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Short communication

Determination of dyes in foodstuffs by capillary zone electrophoresis

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Abstract

A rapid method based on capillary zone electrophoresis coupled with photodiode-array detection has been developed to determine the dyes Tartrazine E-102, Sunset Yellow FCF E110, Amaranth E-123, New Coccine E-124, Patent Blue V calcium salt E-131 and Allura Red AC E-129 in foodstuffs. Separation was done by using a Bare CElect-FS75 CE column, using a 10 m*M* phosphate buffer at pH 11.0. Hydrodynamic injections at 0.5 p.s.i. for 4 s (21 nl of sample) and 20 kV separation voltage were used. The quantitation limits for the six dyes ranged from 3 to 6 μg/ml. A linear relationship between 3 to 95 μg/ml, with correlation coefficient better than 0.995 was obtained. This method has been applied to the determination of the studied dyes in beverages, jellies and syrups.

Keywords: Food analysis; Dyes

1. Introduction

Color is an important characteristic of the food because it allows capturing the desired esthetic quality of a particular food, and this is the reason of the importance of food coloring in food industries. Because some synthetic colors may be pathogenic, especially if they are consumed in excess, they are analyzed and evaluated by both the manufacturer and the Organizations of Health, Food and Agricultural Organization (FAO) and World Health Organization (WHO). Then specific directives in each country strictly regulate the use of synthetic food colors.

As an example, the Food and Drug Administration (FDA) established an acceptable daily intake (ADI) for Tartrazine (E-102) to be 5.0 mg/kg of body weight. Amaranth (E-123) is the most controversial of the color additives used. It had been used in foods since 1908 but is now banned from use in USA. In 1970, two Russian studies alleged that Amaranth was both carcinogenic and embryotoxic. The FDA conducted its own study and confirmed their results. Other studies tried to dispute the findings, but due to unresolved questions about its safety, it was removed from use in food in 1976. Despite USA stance on Amaranth, it is still the most widely used red colorant in the world [1].

Sunset Yellow FCF (E-110) is an azo food dye that has been used since 1929. It gives a reddish-yellow color to foods and drugs. It is used only in small amounts because of its high tinctorial strength. The maximum daily intake established by the FDA is 225 mg for a 60-kg person [1].

In the past, many methods to determine synthetic

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food colorants have been reported [2–6]. In recent years, several papers have described the separation of food dyes by using high-performance liquid chromatography (HPLC) methods [7–9], capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC) [10–16].

The aim of the present work is the optimization of a method to quantify six of the most common food dyes used as additives, Tartrazine (E-102), Sunset Yellow FCF (E-110), Amaranth (E-123), New Coccine (E-124), Allura Red AC (E-129) and Patent Blue V calcium salt (E-131). The proposed method allows the quantification of the analytes in such food samples as beverages, syrups and jellies at ppm levels. In most of the methods reported before cyclodextrins or surface-active agents were used. In the present work the pH and the buffer capacity are enough to obtain good resolution between analytes, and the time of analysis improves the reported before for some dyes [13,14]. Structures, numbers and names of the food dyes used are shown in Fig. 1.

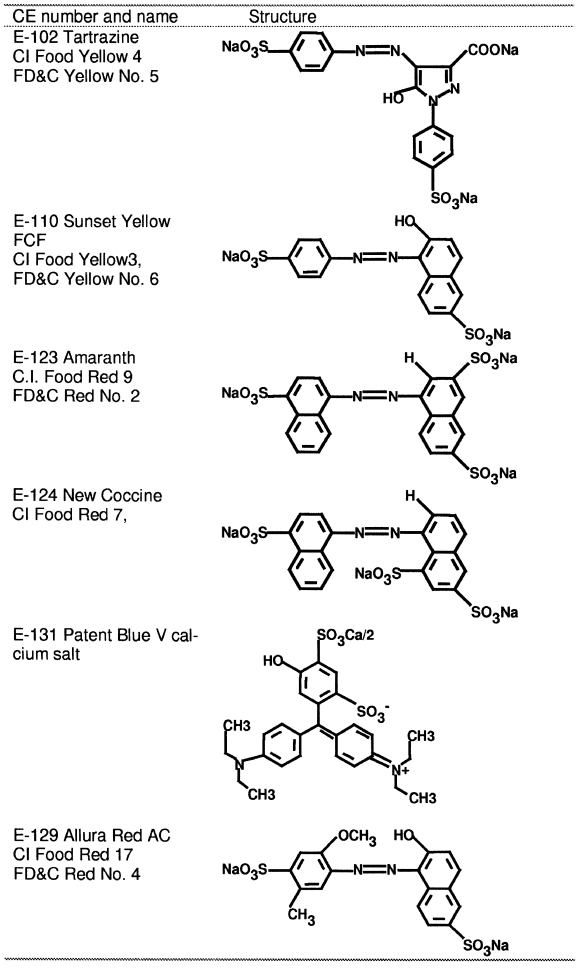

Fig. 1. Structures, numbers and names of the dyes used.

2. Experimental

2.1. Reagents

Ultrapure Milli-Q water from Millipore was used for the preparation of solutions. Dyes were obtained from Aldrich and Fluka. Stock standard solutions between 300 and 630 μg/ml were prepared in Ultrapure Milli-Q water and diluted as required. A mixed standard solution containing the six dyes was used to examine the optimum conditions of separation. The chemicals used for the preparation of the buffer were of analytical-reagent grade. Other reagents used were ortho-phosphoric acid (Fluka) and sodium hydroxide (Merck).

2.2. Apparatus and conditions

Capillary zone electrophoresis was carried out with a P/ACE System 5500 (Beckman, Palo Alto, CA, USA) equipped with a diode array detector. The separation was carried out in a Bare CElect-FS75 CE fused-silica capillary column (Supelco, Bellefonte, PA, USA), 57 cm (50 cm effective length)×75 μm I.D. The capillary was activated by pressure injection of 1.0 *M* sodium hydroxide solution for 20 min followed by a 10-min rinse with Ultrapure Milli-Q water and by a 10-min rinse with the run electrolyte. The electrophoresis buffer was prepared by adding the required amount of sodium hydroxide to 10 m*M* phosphoric acid in order to obtain a pH 11.0. Separation was performed at 25°C and the applied voltage was 20 kV. Injections were done in hydrodynamic mode at 0.5 p.s.i. for 4 s (21 nl of sample). The absorbance from 280 to 600 nm was monitored with an on-column photodiode-array detector. The quantification of the analytes was done measuring the peak areas of the electropherogram at 280 nm.

2.3. Sample preparation

The sample of melon beverage was diluted 1:20 (0.5 g/10 g) and Syrups flavor of strawberry, currant and mint 1:25 (1.0 g/25 g) with Milli-Q water, neutralized with NaOH and centrifuged in order to remove solids. Approximately 1 g of Jellies flavor orange and pineapple were extracted with 10 ml of methanol, centrifuged in order to remove solids, the methanol portion was evaporated to dryness, neutralized and diluted with 5 ml of Milli-Q water. All determinations were carried out in triplicate. Peak identification was done by comparing the migration times and absorption spectra of the samples analyzed with the corresponding to a standard mixture of the dyes studied.

3. Results and discussion

The most important factor in 'traditional' CE is the choice of the buffer pH and the buffering capacity of the buffer at that pH [17]. In the first group of assays, only the pH was varied by using ortho-phosphoric acid at 20 m*M* concentration, and the desired pH values from 6.5 to 11.7 were obtained by the addition of sodium hydroxide. In these conditions the better results were obtained at pH 11 as is shown in Fig. 2, because at pH 6.5 E-123, E-102 and E-124 are not separated well enough, and at pH 8.8 and 9.8 E-129 and E-110 had poor resolution. The concentration of the electrophoretic buffer was studied at 10, 20 and 30 m*M* in order to obtain the optimum ionic strength of electrolyte to an acceptably low current to minimize noise and good peak efficiency. The best results were obtained at 10 m*M*. Three voltage levels were also studied, at 15, 20 and 25 kV. The migration times of the dyes decrease on increasing the applied voltage. A high voltage decreases the migration time of all analytes as well as the resolution between peaks, but also increase the current, therefore 20 kV was selected because we have a good separation in a short time. An electropherogram of a standard and a mint syrup sample under the optimized conditions is shown in Fig. 3, showing that the migration rate increases with decreasing net charge and with increasing molecular size, but molecular size is determinant between dyes

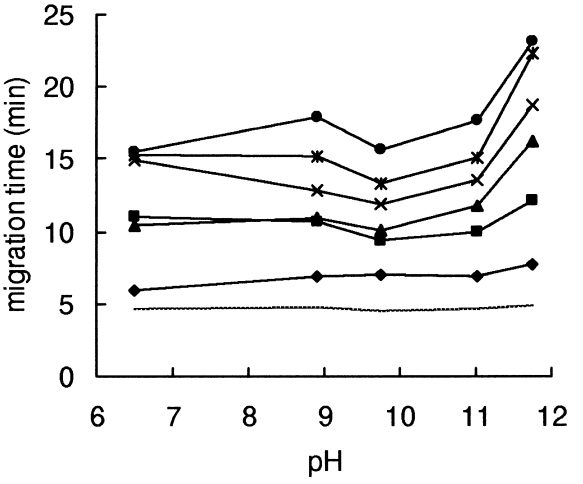

Fig. 2. Effect of pH of the electrophoretic buffer at 20 m*M* concentration and applied voltage 15 kV. E-131 (♦), E-129 (■), E-110 (▲), E-102 (●), E123 (★), E-124 (×), EOF (continuous line).

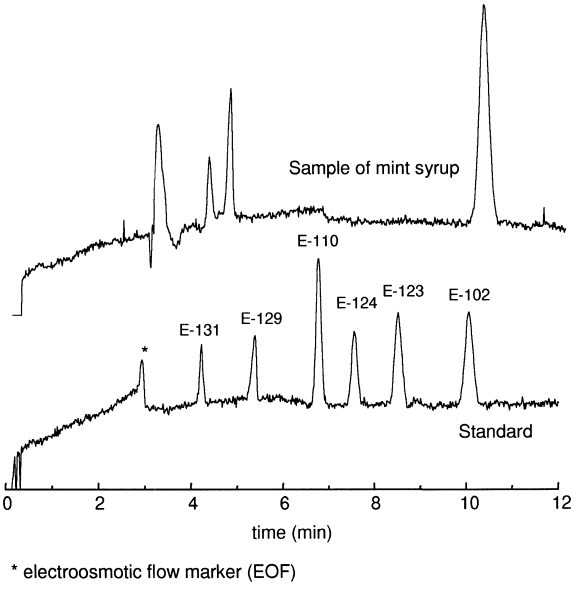

Fig. 3. Electropherogram of the dyes studied under the optimized conditions. Electrophoretic buffer: buffer phosphates 10 m*M*, pH 11.0; applied voltage, 20 kV and λ=280 nm. Hydrodynamic injections at 0.5 p.s.i. for 4 s. All analytes in standard have around 4.5 μg/ml and the sample 9 μg/ml of E-131 and 14.8 μg/ml of E-102.

Table 1
Detection and quantitation limits for the CZE separation of studied dyes at 280 nm

CE name	LOD (μg/ml)	LOQ (μg/ml)
E-102 Tartrazine	1.3	4.4
E-110 Sunset Yellow FCF	1.7	5.5
E-123 Amaranth	1.1	3.6
E-124 New Coccine	1.0	3.3
E-129 Allura Red AC	1.4	4.6
E-131 Patent Blue V calcium salt	1.0	3.2

with equal net charge. The absorption spectra of the dyes present in the mint syrup sample and the corresponding standard dyes were the same.

3.1. Quantification

Calibration plots with correlation coefficient $r^2 \geq 0.995$ were obtained by reporting peak areas as a function of analyte concentrations, at values ranging between 3 and 95 μg/ml. Detection and quantitation limits were calculated as the concentration corresponding to 3 and 10 times the standard error of the intercept obtained by the regression analysis of the calibration line plus the intercept value of the calibration curve [18]. Table 1 reports limit of detection (LOD) and limit of quantitation (LOQ) for each dye. The relative standard deviations (RSD) of the migration times as a measure of buffer stability and peak areas as a measure of accuracy of instrument injection and analyte stability are shown in Table 2. The data for peak areas are from 2 days, in which each standard was injected three times. The concentration obtained for the dyes in the samples analyzed and presented in Table 3 are in accordance with the limits stated in Spanish Boletin Oficial del Estado (BOE) [19].

4. Conclusions

A capillary electrophoresis method was developed for the determination of one sulphonated dye and five sulphonated azo dyes in foodstuffs. The time of analysis is short and it is not necessary the use of surface-active agents or other solvents for separation. The resolution obtained between analytes and the time of analysis improve those reported before for some dyes [13,14]. The method allows analyzing foodstuffs containing ppm levels of the studied compounds. This method gave reliable and reproducible results with a simple sample pretreatment.

Table 2
Stability of CZE separation of studied dyes

CE name	Migration time (min) (RSD%, $n=24$)	RSD% of peak areas ($n=6$)
E-102 Tartrazine	9.97(1.6)	3.1
E-110 Sunset Yellow FCF	6.73(1.4)	2.0
E-123 Amaranth	8.41(1.8)	3.0
E-124 New Coccine	7.49(1.4)	2.3
E-129 Allura Red AC	5.43(1.7)	2.4
E-131 Patent Blue V calcium salt	4.23(1.3)	2.0

Table 3
Concentration (μg/g) obtained for the samples analyzed

Product	E-102	E-110	E-123	E-124	E-129	E-131
Melon beverage	101	210	–	–	–	–
Strawberry syrup	128	–	–	–	–	–
Currant syrup	–	–	–	326	–	–
Mint syrup	118	–	–	–	–	55
Orange jelly	463	262	–	–	–	–
Pineapple jelly	–	13	–	–	–	–

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