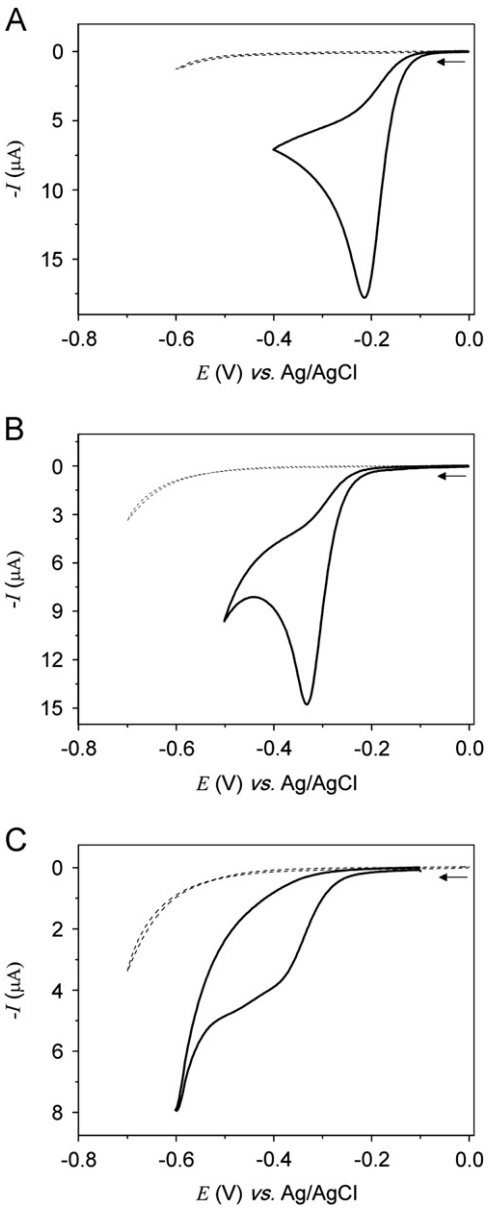

Fig. 2. Cyclic voltammograms (50 mV s^{-1}) in a 0.1 mol L^{-1} H_2SO_4 solution using a cathodically pretreated BDD electrode: dashed line without and solid line with the addition of 0.10 mmol L^{-1} SY (A), 0.10 mmol L^{-1} TT (B), and 0.10 mmol L^{-1} BB (C).

Next, the influence of flow conditions on the MPA response was evaluated by varying the flow rate from 0.45 to 3.5 mL min^{-1}, using solutions of each colorant and applied electrode potentials of −150 mV, for SY, and −450 mV, for TT or BB. The obtained electric currents increased with flow rate up to 2.7 mL min^{-1} and remained constant for higher flow rates; thus, this value of flow rate was selected to assure a good analytical response for the three

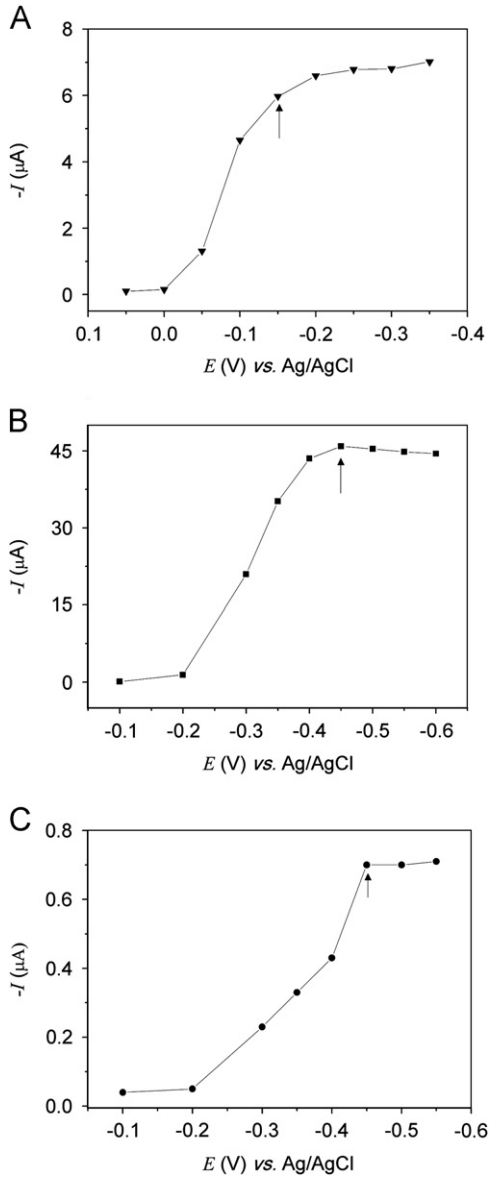

Fig. 3. Hydrodynamic voltammograms for 0.10 mmol L^{-1} SY (A), 0.10 mmol L^{-1} TT (B), and 0.05 mmol L^{-1} BB (C) obtained using a cathodically pretreated BDD electrode. Supporting electrolyte: 0.1 mol L^{-1} H_2SO_4; injected volume = 350 μL; flow rate = 2.7 mL min^{-1}.

food colorants and a high sampling rate (almost 80 determinations per hour).

Lastly, the effect of the injected sample volume was investigated in the range 50–500 μL. The obtained amperometric signal increased with the injected sample volume up to 350 μL; this value was thus selected for all the next measurements.

3.2. Multiple pulse amperometric simultaneous determination of the food colorants

According to the voltammetric characterizations carried out for TT and BB (see Figs. 2 and 3), their reduction potential coincides at approximately −450 mV. Consequently, the simultaneous determination of the three colorants is not feasible due to the overlap of the current peaks for TT and BB. However, electroanalytical procedures could be developed for the simultaneous determination of two pairs of the synthetic food colorants: TT − SY or BB − SY.

Actually, if common chronoamperometry were used, TT or BB could not be quantified in the presence of SY because its reduction potential (−150 mV) is smaller than that of TT or BB (−450 mV). However, the simultaneous quantification of these two pairs of colorants is possible using MPA, with the application of a double-potential waveform at −150 mV and –450 mV, respectively. Although, as can be seen in the hydrodynamic voltammogram for SY (Fig. 3A), a maximum value of current is obtained at a potential of −200 mV, the less negative potential pulse was set at −150 mV to guarantee that no current signal for TT or BB would be present, a necessary condition for the simultaneous determination of the pairs of colorants.

The MPA double-potential waveform used for the simultaneous determination of the two pairs of colorants, TT − SY and BB − SY, is shown in Fig. 4A. In this waveform, $E_{det.1} = -150$ mV (400 ms duration) corresponds to the reduction of SY without any TT or BB interference, while $E_{det.2} = -450$ mV (100 ms duration) corresponds to the reduction of TT and SY or BB and SY. The MPA analytical procedures reported in the literature commonly involve the use of an additional potential pulse to clean the electrode surface, but in our case this was not necessary because no adsorption of the analytes was found on the BDD electrode, which presented good repeatability.

The time periods for application of the two potential pulses were previously optimized (data not shown), and the duration of the pulses at $E_{det.1}$ and $E_{det.2}$ was set at 400 ms and 100 ms, respectively.

Figs. 4B and C show the pulse amperograms obtained at $E_{det.1}$ and $E_{det.2}$ for the injection of three different solutions (each at the concentration 50 μmol L^{-1}): the first solution contains only SY; the second, only TT or only BB; the third, a mixture of TT and SY or BB and SY. As can be apprehended from these figures, at −150 mV only SY is reduced, while at −450 mV the respective pair of colorants is reduced. Thus, the proportionality between current and [SY] is obtained at $E_{det.1}$, whereas that between current and [TT] or [BB] is obtained through adequate current subtractions, i.e.:

$I_{TT} = I_{E_{det.2}} - I_{E_{det.1}}$ or $I_{BB} = I_{E_{det.2}} - I_{E_{det.1}}$. However, the magnitude of the current associated to SY at −150 mV is different from that at −450 mV, because, as can be seen in the hydrodynamic voltammogram of Fig. 3A, the SY current signal continues to increase at electrode potentials more negative than −150 mV, thus causing an increase in the analytical signal (current increase) for the simultaneous determination of this analyte with TT or BB at −450 mV. To take this into account, a correction factor (*CF*) was used that corresponds to the ratio between the currents for SI detected at $E_{det.2}$ and $E_{det.1}$, i.e.:

$$CF = I_{E_{det.2}} / I_{E_{det.1}} \quad (1)$$

The obtained value for *CF* was about 1.5, which indicates that the current signal for SI at $E_{det.2}$ is approximately 50% higher than that at $E_{det.1}$. Thus, the current signals proportional to [TT], [BB], and [SY] were obtained in triplicate ($n = 3$) using the following equations:

$$I_{TT} = I_{E_{det.2}} - I_{E_{det.1}} \times (1.5 \pm 0.1) \quad (2)$$

$$I_{BB} = I_{E_{det.2}} - I_{E_{det.1}} \times (1.5 \pm 0.2) \quad (3)$$

$$I_{SY} = I_{E_{det.1}} \quad (4)$$

Hence, it is perfectly feasible to simultaneously quantify the pairs of food colorants, without interference from SY on TT or on BB as would happen if chronoamperometry were used. Thus, MPA–FIA simultaneous determinations of the TT − SY and BB − SY pairs, at varying concentrations, were performed using the procedure described above (see Fig. 5). The respective analytical curves

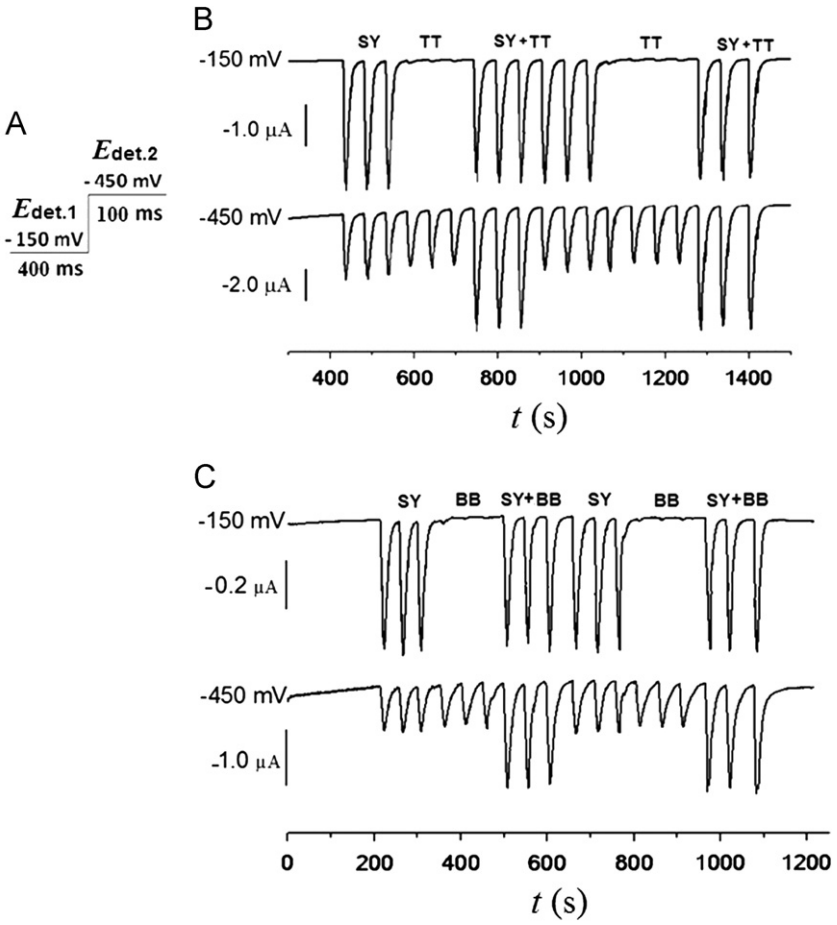

Fig. 4. (A) MPA waveform applied to the cathodically pretreated BDD working electrode as a function of time. (B) Flow-injection pulse amperometric responses in triplicate for solutions containing 50 μmol L^{-1} SY or TT, and both analytes simultaneously at this concentration. (C) Flow-injection pulse amperometric responses in triplicate for solutions containing 10 μmol L^{-1} SY or 50 μmol L^{-1} BB, and both analytes simultaneously at these concentrations. Supporting electrolyte: 0.1 mol L^{-1} H_2SO_4; flow rate = 2.7 mL min^{-1}; injected volume = 350 μL.

obtained for the TT − SY pair presented good linearity in the investigated concentration ranges (5.0–60.0 μmol L^{-1} for TT and 1.0–50.0 μmol L^{-1} for SY), with the following calibration equations (based on the use of Eqs. 2 and 4, respectively):

$$\mathrm{TT}: -\mathrm{I}/\mu\mathrm{A} = -0.316 + 0.108\,[c/(\mu\mathrm{mol\,L^{-1}})]\ (r = 0.999)$$
$$\mathrm{SY}: -\mathrm{I}/\mu\mathrm{A} = 0.0347 + 0.0342\,[c/(\mu\mathrm{mol\,L^{-1}})]\ (r = 0.999)$$

The estimated *LOD* values (3 *S*/*N*) were 2.5 μmol L^{-1}, for TT, and 0.80 μmol L^{-1}, for SY.

In the case of the BB − SY pair, the obtained analytical curves also present a good linearity in the investigated concentration ranges (5.0–60.0 μmol L^{-1} for BB and 1.0–50.0 μmol L^{-1} for SY), with the following calibration equations (based on the use of Eqs. 3 and 4, respectively):

$$\mathrm{BB}: -\mathrm{I}/\mu\mathrm{A} = 0.247 + 0.0949\,[c/(\mu\mathrm{mol\,L^{-1}})]\ (r = 0.995)$$
$$\mathrm{SY}: -\mathrm{I}/\mu\mathrm{A} = 0.0563 + 0.0174\,[c/(\mu\mathrm{mol\,L^{-1}})]\ (r = 0.997)$$

The estimated *LOD* values (3 *S*/*N*) were 3.5 μmol L^{-1}, for BB, and 0.85 μmol L^{-1}, for SY.

The *LOD* values obtained for these colorants are smaller than those previously reported by our laboratory using differential pulse voltammetry [27], which were already the lowest values in the literature. However, lower *LODs* are not necessary for the analysis of colorants in food samples, because their concentrations are usually high; thus, the *LODs* obtained with this proposed method are more than adequate for the analysis of food samples accessible on the Brazilian market. Moreover, the attained analytical frequency is very high (almost 80 determinations per hour) and reagent consumption is low, which are desirable features for routine analysis.

The intra- and inter-day repeatabilities for the two pairs of colorants were determined by successive injections ($n = 10$) of TT and SY or BB and SY, at different concentrations. The obtained intra- and inter-day RSD values for the two pairs of colorants were: (a) TT–SY — 3.3% and 7.5%, for TT, and 3.0% and 8.1%, for SY, respectively; (b) BB–SY—3.8% and 4.9%, for BB, and 6.1% and 6.7%, for SY, respectively.

Additionally, the selectivity of the proposed methods 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, in the concentration ratios (standard solution:interferent) 10:1, 1:1, and 1:10. The comparison of the obtained current signals with those in the absence of each possible interferent allowed us to conclude that these compounds do not significantly interfere with the new methods here reported.

Finally, the proposed flow methods for the simultaneous determination of SY and TT or SY and BB were applied for their determination in the following food samples: powdered juice drinks, gelatins, and nutrient-enhanced sports drink beverages. The transient signals obtained in triplicate for injections of solutions of some of these samples are presented in Fig. 5, whereas the obtained results for all the samples are presented in Tables 1 and 2 comparatively to those obtained using an HPLC

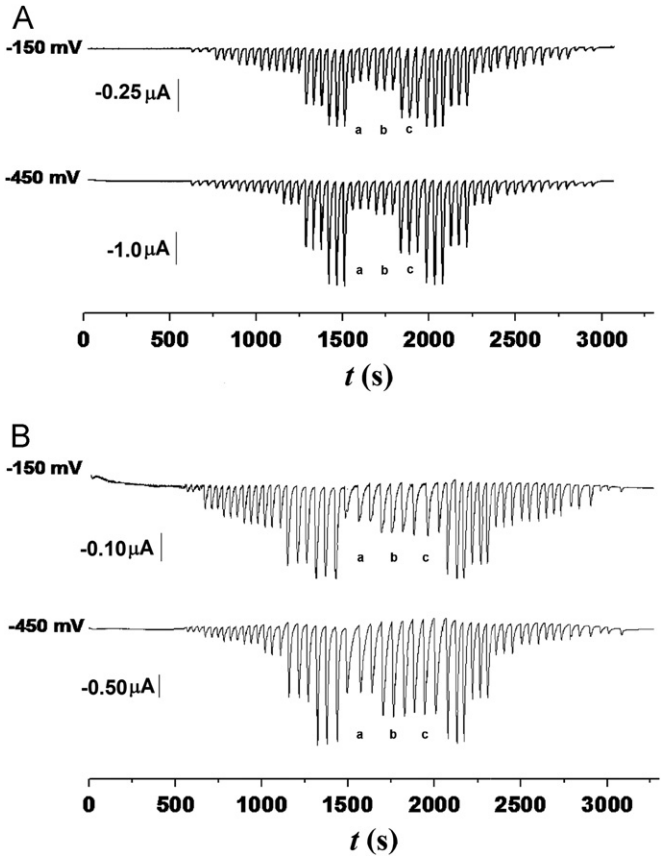

Fig. 5. MPA responses using a cathodically pretreated BDD electrode, after injections of solutions containing: (A) SY (1.0–50.0 μmol L^{-1}) and TT (5.0–60.0 μmol L^{-1}) simultaneously or samples of nutrient-enhanced sports drink beverage (a), gelatin (b), and powdered juice drink (c); (B) SY (1.0–50.0 μmol L^{-1}) and TT (5.0–60.0 μmol L^{-1}) simultaneously or three different gelatin samples (a, b, and c). Supporting electrolyte: 0.1 mol L^{-1} H_2SO_4; flow rate=2.7 mL min^{-1}; injected volume=350 μL.

Table 1
Results obtained in the simultaneous determination of SY and TT in food samples by HPLC and the proposed method (MPA).

Samples	TT		SY		Error 1 (%)[c]	Error 2 (%)[c]
	HPLC[b]	MPA[b]	HPLC[b]	MPA[b]		
Juices[a] (mg 100 g^{-1})						
1	55 ± 2	60 ± 1	49 ± 3	47 ± 3	9.1	−4.1
2	59 ± 4	63 ± 3	34 ± 2	33 ± 2	6.8	−2.9
3	22 ± 3	21 ± 5	8.2 ± 0.5	8.7 ± 0.6	−4.5	6.1
Gelatins (mg 100 g^{-1})						
1	14 ± 2	14 ± 1	3.1 ± 0.6	2.8 ± 0.5	0.0	−9.7
2	5.8 ± 0.3	5.6 ± 0.8	1.2 ± 0.4	1.3 ± 0.6	−3.4	8.3
Drinks[d] (mg 100 mL^{-1})						
1	1.5 ± 0.2	1.6 ± 0.5	0.35 ± 0.04	0.34 ± 0.08	6.7	−2.9
2	0.61 ± 0.06	0.60 ± 0.07	1.7 ± 0.5	1.8 ± 0.5	−1.6	5.9

[a] Powdered juice drinks.
[b] Average of 3 measurements.
[c] Average error (%)=100 × [(MPA value − HPLC value)/HPLC value].
[d] Nutrient-enhanced sports drink beverages.

method [3]—two examples of such chromatograms can be seen in the supplementary data. By analyzing these results (averages of three determinations for each sample, with corresponding standard deviations), one can conclude that the obtained MPA values agree quite well with those obtained using the reference HPLC method. In fact, applying the paired *t*-test to the results obtained by HPLC and the new MPA methods here reported, the resulting *t* values (1.38 for TT and 2.12 for SY or 2.33 for BB and 3.88 for SY) are smaller than the critical ones (2.44, $\alpha=0.05$, or 4.30, $\alpha=0.05$, respectively), indicating that there is no difference between the obtained results, at a confidence level of 95%.

Table 2
Results obtained in the simultaneous determination of SY and BB in gelatin samples by HPLC and by the proposed method (MPA).

Gelatin samples (mg 100 g^{-1})	BB		SY		Error 1 (%)[b]	Error 2 (%)[b]
	HPLC[a]	MPA[a]	HPLC[a]	MPA[a]		
a	10 ± 1	11 ± 3	3.3 ± 3	3.2 ± 2	10	−3.0
b	13 ± 1	14 ± 4	2.4 ± 0.2	2.6 ± 0.8	7.7	8.3
c	9.9 ± 0.5	10 ± 2	3.1 ± 0.4	3.2 ± 0.7	1.0	3.2

[a] Average of 3 measurements.
[b] Average error (%)=100 × [(MPA value − HPLC value)/HPLC value].

4. Conclusions

The obtained results for the new methods here reported allow us to conclude that MPA and FIA can be used along with a cathodically pretreated BDD electrode for the quantitative simultaneous determination of two pairs of food colorants, TT and SY or BB and SY, commonly used as mixtures in different industrialized foods. The obtained detection limits were 0.80 μmol L^{-1} and 2.5 μmol L^{-1} for SY and TT, and 0.85 μmol L^{-1} and 3.5 μmol L^{-1} for SY and BB, respectively. Furthermore, different sample values obtained by these procedures are similar to those obtained using a HPLC method. Additionally, it should be noted that these flow methods are readily applicable to several food products. Most important yet, the proposed methods are simple, quick, and can be carried out with good precision and accuracy.

Acknowledgments

Financial support and scholarships from the Brazilian funding agencies CNPq, CAPES, and FAPESP (Proc. No. 2011/00601-9) are gratefully acknowledged.

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.2012.07.051.

References

[1] W. Sawaya, A. Husain, J. Al-Otaibi, M. Al-Foudari, A. Hajji, Food Control 19 (2008) 98–105.
[2] J.J.B. Nevado, J.R. Flores, M.J.V. Llerena, Fresen. J. Anal. Chem 357 (1997) 989–994.
[3] K.S. Minioti, C.F. Sakellariou, N.S. Thomaidis, Anal. Chim. Acta 583 (2007) 103–110.
[4] ANVISA, Resolution CNNPA n. 44, 〈www.anvisa.gov.br〉 (1978).
[5] Y.N. Ni, Y. Wang, S. Kokot, Talanta 78 (2009) 432–441.
[6] A.H. Aktas, G.P. Ertokus, Rev. Anal. Chem. 29 (2010) 107–115.
[7] T.M. Coelho, E.C. Vidotti, M.C. Rollemberg, A.N. Medina, M.L. Baesso, N. Cella, A.C. Bento, Talanta 81 (2010) 202–207.
[8] N.E. Llamas, M. Garrido, M.S. Di Nezio, B.S.F. Band, Anal. Chim. Acta 655 (2009) 38–42.
[9] M.J. Culzoni, A.V. Schenone, N.E. Llamas, M. Garrido, M.S. Di Nezio, B.S.F. Band, H.C. Goicoechea, J. Chromatogr. A 1216 (2009) 7063–7070.
[10] E. Diacu, C.P. Ene, Rev. Chim-Bucharest 60 (2009) 745–749.

[11] N. Yoshioka, K. Ichihashi, Talanta 74 (2008) 1408–1413.
[12] S.P. Alves, D.M. Brum, E.C.B. Andrade, A.D. Pereira Netto, Food Chem. 107 (2008) 489–496.
[13] A.V. Jager, F.G. Tonin, M.F.M. Tavares, J. Sep. Sci. 28 (2005) 957–965.
[14] N. Dossi, R. Toniolo, A. Pizzariello, S. Susmel, F. Perennes, G. Bontempelli, J. Electroanal. Chem. 601 (2007) 1–7.
[15] K.S. Lee, M.J.A. Shiddiky, S.H. Park, D.S. Park, Y.B. Shim, Electrophoresis 29 (2008) 1910–1917.
[16] D.A. Skoog, F.J. Holler, T.A. Nieman, Principles of Instrumental Analysis, 5th ed., Harcourt Brace College, Philadelphia, PA, 1998.
[17] Y.Z. Song, J.M. Xu, J.S. Lv, H. Zhong, Y. Ye, J.M. Xie, Indian J. Chem. A 49 (2010) 1030–1034.
[18] Y.Z. Song, Can. J. Chem. 88 (2010) 676–681.
[19] M.L.S. Silva, M.B.Q. Garcia, J.L.F.C. Lima, E. Barrado, Talanta 72 (2007) 282–288.
[20] P.L. Lopez-de-Alba, L. Lopez-Martinez, L.M. De-Leon-Rodriguez, Electroanalysis 14 (2002) 197–205.
[21] Y.N. Ni, J.L. Bai, Talanta 44 (1997) 105–109.
[22] Y.N. Ni, J.L. Bai, L. Jin, Anal. Chim. Acta 329 (1996) 65–72.
[23] M.A. Kapor, H Yamanaka, P.A. Carneiro, M.V.B. Zanoni, Eclet. Quim. 26 (2001) 1–14.
[24] S.M. Ghoreishi, M. Behpour, M. Golestaneh, Anal. Methods 3 (2011) 2842–2847.
[25] M. Khanavi, M. Hajimahmoodi, A.M. Ranjbar, M.R. Oveisi, M.R.S. Ardekani, G. Mogaddam, Food Anal. Methods 5 (2012) 408–415.
[26] S.M. Ghoreishi, M. Behpour, M. Golestaneh, Food Chem. 132 (2012) 637–641.
[27] R.A. Medeiros, B.C. Lourencao, R.C. Rocha-Filho, O. Fatibello-Filho, Talanta 97 (2012) 291–297.
[28] O. Chailapakul, P. Ngamukot, A. Yoosamran, W. Siangproh, N. Wangfuengkanagul, Sensors 6 (2006) 1383–1410.
[29] A. Parikh, K. Patel, C. Patel, P. BN, J. Chem. Pharm. Res. 2 (2010) 118–125.
[30] N. Wangfuengkanagul, O. Chailapakul, J. Pharm. Biomed. Anal. 28 (2002) 841–847.
[31] V. Suryanarayanan, Y. Zhang, S. Yoshihara, T. Shirakashi, Sensor Actuat. B-Chem 102 (2004) 169–173.
[32] C. Terashima, T.N. Rao, B.V. Sarada, D.A. Tryk, A. Fujishima, Anal. Chem. 74 (2002) 895–902.
[33] T.A. Ivandini, K. Honda, T.N. Rao, A. Fujishima, Y. Einaga, Talanta 71 (2007) 648–655.
[34] R.C. Matos, M.A. Augelli, C.L. Lago, L. Angnes, Anal. Chim. Acta 404 (2000) 151–157.
[35] W.T.P. dos Santos, E.G.N. de Almeida, H.E.A. Ferreira, D.T. Gimenes, E.M. Richter, Electroanalysis 20 (2008) 1878–1883.
[36] D.T. Gimenes, W.T.P. dos Santos, T.P. Tormin, R.A.A. Munoz, E.M. Richter, Electroanalysis 22 (2010) 74–78.
[37] R.A. Medeiros, B.C. Lourenção, R.C. Rocha-Filho, O. Fatibello-Filho, Anal. Chem. 82 (2010) 8658–8663.
[38] F. Manea, A. Remes, C. Radovan, R. Pode, S. Picken, J. Schoonman, Talanta 83 (2010) 66–71.
[39] W.C. Silva, P.F. Pereira, M.C. Marra, D.T. Gimenes, R.R. Cunha, R.A.B. da Silva, R.A.A. Munoz, E.M. Richter, Electroanalysis 23 (2011) 2764–2770.
[40] Y.V. Pleskov, J. Anal. Chem 55 (2000) 1045–1050.
[41] K. Pecková, J. Musilová, J. Barek, Crit. Rev. Anal. Chem. 39 (2009) 148–172.
[42] S. Jolley, M. Koppang, T. Jackson, G.M. Swain, Anal. Chem. 69 (1997) 4099–4107.
[43] B.C. Lourenção, R.A. Medeiros, R.C. Rocha-Filho, O. Fatibello-Filho, Electroanalysis 22 (2010) 1717–1723.
[44] R.A. Medeiros, R.C. Rocha-Filho, O. Fatibello-Filho, Food Chem. 123 (2010) 886–891.
[45] L.S. Andrade, R.C. Rocha-Filho, Q.B. Cass, O. Fatibello-Filho, Electroanalysis 21 (2009) 1475–1480.
[46] G.R. Salazar-Banda, A.E. de Carvalho, L.S. Andrade, R.C. Rocha-Filho, L.A. Avaca, J. Appl. Electrochem. 40 (2010) 1817–1827.
[47] E.R. Sartori, R.A. Medeiros, R.C. Rocha-Filho, O. Fatibello-Filho, Talanta 81 (2010) 1418–1424.
[48] G.F. Pereira, L.S. Andrade, R.C. Rocha-Filho, N. Bocchi, S.R. Biaggio, Electrochim. Acta (2012), http://dx.doi.org/10.1016/j.electacta.2012.03.157.
[49] G.R. Salazar-Banda, L.S. Andrade, P.A.P. Nascente, P.S. Pizani, R.C. Rocha-Filho, L.A. Avaca, Electrochim. Acta 51 (2006) 4612–4619.
[50] L.S. Andrade, G.R. Salazar-Banda, R.C. Rocha-Filho, O. Fatibello-Filho, in: E. Brillas, C.A. Martínez-Huitle (Eds.), Synthetic Diamond Films: Preparation, Electrochemistry, Characterization and Applications, Wiley, New York, 2011, pp. 181–212.
[51] ICH–International Conference on Harmonisation, Validation of Analytical Procedures: Text and Methodology, 〈www.ich.org〉 (2005).