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# Simultaneous Determination of Food Dyes by First Derivative Spectrophotometry with Sorption onto Polyurethane Foam

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A simple spectrophotometric method is described for resolving binary mixtures of some food dyes: Amaranth, Brilliant Blue, Sunset Yellow and Tartrazine, using the first-derivative spectra with measurements at zero-crossing wavelengths. Analytical curves are linear up to 20 mg $L^{-1}$. Standard deviations of 1.30, 2.22, 1.93 and 0.81% were obtained for synthetic binary mixtures of 2 mg $L^{-1}$ of Amaranth, Brilliant Blue, Sunset Yellow and Tartrazine, respectively. Before the spectrophotometric measurements, the dyes were sorbed onto polyurethane foam and recovered in sodium dodecyl benzene sulfonate solution. Therefore, matrix complexity was eliminated and simple spectra were obtained. The method was very satisfactorily used for determining the colorants in synthetic mixtures, with recoveries in the 96 – 101% range. Detection limit values were dependent on the colorant combination investigated. Commercial products containing binary combinations of these dyes in different ratios (from 1:1 to 1:8) were analyzed. The results were compared with those obtained by HPLC; very similar values were found by the two methods.

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## Introduction

Food quality is closely associated with color and the use of food colorants has been an age-old practice, enhancing the aesthetical appeal of foods. Unfortunately, many of the natural colorants do not have the same stability under processing conditions as the synthetic ones. The use of synthetic organic dyes has been recognised as the most reliable and economical method of restoring or providing color to a processed product. Thus the tendency to include synthetic dyes in commercial products will continue, not always with the correct label designation. However, in order to prevent indiscriminate use, laws and regulations have been developed by many countries limiting the types, purity, uses and amounts of authorized food dyes. The list of permitted synthetic dyes is progressively being reduced and currently, the food dyes permitted in most countries are used when the technological needs are shown and the risks are evaluated.[1–3]

Amaranth, Brilliant Blue, Tartrazine and Sunset Yellow are synthetic organic dyes that can be found in common food products such as beverages, dry mix products, candies, dairy products, sugar confectioneries and bakery products. However, their content must be strictly controlled because they can induce allergies and other diseases in sensitive people. The maximum amounts allowed and the acceptable daily intakes have been decreased.[4] In Brazil, these colorants can be used as food additives at a maximum limit of 0.01 g/100 g of the product to be consumed. Facing with increasing legal restrictions, food dye determination[5] became an analytical challenge.

Different analytical procedures have been used in the identification and quantification of food dyes, mainly chromatography, spectrophotometry and voltammetry.[1,6–10] Dyes are highly absorbing species in the visible region and spectrophotometry is frequently used for their determination. However, severe overlapping is common in mixtures' analysis. Therefore, the direct absorption measurement is not suitable for resolving dye mixtures without a separation step.[11] Attempts to resolve complex spectra by means of different approaches have been made.[4,12–15] Among computer-controlled instruments, derivative techniques and multivariate calibration methods are playing very important roles in the multicomponent analysis of mixtures by UV-VIS absorption spectrophotometry.[16,17] Derivative spectrophotometry is a very useful analytical technique to resolve binary and ternary mixtures with overlapping and has been applied to several analytical problems.[18–22]

In this work, the derivative spectrophotometry is the base of a method for the simultaneous determination of binary mixtures of Amaranth, Brilliant Blue, Tartrazine and Sunset Yellow in commercial foods. In order to minimize possible spectral interferences, sorption onto polyurethane foam was used to free the colorants from the food matrix. Colorants' recovery was carried out using an anionic surfactant.

## Experimental

*Reagents and chemicals*

Amaranth (CI 16185), Brilliant Blue (CI 42090), Sunset Yellow (CI 15985) and Tartrazine (CI 19140) were obtained from Duas Rodas Industry (Santa Catarina, Brazil). The dyes were purified (> 85% purity) by means of successive solvent extraction processes, and dried at 60˚C for 12 h. Working solutions of dyes were prepared freshly from the stock solutions (1000 mg $L^{-1}$), diluting with deionized distilled water. A buffer solution (pH 3.0) was prepared from 1.0 mol $L^{-1}$ acetic acid. All solvents and reagents were of analytical grade. Polyester-

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type polyurethane (PU) foam (d = 60 Kg $m^{-3}$) was washed with water and acetone, and dried before use. The foam pieces were ground in a stainless-steel container with a blender to pass a 150 μm sieve.

In order to achieve all chromatographic assays, the following solutions were also used: $NaH_2PO_4/NaHPO_4$ buffer (0.1 mol $L^{-1}$ and pH = 7.0) and methanol.

*Procedure for dye determination in foods*

A suitable amount of the sample solution was diluted with an equal volume of $CH_3COOH$ 2.0 mol $L^{-1}$, and the pH adjusted to 3.0 with NaOH 1.0 mol $L^{-1}$. Ninety milligrams of PU were added to 10.0 mL of the buffered sample solution, and the mixture was stirred (magnetic stirring) for a period of 20 min. Then, the mixture was centrifuged. The dyes were recovered by mixing the PU with 20.0 mL of 0.25% sodium dodecyl benzene sulfonate (SDBS) aqueous solution, and stirring the mixture for 30 min at 40˚C. The colorants in these sample solutions were then determined as described below. Blanks containing all reagents except the analytes and standard solutions were prepared and treated in the same way as described for the samples.

*First derivative technique*

The absorption spectra of the samples and binary standard mixtures (containing 1 – 20 mg $L^{-1}$ of each dye) were recorded between 400 and 700 nm with a scan rate of 350 nm $min^{-1}$ (Hitachi U-2000 spectrophotometer). First derivative spectra were obtained with an $\Delta\lambda$ = 3 nm and a smoothing over 11 experimental points, using Savitzky–Golay simplified method.[24] The signal at the zero-crossing points for the binary mixtures were measured and, with the help of appropriate working curves, the concentration of each dye in the different mixtures was determined.

*Food sample treatment*

The method was applied to determine Amaranth (A), Brilliant Blue (B), Sunset Yellow (S) and Tartrazine (T) in commercial products.

Gelatine powders (flavors: orange, green apple, lemon, raspberry) were dissolved in hot water, according to the label recommendation; after cooling, they were filtered through a 0.45 μm membrane filter.

Juice powders (flavors: orange, mango, strawberry, grape) were dissolved in water, at room temperature, according to the label recommendation and then they were filtered through a 0.45 μm membrane filter.

*Chromatography procedure*

The determination of A, B, S and T in eight commercial products was also verified by HPLC using a diodo-array detector with measurements at 252 nm (wavelengths where the absorbance was maximal for Amaranth, Sunset Yellow and Tartrazine) and 630 nm (wavelength where the absorbance was maximal for Brilliant Blue) and using a chromatographic column $C_{18}$ as stationary phase and two solutions as mobile phase: $NaH_2PO_4/NaHPO_4$ buffer solution 0.1 mol $L^{-1}$ (90% (v/v) plus methanol 10% (v/v) at pH = 6.0 (as solution A)) and methanol 80% (v/v) plus $NaH_2PO_4/NaHPO_4$ buffer solution 0.1 mol $L^{-1}$ (20% (v/v) at pH = 6.0 (as solution B)). The gradient was carried out varying from 100% of solution A to 100% solution B over 40 min.

The flow rate was kept at 1.0 mL $min^{-1}$; under these conditions, the retention times were 6.5, 8.2, 14.5 and 25.1 min for T, A, S and B, respectively.[14,25]

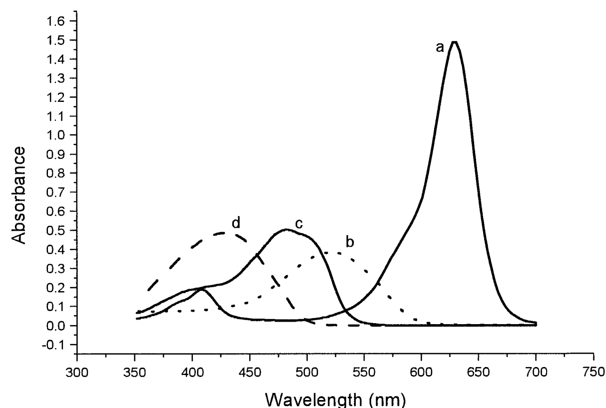


Fig. 1 Absorption spectra in SDBS solution (0.25% m/v). (a) Brilliant Blue; (b) Amaranth; (c) Sunset Yellow and (d) Tartrazine. *C*(dye): 10 mg $L^{-1}$.

## Results and Discussion

*Method development*

A, B, S and T are highly absorbing species in the visible region; the absorption spectra are shown in Fig. 1. In mixtures, the severe overlapping bands prevent conventional spectrophotometry application for quantitative analysis, and derivative techniques have to be used in order to improve the determination of these commonly used food dyes.

However, food samples are frequently complexes, and serious background due to the matrix effect generally appears in the spectrophotometric measurements. The problem in the quantitative determination of synthetic food dyes does not lie in their separation, but rather in the way of their quantitative isolation from the complex matrix. Traditional methods, such as adsorption onto wool or polyamide powder, seem not to be quantitative and can result in dye degradation.[26] PU foams have been used successfully in many separation/preconcentration procedures, once PU can retain both organic and inorganic species.[27] Therefore, in this work the sorption of the selected food dyes onto PU foam was used in order to free the dyes from the food matrix.

The influence of the pH on the absorption spectra of A, B, S and T was studied between pH 0.7 and 12. The spectra of A showed a maximum at 520 nm between 1.0 and 9.0; B showed a maximum at 628 nm between 2.5 and 11; S showed a maximum at 480 nm between 1.0 and 10, and T showed a maximum at 426 nm between pH 2.0 and 11.

The pH-effect on the retention of the colorants onto PU foam was studied from pH 1.0 to 13. A, B, S and T showed maximum adsorption at pH 3.0. There was no or little retention from solutions more basic than pH 6.0. As the solution became more acidic, there was an increase in the sorption process, followed by a decrease as the solution became increasingly more acidic. This decrease in the sorption generally occurred at about pH 2.0. According to the first $pK_a$ values of the dyes investigated,[28] at pH 3.0 the sulfo group is unprotonated and upon protonation of the *N*-group the molecule becomes a neutral zwitterion; therefore, only neutral species can be sorbed. Different buffer solutions were tested, and the acetic acid/sodium acetate buffer solution was found to give the best results.

In order to test the influence of other experimental parameters on the dye sorption onto PU foam, was also studied the amounts

Table 1 Efficiency of surfactants for dyes' recovery from PU foam[a]

| | Mean recovery, % | | | |
|---|---|---|---|---|
| | Triton X-100 | CTAB | SLS | SDBS |
| Amaranth | — | 9.70 | 51.1 | 72.3 |
| Brilliant Blue | — | 9.10 | 40.2 | 59.6 |
| Sunset Yellow | — | 13.8 | 61.7 | 76.1 |
| Tartrazine | — | 14.0 | 60.8 | 77.5 |

a. Experimental conditions: room temperature; C(surfactant), 0.5%; V(surfactant), 20.0 mL; stirring time, 30 min.

Table 2 Recovery of dyes from PU foam using SDBS[a]

| | Mean recovery, % |
|---|---|
| Amaranth | 96.1 |
| Brilliant Blue | 89.8 |
| Sunset Yellow | 96.7 |
| Tartrazine | 96.2 |

a. Experimental conditions: T, 40˚C; stirring time, 30 min; V(SDBS), 20.0 mL.

of the foam and the equilibration times. Ninety milligrams of PU foam were selected as the optimum amount for A, B, S and T sorption. The optimum stirring time, necessary to maximum sorption, was 20 min for all the dyes investigated.

Different solvents were tested for dye recovery. Water was able to poorly recover all the colorants from the PU foam, though by means of excessively long contact times. Dimethylformamide showed very satisfactory recovery efficiency. Twenty minutes of contact time and twenty milliliters of pure solvent gave the best recoveries. However, although very efficient, this procedure has a considerable disadvantage: it requires an organic solvent. Therefore, in order to avoid the use of organic solvents, alternatively four different surfactants were tested: Triton X-100, cetiltrimethylammonium bromide (CTAB), sodium lauryl sulfate (SLS), and sodium dodecyl benzene sulfonate (SDBS). Micellar systems are responsible for enhancement of the solubility of organic compounds in water, for catalysis of many reactions and for alteration of reaction pathways, rates and equilibria. Besides, these systems are convenient to use because they are optically transparent and non-toxic.[29] Surfactants interact with dyes in different ways, which depend on the structure and chemical characteristics of the dye and the structure of the surfactant.

Only the anionic surfactants (SLS and SDBS) were efficient in the colorants' desorption from PU foam; SDBS showed the best efficiency. One hypothesis is an electrostatic interaction between the anionic surfactant and the positive charge of the dyes (*N*-group) favoring desorption from PU foam. Table 1 presents the recovery of A, B, S and T from PU foam using the different surfactants tested.

Some experimental conditions were also studied in order to improve dyes' recovery. The best conditions for colorants recovery from PU foam were: C(SDBS), 0.25%; V(SDBS), 20.0 mL; equilibration time, 30 min; temperature, 40˚C. Table 2 shows the overall efficiency for dye recovery using these optimised conditions; data are averages of three independent measurements. A mean standard deviation of about 3% was observed for all the colorants studied.

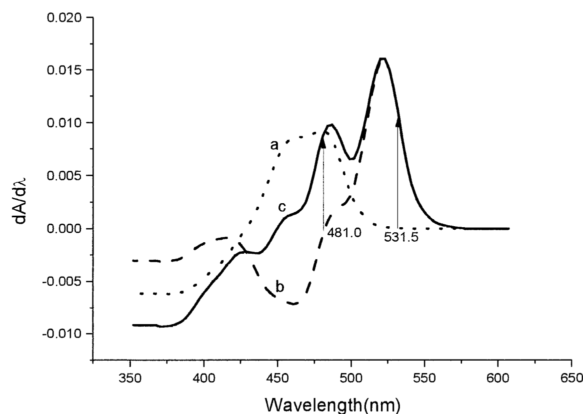


Fig. 2 First-derivative spectra of Tartrazine (a, 10 mg $L^{-1}$), Sunset Yellow (b, 10 mg $L^{-1}$) and their mixture (c). Tartrazine is determined at 481.0 nm; Sunset Yellow is determined at 531.5 nm.

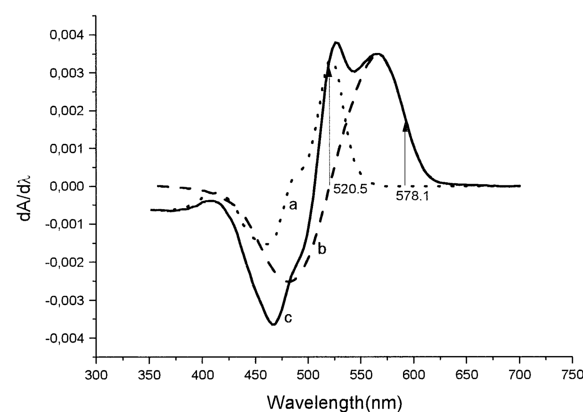


Fig. 3 First-derivative spectra of Sunset Yellow (a, 2 mg $L^{-1}$), Amaranth (b, 5 mg $L^{-1}$) and their mixture (c). Amaranth is determined at 593.0 nm; Sunset Yellow is determined at 520.5 nm.

*Spectrophotometric measurements*

In order for us to improve accuracy and precision, it was necessary to study the main instrumental parameters that affect the shape of the derivative spectra: the scan speed, the smoothing function and the wavelength increment to obtain the derivative spectra ($\Delta\lambda$). These parameters need to be optimized to give well-resolved peaks, improving selectivity and sensibility. Similar analytical signals were obtained for the scan rates evaluated, and an intermediate value (350 nm $min^{-1}$) was chosen. The best $\Delta\lambda$ should consider the noise level, the resolution of the spectrum and the analyte concentration. For all the mixtures studied, a $\Delta\lambda$ of 3 nm was selected as optimum. Due to the extent of noise levels, a smoothing function was used, and 11 experimental points were chosen as optimum values.

According to the proposed method, binary mixtures can be analyzed by measuring the signal at their zero-crossing wavelengths, previously established for each dye present in the mixture. The first derivative spectra of S and T mixture are shown in Fig. 2; S content can be measured at 531.5 nm (zero-crossing point for T), whereas T is determined at 481.0 nm (zero-crossing point for S). For A and S mixture analyses (Fig. 3), A and S can be determined at 578.1 nm and 520.5 nm, respectively. A can be determined in the presence of B by

Table 3 Statistical data for the binary mixtures' analyses

| S-T | | B-T | |
|---|---|---|---|
| $s(S) = 1.0 \times 10^{-4} + 1.1 \times 10^{-3}C(S)$ | ($r^2 = 0.9995$) | $s(B) = -9.0 \times 10^{-5} + 5.2 \times 10^{-3}C(B)$ | ($r^2 = 0.9995$) |
| LD: 0.089 ± 0.002 mg L$^{-1}$ | | LD: 0.017 ± 0.001 mg L$^{-1}$ | |
| $s(T) = -4.10^{-4} + 1.0 \times 10^{-3}C(T)$ | ($r^2 = 0.9991$) | $s(T) = 3.0 \times 10^{-5} + 9.0 \times 10^{-4}C(T)$ | ($r^2 = 0.9994$) |
| LD: 0.557 ± 0.013 mg L$^{-1}$ | | LD: 0.346 ± 0.025 mg L$^{-1}$ | |
| **A-B** | | **A-S** | |
| $s(A) = -3.0 \times 10^{-5} + 5.0 \times 10^{-4}C(A)$ | ($r^2 = 0.9996$) | $s(A) = 7.0 \times 10^{-5} + 6.0 \times 10^{-4}C(A)$ | ($r^2 = 0.9997$) |
| LD: 0.902 ± 0.043 mg L$^{-1}$ | | LD: 0.117 ± 0.001 mg L$^{-1}$ | |
| $s(B) = 4.0 \times 10^{-4} + 3.2 \times 10^{-3}C(B)$ | ($r^2 = 0.9997$) | $s(S) = 1.0 \times 10^{-4} + 1.6 \times 10^{-3}C(S)$ | ($r^2 = 0.9998$) |
| LD: 0.125 ± 0.001 mg L$^{-1}$ | | LD: 0.037 ± 0.002 mg L$^{-1}$ | |

LD (limit of detection) = $3\sigma_B/a$, where $\sigma_B$, standard deviation of blank; $a$, slope of working curve.

Table 4 Determination of dyes in food samples[a] (concentration: mg/g)

| Food sample | Derivative spectrophotometry | | | | HPLC | | | |
|---|---|---|---|---|---|---|---|---|
| | Amaranth | Brilliant Blue | Sunset Yellow | Tatrazine | Amaranth | Brilliant Blue | Sunset Yellow | Tatrazine |
| Gelatine powder (orange) | | | 0.059 ± 0.001 | 0.056 ± 0.001 | | | 0.059 ± 0.014 | 0.057 ± 0.001 |
| Gelatine powder (lemon) | | 0.007 ± 0.001 | | 0.076 ± 0.001 | | 0.007 ± 0.001 | | 0.078 ± 0.003 |
| Gelatine powder (green apple) | | 0.072 ± 0.001 | | 0.400 ± 0.002 | | 0.072 ± 0.001 | | 0.399 ± 0.004 |
| Gelatine powder (raspberry) | 1.407 ± 0.002 | | 0.563 ± 0.004 | | 1.415 ± 0.004 | | 0.586 ± 0.009 | |
| Juice powder (orange) | | | 0.490 ± 0.001 | 0.632 ± 0.002 | | | 0.496 ± 0.017 | 0.623 ± 0.004 |
| Juice powder (mango) | | | 1.182 ± 0.006 | 1.189 ± 0.007 | | | 1.201 ± 0.019 | 1.212 ± 0.058 |
| Juice powder (strawberry) | 3.544 ± 0.014 | | 1.646 ± 0.043 | | 3.556 ± 0.032 | | 1.754 ± 0.081 | |
| Juice powder (grape) | 0.403 ± 0.001 | 0.048 ± 0.001 | | | 0.413 ± 0.029 | 0.050 ± 0.015 | | |

a. Average of three determinations and relative standard deviation.

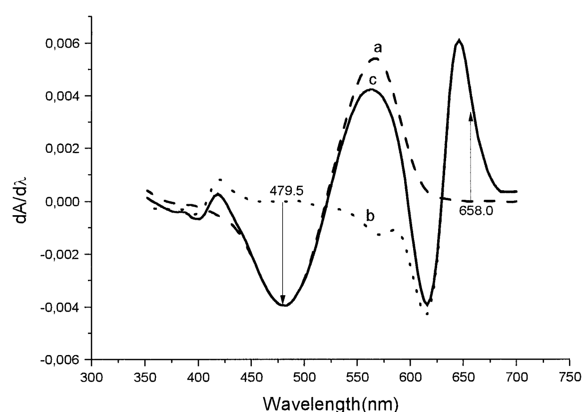


Fig. 4 First-derivative spectra of Amaranth (a, 8 mg L$^{-1}$), Brilliant Blue (b, 1 mg L$^{-1}$) and their mixture (c). Amaranth is determined at 479.5 nm; Brilliant Blue is determined at 658.0 nm.

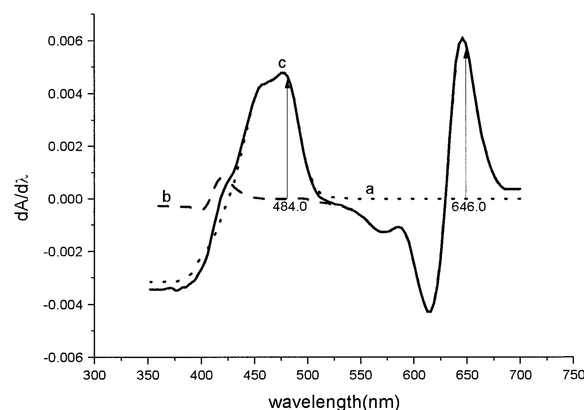


Fig. 5 First-derivative spectra of Tartrazine (a, 5 mg L$^{-1}$), Brilliant Blue (b, 1 mg L$^{-1}$) and their mixture (c). Tartrazine is determined at 484.0 nm; Brilliant Blue is determined at 646.0 nm.

measuring the signal at 479.5 nm, whereas B, in this mixture, is measured at 658.0 nm (Fig. 4). For B and T mixture analyses (Fig. 5), B and T can be determined at 646.0 nm and 484.0 nm, respectively.

Calibration graphs were obtained at the selected wavelengths from synthetic binary mixtures analyses, showing linearity up to 20.0 mg L$^{-1}$. Table 3 shows the analytical parameters obtained from the different calibrations. Very good regression coefficients were reached in all mixtures for the tested concentration range. With regard to detection limits, different

values were obtained for each dye when different mixtures were analyzed. The method was applied to A, B, S and T determinations in synthetic mixtures; good recoveries were obtained, in the range of 96 – 101%. The analytical signals produced by each dye were independent of the concentration of the other dye, since calibration graphs of each colorant in the presence of increasing amounts of the other dye showed similar slopes. Measuring independent samples for each dye on different days checked precision; no differences were observed at a confidence level of 95%. Standard deviations of 1.30, 2.22, 1.93 and 0.81% for A, B, S and T, respectively, were obtained.

Gelatine powder and juice powder samples were prepared as described in the experimental section, and the solutions obtained were then analyzed by the proposed method. Table 4 shows dye contents obtained by the proposed procedure and by the HPLC method. As can be seen, the results obtained by the proposed spectrophotometric method are in agreement with those obtained by the HPLC one.

Tartrazine content in gelatine was high (more than 5 mg/100 g product to be consumed) although lower than the allowed levels (15 mg/100 g product). The analytical results obtained are quite acceptable; nominal contents, provided by the manufacturers, were not available.

## Conclusions

A first derivative technique allowed the simultaneous determination of Amaranth, Brilliant Blue, Sunset Yellow and Tartrazine in binary mixtures with satisfactory detection limits to the food analyses. The method proposed is practical, simple and inexpensive, and was applied to gelatine powder and juice powder analyses. Dye sorption onto polyurethane foam allowed the colorants to be freed from the sample matrix.

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