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Separation of synthetic food colourants by capillary zone electrophoresis in a hydrodynamically closed separation compartment

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Abstract

Separation conditions enabling the complete resolution of eleven permitted synthetic food colourants and some of their subspecies by capillary zone electrophoresis (CZE) were determined. Those conditions involve combining a suitable pH of the carrier electrolyte (pH 6.8) with host–guest complexation effects of β-cyclodextrin. A 300 μm I.D. capillary tube made of fluorinated ethylene–propylene copolymer in a hydrodynamically closed separation compartment was used for the CZE separations. The capillary could accommodate 90-nl sample injection volumes, thus providing limits of detection for the dyes of 11–300 ppb using a photometric detector operating at a wavelength of 254 nm. R.S.D.s of 0.4–3.0% were typical for the determinations of the dyes present in samples at 16 ppm concentrations. Erythrosine, exhibiting residual adsorption, gave more-scattered results under identical working conditions (R.S.D. of ca. 9.0%). The utility of this rapid CZE procedure (migration times of the dyes were 2.5–10.5 min) is illustrated for several practical samples, including soft drink concentrate and liqueur and monitoring of the stability of aqueous solutions of indigo carmine.

Keywords: Capillary electrophoresis; Host–quest complexation; Hydrodynamically closed separation compartment; Food colourants

1. Introduction

The use of synthetic food colourants including some azo dyes, xanthene dyes, triarylmethane and phenylmethane dyes, antraquinones and phenols is restricted by legal regulations of both international and national authorities (see, e.g. Ref. [1]). The lists of permitted food colourants vary in different countries, and the inspection of dyes in food products is strictly controlled by public health institutions. Analytical problems associated with this control range from the determination of dyes in food products and evaluation of food authenticity or adulteration to purity and stability tests of the dyes during processing and storage.

At present, typical analytical schemes employed in the determination of food colourants consist of appropriate sample preparation steps followed by final quantitation procedures. Extraction of the dyes into amyl alcohol [2] or their isolation from solid samples by methanolic or ethanolic solutions of ammonia [2,3] are possible primary sample prepara-

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tion steps. These may be followed by solid-phase extraction on polyamide or Polyclar AT granules [3] or on columns packed with octadecyl-modified silica [4]. These sorbents can also be used in alternative sample preparation procedures for liquid samples.

Although spectrophotometric procedures for mixtures of the dyes (see Ref. [5] and references given therein) are simple and rapid, analytical separation methods based on chromatography principles have a dominant position in practice. Of these, thin-layer chromatography [2,6,7] and, mainly, high-performance liquid chromatography (HPLC) [1,8–10] are preferred for the determination of food dyes.

The ionogenic nature of synthetic food colourants makes high-efficiency capillary electrophoresis (CE) techniques potential analytical alternatives for their determination in food products. So far, however, only capillary isotachophoresis (ITP) [3] and micellar electrokinetic chromatography (MEKC) [11] have been studied in this respect. The former technique was used to separate eight food colourants, but only four could be separated in one analytical run. Nevertheless, it was demonstrated that ITP combined with an appropriate sample pretreatment procedure (extraction followed by a preconcentration on polyamide granules) provides satisfactory analytical results for currently used dye combinations. Seven of the twelve food dyes permitted in Japan could be resolved by MEKC [11].

This work was aimed at investigating the separation of eleven synthetic food colourants, currently permitted in many countries, by capillary zone electrophoresis (CZE). The pH of the carrier electrolyte and host–guest complexations using β-cyclodextrin were the separation parameters studied in this context. We preferred using CZE with a hydrodynamically closed separation compartment to eliminate the negative influences of a varying electroosmotic flow on the migration velocities of the separands caused by changes of ζ-potential (e.g., due to adsorption of matrix constituents [12]). This CZE approach has already been shown to provide very reproducible migration velocities of the separands present in complex matrices such as serum [13], highly proteinous tissue extracts [14] and food [15]. However, it is necessary for a negative impact of the electroosmotic flow on the band broadening in this CZE alternative [16–19] to be eliminated, e.g., via the use of suitable additives in the carrier electrolyte [13–16]. We employed a 300 μm I.D. capillary tube to enhance the load capacity of the column and, consequently, the sample injection volume. In this way we could achieve concentration limits of detection for the analytes in the ppb range for a current photometric detection.

2. Experimental

2.1. Instrumentation

A CS isotachophoretic analyser (Villa-Labeco, Spišská Nová Ves, Slovak Republic) was used in the single-column mode. The separation unit of the analyser consisted of a laboratory-developed CZE injection valve (90-nl sample loop), a column provided with a 0.30 mm I.D. capillary tube made of fluorinated ethylene–propylene (FEP) copolymer (Villa-Labeco) 300 mm in length (240 mm to the detector). The column was provided with a cartridge of an enhanced heat capacity operated at ambient temperature (21–22°C). It was coupled to a UV-M II photometric detector (Pharmacia-LKB, Uppsala, Sweden) operating at 254 nm.

The signal from the photometric detector was registered by a TZ 4200 line recorder (Laboratorní Prístroje, Prague, Czech Republic). In parallel the data were acquired by a 386 DX computer connected to the detector via a UniLab data acquisition unit (Fitek, Šala, Slovak Republic). Software, ITP.Soft-Win, was obtained from KasComp (Bratislava, Slovak Republic).

2.2. Chemicals

Chemicals used for the preparation of the electrolyte solutions were bought from Serva (Heidelberg, Germany), Sigma (St. Louis, MO, USA), Lachema (Brno, Czech Republic) and Merck (Darmstadt, Germany). Polyethyleneglycol 5 000 000 (PEG) obtained from Serva was used as an additive in the carrier electrolyte solutions to suppress electroosmosis. Synthetic food colourants were obtained from Aldrich (Steinheim, Germany) and Aroco (Prague, Czech Republic).

Water from an Aqualabo two-stage demineraliza-

tion unit (Aqualabo, Brno, Czech Republic) was further demineralized by circulation through polytetrafluoroethylene cartridges packed with Amberlite MB-1 mixed-bed ion exchanger (Serva). The electrolyte solutions were prepared from freshly recirculated water and filtered through a 0.8-μm syringe filter (Sigma).

2.3. Samples

Stock solutions of the food colourants (see Table 1) were prepared at 400 ppm concentrations in demineralized water. For CZE experiments they were appropriately diluted with water and a 0.01 mol/l aqueous solution of Na_2SO_4 so that the final concentration of Na_2SO_4 in the injected sample was 1 mmol/l. This electrolyte in the sample solutions was found to minimize adsorptions of the analytes on the walls of the polyethylene sample containers.

Samples of soft drink concentrates and liqueurs were kindly provided by the Specialized Institute of Hygiene and Epidemiology (Banská Bystrica, Slovak Republic). When required, the samples were diluted in the same way as the stock solutions of food colourants.

3. Results and discussion

In order to find separating conditions that provide a complete resolution of the food colourants studied (Table 1) in one CZE run, two approaches were investigated in this work:(1) Separations based on differences in the effective mobilities of the dye constituents originating in their pK values and in the (actual) mobilities of the dye anions. Here, well-known relationships between the effective mobilities, pK values, actual mobilities and pH of the carrier electrolyte [20] were effective.(2) Separations using differences in the effective mobilities of the dye constituents due to different host–guest complexations of the dye anions with β-cyclodextrin. In this instance both the stability constants and actual mobilities of the complex particles influenced the effective mobilities [22,23] of the dye constituents.

Table 1
List of synthetic food colourants

No.	Colourant	C.I.	E No.	Comments
1	Tartrazine	19140	102	Trisodium 5-hydroxy-1-(4-sulphonatophenyl)-4-(4-sulphonatophenylazo)pyrazole-3-carboxylate
2	Ponceau 4R (new coccine)	16255	124	Trisodium 7-hydroxy-8-(4-sulphonato-1-naphthylazo)naphthalene-1,3-disulphonate
3	Amaranth	16185	123	Trisodium 3-hydroxy-4-(4-sulphonato-1-naphthylazo)naphthalene-2,7-disulphonate
4	Brilliant Black BN	28440	151	Tetrasodium 4-acetamido-5-hydroxy-6- [7-sulphonato-4-(4-sulphonatophenylazo)-1-naphthylazo]naphthalene-1,7-disulphonate
5	Sunset Yellow FCF	15985	110	Disodium 6-hydroxy-5-(4-sulphonato-phenylazo)-naphthalene-2-sulphonate
6	Indigo Carmine	73015	132	Disodium 3,3′-dioxo-2,2′-bi-indolylidene-5,5′/7′/-disulphonate
7	Chromotrope FB (carmoisine)	14720	122	Disodium 4-hydroxy-3-(4-sulphonato-1-naphthylazo)naphthalene-1-sulphonate
8	Patent Blue V	42051	131	Sodium compound of [4,4-diethylaminophenyl-(5-hydroxy-2,4-disulphonphenyl-methylidene)-2,5-cyclohexadien-1-ylidene]-diethyl-ammonium hydroxide inner salt
9	Brilliant Blue FCF (erioglaucine)	42090	133	Disodium [4-(N-ethyl-3-sulphonato benzylamino)phenyl]-[4-(N-ethyl-3-sulphonatobenzyl-imino)cyclohexa-2,5-dienylidene]toluene-2-sulphonate
10	Erythrosine (erythrosin B)	45430	127	Disodium 2-(2,4,5,7-tetraiodo-3-oxido-6-oxoxanthen-9-yl)benzoate
11	Quinoline Yellow	47005	104	Sodium salts of mixture of disulphonates (principally), monosulphonates and trisulphonates of 2-(2-quinolyl)indan-1,3-dione

3.1. CZE separations of the food colourants at various pH values

The role of pH in CZE separations of the food colourants was investigated in electrolyte systems 1–4 (Table 2). The studied pH range determined by these electrolyte systems was restricted as xanthene dyes precipitate at a low pH [21] and azo dyes decompose at a high pH [11]. Dependences of the effective mobilities of the dye constituents on the pH of the carrier electrolyte are presented in Fig. 1. Here, the relative values of the effective mobilities were plotted to correct for differences in the migration times (reciprocal of the effective mobilities) due to different conductivities of the carrier electrolytes. Ponceau 4R, which has an effective mobility independent of pH within the pH interval studied, served as a reference constituent. Although in this way the different ionic strength effects of the carrier electrolytes [18,20] were not completely corrected, we can conclude from Fig. 1 that only the effective mobilities of chromotrope FB, patent blue V, erythrosine and quinoline yellow can be significantly influenced via the pH of the carrier electrolyte. Considering a relationship between the effective mobility of the separated constituent, its pK value(s), actual mobilities of the ionic forms involved and pH of the carrier electrolyte [20], it is clear that only these constituents have pK values which can be effectively employed in CZE separations of the food colourants. For quinoline yellow (11a and 11b in Fig. 1 and Fig. 2d) the change in the effective mobility concerned only one of its subspecies (see Table 1), so that at pH 9.28 this dye migrated split into two peaks (Fig. 2d).

CZE separabilities of the food colourants at various pH values are illustrated by electropherograms in Fig. 2. We can see that in each of the electrolyte systems 1–3 pairs of the dyes could not be resolved, and all of them failed to resolve indigo carmine and sunset yellow FCF. Since food products contain only limited numbers of these colourants, this need not be a disadvantage, and the electrolyte systems 1–4 (Table 2) can provide suitable separating conditions in some instances.

In the analysis of practical samples, it is better when the number of migrating matrix constituents (potential interferents) is reduced. This can be achieved by reducing the effective mobilities of weak anionic matrix constituents (ideally to zero effective mobilities), i.e., by performing the CZE separations at a low pH value. Such an approach is applicable for all of the dyes with the exception of erythrosine (Fig. 2a). This xanthene dye forms only slightly soluble erythrosinic acid in the solutions at pH 3–4 [21] and exhibits strong adsorption at pH 4.13 (Fig. 3a). At this pH its adsorption losses were very serious, and the dye could not be detected when

Table 2
Electrolyte systems

Parameter	1	2	3	4	5
Solvent	H_2O	H_2O	H_2O	H_2O	H_2O
Anion	Propionic	MES	TES	Glycine	TES
Concentration (mmol/l)	30	80	30	40	30
Counter-ion	β-alanine	Histidine	Imidazole	BTP	Imidazole
Concentration (mmol/l)	25	8	8	21	8
pH	4.13	5.28	6.84	9.28	6.84
Additive[a]	PEG	PEG	PEG	PEG	PEG
Concentration (%, w/v)	0.2	0.2	0.2	0.2	0.2
Complexing agent	–	–	–	–	β-CD
Concentration (mmol/l)	–	–	–	–	3–9

MES=2-(N-morpholino)ethane sulphonic acid; TES=N-Tris(hydroxymethyl)methyl-2-aminoethane sulphonic acid; BTP=1,3-Bis[tris-(hydroxymethyl)-methylamino]propane; PEG=polyethyleneglycol; β-CD=β-cyclodextrin.

[a] The capillary tube was washed before a series of experiments with a 0.5% (w/v) aqueous solution of methylhydroxyethylcellulose 30000 (Serva).

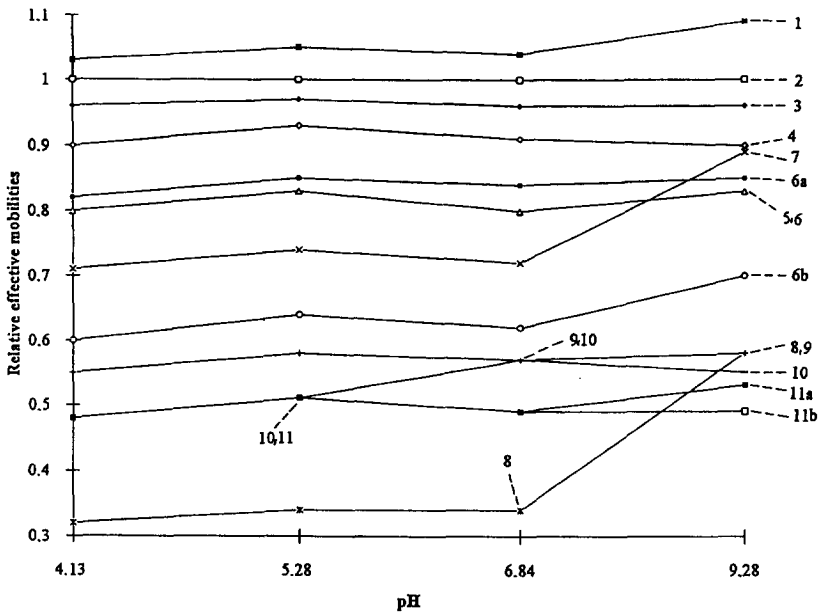

Fig. 1. Dependence of the relative effective mobilities of the food colourants on the pH of the carrier electrolyte. Ponceau 4R served as a reference constituent in calculating the values of the relative effective mobilities. The experiments were carried out in the electrolyte systems 1–4 (Table 2) with a 150 μA driving current. For the assignments of the colourants, see Table 1.

present in the injected sample at 20 ppm (Fig. 2a). Also at pH 5.28 its tailing was considerable (Fig. 3b). Improved peak shapes were obtained when the pH of the carrier electrolyte was 6.84 or higher (Fig. 3c and d). The data in Fig. 1 indicate that this improvement is coincidental with an enhanced ionization (higher negative charge) of the dye.

3.2. CZE separations of the food colourants via host–guest complexation

Dependence of the effective mobilities of the dye constituents on the concentration of β-cyclodextrin in the carrier electrolyte is plotted in Fig. 4. These plots show that this complexing agent influenced the effective mobilities of the dyes in a differentiating manner. It is apparent [23] that the stability constants and the ionic (actual) mobilities of the dye anions and their complexes are responsible for the effect. At the concentrations of β-cyclodextrin offering a complete CZE resolution of the food colourants (5–7 mmol/l), the course of the dependence allows a certain classification of their contributions to the effective mobilities of the dye constituents to be made. (i) The actual mobilities of the dye anions at pH 6.84 determine the effective mobilities of erythrosine and Ponceau 4R (no detectable influence of β-cyclodextrin). (ii) Both the stability constants and the actual mobilities of the complexes determine the effective mobilities of tartrazine and sunset yellow FCF. (iii) The actual mobilities of the complexes determine the effective mobilities of the rest of the food colourants (the plots at 5–7 mmol/l concentrations of β-cyclodextrin are flat). Here, however, we have to keep in mind that a relative scale for the effective mobilities was employed and, at least, ionic strength effects could not be corrected for completely [18,20].

From the dependences in Fig. 4 and the electropherograms in Fig. 5, we can conclude that the most significant effects of β-cyclodextrin were found for tartrazine, chromotrope FB and sunset yellow FCF. To a lesser extent it influenced the migration properties of brilliant black BN, quinoline yellow and brilliant blue FCF. β-Cyclodextrin enabled the resolution of sunset yellow FCF and indigo carmine (inseparable in the electrolyte systems without β-cyclodextrin as shown in Fig. 2). Resolution of the

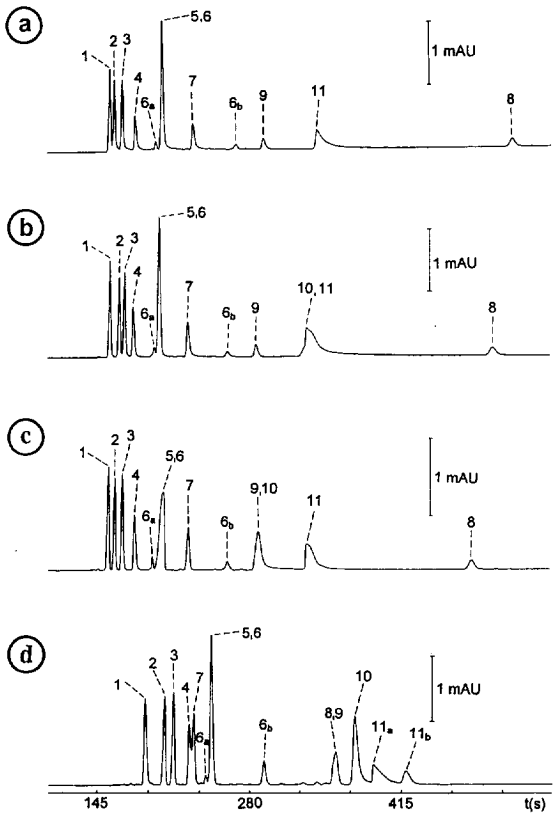

Fig. 2. Electropherograms from the separations of the synthetic food colourants at various pH: (a) pH 4.13, (b) pH 5.28, (c) pH 6.84, (d) pH 9.28. The driving current was 150 μA. For the peak assignments see Table 1, and detail compositions of the electrolyte systems are given in Table 2. The concentrations of the analytes were 20 ppm.

subspecies of quinoline yellow (11a and 11b in Fig. 4 and Fig. 5) and brilliant blue FCF (9a and 9b in Fig. 4 and Fig. 5) can also be ascribed to the presence of β-cyclodextrin in the carrier electrolyte.

These differences in the complexing effect of β-cyclodextrin are due to the various ways in which the dye anions fit the cavity of the complexing agent [22,23]. When we consider intramolecular interactions involved in these complexation reactions (see, e.g. Refs. [22,23] and references given therein) it is apparent that there is no simple explanation of the differentiating effect of β-cyclodextrin on the molecular level. Here, however, only a detailed study with appropriately chosen series of model compounds can provide the relevant information.

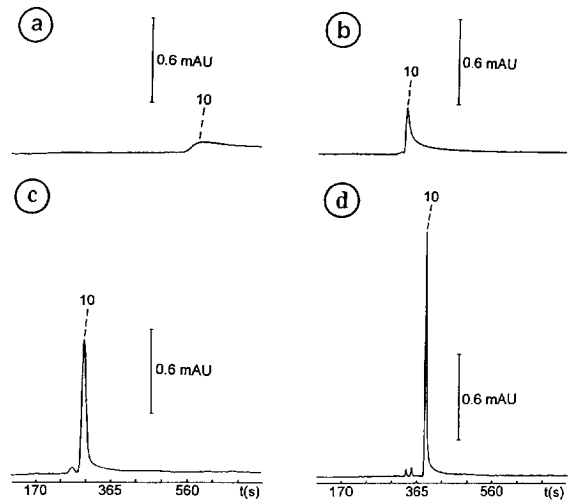

Fig. 3. Profiles of the erythrosine zone at various pH values: (a) pH 4.13, (b) pH 5.28, (c) pH 6.84, (d) pH 9.28. The driving current was stabilized at 150 μA. For the peak assignments see Table 1, and detail compositions of the electrolyte systems are given in Table 2. The concentration of erythrosine was 80 ppm.

3.3. Some analytical performance characteristics and examples of analytical applications

The separation efficiencies of the dyes as obtained in the electrolyte systems with and without β-cyclodextrin are summarized in Table 3. These data show that the complexing additive positively influenced the separation efficiencies of indigo carmine and erythrosine. On the other hand, the separation efficiencies of tartrazine and amaranth were significantly lower in its presence. The origins of these effects are unknown. The following factors probably could play certain roles. (i) Some of the impurities present in the dyes are overlapped by the peaks of the main components, as found for brilliant blue FCF and quinoline yellow (see Fig. 5a and b). (ii) A reduced adsorption dispersion [24] can explain a considerable increase of the efficiency for erythrosine in the presence of β-cyclodextrin. (iii) Rates of the complex equilibria can be comparable with the migration velocities, and this can result in kinetic effects known as electrodiffusion [25]. This can explain reduced efficiencies for tartrazine and amaranth in the presence of β-cyclodextrin.

The migration times of the dyes were characterized by the relative standard deviations in the range

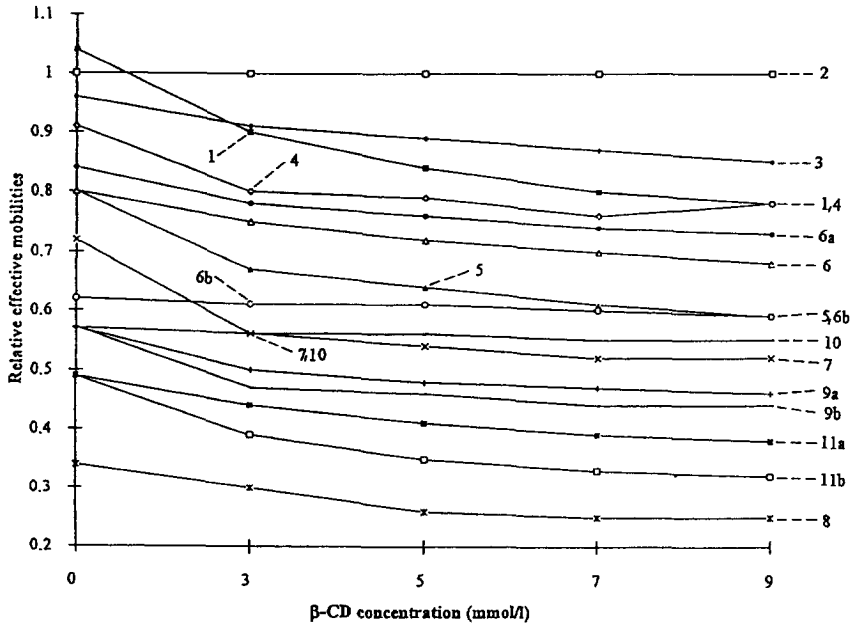

Fig. 4. Dependence of the relative effective mobilities of the food colourants on the concentration of β-CD in the carrier electrolyte. Ponceau 4R served as a reference constituent. The experiments were carried out in electrolyte system 5 (Table 2) with a varying concentration of β-CD and a 150-μA driving current. For the assignments of the colourants, see Table 1.

of 0.5–1.0%, with the higher values being typical for the most mobile constituents.

The limits of detection (Table 3) were estimated as proposed for elution chromatography [26]. The values obtained under our conditions are considerable lower than those reported for MEKC in a 75 μm I.D. capillary tube [11]. Undoubtedly, this is at least in part due to the higher load capacity of the 300 μm I.D. column, allowing the injection of 90-nl sample volumes without any detectable band broadening caused by the injection [18]. A longer path length in the detection cell was also favourable for such an I.D. of the column.

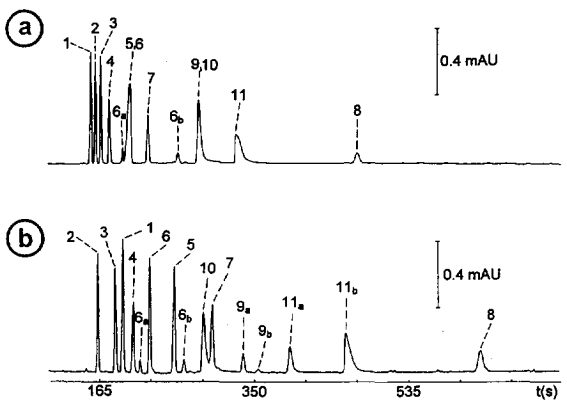

Fig. 5. Electropherograms from the separations of the synthetic food colourants at pH 6.84 without (a) and with (b) β-CD (5 mmol/l). The driving current was 150 μA. For the peak assignments see Table 1, and the composition of the electrolyte system is given in Table 2. The concentration of the analytes in the injected model mixture (90 nl) was 20 ppm.

Reproducibilities in the peak-area measurements for 16 ppm concentrations of the dyes are given in Table 3. With the exception of erythrosine, both manual and automatic procedures gave satisfactory results. A higher scatter of the data for erythrosine can probably be ascribed to higher uncertainties in finding the end of the peak due to a residual adsorption of the dye. In general, agreements of the data for the manual and automatic procedures were acceptable. Somewhat higher dispersions in the automatic peak-area measurements for less mobile dyes are probably due to higher uncertainties in identifying the start and the end of the CZE peaks using current elution chromatography approaches in the data analysis [27].

Table 3
Limits of detection, separation efficiencies and reproducibilities of the peak areas of the dyes

Constituent[a]	Detection limit[b] (ppb)	Separation efficiency[c] (N/m)		R.S.D. of the peak area[d] (%)	
		System No. 3	System No. 5 (7 mmol/l β-CD)	Automatic	Manual
1	29	113 000	50 000	0.4	0.4
2	33	134 000	163 000	0.3	0.9
3	11	145 000	46 000	1.2	1.0
4	49	111 000	101 000	2.3	0.8
5	33	181 000	154 000	0.7	0.6
6	68	70 000	144 000	0.9	1.1
7	66	128 000	137 000	1.5	3.0
8	267	129 000	113 000	4.8	0.9
9a	301	98 000[e]	149 000	2.5	0.5
9b	–	–	129 000	–	7.9
10	51	116 000	186 000	13.8	9.1
11a	160	30 000[e]	115 000	6.0	1.5
11b	85	–	72 000	5.4	1.3

R.S.D.=relative standard deviation.
[a] For the assignments of the constituents see Table 1.
[b] The average values calculated from the data obtained for three repeated measurements.
[c] N/m=number of theoretical plates per meter.
[d] The average values calculated for three repeated measurements for 16 ppm concentrations of the analytes.
[e] Evaluated for single peaks of 9 and 11 (see Fig. 5a).

Electropherograms in Fig. 6 were obtained from the separations of food colourants present in practical samples. Liquid samples were preferred for these illustrations as no sample preparations (only dilutions) were required before the CZE runs. The claimed colourants were detected in both samples, and from the electropherograms it is apparent that a minimum number of migrating constituents was detected. A liqueur sample containing 30% (v/v) of ethanol had to be diluted as a higher concentration of ethanol in the injected sample pulse led to a local overheating, sometimes associated with a bubble formation in the injection valve followed by an interruption of the driving current.

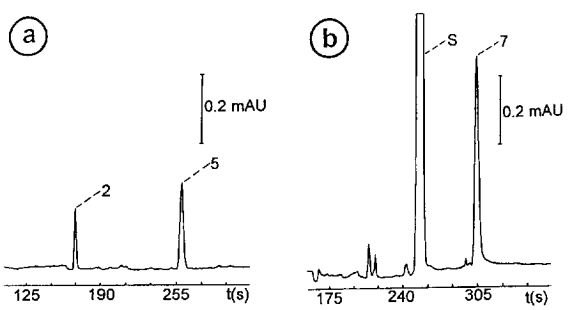

Fig. 6. CZE separations of the colourants present in food products. (a) Aperol liqueur (5-fold dilution). (b) Malina Sandy soft drink concentrate (2-fold dilution). The separations were carried out in the electrolyte system 5 (Table 2) with a 6 mmol/l concentration of β-CD. The driving current was 150 μA. For the peak assignments see Table 1.

Besides the food colourants soft drink concentrates may contain sorbic acid (food preservative) at considerably higher concentrations. This acid (S, in Fig. 6b), having a high molar absorptivity at the detection wavelength used in this work, should be considered as a potential interferent in the analysis of dyes. Under our working conditions its migration position was identical to that of sunset yellow FCF. This peak overlap problem can probably be easily solved by using a more appropriate detection wavelength.

The purities of the food colourants are also of current analytical concern [1]. From the results presented in the previous part it is apparent that under our working conditions, subspecies (impurities) present in some dyes (indigo carmine, brilliant blue FCF, quinoline yellow) could be separated. Although no special attention was paid to this subject, a series of electropherograms in Fig. 7 is

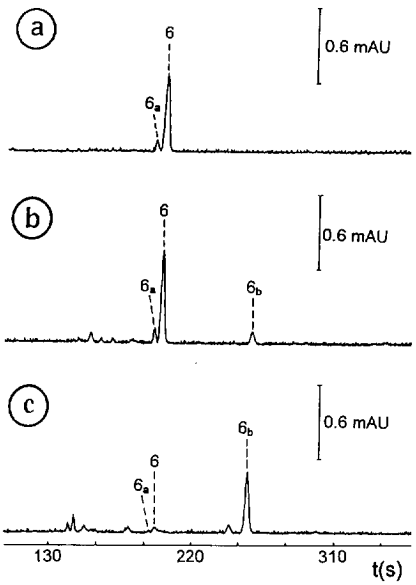

Fig. 7. CZE monitoring of an aqueous solution of indigo carmine (6). (a) Freshly prepared solution. (b) The same solution as in (a) after one month. (c) The same solution as in (a) after three months. The concentration of indigo carmine in the solution was 20 ppm. The driving current was 150 μA, and the separations were carried out in the electrolyte system 3 (Table 2).

given to illustrate the possibilities of CZE in this area. Here, CZE monitoring of an aqueous solution of indigo carmine was carried out to identify the origin of the impurities currently detected in the dye solution during its storage. It is worth mentioning that the main component disappeared in the stock solution when stored for a longer time.

4. Conclusions

By minimizing the adsorption of erythrosine via an appropriate choice of pH, the effective mobilities of the studied food colourants and some of their subspecies could be optimized via differentiating complexations of β-cyclodextrin in such a way that their complete resolutions were possible in one CZE run. The use of a 300 μm I.D. capillary tube was effective in enhancing the sample injection volume up to 90 nl so that the limits of detection in the range 11–300 ppb could be achieved without any pre-column concentration. Such an I.D. is less favourable as far as thermal dispersion is concerned [18]. However, the separation efficiencies as currently achieved in our experiments (typically, $1.3–1.5 \cdot 10^5$ theoretical plates per meter) were influenced to tolerable extents by the thermal effects. Considering the results related to the quantitations (reproducibilities in the peak-area measurements), short (2.5–10.5 min) and reproducible migration times of the dyes, it is apparent that CZE is a promising alternative to the determination of synthetic food colourants. However, detail studies which focus on various food matrices will be essential in proving these expectations.

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