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Short communication

Determination of synthetic colourant food additives by capillary zone electrophoresis

Huwei Liu, Tao Zhu, Yingnan Zhang, Shize Qi, Aijin Huang, Yiliang Sun*

Department of Chemistry, Peking University, Beijing 100871, China

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Abstract

A capillary zone electrophoresis method for the separation of six synthetic food colourants is proposed. A background electrolyte solution consisting of 20 m*M* borate buffer adjusted to pH 7–9 and electromigration injection at 4 kV for 14 s were utilized. A baseline separation of six synthetic colourants commonly used as food additives can be achieved within 10 min, and a linear relationship between the concentration and peak area for each of these pigments was obtained in the concentration range 2–50 ppm, with a correlation coefficient greater than 0.995. The selection of the analytical conditions and the experimental reproducibility are discussed.

1. Introduction

For economic reasons, brightly coloured and stable synthetic colourants have been widely used as food additives (single component or mixtures). However, some synthetic colourants may be pathogenic, especially if they are consumed in excess. Therefore, safety data for every synthetic colourant food additive have been repeatedly determined and evaluated by the Food and Agricultural Organization (FAO) and World Health Organization (WHO). The use of synthetic colourants as food additives is strictly controlled by laws and regulations. About ten kinds of synthetic colourants are permitted to be used as food additives in most countries, including China and Japan, and their maximum permissible amounts in food are rigidly specified in order to safeguard consumers' interests. In China, the permissible contents of the colourants are not higher than 0.05 g/kg for amaranth (Food Red No. 2), Ponceau 4R (Food Red No. 102) and erythrosine (Food Red No. 3), 0.1 g/kg for tartrazine (Food Yellow No. 4), sunset yellow (Food Red No. 5) and indigo carmine (Food Blue No. 2) and 0.025 g/kg for brilliant blue (Food Blue No. 1) [1]. As a result, the analysis of synthetic colourants in food is very important.

In the past, many methods to determine pigments in food have been reported [2,3], including chromatographic methods, such as column chromatography and TLC, spectrophotometric methods, such as dual-wavelength and derivative spectrophotometry, electrochemical methods, such as polarographic analysis, voltammetry and ion-selective electrode analysis, and chemometrics, such as factor analysis and multiple linear regression. In general, chromatographic meth-

* Corresponding author.

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ods, especially HPLC [4–8], and chemometric methods, e.g., Kalman filtering spectrophotometry [9,10], fuzzy linear programming [11] and progressive regression analysis [12], are more practical than others as routine methods.

In recent years, high-performance capillary electrophoresis (HPCE) has received wide acceptance not only for biomacromolecules but also for organic and inorganic ions [13]. Several papers [14–18] have described the separations of aromatic sulfonic acid and related dyes by the use of capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC). For example, Suzuki et al. [19] reported the simultaneous separation of seven red dyes by capillary electrophoresis with sodium dodecyl sulfate (SDS) or β-cyclodextrin (β-CD) as buffer additive. Nevertheless, the application of HPCE to the analysis of synthetic food dyes with different colours, has not yet been reported. In this paper, a CZE method for separating six synthetic pigments commonly used as food additives was developed. It was demonstrated that this method is simple, reliable, and sensitive enough to be used in the determination of synthetic colorant additives in some food samples.

2. Experimental

2.1. Instrumentation

A modular CE system [20] was used, consisting of a laboratory-made high-voltage supply, a CV^4 CE absorbance detector (ISCO, Lincoln, NE, USA) and an HP 3394 integrator (Hewlett-Packard, Avondale, PA, USA). A fused-silica capillary of 375 μm O.D., 75 μm I.D., 60 cm total length and 40 cm effective length was provided by Yongnian Optical Fiber Factory (Hebei Province, China). Before use, the capillary was conditioned by flushing with 0.1 *M* NaOH for 30 min, then with redistilled water for 15 min and finally with the background electrolyte (BGE) solution for 15 min.

2.2. Sample preparation

Commercial pigment standards (Shanghai Institute of Dyestuff Chemistry, Shanghai, China) were first dissolved in redistilled water to give standard solutions with concentrations of about 1 mg/ml. Mixed sample solutions with concentrations ranging from 2 to 50 μmol/ml were prepared by dissolving a certain amount of the standard solution in the BGE solution. Each of the standard samples contained 20 ppm of phenol, which was used as marker for electroosmotic flow (EOF) determination.

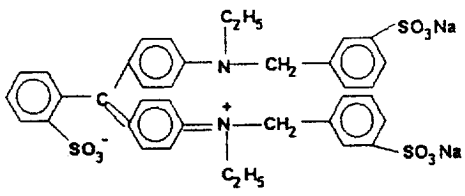
2.3. Experimental conditions

The BGE solution consisted of 20 m*M* borate buffer adjusted to pH 7–9 to facilitate sampling of the analytes that contain carboxylic or sulfonic groups in their molecules, forming multiply charged anions. The experimental parameters were as follows: electromigration injection, 4 kV for 14 s; analytical voltage, 25 kV; detection wavelength, 220 nm; and sensitivity, 0.005 AUFS. The CE systems was operated manually and the capillary was kept at ambient temperature (18°C) with forced air ventilation, in all experiments, the capillary was flushed with BGE solution for 1.0 min before each injection and the solution in the solvent reservoirs was renewed after every five injections to improve the experimental reproducibility.

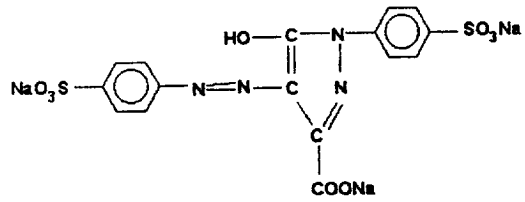
3. Results and discussion

3.1. Selection of a suitable capillary and injection conditions

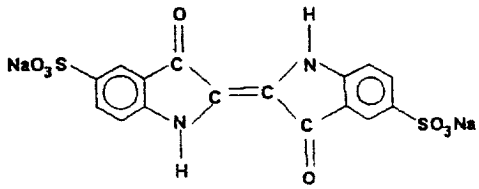
All six synthetic pigments studied contain carboxylic or sulfonic acid groups in their molecules, as illustrated in Fig. 1. In borate buffer solution they can dissociate into multiply charged anions with high negative electrophoretic mobilities. For electromigration injection at the anode, the EOF should be large enough to facilitate the introduction of these anionic ana-



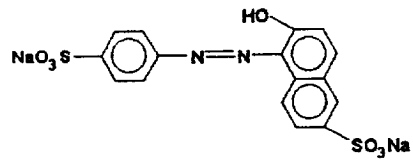
Brilliant blue



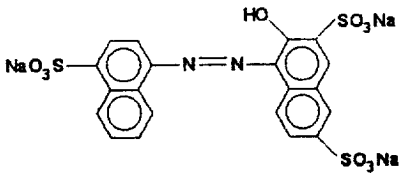
Tartrazine



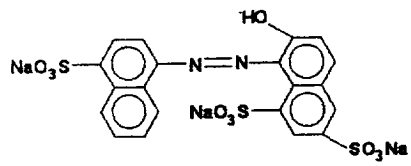
Indigo carmine



Sunset yellow



Amaranth



Ponceau 4R

Fig. 1. Structures of the synthetic food colourants studied.

lytes. If the EOF of the capillary is not sufficiently high, the resultant mobility of some or even all analyte species may become negative, making the separation impossible. Kohr and Engelhardt [21] reported that the EOFs of capillaries varied from manufacturer to manufacturer, and even from batch to batch. Therefore, it is necessary to use a separation capillary of sufficiently high EOF. On the other hand, when hydrodynamic injection was tried, although all the components can be introduced into the column inlet at the moment of injection without discrimination, some or even all of the components already introduced into the column inlet would move backwards into the anode reservoir immediately after the application of the run voltage. Therefore, for a capillary with an insufficiently high EOF, neither electrokinetic nor hydrodynamic injection can be used with success. In addition, sample introduction at the cathode reservoir was also tried on polarity reversal, but the results demonstrated a poor separation of some test colourants such as amaranth and Ponceau 4R. As is well known, columns with a higher silanol group density on the inner surface give rise to higher EOF at high pH. Our experience showed that the activity and thus the EOF of the column from Yongnian Optic Fiber Factory are usually higher than those provided by the Hewlett-Packard column under

the same experimental conditions. In our case with borate buffer as BGE solution (pH 9.0), an EOF mobility higher than $1.5 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1} (measured with phenol as a marker) can allow the successful electrokinetic injection and subsequent CZE separation of the food colourants studied.

The electroosmosis of a fused-silica capillary is determined not only by the intrinsic properties of bare fused-silica capillary tubing, but also by the composition and pH of the background electrolyte solution. Thus, for the successful separation of an anionic sample, the capillary column, the run buffer and its pH must be carefully optimized to obtain a sufficiently large EOF. This seems to be a prerequisite for the separation of anions by CZE and is especially important for multiply charged anions of low molecular mass with high negative electrophoretic mobility. Regarding the injection conditions, our experiments indicated that the amount of sample injected is directly proportional to the injection voltage and time, and that a lower voltage and longer injection time are preferable to improve the reproducibility when manual injection is used. In our case, injection conditions of 4 kV for 14 s were found to be satisfactory.

3.2. Separation

All six food colourants studied contain two to three sulfonic acid groups in their molecules, and at pH >3–4 they dissociate completely into sulfonate anions. For tartrazine there is an additional carboxylic group in the molecule that will completely dissociate at pH >7. Thus, in the pH range 7–9 where the EOF is large enough to facilitate the separation, indigo carmine and sunset yellow form divalent anions and the other four colourants form trivalent anions. With a further increase in the solution pH, the phenolic groups in sunset yellow, amaranth and Ponceau 4R begin to dissociate. For the two positional isomers, amaranth and Ponceau 4R, variation of pH might be a useful parameter to effect their separation. As was pointed out above, working with a column of high EOF is a prerequisite for the successful separation of multiply charged anions by CZE with EOF in the normal direction, whereas the optimizations of pH of the BGE solution and the analytical voltage are only less difficult once a proper capillary column has been chosen. Two buffer systems, phosphate and borate, with pH 7.0–9.8 and concentrations of 20 and 50 m*M*, were compared, showing that the six synthetic colourants could be completely separated at pH 7–9. As expected, the migration order remains unchanged for the six colourants in this pH interval. With pH increase above 9.3, the migration order of the last two peaks (tartrazine and an unidentified peak) became reversed, and above pH 9.4 amaranth and Ponceau 4R could not be separated from each other. This implies that dissociation of the phenolic groups in the two positional isomers begins to affect their effective mobilities at pH >9. In order to make the EOF sufficiently high, a 20 m*M* borate buffer solution adjusted to pH 9.0 was used as the BGE and the analytical voltage was set to 25 kV. Fig. 2 is a typical electropherogram of the six synthetic colourants, indicating that a simultaneous analysis of the six pigments could be achieved within 10 min, and a baseline separation could be obtained even for the two posi-

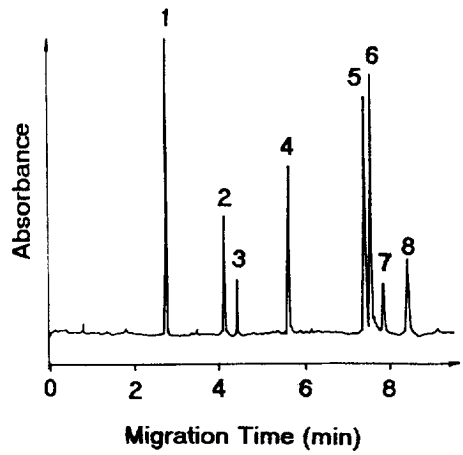


Fig. 2. Typical electrophoregram of a mixture of six synthetic pigments. pH = 9.0; for other conditions, see text. Peaks and concentrations: 1 = phenol (20.0 ppm), 2 = brilliant blue (21.4 ppm), 3 = indigo carmine (20.0 ppm), 4 = sunset yellow (24.7 ppm), 5 = amaranth (15.2 ppm), 6 = Ponceau 4R (18.3 ppm), 7 = tratrazine (21.7 ppm); 8 = unknown.

tional isomers amaranth and Ponceau 4R. The CZE method, with good peak shape and without using additives, is relatively simple compared with the CE method described by Zuzuki et al. [19]. In Fig. 2, peak 8 may be a component of indigo carmine, which remains to be identified.

3.3. Quantification

HPCE is one of the most powerful separation methods and has many potential uses in various fields, although some problems remain to be solved for accurate and precise quantitative analysis. One of them is the poor reproducibility, which has already been overcome to a great extent by instrumental automation. Although the CE system developed in our laboratory is manually operated and the temperature is controlled with forced air ventilation, the reproducibility (R.S.D.) for the migration time was below 1.0% and for peak area of the six analytes was 0.5–5.0%, which seem to be tolerable for routine food analysis for pigment additives.

Another problem associated with quantification by CE is its narrow dynamic range. It was found that when using the external standard method in order to extend the dynamic range of the determination, the ionic strength of the sample solution should be nearly equal to that of the BGE solution. When sample solutions were prepared by dissolving analytes in pure water, poor reproducibility and a very narrow dynamic range (e.g., 2–20 ppm for brilliant blue) were obtained. However, when these sample solutions were prepared with the BGE solution, both the reproducibility and the dynamic range (e.g., 2–50 ppm for brilliant blue) were significantly improved. The linear regression equations for these pigments under our conditions are summarized in Table 1, indicating that the correlation coefficients between the concentration of each pigment and its peak area are larger than 0.995 in the concentration range 2–50 ppm. The R.S.D. of the method was about 3% and the minimum detectable amount at a signal-to-noise ratio of 3 for all six synthetic colourants was 3 ppm. According to Heiger [22], quantitative analysis in CE with electrokinetic injection should be corrected by dividing the integrated peak areas by the corresponding migration times. However, for the external standard method of quantification this correction can be neglected. In fact, the reproducibility and linearity range of determination did not improve after making such a correction.

Table 1
Regression equations for six synthetic food colourants in the concentration range 2–50 ppm

Colourant	a	b	r
Brilliant blue	3530.04	−10679.1	0.99778
Indigo carmine	1669.30	−4873.26	0.99898
Sunset yellow	7099.71	−33844.6	0.99752
Amaranth	6901.51	2572.56	0.99622
Ponceau 4R	8472.33	6020.40	0.99599
Tratrazine	6769.94	−24133.8	0.99786

$y = ax + b$, where y is the amount of colorant in ppm, x is the corresponding peak area in integrated unit (μV s) and r is the correlation coefficient.

4. Conclusion

The CZE method developed in this work was successfully used to separate and determine six synthetic pigments widely utilized as food additives with acceptable reproducibility, peak shape and accuracy. The injection and analysis of the pigment samples require a high EOF. The linear dynamic range extends from 2 to 50 ppm when the sample is dissolved in the BGE solution. The method is simple, effective, inexpensive and competitive with other existing methods, and shows acceptable reproducibility and accuracy. Its limit of detection and dynamic range are better than those of the HPLC method [6].

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