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# Improvement in the determination of food additive dyestuffs by capillary electrophoresis using β-cyclodextrin

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**Abstract**

The determination of seven food additive dyestuffs was investigated by capillary electrophoresis. When β-cyclodextrin was introduced into the carrier electrolyte, the apparent mobility was increased, leading to 9.5–39% lower migration times due to the increase in the solute's mass after inclusion complex formation. The reproducibility and peak shape were improved because interaction between the solute and the capillary wall was alleviated. The effects of β-cyclodextrin on the migration time, elution order, peak shape and reproducibility of food additive dyestuffs are discussed in terms of providing a considerable advantage for determining organic anions by capillary electrophoresis. Sequential injection of dyestuffs and β-cyclodextrin into a capillary electrophoresis column was found to be a simple and rapid method for a qualitative comparative study of inclusion complexation phenomena.

## 1. Introduction

Cyclodextrins (CDs) are well known carrier electrolyte additives in high-performance capillary electrophoretic (HPCE) techniques for improving separation efficiency [1–3] and have been recognized as successful chiral selectors during the last few years [4]. Since research has been concentrated on the above aspects, other applications of CDs based on their inclusion complexation ability with a large number of aromatic ring-containing compounds have yet to receive adequate attention. The role of β-CD in decreasing analysis times and improving the reproducibility during the determination of organic anions, specifically dyestuff food additives, was investigated in this work.

High-performance liquid chromatography (HPLC) [5,6] and thin-layer chromatography (TLC) [7] are the most popular methods for the determination of dyestuffs. Although HPLC methods can be performed with acceptable sensitivity, most of them are time consuming and suffer from a poor separation ability for the simultaneous determination of a broad range of dyestuffs, and they are not amenable to further methodological development. The appearance of capillary electrophoresis (CE) in the field of separation science [8] with its intrinsic capabilities, i.e., high resolution efficiency and short analysis times, ease of setting up, the small volumes required, the numerous modes for varying the selectivity and the wide range of applica-

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tion [9,10], have stimulated many researchers to examine this technique [11].

Some papers on the determination of dyestuffs by CE have been published [12–17]. In this report, we present a study on the simultaneous determination by CE of seven food additive dyestuffs using a method that takes advantage of inclusion complexation with β-CD. The effects of β-CD on analysis time, elution order, peak shape, and reproducibility were investigated. With sequential injection of dyes and β-CD into a CE column, an unusual band broadening was observed, which can be considered in terms of the formation of a mobility gradient along the sample zone due to inclusion complexation. By observing the extent of band broadening, comparison of complex formation ability with β-CD among the various dyes is feasible.

## 2. Experimental

### 2.1. Instrumentation

A high-voltage power supply (Model HCZE–30 PNO.25-LDS; Matsusada Precision Devices) was used to generate an electric field up to 30 kV. Separations were performed at 25 kV. For sequential injection studies, a 20 kV electric field was applied. On-column detection of the separated peaks was performed at 245 nm with a Model 875-CE UV–Vis detector (Jasco). Electropherograms were processed and recorded on a Chromatopac model CR3A instrument (Shimadzu). Separations were performed using a fused-silica capillary tube (77 cm long, 60 cm to the detector ×50 μm I.D. and 375 μm O.D.). For sequential injection, a 60 cm long capillary, 40 cm to the detector, was used. Capillaries were obtained from GL Sciences (Tokyo, Japan). Each new capillary was conditioned by flushing for 30 min with 1 *M* NaOH and for another 30 min with a carrier electrolyte containing 20 m*M* sodium tetraborate adjusted to pH 7.5 prior to use. For sequential injections studies, 10 m*M* sodium tetraborate of the same pH was used. Between two runs, when β-CD was absent from the carrier electrolyte, the capillary was flushed with NaOH and conditioned for 5 min with electrolyte buffer. Hydrodynamic injection was performed by raising the anodic end of the capillary 10 cm higher than the level of the cathodic vial for 5 s.

### 2.2. Chemicals and reagents

All reagents were of analytical-reagent grade if not stated otherwise. All solutions including carrier electrolytes and standards were prepared using 18-MΩ water generated by a Milli-Q laboratory water purification system (Millipore, Bedford, MA, USA). Dyestuff food additives were received as a gift from the National Institute of Health Sciences, Japan. Stock standard solutions were prepared by dissolving the dyestuffs at $1 \cdot 10^{-4}$ *M* in distilled water and were diluted to the appropriate concentration with the same solvent prior to use.

## 3. Results and discussion

In CE, the relative electrophoretic mobility ($\mu_{rel}$), depends on charge ($Z$) and molecular mass ($M_r$) and is estimated with the following equation [18]:

$$\mu_{rel} = ZM_r^{-2/3} \quad (1)$$

Owing to the dependence of mobility on charge density, any manipulation of $M_r$ can affect the mobility and consequently the migration time. Potential approaches are derivatization and complexation. In this regard, to improve the determination of dyestuffs, we checked the effect of β-CD, which has a relatively high molecular mass and a well known ability to form inclusion complexes with organic compounds containing aromatic rings. Although β-CD is neutral, it could alter the electrophoretic mobility of analytes based on differing molecular masses of the complexed and free solutes. New patterns of elution order and resolution were observed, which are dictated by how and to what extent one solute can be included in the β-CD cavity. We investigated the behaviour of the seven food

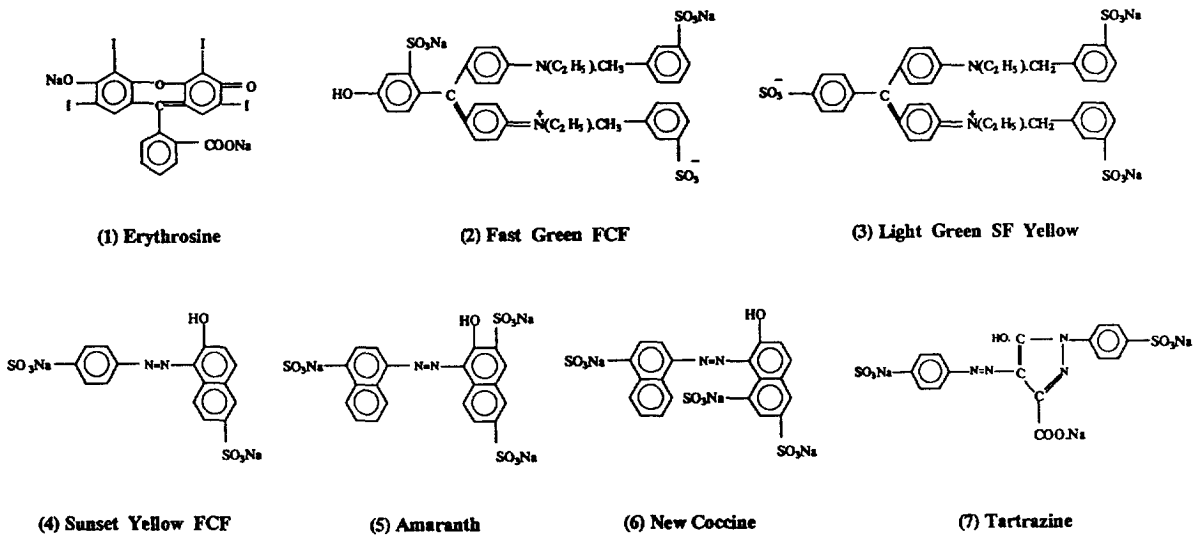


Fig. 1. Structures of the dyestuff food additives studied.

additive dyestuffs shown in Fig. 1 in the presence and absence of β-CD in carrier electrolyte. For this purpose, several analyses were performed at different concentrations of β-CD and the resulting electropherograms are shown in Fig. 2. The migration time, elution order, peak shape and reproducibility were altered as a result of differing extents of complexation of the solutes with β-CD. These parameters are discussed separately.

### 3.1. Migration time

Owing to formation of inclusion complexes between the dyes and β-CD, which have higher molecular masses than free dyes, the migration time decreased in all cases (see Fig. 2). Since the complex formation constants varied from solute to solute, the retention time was reduced to different extents. With a 20 m*M* concentration of β-CD in the carrier electrolyte, the greatest decreases were 39% in amaranth and 38% in Sunset Yellow FCF. The migration times of New Coccine, Fast Green FCF, Light Green SF Yellow and tartrazine decreased by 34.6, 34.0, 34.0 and 30.0%, respectively. The migration time of erythrosine decreased by only 9.5%. This small decrease in migration time is evidence of poor complexation with β-CD. The presence of bulky iodine atoms in the erythrosine molecule may explain this behaviour. Another observation is that although the elution window of dyestuffs decreased from 10 to 5.5 min, complete resolution between peaks could still be established. Narrowing of the elution window not only failed to disturb the resolution between peaks, but even improved it in the case of isomeric pairs: amaranth and New Coccine. Amaranth, which is different from New Coccine only in the position of the sulfonate group (Fig. 1), showed a stronger tendency for inclusion in the β-CD cavity and eluted earlier (39 and 34.6% decrease in migration times for amaranth and New Coccine, respectively). Taking the above results into consideration, a short and qualitative discussion is given below.

The relative stabilities of β-CD and the dyes studied are governed by factors such as hydrophobic interaction and space-filling ability of molecules. Depending on the size and geometry of dyes, in relation to the dimensions of the β-CD cavity, substantial differences in the migration behaviour of dyes were observed. For example, amaranth and Sunset Yellow, bearing a similar naphthonoid moiety, showed the strongest binding, which could be explained as being due to the ready inclusion of this part of molecule in the β-CD cavity via Van der Waals

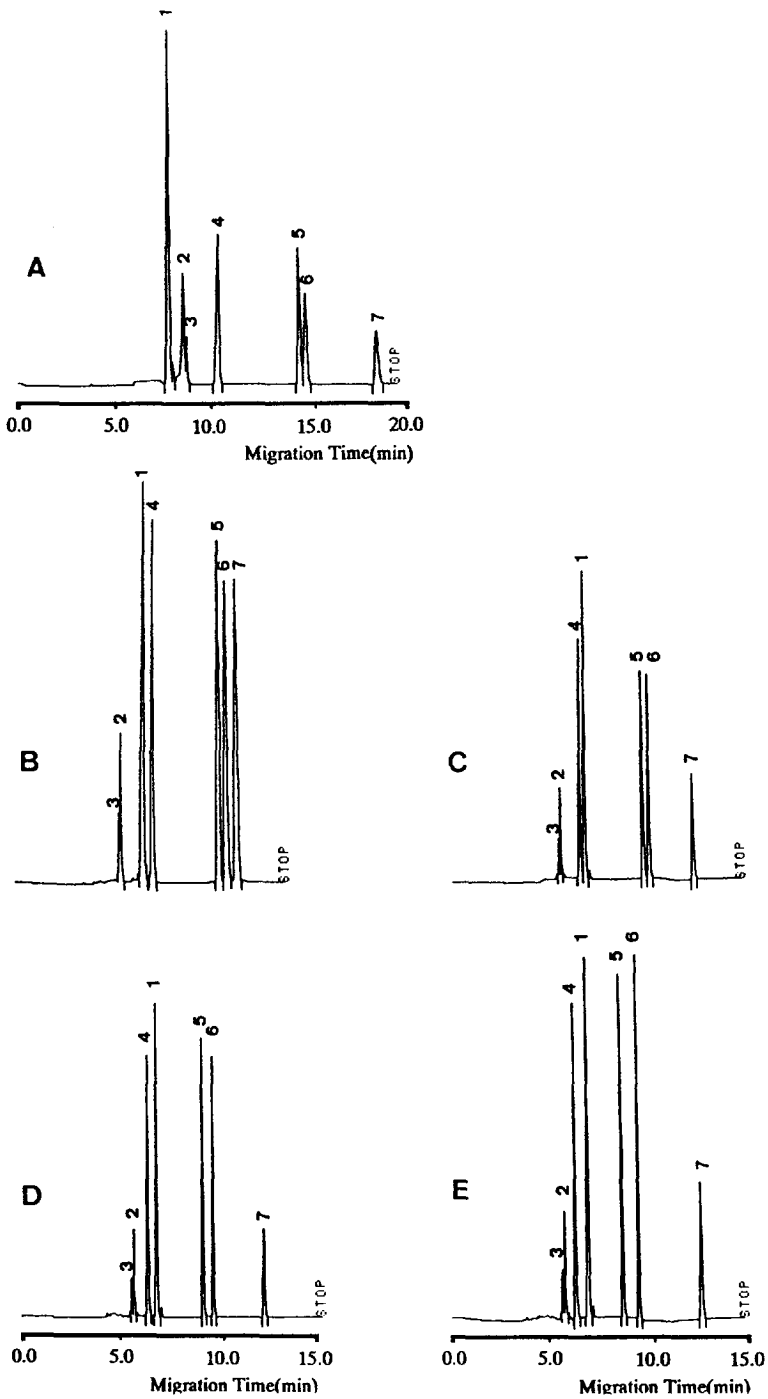


Fig. 2. Electropherograms of the separated dyestuffs and the effect of β-CD concentration on the migration time, separation and elution order. Mixtures of the dyestuffs, each at $10^{-5}$ *M*, were injected hydrodynamically for 30 s. Electropherograms: (A) 0, (B) 5, (C) 10, (D) 15 and (E) 20 m*M* β-CD in the carrier electrolyte. See Experimental for other conditions and Fig. 1 for peak identification.

interactions and cavity size considerations. The isomeric pair of amaranth, i.e., New Coccine, however, showed a weaker interaction owing to the different position of the sulfonate group in the naphthonoid part of the molecule. Again, Fast Green FCF and Light Green SF Yellow showed similar behaviours towards the β-CD cavity owing to the similarity of the included part of these molecules inside the β-CD cavity. In the case of tartrazine, a decrease in the hydrophobicity of molecule tends to weaken the interaction with β-CD. Finally, erythrosine as mentioned previously, showed the weakest interaction with β-CD owing to the size effect.

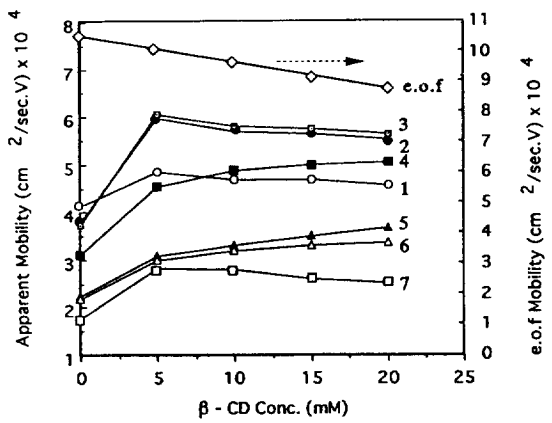


Fig. 3. Change in apparent electrophoretic mobility of dyestuffs as a function of β-CD concentration.

### 3.2. Elution order

A change in the elution order of the dyestuffs was observed after the addition of β-CD to the carrier electrolyte (Fig. 2). The tendency for inclusion in the β-CD cavity appears to influence the elution order of dyestuffs. The apparent mobility ($\mu_{app}$) of dyestuffs were calculated using the equation [11]

$$\mu_{app} = L_d L_t / t_{app} V \quad (2)$$

where $L_d$ is the distance from the injector to the detector (cm), $L_t$ is total capillary length (cm), $t_{app}$ is the apparent or observed migration time of dyestuffs (s) and $V$ is the applied voltage (volts). In Fig. 3, the change in the apparent mobility of the dyestuffs is plotted as a function of β-CD concentration. When the concentration of β-CD in the carrier electrolyte increased, the apparent mobility values changed with varying patterns. This phenomenon can change the elution order of the dyestuffs, e.g., in the presence of 20 m*M* β-CD the elution order of erythrosine shifted from the first to the fourth peak. Fast Green FCF and Light Green SF Yellow were also eluted before erythrosine, owing to a greater tendency for inclusion complex formation. On the other hand, the elution order of erythrosine and Sunset Yellow SCF was reversed when the concentration of β-CD was increased from 5 to 10 m*M* (see Fig. 2). At this concentration, the interaction of Sunset Yellow SCF with β-CD was strong enough to change the elution order. In addition, higher concentrations of β-CD up to 20 m*M* were necessary for a better resolution between the peaks of erythrosine and Sunset Yellow SCF, which was decreased in 5 m*M* β-CD in the carrier electrolyte. It should be noted that the apparent mobility of erythrosine, Fast Green FCF, Light Green SF and tartrazine showed slight decreases when the concentration of β-CD was increased from 10 to 20 m*M*. These decreases in mobilities came from the decrease in electroosmotic flow due to the increase of the carrier electrolyte viscosity.

### 3.3. Peak shape and reproducibility

One of the drawbacks in HPCE is adsorption of analytes by silanol groups on the capillary wall. These groups can be positively charged as $SiOH_2^+$, neutral as SiOH or negatively charged as $SiO^-$, depending on the pH of the carrier electrolyte. The surface exhibits strong adsorption of many compounds, resulting in peak tailing and poor reproducibility of migration times. Besides static or dynamic coating of the interior capillary to diminish the capillary wall effect [19,20], as a general procedure it is recommended to wash out the capillary prior to the next analysis as a capillary wall conditioning step to maintain reproducible results. In this study, owing to inclusion complexation, some part of

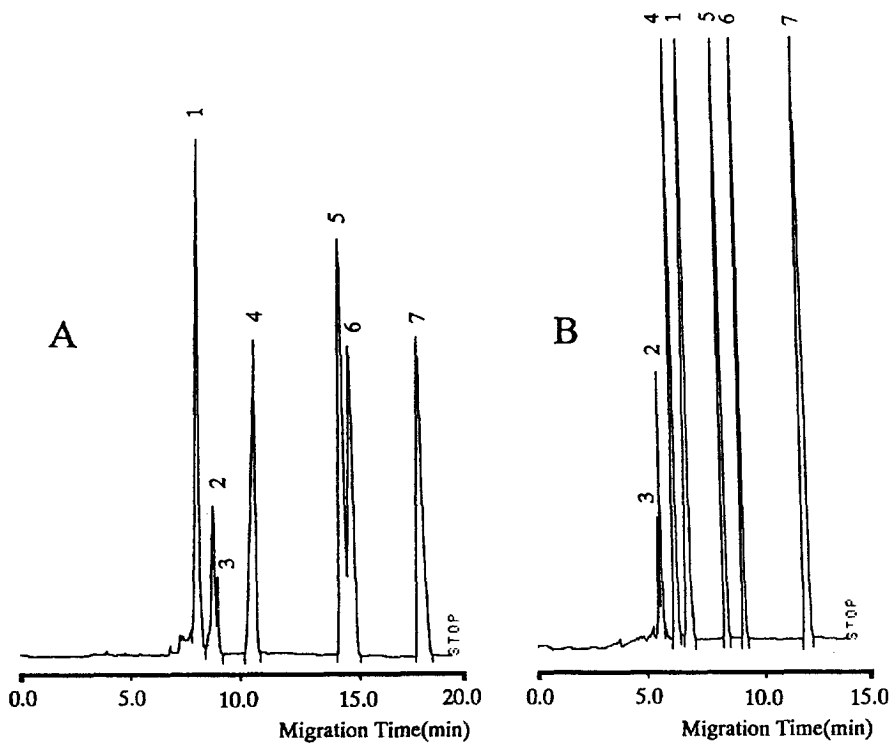


Fig. 4. Effect of β-CD on peak shape of separated dyestuffs; $10^{-4}$ *M* dyes were injected. (A) 0 and (B) 20 m*M* β-CD. See Experimental for other conditions and Fig. 1 for peak identification.

the dyestuffs (yet to be identified) was included in the β-CD cavity and the solute–capillary interaction seems to have been diminished. This effect can be seen in Fig. 4, where in electropherogram B sharper peaks and better resolution are obtained. The peak width at half-height for each solute were calculated from the data taken from electropherograms A and B in Fig. 4 and provided in Table 1. Decreased peak widths and improved peak symmetry in the presence of β-CD could be evidence for lessening of the interaction with the capillary wall.

Table 1
Effects of inclusion complexation with β-CD on peak area, peak height and peak width at half-height (all in arbitrary units) of dyestuffs shown in Fig. 1

| Compound | Without β-CD | | | With 15 m*M* β-CD | | |
|---|---|---|---|---|---|---|
| | Peak area | Peak height | Peak width | Peak area | Peak height | Peak width |
| Erythrosine | 29450 | 3171 | 2.32 | 46003 | 5203 | 2.21 |
| Fast Green FCF | 8357 | 855 | 2.44 | 1880 | 1875 | 1.08 |
| Light Green SF Yellow | 1767 | 424 | 1.04 | 3500 | 978 | 0.89 |
| Sunset Yellow FCF | 26141 | 1990 | 3.28 | 34985 | 5001 | 1.75 |
| Amaranth | 41387 | 2679 | 3.86 | 37569 | 6070 | 1.55 |
| New Coccine | 29187 | 1987 | 3.67 | 46069 | 9278 | 1.24 |
| Tartrazine | 34183 | 2049 | 4.17 | 51203 | 4599 | 2.78 |

In order to explain the effect of complexation on reproducibility, two sets of experiments with five consecutive analyses were carried out. In the first set, β-CD was present in the carrier electrolyte without the washing step between runs. In the second set, β-CD was absent and the washing step was also omitted. The ordinary procedure without β-CD in the carrier electrolyte but with the washing step between runs was also performed for comparison and the results are given in Table 2. The standard deviation for the migration times of the first set was comparable to that for the ordinary procedure. This comparative study indicates the merit of β-CD as an additive to the carrier electrolyte in CE to counteract the adverse capillary wall effect.

### 3.4. Sequential injection study

CE can be used for electrophoretically mixing spatially distinct zones of chemical reagents in which ethanol was determined by enzymatic reaction [21]. In our study, selected dyestuffs and β-CD were sequentially injected into a capillary column through the following steps to obtain information about the extent of interaction between the dyes and β-CD. First a mixture of dyes including erythrosine, Sunset Yellow FCF and amaranth was injected. The power supply was turned on to move the sample zone electrophoretically towards the cathodic end. Then the power supply was turned off and β-CD was injected. Again the power supply was turned on and the main electrophoresis was started. These steps were repeated with different concentrations of β-CD and the electropherograms were recorded. As shown in Fig. 5, the electropherograms obtained had broader than normal CE peaks. The extent of band broadening was different for each solute. One explanation for this observation is that, since β-CD is neutral and the dyestuffs are anionic, after few seconds the β-CD zone can overtake the dyestuff zone and interact with solutes for inclusion complex formation. Any solute that can enter the β-CD cavity due to an increase in molecular mass will move faster than free solutes. By means of this process a velocity difference generated along the sample zone tends to form a broader band. This phenomenon is shown schematically in Fig. 6. Owing to the probability factor, higher β-CD concentrations are more favoured for inclusion complexation, so a wider sample zone and broader bands can be expected when the concentration of β-CD is increased (see Fig. 5). When water was injected instead of β-CD, band broadening was not observed, showing that the major source of band broadening comes from inclusion complex formation. This study also showed that, since in one mixture solutes with different complexation abilities have

Table 2
Reproducibility of migration times (min) for successive analyses with and without a washing step between runs in comparison with the condition of the presence of β-CD in the carrier electrolyte

| Compound | No additive | | | 15 m*M* β-CD | |
|---|---|---|---|---|---|
| | Mean | S.D.[a] | S.D.[b] | Mean | S.D.[a] |
| Erythrosine | 7.45 | 0.38 | 0.03 | 6.59 | 0.05 |
| Fast Green FCF | 8.13 | 0.26 | 0.04 | 5.47 | 0.05 |
| Light Green SF Yellow | 8.28 | 0.28 | 0.03 | 5.36 | 0.03 |
| Sunset Yellow FCF | 9.87 | 0.61 | 0.04 | 6.16 | 0.07 |
| Amaranth | 13.73 | 0.61 | 0.05 | 8.79 | 0.10 |
| New Coccine | 14.04 | 0.33 | 0.03 | 9.32 | 0.05 |
| Tartrazine | 17.50 | 0.33 | 0.03 | 11.83 | 0.06 |

[a] No washing step between runs.
[b] With washing step between two runs.

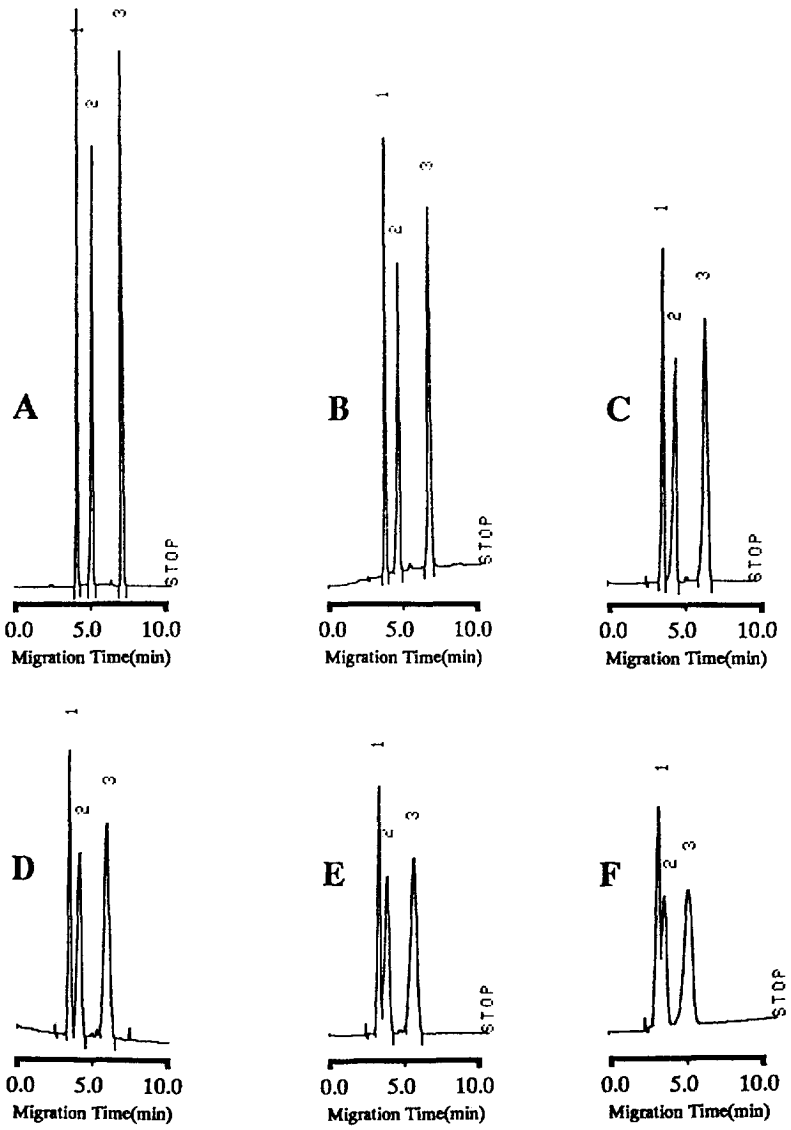


Fig. 5. Electropherograms obtained from sequential injection of dyes and β-CD into a CE column. Peaks: 1 = erythrosine; 2 = Sunset Yellow FCF; 3 = amaranth. Electropherograms: (A) injection of dye mixture only; (B–F) injection of dye mixture followed by injection of (B) 5, (C) 10, (D) 15, (E) 20 and (F) 25 m*M* β-CD. See Experimental for other conditions.

weaker or stronger interactions with β-CD, various extents of band broadening for each peak could be expected. Amaranth, for example, with its relatively high ability for complex formation, showed the broadest band (see Fig. 5). In fact, the extent of band broadening observed is directly related to the ability of dyestuffs to form inclusion complexes with β-CD. The sequential injection method can thus provide an easy and rapid method for qualitative comparison of the inclusion complex formation ability of dyestuffs with β-CD. Generally this method can be ap-

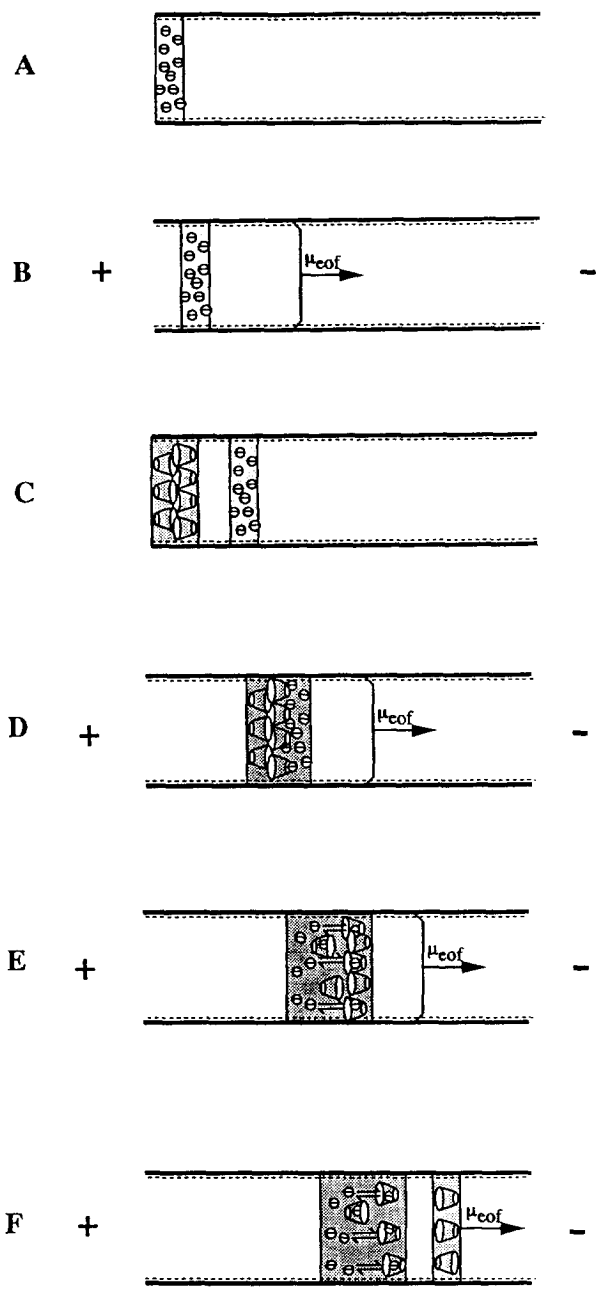


Fig. 6. Schematic representation of different steps in the sequential injection of β-CD and dyestuffs. (A–C) spatial injection of solute and reagent; (D) overtaking of the dyestuff zone by β-CD during the electrophoretic run; (E) equilibriation for inclusion complex formation; (F) separation of β-CD zone from dyestuff zone, which becomes broad owing to the formation of a velocity gradient along the solute zone.

plied in any comparative study when the interaction between reagent and solutes can affect the solute's electrophoretic mobility.

## 4. Conclusion

We have described the advantages of host–guest complexation of food additive dyestuffs with β-CD for the simultaneous determination of these compounds in CE. The complexation with β-CD decreases the charge density and the electrophoretic mobility of the dyestuffs. Consequently, the dyestuffs eluted faster and migration times were decreased by 9.5–39% according to ability of the solutes for complex formation. Although the elution window decreased from 10 to 5.5 min, no loss in separation efficiency was observed. The resolution between the isomeric pairs amaranth and New Coccine was even improved by selective complexation with β-CD. Owing to the diminished interaction of solutes with the capillary wall and faster elution, sharper peaks and improved reproducibility in migration times were observed, which are key points for quantitative studies. Introduction of the sequential injection method described here showed the feasibility of qualitative comparative studies of inclusion complexation phenomena. The extent of band broadening for each solute is directly related to the extent of complexation ability of the solute with β-CD.

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