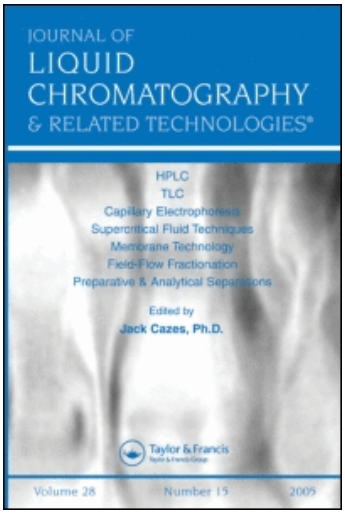

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Analytical Assessment of a Home Made Capillary Electrophoresis Equipment with Linear Charge Coupled Device for Visible Light Absorption Detection in the Determination of Food Dyes

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Abstract: The performance of modular home made capillary electrophoresis equipment with spectrophotometric detection, at a visible region by means of a miniaturized linear charge coupled device, was evaluated for the determination of four food dyes. This system presents a simple but efficient home made cell detection scheme. A computer program that converts the spectral data after each run into the electropherograms was developed to evaluate the analytical parameters. The dyes selected for analytical evaluation of the system were Brilliant Blue FCF, Fast Green FCF, Sunset Yellow FCF, and Amaranth. Separation was carried out in a 29 cm length and 75 μm I.D fused silica capillary, using 10 mmol L^{-1} borate buffer at pH 9, with separation voltage of 7.5 kV. The detection limits for the dyes were between 0.3 and 1.5 mg L^{-1} and the method presented adequate linearity over the ranges studied, with correlation coefficients greater than 0.99. The method was applied for determination and quantification of these dyes in fruit juices and candies.

Keywords: Capillary electrophoresis, Food dyes, Instrumentation, Miniaturization, Spectrophotometric detection

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INTRODUCTION

Many advances in conventional analytical separation methods, such as high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) have been made towards miniaturization. In order to comply with the current miniaturization demands in these advances some research groups have developed home made systems. Aiello and McLaren developed a small volume UV/Vis flow cell as part of an optical multichannel μ-HPLC detector, dedicated for the measurement of organic aldehydes and ketones in tropospheric air.[1] Their cell was made from two blocks of stainless steel with a 12 mm path length and 30 nL detection volume, and it was coupled to the detection system via fiber optics. The transmitted light comes from a 30 W deuterium lamp and was coupled into a S2000 Ocean Optics miniature fiber optic spectrometer with a 2048 element linear CCD detector. The system consisted of two micropumps, mixer, and controller, and a 1.0×250 mm C_{18} column. The absorbance S/N ratio has been increased through the use of large bandwidth total absorbance signals (TAS), and the operational TAS bandwidth used allowed a reasonably accurate calculation of concentration for organic carbonyl hydrazones. The instrumental detection limit corresponds to 5–8 ppt of organic carbonyl in air for a 2 L air sample collected on the cartridge.

Adaptation of the capillary electrophoresis technique to miniaturization purposes is somehow straightforward and a few home made systems were described in literature. King et al. described the application of a UV light emitting diode (LED) source to indirect absorbance detection of inorganic anions in water.[2] The LED used had an emission maximum at 379 nm, measured with an Ocean Optics S 1000 diode array fiber optics visible spectrophotometer. These values match closely the absorbance maximum for chromate, which was used as the background electrolyte, improving the detection limits. The noise, sensitivity, and linearity of the LED detector were evaluated and compared with the standard mercury lamp, exhibiting up to 70% decrease in noise, up to 26.2% increase in sensitivity, and over 100% increase in linear range, while keeping the cost low.

Casado-Terrones et al. compared two capillary electrophoresis systems, one commercial (LIF) and one home made for the determination of derivatized aminated compounds with fluorescein isothiocyanate (FITC) in brewing samples.[3] The home made device used a blue light emitting diode (Ocean Optics LS-450 Blue-LED Pulsed Light Source) as the light source and a charge coupled device (AvaSpec-2048 CCD) as the detection system. The cell detection presents a T shape and permitted an optimum alignment of the probe and capillary, without focusing optics. The effect of pH on the reversed EOF and resolution

of the peaks was studied, testing three different buffers, among them sodium borate, which gave the best separation and a satisfactory analysis time. Resolution achieved with the home made instrument was poorer than that with the commercial device, which could be explained by the larger detection window used in the LED system, with additional band broadening coming from the lack of refrigeration. LODs determined with this system were 1.8 worse than those obtained with the LIF system, concluding that improvements will be necessary, and the use of the system depends on the complexity of the sample and the analytical requirements.

Munoz et al. described a method based on capillary zone electrophoresis with contactless conductivity detection for the determination of some cations and anions in ethanol fuel.[4] The samples were injected by gravity from a height of 100 mm for 30 s, but tests with the direct injection of untreated samples showed a marked lack of repeatability, that could be resolved by the evaporation of ethanol. The method presented relative standard deviations ($n = 4$) below 0.8%, and detection limits that satisfy limits established by Agência Nacional do Petróleo (ANP) for ethanol fuel analysis. The method has a lower cost and shorter analysis time, if compared to ion chromatography, the method described by ANP.

The use of food colorants dates back from antiquity, and nowadays they have been used to preserve the organoleptic characteristics of any kind of food. However, sometimes dyes are used to cover up bad food quality, degraded or old food, and to simulate food with higher nutritional values.[5] So low cost and high resolution equipment dedicated for routine analyses have to be developed to control the limits stated by each country legislation. Based on the requirement of our samples of interest and the relative easy adaptation of the capillary electrophoresis technique to the current miniaturization efforts, our group designed and built a CE instrument equipped with a miniaturized linear charge coupled device (CCD) for spectrophotometric detection in the visible region. This system uses a spectrophotometer manufactured by Ocean Optics® with the cell detection and the control of high voltage power supply designed and built in our laboratory. The spectrophotometer used was originally designed by the manufacturer for single spectrum data acquisition and did not present either real time monitoring or continuous data acquisition, which motivated us to create a post-run computer program that converts the streaming data into the electropherograms, using software based in an open source operating system. The low price of this system (around US$ 5,000) becomes very attractive for routine analysis applications and setup in laboratories with limited resources. To evaluate this system we studied four synthetic food dyes allowed by Brazilian legislation to assess instrument and method validation.

Food dyes can be classified as synthetic or natural compounds and can also be used as mixtures. Dyes guarantee color uniformity, restore

the original color when it is destroyed during processing or storage, and increase the appeal of colorless foodstuffs. Synthetic dyes in food are predominantly azo and triarylmethane dyes and they are mostly acidic or anionic dyes containing carboxylic acid, sulfonic acid, or hydroxyl groups, which form negatively charged colored ions at basic pH ranges. Some of them may be toxic if consumed in large amounts.[6] Safety data for every synthetic colorant food additive have been determined and evaluated by the Food and Agricultural Organization (FAO) and World Health Organization (WHO). In Brazil 11 synthetic dyes are allowed: Brilliant Blue FCF (E133), Indigotine (E132), Sunset Yellow FCF (E110), Tartrazine (E102), Allura Red AC (E129), Erythrozine (E127), Ponceau 4R (E124), Amaranth (E123), Fast Green FCF (E143), Patent Blue (E131) and Azorrubine (E122), and the regulation for using food additives is made by Agência Nacional de Vigilância Sanitária (ANVISA).[7]

Various analytical techniques have been used to analyze colorants, such as thin layer chromatography, derivative spectrophotometry, and electrochemical methods.[8–10] The most used technique to separate food dyes is high performance liquid chromatography, generally coupled to diode arrays (HPLC-UV) to confirm the identity of the analyte.[11,12] In recent years, the use of electrophoretic techniques to analyze these dyes has increased. Capillary micellar electrokinetic chromatography (MEKC) methods have been successfully used as an alternative to reversed phase liquid chromatography (RP-HPLC). MEKC was applied to the determination of artificial sweeteners, preservatives, and colors, used as additives in carbonated soft drinks. Good resolution was achieved in a 15 min run by employing 20 mmol L^{-1} carbonate/hydrogen carbonate buffer at pH 9.5 as the aqueous phase and 62 mmol L^{-1} sodium dodecyl sulfate as the micellar phase.[13] Also using MEKC, Jager et al. demonstrated complete resolution in the separation of 11 dyes, which was achieved in less than 9 min with a well elaborated electrolyte, consisting of 7.5 mmol L^{-1} tetraborate at pH 10.1, 10 mmol L^{-1} Brij, and 15% ACN, with migration time and peak area repeatabilities (RSD) better than 1.6% and 5%, and the detection limits ranged from 0.47 to 2.3 mg L^{-1}.[14]

The use of free solution capillary electrophoresis (FSCE) has also been presented and this method was applied successfully in the determination of synthetic dyes in drinks, ice creams, jellies, sweeteners, syrups, and preservatives using UV detector coupling with diode array detector (DAD).[15,16] A method for determination of 12 dyes was used to evaluate their presence in alcoholic beverages.[17] Nevado et al. developed a method for the separation of seven dyes using a 15 mmol L^{-1} borate buffer at pH 10.5 with detection limits ranging from 0.35 to 2.12 mg L^{-1}, data comparable with those previously obtained by HPLC–UV.[6]

Despite some authors having mentioned that the reproducibility of sample injection in FSCE is worse than in HPLC-UV,[18,19] the best limits of quantification were achieved using large volume sample stacking, allowing the quantification of dyes in soft drinks, jellies, and milk beverages, that enabled the determination of colorants in food samples in concentrations as low as $0.1 - 0.5\,\mathrm{mg\,L^{-1}}$.[20]

To develop the method in our home made system we chose four food dyes, namely Brilliant Blue FCF (disodium salt of ethyl[4-[ethyl (m-sulfobenzyl)amine]-2,5-ciclohexadiene-1-ilydene](m-sulfobenzyl ammonium hydroxide – E133), Sunset Yellow FCF (disodium salt of 6-hydroxy-5-[(4-sulfophenyl)azo]-2-naphthalenesulfonic acid – E110), Amaranth (trisodium(4E)-3-oxo-4-[(4-sulfonato-1-naphthyl)hidrazono] naphthalene-2,7-disulfonate – E123), and Fast Green FCF (ethyl-[4-[[4-ethyl-[(3-sulfophenyl)methyl]amino]phenyl]-(4-hydroxy-2-sulfophenyl) methylidene]-1-cyclohexa-2,5-dienylidene]-[(3-sulfophenyl)methyl]azanium – E143). The method was applied to the determination of these dyes in commercial food samples. Structures of the dyes and their codes for food additives (E numbers), according to the International Numbering System (INS), are shown in Figure 1.

This system can perform very well for specific routine analysis, and because of its low cost, can be dedicated for single applications, while

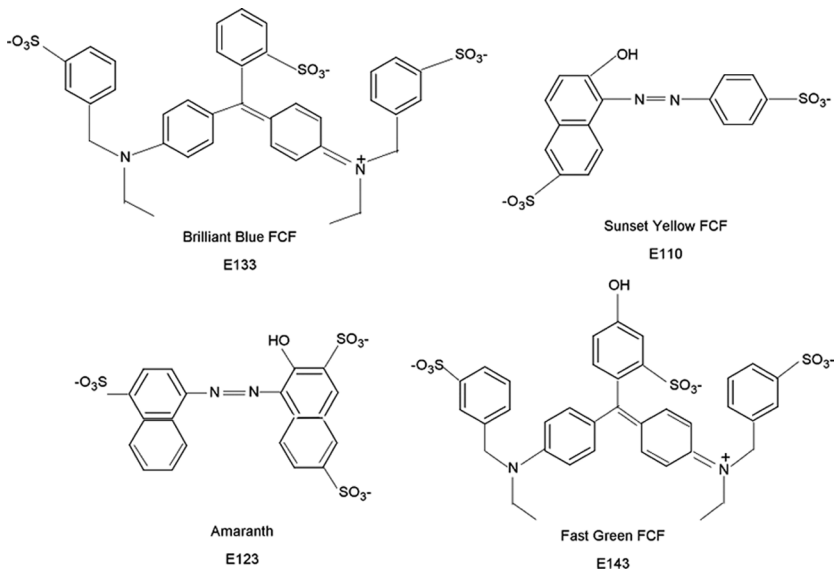

Figure 1. Chemical structures, E numbers, and names of the dyes used in this study.

commercial systems are best suited to versatile and general method developments.

EXPERIMENTAL

Reagents

Purified water from a MilliQ-Synthesis system (Millipore, Billerica, MA, USA) was used to prepare solutions and the dyes were purchased from Aldrich (Toronto, ON, Canada) and Sensient do Brasil (São Paulo, SP, Brazil). Stock standard solutions of Brilliant Blue FCF ($7.9\,g\,L^{-1}$), Sunset Yellow FCF ($4.5\,g\,L^{-1}$), Amaranth ($6.0\,g\,L^{-1}$), and Fast Green FCF ($8.1\,g\,L^{-1}$) were prepared. A mixed solution was used to evaluate the optimum conditions of separation.

The electrophoresis buffer was $10\,mmol\,L^{-1}$ borate (Mallinckrodt, Paris, France); the pH was adjusted to 9 by the addition $0.5\,mol\,L^{-1}$ boric acid. The buffer was filtered using a 0.22 μm filter (Corning Inc, Berlim, Germany) before use. Solutions of 0.1 and $1.0\,mol\,L^{-1}$ NaOH were prepared for capillary conditioning. All the solutions were kept in the refrigerator at 4°C, throughout the work. Room temperature was kept ca. 22°C.

Instrumentation and Operating Conditions

The CE system built and assembled in our laboratory consists of an adaptation of a PC2000 PC Plug-in Spectrometer with 2048 element linear CCD array fiber optic spectrometer mounted on a 1 MHz ISA-bus A/D converter purchased from Ocean Optics (Dunedin, FL, USA). This spectrometer A/D converter combination fits into ISA slotted PCs. The PC2000 multiple channel device allows users to configure systems to detect a wavelength range of 350–1000 nm. The equipment has an on-column home made cell detection that is made from bakelite material. The capillary was fixed with an optical aligner obtained from Agilent Technologies (Santa Clara, CA, USA). This cell is connected by an optic fiber with the visible light provided by a 2 W tungsten mini lamp from Ocean Optics (Dunedin, FL, USA), and the mini spectrophotometer is installed on the board of the computer. The intensity of the lamp is adjusted using a variable density optic filter from Edmund Optics (Barrington, NJ, USA). The system presents a CZE 30PN10 high voltage power supply (0–30 kV) purchased from Spellman (Hauppauge, NY, USA) controlled by a home made device. This external controller presents switchable polarity that can be adjusted to each analysis, as well

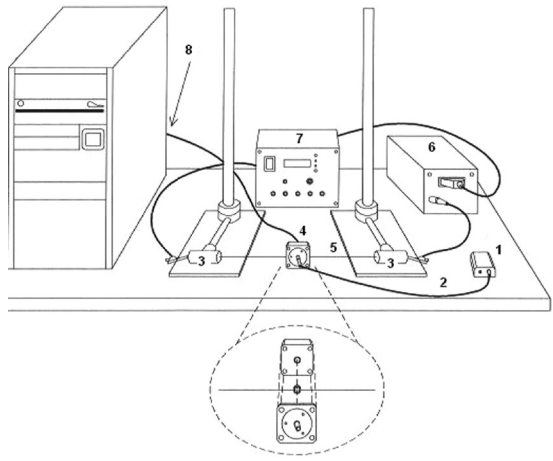

Figure 2. Schematic diagram of the modular home made CE system. (1) Tungsten lamp, (2) optic fibers, (3) buffer reservoirs, (4) on-column detection cell, (5) electrophoresis capillary, (6) high voltage (HV) power supply, (7) external HV controller, (8) on-board CCD spectrophotometric detector.

as buttons to control the voltage and the current. The diagram of the modular CE system is shown in Figure 2.

Originally, this spectrophotometer was only suitable for single spectrum data acquisition and did not present continuous acquisition, such as needed in separations. The data acquired using the multi-channel scanning capability of the PC2000 was automatically de-interlaced (i.e., separated into individual spectra for each channel) by Ocean Optics software drivers. An adaptation of software was necessary to convert the multiple spectral data of a run into only one data matrix. The acquisition system records the spectrum data points and a post-run program convert this data into a matrix of time versus wavelength, which was written in house. This program (SoCE – Software for Capillary Electrophoresis) is a multi-platform software that was developed using JAVA, which is supported by many operating systems and is an open source system. The software has the following functions: 1) Data acquisition; that is responsible to read the files that was acquired by the spectrophotometer and inserts the read data in a meta object that can be easily manipulated by the user. 2) Creating data matrix; this feature creates a table (matrix), which contains all the data supplied by data acquisition, in which each line of the table represents the acquisition time and each column represents wavelengths with the values being the absorbance. These data can be exported to a text file, and imported by other software such as Origin or LabPlot. 3) Electropherogram; allows the visualization of the

electropherograms in a single or in a range of wavelengths. This module allows the integration of peak areas, made by peak selection. 4) Spectrum; allows the visualization of the spectrum in each time point, which has the same interface and functionalities as the electropherogram function.

All separations were performed in a fused silica capillary with 29 cm (16 cm effective length) and 75 μm I.D. The applied voltage was 7.5 kV, resulting in an electric field of 250 $V\,cm^{-1}$ and a current of 26 μA. Temperature was not controlled in the equipment, but the room temperature was kept ca. 22°C.

The capillary was conditioned daily prior to the first run, with 1.0 $mol\,L^{-1}$ NaOH for 10 min, 0.1 $mol\,L^{-1}$ NaOH for 10 min, Milli-Q water for 10 min, and then the electrolyte solution for 5 min. Between sample injections, the capillary was rinsed with 0.1 $mol\,L^{-1}$ NaOH for 2 min, Milli-Q water for 2 min, and then the electrolyte solution for 1 min. Samples were injected manually by siphoning at a height difference of 2.5 cm for 10 sec.

The absorbance from 400 to 800 nm was monitored and the quantification of the analytes was carried out, measuring the peak areas of the single channel electropherograms at the maximum wavelength of each dye that was in the range of 480–530 nm for Sunset Yellow FCF and Amaranth and 580–630 nm for Brilliant Blue FCF and Fast Green.

The spectrum of each dye and the experimental determination of each maximum wavelength were obtained aspirating each dye solution with a 50 μL syringe (Hamilton, Reno, NV, USA) connected to the capillary, by a teflon tube purchased from Sigma (Toronto, ON, Canada) with 370 μm I.D. until they fill the capillary, reaching the detector. These spectra were compared with those obtained in 1 mL quartz cuvette on a Hitachi U-2800 UV spectrometer (Tokyo, Japan). Triplicate injections of the analytical standards were performed and the average peak areas were used in the quantification.

The method and instrument was validated in respect to linearity, limits of detection and quantification, precision, expressed by the RSD of inter- and intra-day repeatabilities, and accuracy measured by recovery studies.

Sample Preparation

The powdered samples of grape and tangerine juices were prepared according to the dilution indicated by the manufacturers to prepare 1 L of juice (40 g of powder per 1 L of water), and then centrifuged in order to remove any remaining solids. The samples of mango and mint candies were ground and 4 mL water added, and then heated until complete dissolution. All determinations were carried out in triplicates.

RESULTS AND DISCUSSION

The idea of the development of small equipment with detection in visible region was possible for dedicated routine analysis of dyes, such as in foods. Another dedicated application would be in the field of multiplexed genetic analyses of DNA intercalated dye complexes, with reduced cost and time when compared with the available commercial CE instruments or even the expensive genetic analyzers.[21] Preliminary analysis to evaluate the fidelity of the spectra obtained by the home made system, and the electrophoretic conditions to separate the dyes were made.

Spectral Quality

To evaluate the spectral quality of this miniaturized flow cell spectrophotometer, each spectrum was compared to a reference spectrum obtained in a bench top spectrophotometer. The spectrum comparison is shown in Figure 3.

Differences in the absorbance for both systems are due to the difference of the path length, which is 1 cm in the spectrophotometer and 75 μm in the home made equipment, and the concentrations were different. It is possible to note that the maximum wavelength for each dye is about the same in both systems. Moreover, we can see all the spectral features for all dyes, even at the lower wavelength region in which the light source provides a poor output. We could detect higher noise in such lower wavelengths but the signal to noise ratio was still acceptable, and all spectral features were preserved.

Electrophoretic Parameters

The concentration of borate buffer was slightly varied between 10 and 20 mmol L^{-1} in order to verify the effect of the ionic strength of the electrolyte. Increasing the ionic strength of the separation buffer has the effect of decreasing the electroosmotic flow, hence, increasing the analysis time for anionic analytes. Another effect of increasing the ionic strength, for a given voltage, is increased heating, owing to the higher currents and the consequent inability to dissipate such heat. The Joule heating effect could be observed as baseline noise because the system did not have a forced heat dissipation unit.[22] The concentration of the run buffer can also affect peak symmetry, because the presence of high concentration of solute ion relative to the concentration of run buffer ions may distort the electric field, causing distortion of peak shapes.[23] The buffer of choice was 10 mmol L^{-1} borate at pH 9 because of the

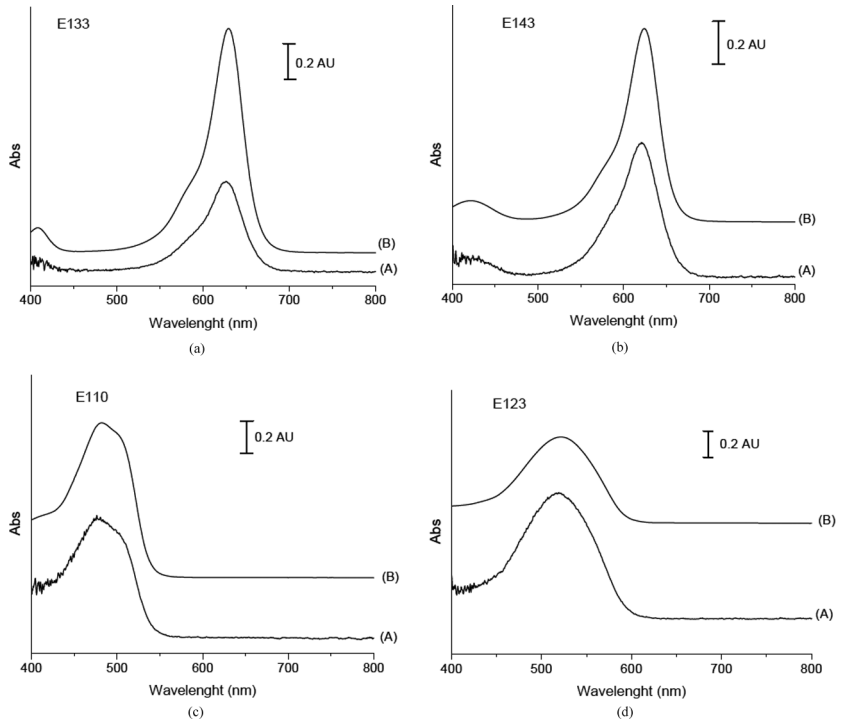

Figure 3. Spectra of the dyes obtained in (A) the home made CE system: 1 mM E133, 1 mM E143, 5 mM E110, and 5 mM E123; (B) the Hitachi U-2800 spectrophotometer: 0.05 mM each dye.

lower analysis time provided, while maintaining a good peak shape and a good signal-noise ratio.

An increase in voltage increases electroosmotic flow and reduces migration times, leading to shorter analysis times and higher efficiencies. However, excessive heat is produced causing an increase in current, which can be deleterious to the analysis resulting in broader peaks or even the formation of bubbles in the capillary. The maximum voltage that should be used for a given set of conditions can be determined from a graph of observed current versus applied voltage (Ohm's law plot), and it is indicated by the point at which non-linearity occurs.[23] The applied voltage of 7.5 kV used during the analysis was chosen according to the Ohm's plot experiments (data not shown).

Electropherograms of a standard mixture is shown in Figure 4, demonstrating the versatility of the detection system from universal to selective, by selecting the proper wavelength conditions, as expected from an array detector.

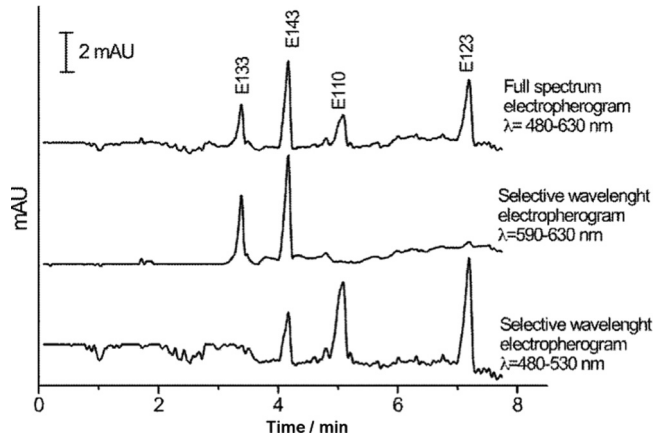

Figure 4. Electropherograms of the separation of four food dyes. Composition of the mixture: 63.4 mg L^{-1} E133, 64.7 mg L^{-1} E143, 36.2 mg L^{-1} E110, and 48.4 mg L^{-1} E123. Analysis conditions: 10 mmol L^{-1} borate, pH 9; V = 7.5 kV; injection of 10 sec at 2.5 cm height. Capillary dimensions: 29 cm length and 75 μm I.D.

All four dyes were separated in less than 8 min and the peaks presented regular shapes showing good resolution, which varied for 3.71 in the first pair of peaks to 9.22 in the last pair of peaks, and efficiency, which ranged from 17,559 plates m^{-1} for E133 to 49,665 plates m^{-1} for E123.

Validation of the Method

The linearity of the analytical curve was studied over a range of 6–400 mg L^{-1} for E133 and E143, 4–250 mg L^{-1} for E110, and 5–300 mg L^{-1} for E123. The linearity was determined within four repeated injections at six concentration levels of each dye. Table 1 presents the linear regression equations for their analytical calibration curves. The coefficients of correlation between the concentration of each dye and its peak area are greater than 0.99 in the concentration range studied for each dye.

An evaluation of the instrument detectability was made by determining the values of the LOD calculated by the ratio of 3 times the standard deviation of the baseline from the blank (n = 10) and the intercept of every calibration equation. The LOQ were calculated in the same way, but using 10 times the standard deviation of the peak area of the blank. The values are shown in Table 1. The obtained results can be compared with those related to other authors, as discussed in the

Table 1. Figures of merit and linearity from the analytical calibration curves for the studied dyes by the equation of the peak area (y) *versus* concentration (x), and the instrument limits of detection (LOD) and quantification (LOQ)

Dye	Equation	Coefficient of correlation (r)	LOD ($mg\,L^{-1}$)	LOQ ($mg\,L^{-1}$)
E133	$y = -0.0418 + 0.0019x$	0.9988	0.5	1.7
E143	$y = -0.0371 + 0.0026x$	0.9984	0.3	1.0
E110	$y = -0.0058 + 0.0017x$	0.9931	1.5	5.0
E123	$y = -0.0093 + 0.0021x$	0.9973	1.2	4.0

introduction.[6,14] It is noteworthy to recall that the optical setup is the simplest possible, the light is brought in and out of the capillary (sample path length) by optical fibers without any focusing lenses. Yet the system matched the sensitivity of commercial CE equipments as reported in the literature. If lower limits of detections are needed, there is still room for improvement by using fiber optics with built in lenses for better light collection.

The precision of the method for the determination of the dyes was expressed by the relative standard deviation (RSD). Six injections of the dyes were carried out sequentially in two days, and it was possible to calculate the intra-day and inter-day repeatability of migration times as a measure of buffer stability and peak areas as a measure of the manual precision injection. Table 2 reports such data.

One of the problems related with capillary electrophoresis in quantitative analysis was the poor reproducibilities of sample introduction that has already been reduced to a great extent by instrumental automation since the late 1980s. Lavorante et al. proposed an interface to substitute gravity injection by pressurized hydrodynamic injection to minimize usual measurement errors from home made systems, achieving RSD $< 5\%$ using the proposed system against 7% using gravity injection mode,

Table 2. Results of validation studies for precision of the method in intra-day and inter-day analysis

	Intra-day repeatability (n = 6)		Inter-day repeatability (n = 12)	
Dye	RSD (%) migration time	RSD (%) peak area	RSD (%) migration time	RSD (%) peak area
E133	5.5	11.4	6.0	14.4
E143	5.7	10.1	6.9	13.5
E110	6.3	13.8	8.6	18.0
E123	5.5	10.8	8.0	11.6

Table 3. Recoveries (%) for standard mixtures of the studied dyes at three different concentration levels

Composition of standard mixtures ($mg\,L^{-1}$)				Recovery (%) (RSD (%)*)			
E133	E143	E110	E123	E133	E143	E110	E123
23.8	24.3	13.6	18.1	109.6 (2.6)	104.3 (3.5)	77.4 (8.0)	88.2 (10.8)
31.7	32.3	17.0	24.2	97.8 (0.8)	81.0 (13.9)	94.6 (5.9)	85.1 (1.8)
39.6	40.4	22.6	30.2	102.2 (1.8)	95.8 (3.1)	88.0 (15.6)	92.8 (15.6)

*Mean of three determinations.

considering measurements in terms of signal magnitude and migration times.[24] Our system provided values of RSD lower than 8.6% for the migration time and the values to peak area were between 10 and 18%, due to the manual injection. The difficulty of placing the sample vial exactly at the same height every injection and the time to move the vial up and down is greatly worsened when working in a system such ours, because of the short capillary length. Therefore, our system would require further improvements in the injection mechanism, if the capillary length is to be kept short. Nevertheless, the numbers was still suitable for method validation.

The recovery studies of the method were evaluated by the addition of known amounts of standard solutions of each dye, in three different levels, to a sample of mint candy to assess the accuracy of the method. Table 3 presents the composition of the mixtures and the values of recovery.

The recoveries varied from 77.2 to 109.6% for all four dyes, however there was no bias related to the concentration, therefore the method and the instrument can comply with most regulatory demands.

Samples Analysis

The viability of this home made CE instrument was finally evaluated for the analysis of dyes in food samples. Our system and method were subjected to the determination of food colorants in two samples of fruit juice powder and two samples of candies. All determinations were carried out in triplicates. The concentrations obtained in the samples analyzed were presented in Table 4, and they are in accordance with the limits stated by the Resolution Numbers 387 and 389 from ANVISA,[7] in Brazil.

Figure 5 presents an average example of the electropherograms obtained from these samples.

Table 4. Concentration of dyes obtained in food samples* and limits stated by ANVISA

Dye	ANVISA R #389 Juice powders ($mg\,L^{-1}$)	Grape juice ($mg\,L^{-1}$)	Tangerine juice ($mg\,L^{-1}$)	ANVISA R #387 Candies ($mg\,kg^{-1}$)	Mango candy ($mg\,kg^{-1}$)	Mint candy ($mg\,kg^{-1}$)
E133	100	13.9 ± 0.7	–**	300	–	16.1 ± 0.8
E143	50	–	–	300	–	–
E110	100	–	21.6 ± 1.3	100	7.9 ± 0.5	–
E123	50	–	–	100	–	–

*Mean of four determinations.
**not present in this matrix.

All the dyes obtained were described in the package label from each sample. Some differences in migration times can be explained by the fact that the system did not have any control of temperature, and by differences in chemical composition of the samples (sample viscosity due to variable sugar content). We can observe that the migration times of EOF (negative peak corresponding to colorless neutral compounds present in the samples at the studied pH) and, consequently, the migration time of the analytes from candy samples are longer than from

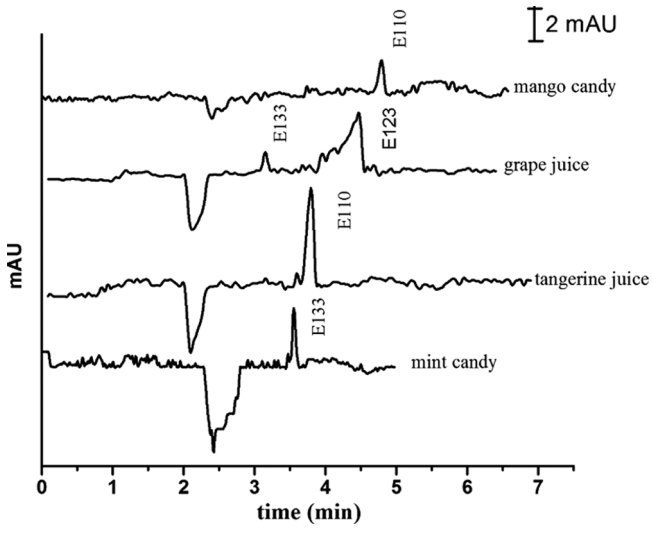

Figure 5. Full spectrum electropherograms of food samples. Analysis conditions were the same as in Fig. 4. $\lambda_{detection} = 480-630$ nm.

the juices, which can be because of the greater viscosity of the candies samples. This effect can be observed for E110 and E133 in both samples. These values can be corrected by the calculation of the apparent electrophoretic mobility, which is $-21.8 \pm 0.1 \times 10^{-5}\,cm^2\,s^{-1}\,V^{-1}$ for E110 in tangerine juice and $-20.5 \pm 0.1 \times 10^{-5}\,cm^2\,s^{-1}\,V^{-1}$ for E110 in mango candy. Considering E133 the values of apparent electrophoretic mobilities are $-15.9 \pm 0.1 \times 10^{-5}\,cm^2\,s^{-1}\,V^{-1}$ for grape juice and $-13.6 \pm 0.1 \times 10^{-5}\,cm^2\,s^{-1}\,V^{-1}$ for mint candy. Despite of the differences from sample to sample and from sample to standards, the identity of each sample was confirmed by analyte spiking and spectral confirmation. Additionally, the peak in 4.5 min present in grape juice corresponds to Allura Red AC (E129), according to the manufacturer information. It could be identified because a confirmatory test with this dye was made (not presented here), and besides the visible spectrum, the migration time and the front tailing peak are features observed for this dye.

CONCLUSIONS

A capillary electrophoresis method was described for the determination of four synthetic dyes, two azo and two triarylmethane dyes, allowed in Brazil, using modular home made equipment. The instrument and dedicated software were tested thoroughly and was reliable enough to be applicable in routine analytical analysis. The instrument preserved the spectral features of all analytes tested and the sensitivity was adequate given the optical path and optical settings available. Complete separation of the four dyes was achieved in less than 8 min. The detection limits obtained for the four dyes were between 0.3 and 1.5 $mg\,L^{-1}$ for different dyes, and the method presented good linearity, with correlation coefficients better than 0.99. The method presented acceptable precision of migration time measurements, less than 9%, and studies of repeatability of the peak areas obtained by the manual injection varied from 10 to 18%. Samples of fruit juices and candies were analyzed, and the concentrations measured are in accordance with the limits stated by ANVISA Brazilian regulation. The capillary electrophoresis method made possible the injection of the samples without any sample clean up procedure. The samples were only diluted in water and filtered before analysis.

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