High-Performance Capillary Electrophoretic Analysis of Synthetic Food Colorants

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Key Words

Capillary electrophoresis
Cyclodextrin mobile phase additives
Synthetic food colorants
Selectivity

Summary

A high-performance capillary electrophoresis method with diode-array detection has been developed for analysis of synthetic food colorants. The influence of buffer composition on the separation of the food colorants was examined, as were the effects of α-, β- and γ-cyclodextrins on analyte migration behavior. Eight food colorants were completely separated within 10 min using pH 9.5 borax – NaOH buffer containing 5 mM β-cyclodextrin. Experimental results indicate that the relative standard deviations of analyte migration times were < 0.88% under the optimized separation condition. Correlation coefficients of the linear calibration plots of the analytes exceeded 0.998. The method was suitable for determination of the quantities of synthetic food colorants in ice cream bars and fruit soda drinks.

Introduction

Synthetic food colorants are extensively used as food additives to enhance sensory quality and the desire to purchase because they can withstand a range of temperatures, light, and redox reagent conditions. Synthetic food colorants can be classified on the basis of their chemical structures; they include azo dyes, triarylmethane dyes, phenylmethane dyes, xanthene dyes, quinoline dyes, anthraquinones, and phenols; among these, yellow and red dyes are predominantly used. Some synthetic food dyes and their impurities might be carcinogenic [1] and the colorants permitted as food additives vary in different countries. Government regulations in the European Union (EU), the United States (US), and some other countries authorize the use of eight food colorants as food additives: new coccine, erythrosine, allura red AC, tartrazine, sunset yellow FCF, brilliant blue FCF, indigo carmine, and fast green FCF. Table I lists their numbers as used in Color Index (CI), EU, and US [1, 2].

The toxicity of food colorants to man accounts for the need to develop efficient analytical methods for determination of the level of synthetic colorants in food products. The analytical method can also provide useful information about food quality during the manufacturing process.

Chromatographic methods, including thin-layer chromatography and high-performance liquid chromatography (HPLC), have been widely employed in recent years to determine synthetic colorants in soft drinks and other food products [1, 3–6]. HPLC methods, particularly ion-pair chromatography, are suitably selective for determination of synthetic colorants. Capillary electrophoresis (CE) is a highly effective technique for analysis of a variety of compounds in different fields [7–13]. The amounts of organic solvent used and hazardous waste produced by CE analysis are minimal. In addition, CE is suitable for analysis of ionic species and hydrophilic compounds. That synthetic food colorants are hydrophilic compounds explains why CE is a promising alternative for their separation. Previous investigators have used CE for analysis of a variety of food additives, including preservatives, sweeteners, and synthetic food colorants [2, 14–18]. Herein, food colorants are of particular interest. Suzuki et al. employed CE for simultaneous determination of seven red dyes [2] and Liu et al. have separated food dyes of three different colors by CE with a borate buffer [18]. These studies, however, investigated dye standards only and to the best of our knowledge the feasibility of applying CE to the analysis of synthetic food colorants of different color in real food products has not been explored.

In this paper, not only do we present a CE method for separation of eight synthetic food colorants of five different colors but also describe the determination of the analytes in different food products. The influences of buffer pH and different cyclodextrins on the separation are also investigated.

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Table I. The different numbers used to denote the eight synthetic food colorants.

Colorant	Color	CI No.	EU No.	US No.
New coccine	Red	16255	E124	
Erythrosine	Red	45430	E127	FD&C Red No. 3
Allura red AC	Red	16035	E129	FD&C Red No. 4
Tartrazine	Yellow	19140	E102	FD&C Yellow No. 5
Sunset yellow FCF	Orange	15985	E110	FD&C Yellow No. 6
Brilliant blue FCF	Blue	42090	E133	FD&C Blue No. 1
Indigo carmine	Blue	73015	E132	FD&C Blue No. 2
Fast green FCF	Green	42053		FD&C Green No. 3

Experimental

Apparatus

All experiments were performed with a Beckman P/ACE 5500 capillary electrophoresis system (Fullerton, CA, USA). A diode-array detector was employed for detection. Instrument control and data analysis were performed by use of System Gold software (Beckman) on a 486-personal computer. Separations were performed in a 47-cm length (40 cm to detector) of 50-μm i.d. fused-silica capillary tubing (Polymicro Technologies, Phoenix, AZ, USA). The capillary column was locally assembled in the cartridge format. The temperature of the capillary tubing was maintained at 25 °C during electrophoresis. The electrophoretic separation was performed with an electric potential of 20 kV. Samples were pressure-injected at 0.034 bar (0.5 p.s.i.) for 3 s.

Chemicals

New coccine, erythrosine, tartrazine, indigo carmine, and fast green FCF were purchased from Sigma (St Louis, MO, USA) and allura red AC, sunset yellow FCF, and brilliant blue FCF from Tokyo Chemical Industry (Tokyo, Japan). γ-Cyclodextrin was purchased from Nacalai Tesque (Kyoto, Japan); α- and β-cyclodextrin, borax, and benzyl alcohol were obtained from Sigma. Other chemicals were analytical grade. Food samples were obtained commercially. Soda was canned by Swire Coca-Cola Taiwan Ltd. Water was purified with a Milli-Q system (Millipore, Bedford, MA, USA).

Preparation of Standards and Samples

Standard solutions of eight food colorants (3 mg mL^{-1}) were prepared in deionized water and stored at 4 °C. Sample solutions of different concentration were prepared by diluting standard solutions with deionized water. Benzyl alcohol was added to standard solutions as an EOF marker. Samples of ice cream bars were melted at room temperature and the melted solutions injected directly and analyzed without any extraction procedure. Before analysis, samples of fruit soda drink were degassed by sonication.

Procedure

Running buffer solutions were prepared by dissolving β-cyclodextrin in 0.025 M borax – NaOH buffer solution (pH 9.5) prepared by mixing appropriate amounts of 0.1 M borax and 0.1 M NaOH. Buffer and sample solutions were prepared with deionized water. The separation capillary was rinsed sequentially with 0.1 M NaOH, deionized water, and running buffer solutions before each separation.

Results and Discussion

Figure 1 shows the chemical structures of the eight food colorants. They are water-soluble dyes with two or three sulfonic groups on the benzene ring in their structures, except for erythrosine, which has a carboxyl group. Those compounds, although of different colors, generally have maximum absorbance around 200 nm. The colorants can carry more than one negative charge and form anions in water because of the dissociation of their sulfonic or carboxyl groups. Because of their molecular structures, in this study aqueous buffer solution was selected for separation of the colorants.

Figure 2 depicts the separation of the colorants using buffers of three different pH. Because the analytes are water-soluble, the standard was prepared in aqueous solution. Because the electroosmotic flow (EOF) signal was not apparent in the electropherogram, benzyl alcohol was added as the EOF marker. According to these results buffer pH significantly influences analyte separation. More specifically, resolution of the analytes and migration times increased with an increasing pH. Because the electrophoretic migration of the analytes is in a direction counter to the electroosmotic flow, increasing buffer pH significantly reduced net migration velocities (toward the cathode) of five analytes – tartrazine, new coccine, sunset yellow FCF, allura red AC, and fast green FCF. This phenomenon is probably a consequence of the hydroxyl group common to each analyte and of the EOF flow. Dissociation of the hydroxyl group increases at a higher pH values and/or with decreasing EOF flow, leading to a lower net migration velocity.

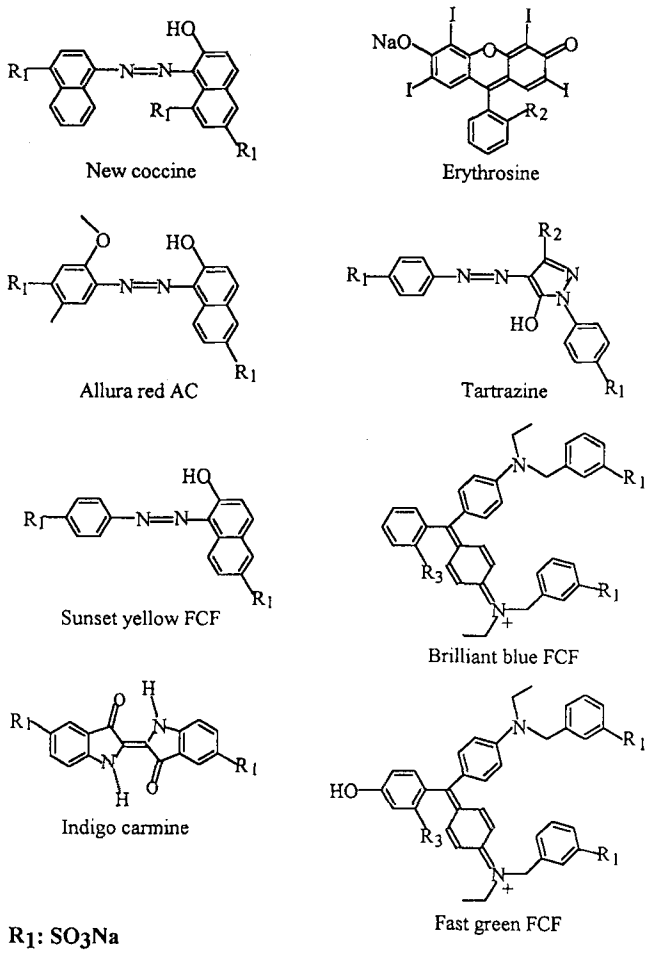


Figure 1
Structures of eight synthetic food colorants.

As Figure 2 indicates, six analytes were resolved by use of pH 10.0 buffer. The tartrazine peak tailed at pH 10.0, however, possibly because of isomerization or dissociation of the phenolic group on the pyrazole ring. For the purpose of high separation efficiency pH 9.5 buffer was chosen for further studies. Modifiers must be added to the pH 9.5 borate buffer to yield satisfactory resolution of indigo carmine, allura red AC, and sunset yellow FCF.

The Effects of Cyclodextrins on the Separation

Taking the chemical structures of the analytes into consideration, cyclodextrins with various cavity sizes were selected as modifiers to enhance the resolution and separation efficiencies. Neutral cyclodextrins, such as α-, β-, and γ-cyclodextrins, were first employed in the next study. These cyclodextrins had the same migration velocity as the electroosmotic flow in the pH range 8.4 to 10.0, because cyclodextrin pK_a values are all above 12.0. In addition, the migration windows of analytes increased with increasing pH.

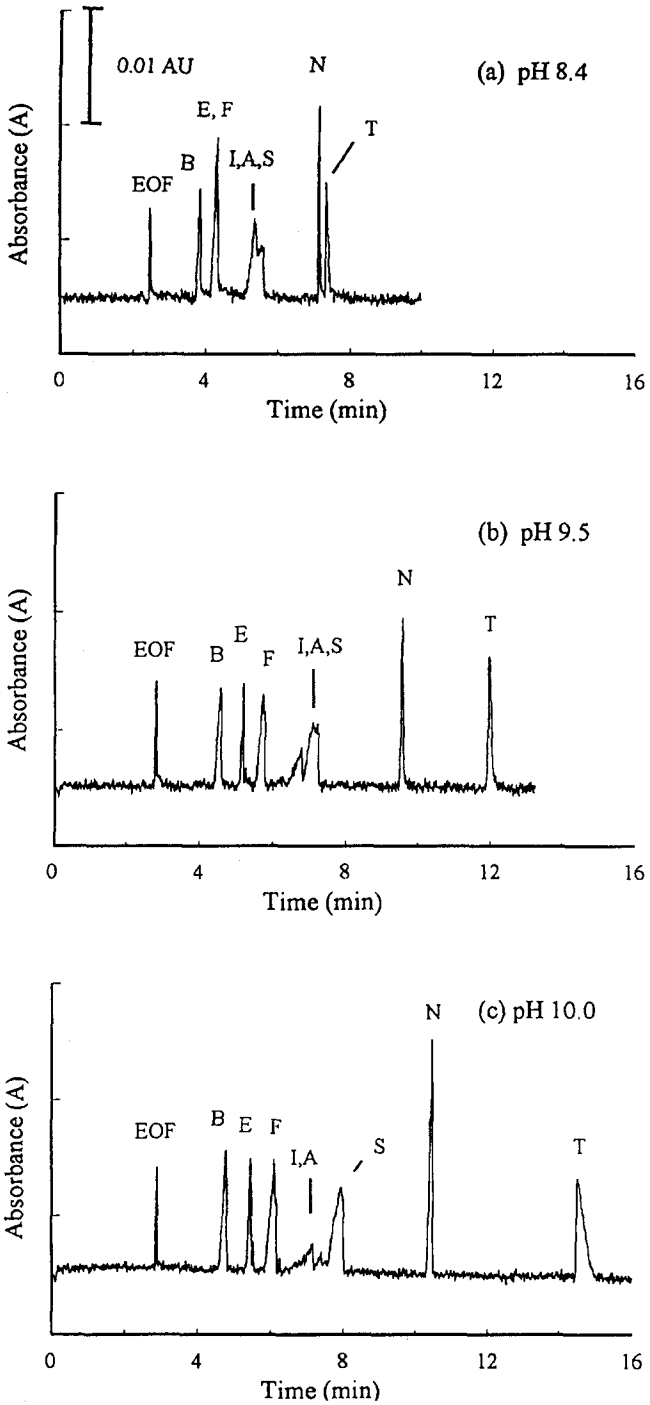
Figure 3 depicts the separation of synthetic food colorants by pH 9.5 borax – NaOH buffers containing three different cyclodextrins. Experimental results indicated that although addition of β- or γ-cyclodextrin affected the separation, addition of α-cyclodextrin had no effect, implying that cyclodextrin cavity size markedly influenced the separation.

Figure 3 shows that the allura red AC peak was quite sharp in running buffer containing γ-cyclodextrin. The net migration velocity of allura red AC increased with increasing γ-cyclodextrin concentration, because allura red AC could form an inclusion complex with γ-cyclodextrin. As Figure 3b reveals, adding β-cyclodextrin to the buffer not only yielded a better resolution but also resolved some impurities present in the food colorants. A mixture of β- and γ-cyclodextrins was, therefore, added to the running buffer in an attempt to improve separation efficiency. Although the separation of allura red AC was improved by addition of 2 mM β-cyclodextrin and 0.5 mM γ-cyclodextrin to the buffer, the resolution of the other analytes was unsatisfactory. β-Cyclodextrin alone was, therefore, selected for further examination of its effectiveness.

Figure 4 illustrates the effect of β-cyclodextrin, at concentrations ranging from 1 to 7 mM, on analyte migration behavior. Increasing the concentration of β-cyclodextrin altered the order of migration of the analytes and increased the net migration velocities of tartrazine and sunset yellow FCF by more than 35 %. This faster migration implies that these analytes interact more closely with β-cyclodextrin than do the other six analytes. These stronger interactions probably occur because the substituents on the benzene rings of tartrazine and sunset yellow FCF are in the *para* position and so these two analytes interact similarly with β-cyclodextrin. Nevertheless, the migration times of erythrosine and new coccine changed only slightly. Their molecular sizes and the positions of hydrophilic substituent result in their interactions with β-cyclodextrin being much weaker than those of the other analytes.

The eight synthetic colorants were sufficiently separated with only 2 mM β-cyclodextrin added to the buffer. Under these conditions, however, allura red AC and the impurity in indigo carmine were not adequately separated. In addition, fronting was apparent on the peaks of indigo carmine and allura red AC; this again affected peak resolution. When the concentration of β-cyclodextrin exceeded 5 mM, the resolution of indigo carmine and erythrosine was less than 1.5 whereas when the concentration of β-cyclodextrin was below 5 mM, the resolution of allura red AC and the impurity in indigo carmine was less than 1.0. The concentration of β-cyclodextrin was, therefore, critical to the resolution of all the peaks. As shown in Figure 4, addition of 5 mM β-cyclodextrin seemed to be the best choice and this concentration was selected for subsequent analysis.

Figure 5 shows the separation electropherogram of the eight synthetic colorants under the optimized condi-



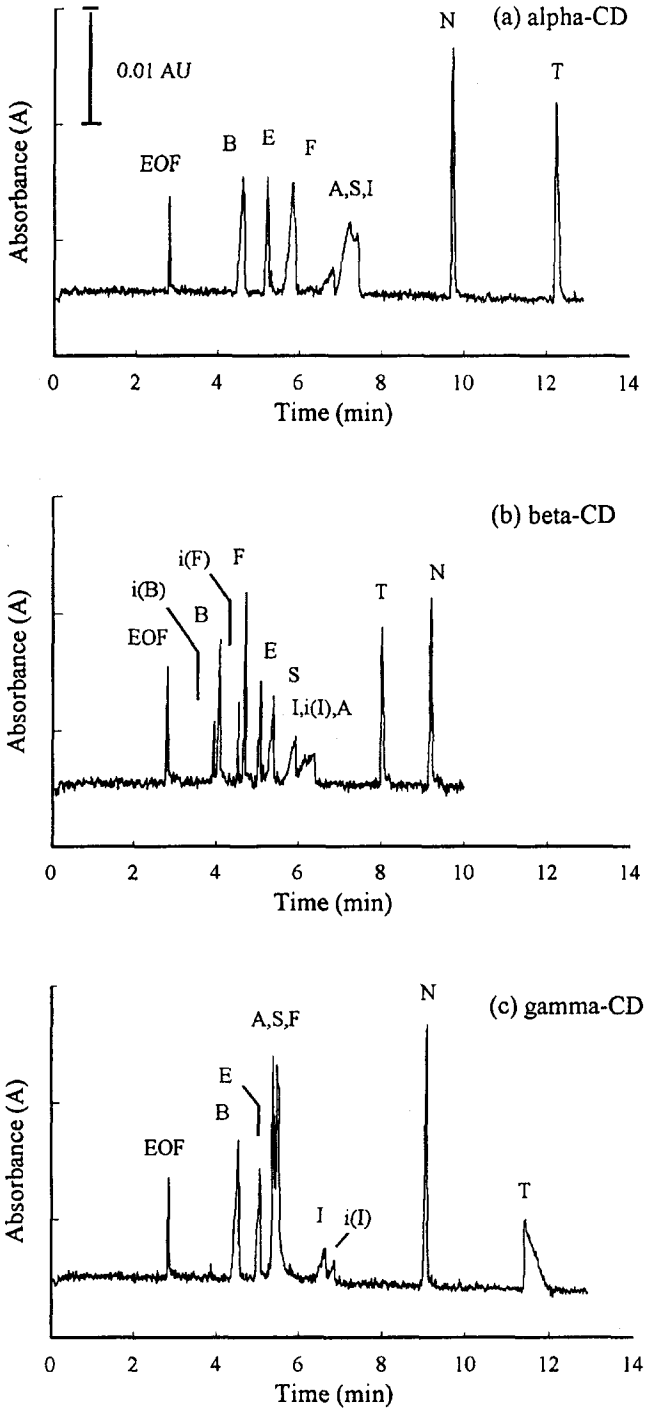


Figure 2

Separation of synthetic food colorants using (a) pH 8.4, (b) pH 9.5, and (c) pH 10.0 borate buffers. Conditions: separation capillary, 47 cm (40 cm to detector) × 50 µm i.d.; applied voltage, 20 kV; pressure injection, 3 s; detection wavelength, 200 nm; capillary temperature, 25 °C. Peaks: **EOF** = electroosmotic flow; **B** = brilliant blue FCF; **E** = erythrosine; **F** = fast green FCF; **I** = indigo carmine; **A** = allura red AC; **S** = sunset yellow FCF; **N** = new coccine; **T** = tartrazine.

Figure 3

Separation of synthetic food colorants using 0.025 M borax – NaOH buffer (pH 9.5) containing 2 mM α-cyclodextrin (a), β-cyclodextrin (b), or γ-cyclodextrin (c). Other conditions as for Figure 2. Peaks: **EOF** = electroosmotic flow; **B** = brilliant blue FCF; **i(B)** = impurity in brilliant blue FCF; **E** = erythrosine; **F** = fast green FCF; **i(F)** = impurity in fast green FCF; **I** = indigo carmine; **i(I)** = impurity in indigo carmine; **A** = allura red AC; **S** = sunset yellow FCF; **N** = new coccine; **T**= tartrazine.

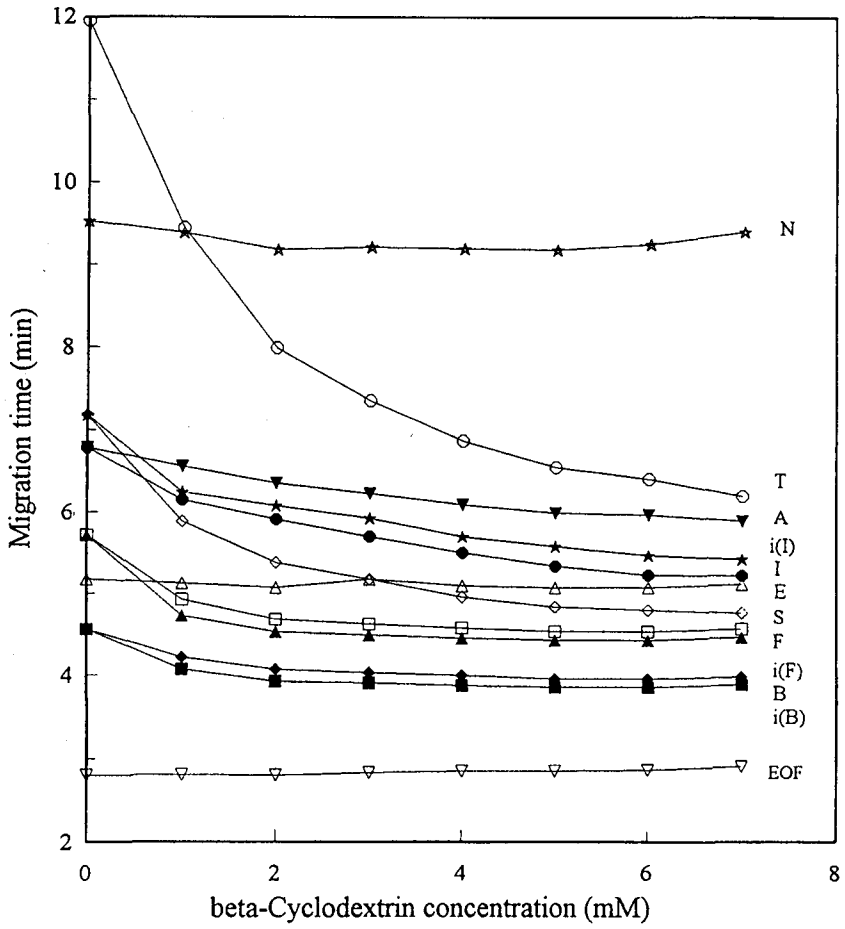


Figure 4

Effect of the concentration of β-cyclodextrin on the separation of synthetic food colorants. Conditions: separation solution, β-cyclodextrin in 0.025 M borax – NaOH buffer, pH 9.5. Other conditions as for Figure 3. EOF (▽), electroosmotic flow; i(B) (■), impurity in brilliant blue FCF; B (◆), brilliant blue FCF; i(F) (▲), impurity in fast green FCF; F (□), fast green FCF; S (◇), sunset yellow FCF; E (△), erythrosine; I (●), indigo carmine; i(I) (★), impurity in indigo carmine; A (▼), allura red AC; T (○), tartrazine; N (☆), new coccine.

tions. With use of 0.025 M borax – NaOH buffer (pH 9.5) containing 5 mM β-cyclodextrin the separation was complete within 10 min. Under these conditions, impurities in brilliant blue FCF, fast green FCF, and indigo carmine were also resolved. Table II lists the average migration times, reproducibilities (relative standard deviations) of the migration times, and the linearities of the calibration graphs for the eight analytes. The average migration times were means of seven inter-day replicates. The relative standard deviations (*RSD*) of the migration times were less than 0.88 % under the optimized separation conditions. Peak areas from the electropherogram were used for quantitation of the analytes. Calibration graphs were constructed from triplicate measurements at each concentration in the range from 10 to 300 μg mL^{-1} for those analytes. Notably, the correlation coefficients of the linear calibration graphs exceeded 0.998. The method of analysis proposed herein has the advantages of high reproducibility, fast analysis, and a relatively simple running buffer system. It can, moreover, be employed for qualitative and quantitative analysis of the colorants.

Determination of Food Colorants in Selected Food Products

Figure 6 shows the analysis of synthetic food colorants in real food products. The ice cream bars were melted at room temperature to prevent decomposition of the colorants. The sample solution was then injected directly without extraction or filtration. As Figures 6a and 6b reveal, synthetic colorants (i.e., tartrazine and new coccine) could be identified in the ice cream bars by the method of standard addition. The UV-Vis spectra of the analytes, acquired by use of the diode-array detector, were used for further confirmation of the identities of the food colorants. The main ingredients of the ice cream bars, i.e. sugar, flavors, and other additives, were sufficiently separated from the colorants. Such a discrete separation probably occurs as a result of the differ-

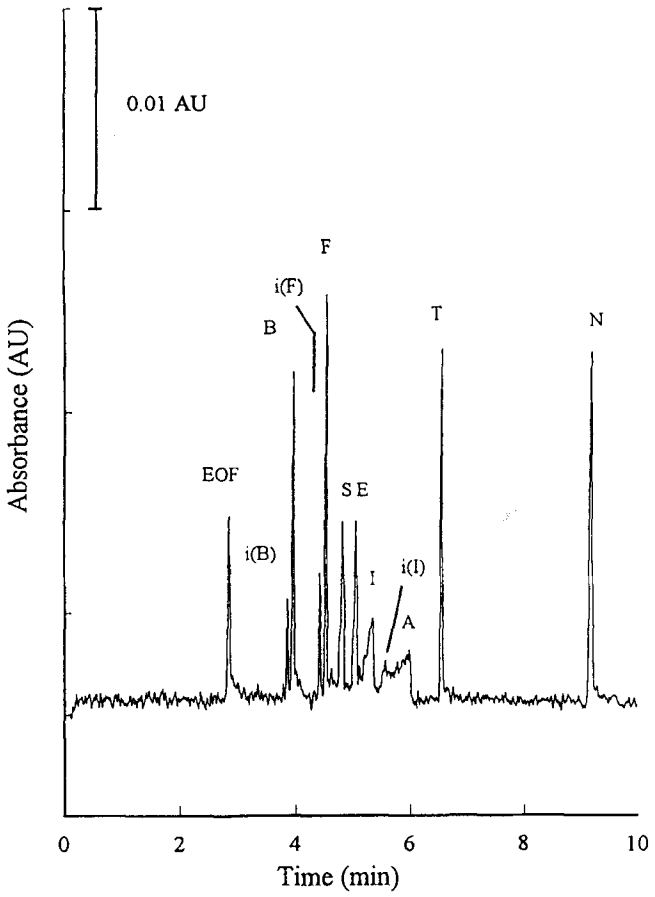


Figure 5
Electropherogram obtained for synthetic food colorants under the optimized conditions: separation solution, 0.025 M borax – NaOH buffer containing 5 mM β-cyclodextrin, pH 9.5; concentration of each analyte, 120 μg mL^{-1}. Other conditions and peak identities as for Figure 3.

ent net migration velocities or different absorption wavelengths of the various ingredients. Table III summarizes the quantitative results obtained for synthetic colorants in ice cream bars. These experimental results demonstrate that the CE method is suitable for efficient and convenient analysis of real food products.

Figure 7 depicts electropherograms of the synthetic colorants present in fruit soda drinks. The large amount of gas always present in solution of soda drinks must be removed by sonication to eliminate the signals from the bubbles and to maintain the stability of the electric current during separation. Interestingly, the separation efficiencies in Figure 7 exceed those in Figure 5, probably because of the different matrixes in the standards and samples. It is noteworthy that other components of the soda drinks did not interfere with the determination of the food colorants.

Table III lists the quantities of food colorants in three different fruit soda drinks. The relative standard deviations are presented to demonstrate the precision of quantitative analysis. In contrast with HPLC analysis [3, 4], sample extraction or filtration is not necessary before CE analysis. These results confirm that the CE method described herein is suitable for analysis of food colorants in soda drinks.

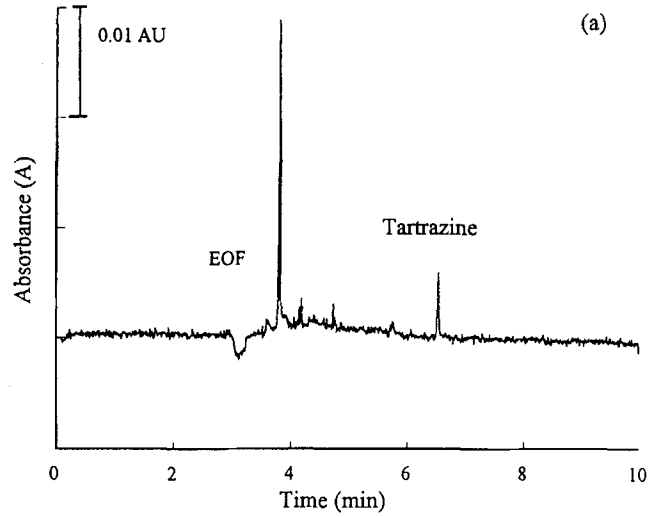
Conclusion

A HPCE method has been successfully developed for the separation of the synthetic food colorants. The migration behavior of the analytes is greatly influenced by buffer pH and the presence of cyclodextrins. Samples of ice cream and fruit soda drinks do not need any filtra-

Table II. Average migration times, reproducibilities (*RSD*) of migration times, and the linearities of calibration graphs for the synthetic food colorants.[a]

Colorant	Migration time (min)	*RSD* (%)	Slope ($\times 10^{-2}$)	Intercept ($\times 10^{-2}$)	Correlation coefficient of calibration graph (*r*)
Brilliant blue FCF	3.94	0.29	0.3361	–0.8629	0.999
Fast green FCF	4.52	0.29	0.5198	–0.3242	0.999
Sunset yellow FCF	4.83	0.49	0.4434	0.9482	0.999
Erythrosine	5.06	0.53	0.4085	–1.0044	0.999
Indigo carmine	5.37	0.83	0.4153	0.0622	0.998
Allura red AC	6.06	0.88	0.5781	–1.1104	0.999
Tartrazine	6.57	0.58	0.3923	0.3581	0.999
New coccine	9.17	0.62	1.0731	2.0141	0.999

[a]Values are means of seven inter-day replicates.



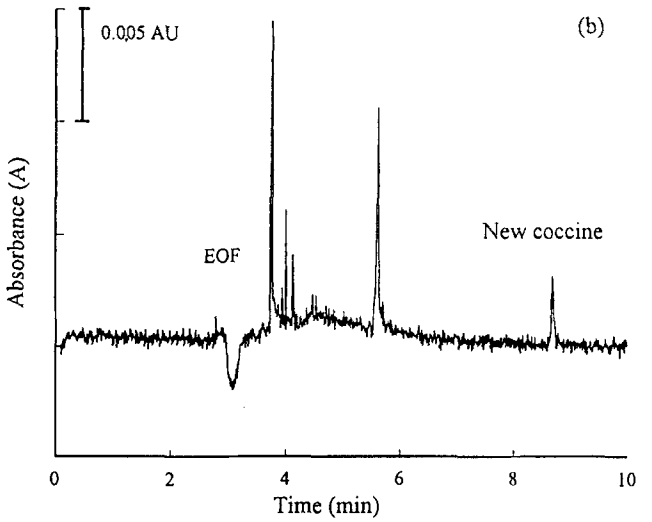
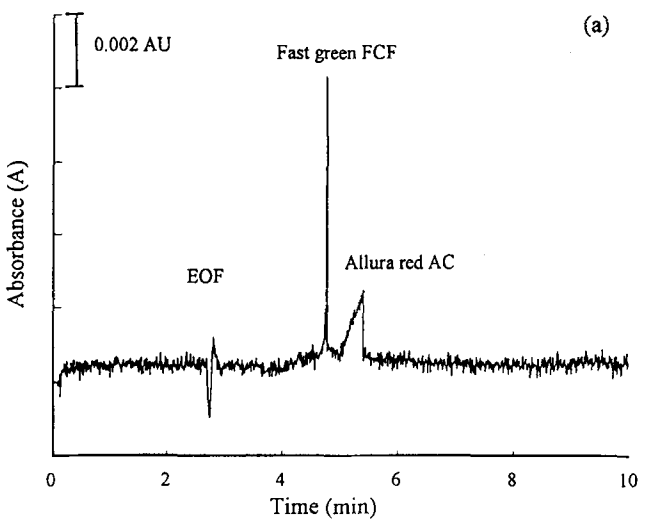


Figure 6
Electropherogram of synthetic food colorants in two different ice cream bars. Conditions and peak identities as for Figure 5.



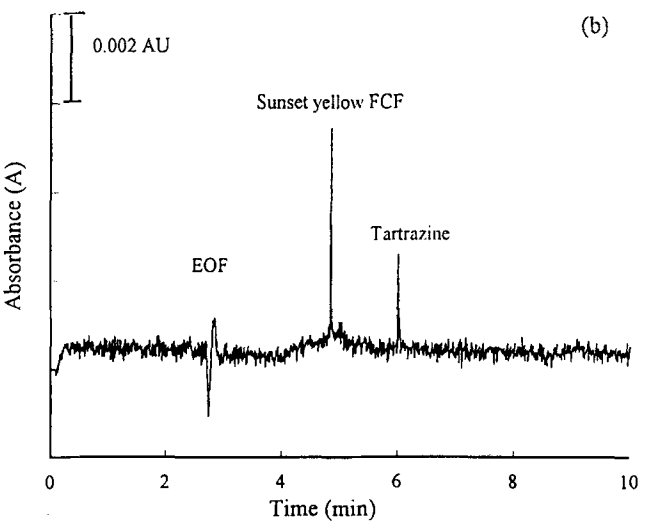


Figure 7
Electropherogram of synthetic food colorants in (a) grape soda and (b) mango soda. Conditions and peak identities as for Figure 5.

Table III. Content of synthetic food colorants in selected food products.[a]

Sample	Colorant	Concentration ($\mu g\ mL^{-1}$) and (*RSD*, %)
Ice cream bar A	Tartrazine	27.88 (5.37)
Ice cream bar B	New coccine	21.41 (3.54)
Grape soda drink	Fast green FCF	9.38 (7.38)
	Allura red AC	25.32 (4.95)
Mango soda drink	Sunset yellow FCF	9.34 (3.81)
	Tartrazine	9.66 (4.85)
Pineapple soda drink	Tartrazine	11.94 (3.29)

[a]Values are means of triplicate determinations.

tion or extraction before CE analysis, i.e. the method has the merit of enabling analysis of real samples with limited preparation steps and so the pretreatment time is dramatically reduced. No solvent is necessary for extraction. Direct injection of real samples does not clog the separation capillary. These advantages emphasize that this HPCE method is a simple, rapid, and effective technique for analysis of synthetic food colorants in real food products.

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