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Determination of synthetic food dyes by capillary electrophoresis

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Abstract

A method for the determination of synthetic tar dyes used as food additives using capillary electrophoresis with photodiode-array detection was investigated. The dyes Erythrosine (R-3), Phloxine (R-104), Rose Bengal (R-105), Acid Red (R-106), Amaranth (R-2), New Coccine (R-102) and Allura Red AC (R-40) were separated on a capillary column (50 cm × 75 μm I.D.) and identified from the absorbance spectra of each peak. The electrophoresis buffer used was a mixture of 25 m*M* sodium phosphate buffer and 25 m*M* sodium borate buffer (1:1) (pH 8.0) containing 10 m*M* sodium dodecyl sulfate (SDS). Substitution of β-cyclodextrin for SDS in the electrolyte buffer was effective for the separation of R-2 and R-102. This modified method could be employed as an additional assay method for these two dyes.

1. Introduction

Food dyes including synthetic colours are used under governmental regulations all over the world, and the kinds and numbers of permitted dyes vary from country to country. In Japan, the following twelve synthetic tar dyes are permitted for use as food additives: Erythrosine [Color Index (C.I.) No. 45430, R-3], Phloxine (C.I. 45410, R-104), Rose Bengal (C.I. 45440, R-105), Acid Red (C.I. 45100, R-106), Amaranth (C.I. 16185, R-2), New Coccine (C.I. 16255, R-102), Allura Red AC (C.I. 16035, R-40), Tartrazine (C.I. 19140, Y-4), Sunset Yellow FCF (C.I. 15985, Y-5), Fast Green FCF (C.I. 42053, G-3), Brilliant Blue FCF (C.I. 42090, B-1) and Indigo Carmine (C.I. 73015, B-2). With respect to food hygiene, simple and rapid methods for the determination of dyes are required for the inspection of proper use and for the assurance of food safety.

Paper chromatographic [1], thin-layer chromatographic [1,2] and high-performance liquid chromatographic (HPLC) methods have been reported, and HPLC methods were found to be superior in sensitivity. However, most of methods were not suitable for routine analysis, e.g., operation of the solvent gradient system is complicated [3,4], ion-pair chromatography [5] for the simultaneous determination is time consuming and the detection of various dyes needs different conditions [6].

Recently, capillary electrophoresis has been developed as a rapid method with high resolution [7,8]. Electrophoretic mobility, which is

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expressed as a function of the ratio of net charge to molecular size, plays an important role in separations by capillary electrophoresis. Although the dyes are divided into several groups based on their chemical structures, all of them have effective charge under the conditions used in this study.

In capillary electrophoresis, measurement with a single ultraviolet absorbance wavelength was insufficient for the identification of analytes because of the relatively poor reproducibility. Also, the extremely small amount of sample subjected to capillary electrophoresis makes the recovery of the analytes for application in further analyses impossible. Capillary electrophoresis with novel on-column detection techniques have been actively investigated, and recently photodiode-array detection with a very fast response time has been introduced [9,10].

In this study, we investigated a new approach for the simultaneous determination of the synthetic dyes by capillary electrophoresis. Spectral peak information using photodiode-array detection was utilized as a powerful means for the easy identification of seven red dyes.

2. Experimental

2.1. Chemicals

Sodium dodecyl sulfate (SDS) was purchased from Nacalai Tesque (Kyoto, Japan). Sodium tetraborate, sodium borate, sodium dihydrogenphosphate, sodium hydrogenphosphate and β-cyclodextrin were obtained from Wako (Osaka, Japan).

Dyes R-3, R-104, R-105, R-106, R-2, R-102 and R-40 (Fig. 1) were obtained from Tokyo Chemical Industry (Tokyo, Japan). Standard solutions of 1000 μg/ml were prepared in pure water and diluted as required. A mixed standard solution containing 10 μg/ml of each of the seven dyes was used to examine the optimum conditions of electrophoretic separation. Sudan III was a product of Wako and dissolved in

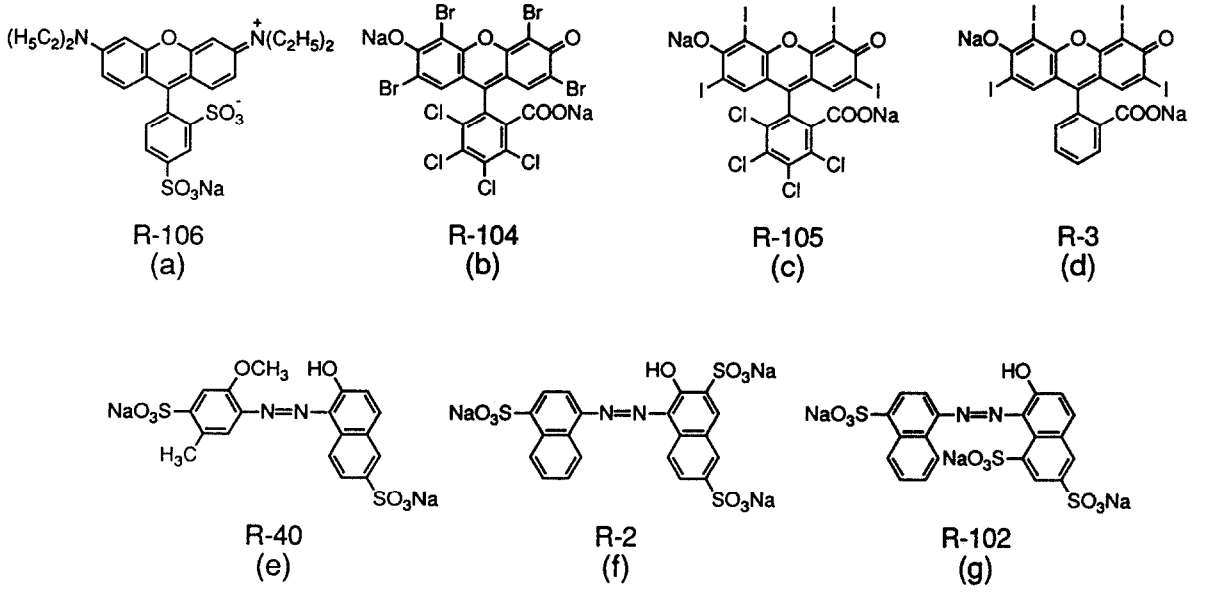

Fig. 1. Structures of the synthetic red dyes studied. R-106 (a), R-104 (b), R-105 (c) and R-3 (d) are classified as xanthene dyes and R-40 (e), R-2 (f) and R-102 (g) as azo dyes.

electrophoresis buffer. All other chemicals were of analytical-reagent grade.

2.2. Apparatus and conditions

Electrophoretic separations were carried out on a CAPI-3000 system (Otsuka Electronics, Osaka, Japan) with a 75 μm I.D. capillary of synthetic fused silica (Otsuka Electronics). The capillary was 50 cm in total length and 37.5 cm to the detector. Separations were performed at 10 kV with a capillary temperature of 25°C. Absorbance from 190 to 600 nm was monitored with an on-column photodiode-array detector. Injections were hydrostatic and performed by raising the sample vial 20 mm above the level of the destination vial for 20 s. The calculated injection volume was ca. 12 nl. After each run, the capillary was flushed with water followed by electrophoresis buffer. Washing with sodium hydroxide solution or aqueous methanol was carried out when needed.

An electrophoresis buffer was prepared by combining equal volumes of 25 m*M* sodium phosphate buffer (pH 8.0) and 25 m*M* sodium borate buffer (pH 8.0). For the simultaneous separation of the seven dyes 10 m*M* SDS was added and for the separation of R-2 and R-102 10 m*M* of β-cyclodextrin was combined with the mixed buffer. Buffer solutions were filtered through a 0.5-μm Millipore filter (Nihon Millipore, Tokyo, Japan) and degassed by sonication before use.

Absorbance spectra of the dyes dissolved in electrophoresis buffer were measured with a UV260 spectrophotometer (Shimadzu, Kyoto, Japan) with a 1-cm path-length quartz cell to identify the peaks obtained.

3. Results and discussion

3.1. Simultaneous separation of seven red dyes

Some xanthene dyes precipitate under acidic conditions and some azo dyes become labile at higher pH [1]. Therefore, measurements were carried out around neutral pH.

The separation of the seven red dyes by capillary zone electrophoresis (CZE) was investigated using phosphate and/or borate buffer. The peaks obtained in the electropherogram were easily identified with the absorbance spectra measured with the photodiode-array detector.

In the neutral pH range, seven dyes behaved as anions and migrated after the neutral substances, because the observed migration rate of the dyes was lower than the electroosmotic flow-rate. The migration order of the dyes was explained by the difference in the electrophoretic mobilities attributed to their chemical structures. The migration rate increases with decreasing net charge and with increasing molecular size, but molecular size is the determinant when the dyes possess equal net charge. In other words, an anion with higher electrophoretic mobility moves more slowly towards the cathode, near which the detection window is located.

Relatively good separation was obtained under electrophoresis buffer conditions of pH 7–8 with 25 m*M* sodium phosphate, of pH 8–9 with 25 m*M* sodium borate and of pH 8.0 with phosphate–borate (1:1) (Fig. 2). As shown in Fig. 2,

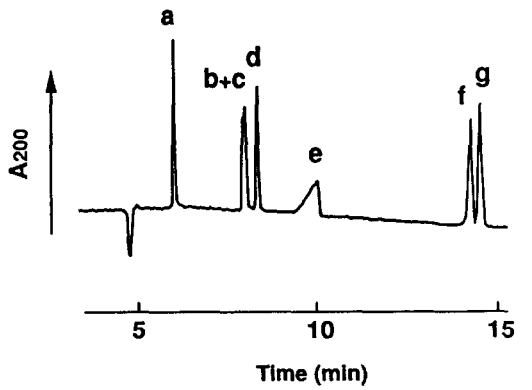

Fig. 2. Electropherogram at 200 nm of the seven red dyes. Buffer: mixture of equal volumes of 25 m*M* sodium phosphate (pH 8.0) and 25 m*M* sodium borate (pH 8.0). For peak identification, see Fig. 1. For other analytical conditions, see Experimental.

the azo dyes R-40, R-2 and R-102 migrated after xanthene dyes. The effect of sulfonic groups in azo dyes on the migration could be greater than that of the effective anionic charge of xanthene dyes. The substituted analogues R-105 and R-104 were hardly separated under the conditions applied. Being larger than R-104 in molecular size, R-105 migrated faster as judged from a spectral resolution of the combined peak. Similarly, R-3, which is dechlorinated molecule R-105, also migrated after R-104 and R-105. In addition, the structural isomers R-2 and R-102 were not separated satisfactorily at higher pH such as pH 9.0 with phosphate buffer, whereas R-104 and R-105 were slightly separated. No influence of phosphate and borate buffer ratio, pH or the addition of an organic solvent such as methanol on the simultaneous separation of R-104–R-105 and R-2–R-102 was observed.

Terabe et al. [11] introduced micellar electrokinetic chromatography (MEKC). The separation of analytes was mainly achieved by the difference in partitioning between the micellar phase and the aqueous phase and also according to electrophoretic mobility. This method was reported to have advantages over capillary zone electrophoresis in the separation of both electrically neutral and ionic substances [12].

The effect of adding SDS to 25 m*M* sodium phosphate buffer (pH 8.0) is shown in Fig. 3. The analogous xanthene dyes R-3, R-104 and R-105 were clearly separated and migrated in that order. The migration order of dyes was explained in terms of degree of hydrophobicity and agreed with the results obtained by reversed-phase HPLC [5]. The migration time of R-106 was greatly influenced by SDS concentration. There might be an interaction between the positive charge of R-106 and micelles. In contrast, no effect of SDS on the migration time and the peak shape of R-40, R-2 and R-102 was observed. Less partitioning of these dyes to the micelles could be attributed to peripheral sulfonic groups in the molecule. To improve the separation and the peak shape of R-2 and R-102, the effect of the ratio of mixing 25 m*M* sodium phosphate buffer and 25 m*M* sodium borate buffer was studied. As shown in Fig. 4, equal

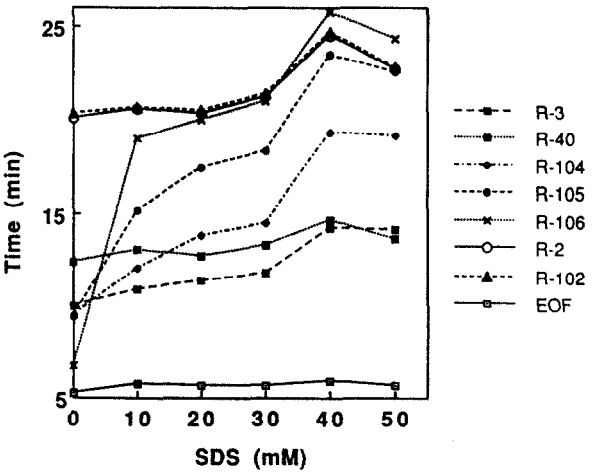

Fig. 3. Effect of SDS on migration times of the seven red dyes. SDS was added to 25 m*M* sodium phosphate buffer (pH 8.0). EOF indicates the position of water. For other analytical conditions, see Experimental.

volumes of the two buffers, addition of 10 m*M* SDS and a pH of 8.0 gave a favourable separation in an acceptable operating time.

An electropherogram at 200 nm and absorbance spectra obtained under the above conditions are shown in Fig. 5. The seven red dyes were separated within 20 min with good reproducibility. The relative standard deviations of the migration time and the peak height were 0.3–0.7% and 2.3–5.1%, respectively ($n = 8$). The

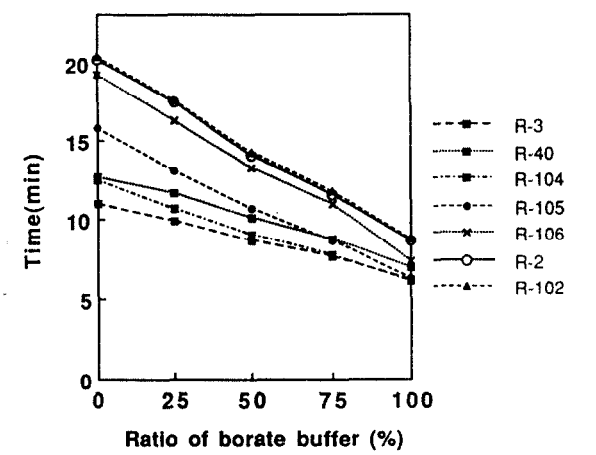

Fig. 4. Effect of phosphate and borate buffer ratio on the separation of the seven red dyes. SDS concentration = 10 m*M*. For other analytical conditions, see Experimental.

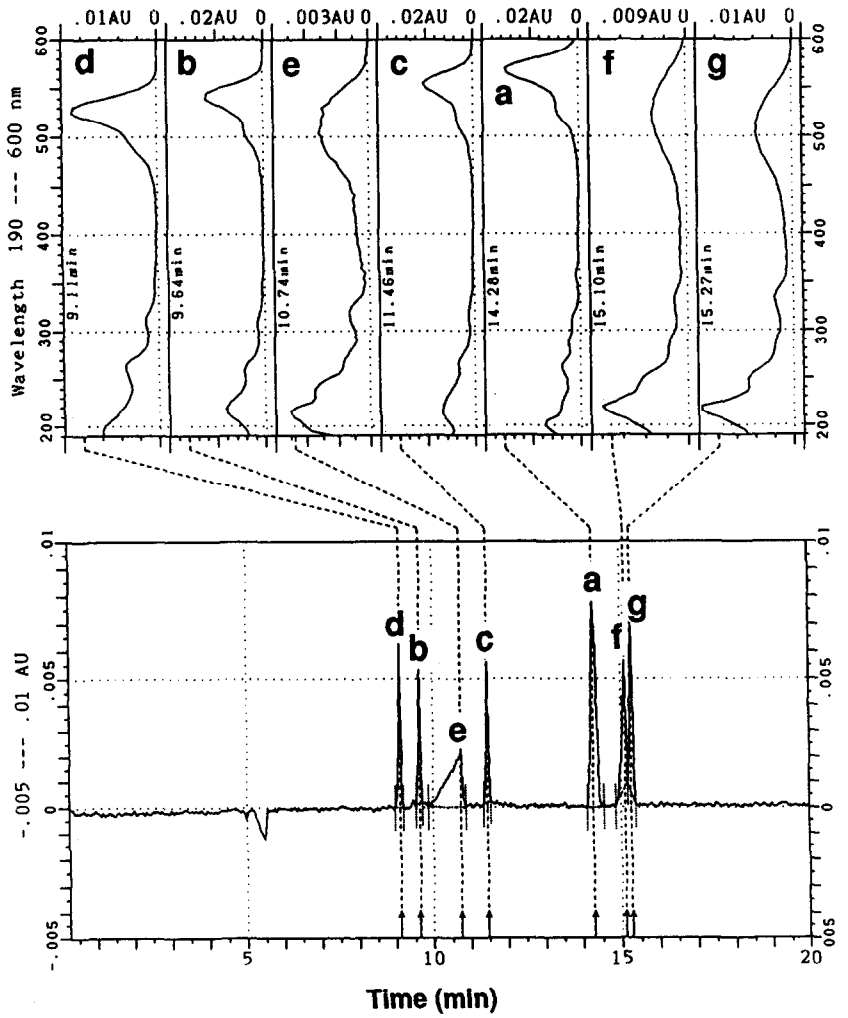

Fig. 5. Electropherogram at 200 nm and the absorbance spectra of the seven red dyes by MEKC. For peak identification, see Fig. 1. For analytical conditions, see Experimental.

detection limits of the dyes were ca. 1 μg/ml with a signal-to-noise ratio of 3, and the calibration graphs were linear up to 30 μg/ml. The observed electric current was constant between 26 and 27 μA.

No effect of pH, buffers, SDS or sample concentration was observed on the peculiar fronting peak of R-40 shown in Figs. 2 and 5. Peak deformations are often explained by conductivity differences between the zones of analytes and the carrier electrolytes [8,13]. However, the explanation might not be applicable for R-40, because the other six dyes showed good peak shapes. Interaction of R-40 with the capillary wall might be responsible for this peak deformation and further investigation is required for the clarification of this point.

3.2. Separation R-2 and R-102 with β-cyclodextrin

R-2 and R-102 moved in close proximity in MEKC with SDS, as described above. No improvement in separation was demonstrated

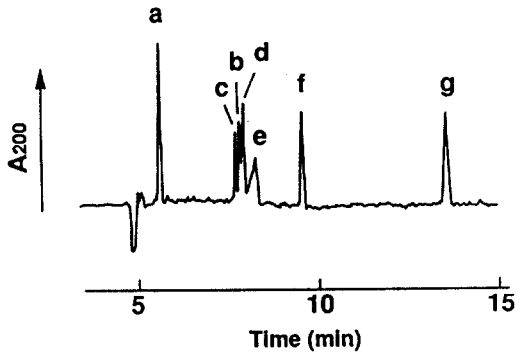

Fig. 6. Effect of β-cyclodextrin on the separation of the seven red dyes. The electropherogram at 200 nm is shown. For peak identification, see Fig. 1. For analytical conditions, see Experimental.

either by raising the capillary temperature or an addition of tetraalkylammonium salts to the buffer, which promote the separation of ionic substances in MEKC [14].

The structural difference between R-2 and R-102 is the site of the sulfonic group. Cyclodextrins have been reported to provide additional selectivity for isomers [15,16]. Therefore, the separation of the seven red dyes with addition of 10 m*M* β-cyclodextrin was examined (Fig. 6). In contrast to SDS, β-cyclodextrin greatly affected the migration behaviour of R-2 and R-102, and therefore the separation of these two dyes was successfully achieved. The effective net charge or ionic radius of inclusion complexes appears to be greatly influenced by the site of a sulfonic group. In contrast, little effect of β-cyclodextrin on the separation of xanthene dyes was observed, but the separation of R-104 and R-105 was perceptible. Therefore, the effects of various concentrations of β-cyclodextrin and the combination of β-cyclodextrin and SDS were further investigated for the simultaneous separation of xanthene dyes along with R-2 and R-102, but in vain.

4. Conclusions

A method for the determination of synthetic dyes used as food additives was investigated using capillary electrophoresis with multi-wavelength detection. In this study, the separation of seven synthetic red dyes, all permitted as food additives in Japan, was examined. They were separated under optimized conditions in the presence of SDS within 20 min and the results were in accord with those of HPLC. For confirmative analysis, addition of β-cyclodextrin to the electrolyte buffer significantly improved the separation of R-2 and R-102 and gave satisfactory results.

Five synthesized tar dyes, Y-4, Y-5, G-3, B-1 and B-2, are also permitted in foods in Japan. Preliminary experiments by MEKC showed comigration of some of these dyes with red dyes, but the absorbance spectra of each dye proved to give good resolution. The simultaneous determination of all twelve dyes is under investigation.

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