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Determination of synthetic colours in confectionery and cordials by micellar electrokinetic capillary chromatography

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Abstract

A rapid method for the determination of a number of commonly used synthetic colours permitted in confectionery and cordial in Australia by micellar electrokinetic capillary chromatography (MEKC) is described. The synthetic colours, green S, brilliant blue, erythrosine B, allura red, indigo carmine, sunset yellow, azorubine, amaranth, ponceau and tartrazine are well separated in less than 20 min using a 65 cm × 50 μm uncoated fused-silica capillary column with a buffer comprising of 15% acetonitrile and 85% 0.05 *M* sodium deoxycholate/0.005 *M* potassium dihydrogenorthophosphate/0.005 *M* sodium borate pH 8.6 operating at 30 kV. The compounds are detected with UV detection at 214 nm. Quinoline yellow and brilliant black can also be determined using this system. The levels of synthetic colours in a variety of confectionery items and cordials were in good agreement with those determined by high-performance liquid chromatography (HPLC). The level of reporting for the MEKC procedure is 5 mg/kg. This procedure is faster and less costly to operate than the HPLC method currently used in our laboratory.

1. Introduction

Analytical procedures based on the relatively new technique of micellar electrokinetic capillary chromatography (MEKC) are rapidly gaining acceptance as rugged analytical methods [1–5]. MEKC separations exhibit superior resolution to high-performance liquid chromatography (HPLC) separations, have the same order of repeatability and are faster and less costly to operate than HPLC methods [6]. MEKC was introduced by Terabe et al. [7] in 1984. With this technique, an electrophoretic buffer is modified with an ionic surfactant to provide a phase for chromatographic separation. Both anionic and cationic surfactants have been used as micelle modifiers to separate mixtures that cannot be easily separated using traditional capillary zone electrophoresis (CZE) [6]. Sodium dodecyl sulphate (SDS) and cetyltrimethylammonium bromide (CTAB) are commonly used as surfactants for MEKC. Bile salts (e.g. sodium cholate, sodium deoxycholate, sodium taurodeoxycholate) can also be used as anionic surfactants. These compounds exhibit different structural and aggregation properties to the other anionic surfactants and so affect the separations differently [8]. Nishi et al. [9] reported the separation and determination of the ingredients of a cold medicine by MEKC using sodium cholate and sodium deoxycholate as buffer modifiers. The separations were superior to when SDS was used as the

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buffer modifier. We recently reported that buffers containing sodium deoxycholate gave better separation of *l*-ascorbic acid and *d*-erythorbic acid in fruit juices and wines than buffers modified with SDS when analysed by MEKC [5]. The addition of an organic solvent, (e.g. methanol, acetonitrile, tetrahydrofuran, dimethyl sulphoxide, dimethylformamide) to the buffer can also affect the separation of complex mixtures and buffers modified with various solvents have been used in a number of applications [6]. We have separated heroin, cocaine and related compounds in illicit drug seizures with buffers modified with acetonitrile [1,2]. We have also used a buffer modified with dimethyl sulphoxide to achieve the separation of a number of amphetamines [10], and we are currently investigating the separation of morphine alkaloids with a buffer modified with dimethylformamide [11]. Texts by Li [6], Weinberger [12] and by Kuhn and Hoffstetter-Kuhn [13] provide detailed descriptions of MEKC as well as many other examples of this technique.

A number of methods for the quantitative determination of synthetic colours using HPLC with UV–Vis detection as the determinative step have been reported in the literature [14–16]. Clarke and Wells [17] recently reported the separation of a number of synthetic colours by MEKC using a 75 cm × 50 μm I.D. uncoated fused-silica capillary column with a buffer comprising of 15% acetonitrile and 85% 0.05 *M* SDS/0.01 *M* sodium borate pH 9.2. The compounds were detected by UV at 214 nm. The separation was used to demonstrate the excellent separating potential of MEKC but was not used to quantitatively determine the levels of the colours. The related technique of isotachophoresis with conductivity detection has been used to quantitatively determine a number of synthetic colours in foods [18].

This paper describes the separation of green S, brilliant blue, erythrosine B, allura red, indigo carmine, sunset yellow, azorubine, amaranth, ponceau and tartrazine by MEKC using a phosphate/borate buffer modified with sodium deoxycholate and acetonitrile with the subsequent quantitation of the colours in some confectionery and cordial samples available in Australia. Azorubine, which is rarely used as an artificial colorant, was used as the internal standard for these determinations. Two other artificial colours, quinoline yellow and brilliant black, can also be determined using this electrophoretic system.

2. Experimental

2.1. Reagents

Amaranth, tartrazine, erythrosine B, indigo carmine, sodium deoxycholate, sodium cholate and SDS were obtained from Sigma (St. Louis, MO, USA). Brilliant blue, allura red and azorubine were obtained from Tokyo Kasei Kogyo (Tokyo, Japan). Green S, sunset yellow, ponceau, quinoline yellow, brilliant black, brown HT and chlorophyll copper complex were gifts from Dr. David Briggs and Ms. Geraldine Keogh, Faculty of Health and Behavioural Sciences, Deakin University, Geelong, Australia. Tetra-*n*-butylammonium hydroxide solution (40%) was obtained from Fisons (AAG) (Homebush, Australia). Tetrabutylammonium phosphate was obtained from Ajax Chemicals (Auburn, Australia). All other chemicals and solvents were analytical-reagent grade or HPLC grade and used without further purification.

2.2. MEKC buffer

A 2.16-g amount of sodium deoxycholate was dissolved in 100 ml of a 1:1 mixture of 0.01 *M* sodium borate and 0.01 *M* potassium dihydrogenorthophosphate. The pH of the solution was 8.6. A 15-ml volume of acetonitrile was added to 85 ml of the buffer and the solution filtered through a 0.8-μm PTFE filter disc before use.

2.3. Apparatus

MEKC

The samples were analysed with an uncoated fused-silica capillary column (65 cm × 50 μm I.D.) with an effective length to the detector of

40 cm (Polymicro Technologies, AZ, USA), using an Isco Model 3140 electropherograph (Isco, Lincoln, NE, USA) operating at 30 kV and at 25°C. The sample solutions were loaded under vacuum (vacuum level 2, 10 kPa s) and the colours were detected at 214 nm at 0.005 AUFS. The detector response was linear to 100 μg/ml. The capillary was flushed with running buffer for 2 min between analyses. Also, the running buffer was replaced after fifteen analyses. The capillary was cleaned on a weekly basis by washing with 0.1 *M* sodium hydroxide for 10 min followed by deionised water for 10 min before filling with running buffer. Electropherograms were recorded with either the ICE data management and control software supplied with the electropherograph or a HP 3350 laboratory data system (Hewlett-Packard, Palo Alto, CA, USA).

HPLC

The analyses were performed with a Model 600E HPLC pump, Model 712 WISP and a Model 490 programmable multiwavelength UV detector using a 4-μm C_{18} NovaPak 8 × 10 cm Radial-Pak cartridge equipped with a C_{18} precolumn (Waters Chromatography Division of Millipore, Milford, MA, USA) using gradient elution. The solvents were degassed before use.

Solvent A was deionised water. Solvent B was methanol and solvent C was 0.1 *M* tetra-*n*-butylammonium hydroxide in deionised water, neutralised with saturated potassium dihydrogenphosphate. Eluent flow-rate was 1.0 ml/min for 9 min, 1.5 ml/min from 10 to 15 min and returning to 1 ml/min after 17 min. The eluent composition was 45% A, 50% B and 5% C for the first 5 min (curve 1), changing to 15% A, 80% B and 5% C to 6 min (curve 6). This eluent composition was maintained to 15 min when it was changed to 45% A, 50% B and 5% C (curve 6) to 17 min. The system was allowed to equilibrate for 20 min before the next sample solution was injected onto the column. The column eluate was monitored at two wavelengths; 475 nm for the yellow and red dyes and 600 nm for the blue and green dyes. The detector output was set at 0.2 AUFS. The chromatograms were displayed on a dual-pen Omniscribe chart recorder (Houston Instruments, USA). Peak areas obtained from a HP 3350 laboratory data system were used in the calculations.

2.4. Samples and standards

MEKC

Samples.

The confectionery and cordials were products available for purchase in Australia and were analysed within the recommended "use by" dates. The confectionery was pulverised in either a commercial food processor or with a mortar and pestle. A 5-g amount of the powdered confectionery sample was weighed accurately and mixed with 25 ml methanol–water (20:80) and the mixture shaken vigorously for 5 min. The solution was centrifuged at 1000 *g* for 5 min and the supernatant filtered through a 5-μm cellulose acetate filter disc. A 1-ml volume of 0.05 *M* tetra-*n*-butylammonium phosphate solution was added to the supernatant and the solution passed through a C_{18} Sep-Pak cartridge which had been previously activated with methanol followed by 1% acetic acid in water. The retained colours were washed with 4 ml of water and then eluted from the C_{18} Sep-Pak cartridge with methanol. The methanol was removed with a stream of nitrogen. A 0.1-ml volume of azorubine solution (1000 μg/ml) was added to the residue and the mixture diluted to 2 ml with deionised water. The solutions were filtered through a 0.45-μm cellulose acetate filter disc before analysis. The cordials were treated in the same way except that 5 ml was used.

Standards.

Standard solutions were prepared in deionised water with azorubine as the internal standard at a final concentration of 50 μg/ml. The solutions were filtered through a 0.45-μm cellulose acetate filter disc before analysis.

HPLC

The sample solutions were prepared as described for the MEKC analyses, except that the final volume was 20 ml. The standard solutions

were prepared by dissolving the colours in deionised water. No internal standard was used for the HPLC analyses.

3. Results and discussion

Clarke and Wells [17] recently reported the separation of a number of synthetic colours using a 75 cm × 50 μm I.D. uncoated fused-silica capillary column with a buffer comprising of 15% acetonitrile and 85% 0.05 *M* SDS/0.01 *M* sodium borate with an applied voltage of 25 kV and a temperature of 25°C. We attempted to use this buffer with a 65 cm × 50 μm I.D. uncoated fused-silica column and an applied voltage of 30 kV to separate green S, brilliant blue, erythrosine B, allura red, indigo carmine, sunset yellow, azorubine, amaranth, ponceau and tartrazine. These compounds are the most commonly used synthetic food colours permitted for use within Australia [19]. The separation is displayed in Fig. 1A. This buffer was unsuitable as azorubine, amaranth and ponceau comigrated. However, the other compounds were well separated within 15 min. Separation was complete when 0.01 *M* sodium borate was replaced with a 1:1 mixture of 0.01 *M* potassium dihydrogenorthophosphate and 0.01 *M* sodium borate pH 8.6 (Fig. 1B). A similar separation was achieved when SDS was replaced with sodium deoxycholate (Fig. 1C). The peak shape for sunset yellow improved using this buffer, however the peak shape for tartrazine now showed tailing. Peak shapes similar to tartrazine are not uncommon in capillary electrophoretic separations, and can be used for quantitative determinations [20]. Sodium cholate was unsuitable as a micelle modifier as tartrazine migrated as a very broad peak and allura red split into two poorly resolved peaks (Fig. 1D). The peak shapes change from the SDS buffer to the sodium deoxycholate and sodium cholate buffers. These changes could be due to the interactions between the analytes and the micelle/organic solvent in the buffer. We use a number of MEKC/CZE methods in our laboratory in which the analytes are dissolved in water without any problems with poor peak shapes.

The buffer containing sodium deoxycholate as the micelle modifier was used for the quantitative analyses as this gave the best separation and the best peak shapes for the majority of the compounds. The separation of the ten compounds and the ratio of the migration times to the migration time of the internal standard (azorubine) were reasonably consistent over twenty repetitive injections using this buffer. Also, the peak shapes were maintained over twenty repetitions. This was in contrast to the buffer modified with SDS, where allura red and indigo carmine became quite broad with time. The repeatability data (relative standard deviations, R.S.D.s) for seven consecutive injections of a number of standards of different concentrations were satisfactory (e.g. green S, 10 μg/ml, R.S.D. 4.3%; 25 μg/ml, R.S.D. 2.0%; 50 μg/ml, R.S.D. 2.1%; 100 μg/ml, R.S.D. 2.1%; tartrazine, 10 μg/ml, R.S.D. 4.1%; 25 μg/ml, R.S.D. 2.8%; 50 μg/ml, R.S.D. 1.9%; 100 μg/ml, R.S.D. 2.5%).

The addition of 15% acetonitrile and 0.05 *M* sodium deoxycholate to the phosphate/borate buffer was necessary to achieve the desired separation. Electropherograms showing the separation of the colours with the different buffers are displayed in Fig. 2. Replacing acetonitrile with either methanol or dioxane in the buffer resulted in poor peak shapes and long run times. Dimethylformamide and dimethyl sulphoxide, which have been used successfully in our laboratory to assist in the separation of complex mixtures [10,11] were unsuitable as they absorb UV light at the detection wavelength used for this analysis (214 nm). The compounds were detected at a common wavelength of 214 nm as it was not possible to detect the synthetic colours in the visible region of their spectra with the instrument used for the analyses.

A range of confectionery items and cordials were analysed by MEKC and the levels of the colours in the products were compared with the levels obtained from the HPLC procedure that is currently used in our laboratory [21]. Azorubine was not present in any of the confectionery samples and so was used as the internal standard for the MEKC analyses. Azorubine was also

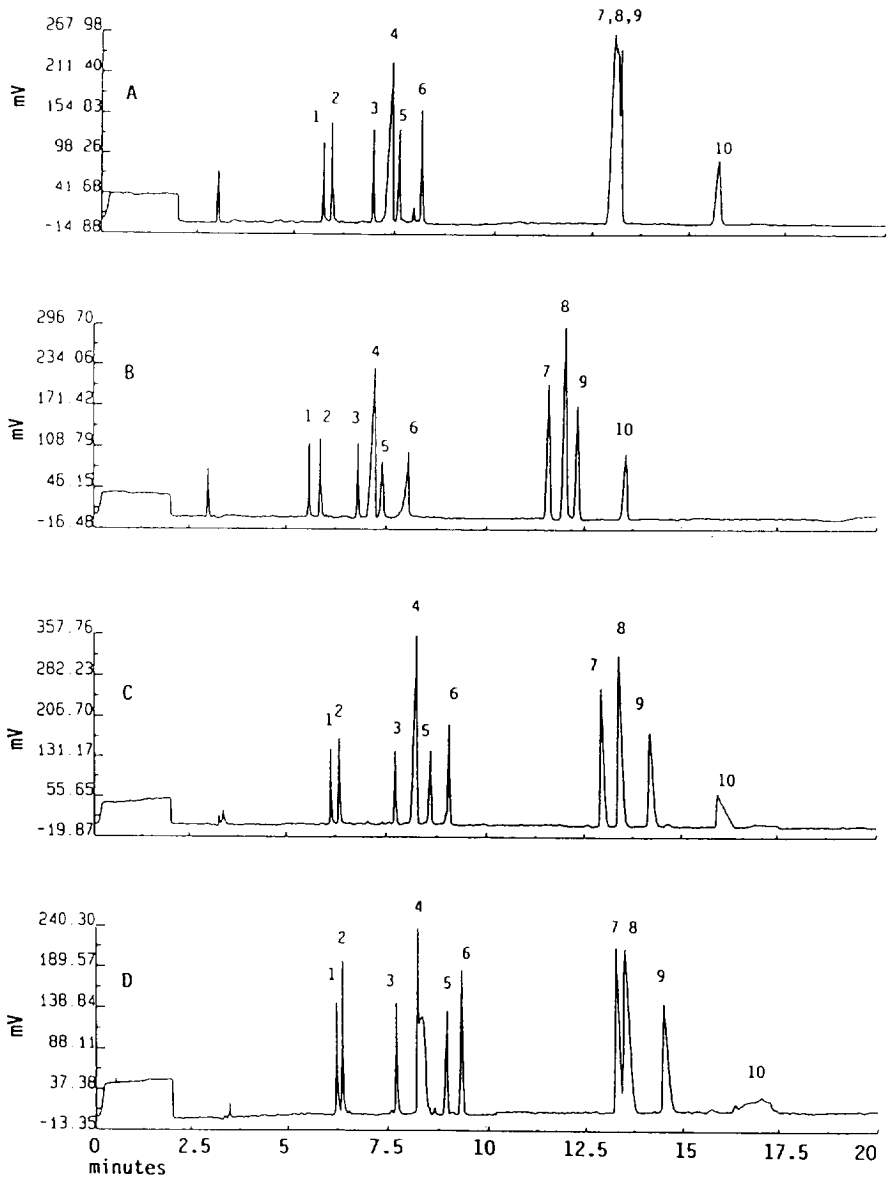

Fig. 1. Electropherograms showing the separation of green S (1), brilliant blue (2), erythrosine B (3), allura red (4), indigo carmine (5), sunset yellow (6), azorubine (7), amaranth (8), ponceau (9) and tartrazine (10) for (A) 15% acetonitrile and 85% 0.05 *M* SDS/0.01 *M* sodium borate buffer, (B) 15% acetonitrile and 85% 0.05 *M* SDS/0.005 *M* potassium dihydrogenorthophosphate/0.005 *M* sodium borate buffer, (C) 15% acetonitrile and 85% 0.05 *M* sodium deoxycholate/0.005 *M* potassium dihydrogenorthophosphate/0.005 *M* sodium borate buffer and (D) 15% acetonitrile and 85% 0.05 *M* sodium cholate/0.005 *M* potassium dihydrogenorthophosphate/0.005 *M* sodium borate buffer.

used as the internal standard for the cordials, except for one sample, where sunset yellow was used as the internal standard.

The levels of synthetic colours in five samples of confectionery and three cordials were in good agreement with the HPLC data. The instrument repeatability data (R.S.D.s) for the standard and sample solutions for the MEKC determinations

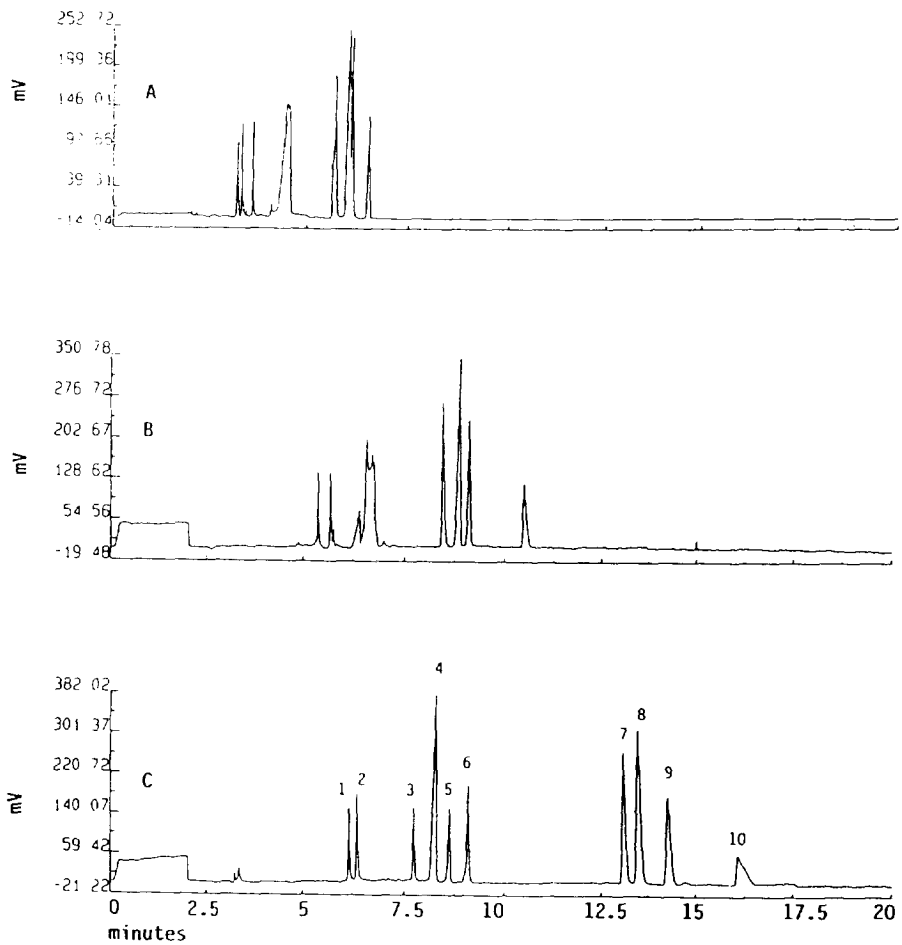

Fig. 2. Electropherograms showing the separation of green S (1), brilliant blue (2), erythrosine B (3), allura red (4), indigo carmine (5), sunset yellow (6), azorubine (7), amaranth (8), ponceau (9) and tartrazine (10) for (A) 0.005 *M* potassium dihydrogenorthophosphate/0.005 *M* sodium borate buffer, (B) 0.05 *M* sodium deoxycholate/0.005 *M* potassium dihydrogenorthophosphate/0.005 *M* sodium borate buffer and (C) 15% acetonitrile and 85% 0.05 *M* sodium deoxycholate/0.005 *M* potassium dihydrogenorthophosphate/0.005 *M* sodium borate buffer.

were also satisfactory. The electropherograms of the cordials were more complex than the confectionery due to the other additives present in samples. All of the cordials contained benzoic acid as a preservative. Benzoic acid and sorbic acid, which are permitted for use as preservatives in Australia [19] were well separated from the synthetic colours, however, the artificial sweetener saccharin, which is present in one sample of cordial, has the same migration time as allura red. Changing the detection wavelength to the absorption maxima for the colours in the visible region would eliminate these other components from the electropherograms. Citric acid, which is also present in the cordials, does not absorb strongly enough at 214 nm to cause a problem with the analyses.

Quinoline yellow, brilliant black, brown HT and chlorophyll copper complex are also permitted for use as synthetic colours in Australia [20]. Quinoline yellow appears as two peaks with similar migration times to green S and brilliant blue, while brilliant black migrates between amaranth and ponceau. The two peaks for quinoline yellow correspond to the two components in the synthetic colour preparation [21].

The ratio of the migration times of quinoline yellow and brilliant black to the internal standard (azorubine) are consistent over 20 repetitive injections. Both quinoline yellow and brilliant black are linear to 100 μg/ml and could therefore be determined quantitatively by this procedure. Brown HT and chlorophyll copper complex are not pure compounds and so appear as a mixture of peaks.

The analysis time is reduced from 37 min for HPLC to 18 min for MEKC procedure. Also, the procedure is less costly to operate than HPLC as MEKC is frugal in its use of chemicals and solvents and no costly columns are employed.

4. Conclusions

A rapid method for the separation and determination of commonly used synthetic colours in confectionery and cordials by MEKC is described. The separation of the compounds and the peak shapes were more consistent with a buffer modified with sodium deoxycholate than with a buffer modified with SDS. The instrument repeatability was acceptable, and the levels of the synthetic colours were in good agreement with the levels determined by the HPLC procedure currently used in our laboratory. The MEKC procedure is also faster and less costly to operate than the HPLC method.

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