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Isotachophoresis of some synthetic colorants in foods

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ABSTRACT

An isotachophoresis study of fifteen food products coloured with synthetic colorants showed that this method is suitable for the determination of dyes in foodstuffs.

INTRODUCTION

Food additives are incorporated in food products to improve their sensory qualities [1,2]. The concentrations of these additives must be carefully controlled as they may have various harmful effects to human health. Studies on health-related aspects of the use of synthetic colorants have been reported [3–6]. At present, the following synthetic substances are approved in Czechoslovakia: tartrazine, Sunset Yellow FCF, cochineal red, Ponceau 6R, amaranth, erythrosine, indigotine, Patent Blue V and Brilliant Black BN [7]. All of these are acidic and water-soluble.

Acidic dyes can be isolated by amyl alcohol extraction followed by multiple aqueous extraction [8]; for solid samples ammoniacal methanol or ethanol solutions are used [9]. Other frequently used methods include adsorption on polyamides [8], kaolin, natural aluminosilicate, activated carbon [10] and Polyclar AT [11,12].

For thin-layer chromatography, various supports are used, *e.g.*, paper, starch, cellulose, alumina, magnesium oxide, silica gel, polyamides, ion-exchange resins, etc. Separations of synthetic dyes have been effected with various developing systems, from universal, *e.g.*, 2% sodium citrate in 5% ammonia, to specific, *e.g.* *n*-butanol–acetic acid–water (1:1:1) [13–15]. Dyes have been identified by spectral methods (UV and visible), from the characteristics of line spectra and by fluorescence. Methods have also been elaborated for the determination of binary dyestuff mixtures spectrophotometrically, using computer-aided numerical evaluation [16–18].

Some workers [19–21] have used high-performance liquid chromatography, including the use of diode-array detectors, to determine synthetic dyes. Barros *et al.* [22] used differential-pulse polarography.

The utilization of capillary isotachophoresis in the determination of food additives has been reported by Zuche and Gruending [23], who determined synthetic sweeteners, Klein and Stoya [24], acesulpham K, Kvasnička [25], aspartame, Kaiser

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and Hupf [26,27] and Nierle [28], conserving agents, and Hiroaka and Kunijiro [29,30], who attempted the separation of flavonoid pigments and anthocyanines.

EXPERIMENTAL

We used a CS ZKI 001 isotachophoretic analyser (Spišská Nová Ves, Czechoslovakia) with a conductivity detector and a TZ 4200 double-line recorder (Laboratorní přístroje, Prague, Czechoslovakia), and several electrolytic systems as shown in Table I. The samples were diluted with water according to their colour content and injected into the column using the four-way valve of the instrument. The duration of the analysis was 30–40 min, depending on the electrolytes used. The synthetic colorants were identified using standards. Samples of synthetic colorants were supplied by the producers. Their purity was not specially controlled and for quantitative evaluation we took the longest zone of a revelant colorant such as that of a pure colorant. Quantitative analysis was performed using the calibrating.

Certain food products (powdered puddings, syrups) could not be analysed by capillary isotachophoresis without prior separation of the synthetic colorants from them, using either extraction [methanol–ammonia solution (95:5)] from powdered samples (this method is not quantitative and standard additions must be used in the analysis), extraction into *n*-amyl alcohol (liquid samples) or adsorption on powdered or granulated polyamides (using 50 ml of sample with 2.5 ml of acetic acid and 25 g of granulated polyamides, the mixture being shaken for 20 min until loss of colour; subsequently, the adsorbed granulate was extracted three times with methanol ammonia solution, the extracts were combined, concentrated on a water-bath and adjusted to 10 ml before measurement).

RESULTS AND DISCUSSION

First, model solutions using standards prepared from synthetic colorants

TABLE I

COMPOSITIONS OF ELECTROLYTE SYSTEMS (ES) AND CONDITIONS OF SEPARATION

Parameter[a]	ES I	ES II	ES III	ES IV
Concentration of leading electrolyte (hydrochloric acid) (*M*)	10^{-2}	10^{-2}	10^{-2}	10^{-2}
Counter ion	β-Alanine	ε-ACA[b]	Histidine	Histidine
pH	3.5	4.5	6.0	6.0
Additive MHEC[c] (%)	0.1	0.1	0.1	0.1
Terminating electrolyte	Acetic acid	Caproic acid	Caproic acid	MES[d]
Concentration (*M*)	5.10^{-3}	5.10^{-3}	5.10^{-3}	5.10^{-3}, histidine 5.10^{-3}, pH 5–6

[a] Other conditions: driving current, 300 μA in the preseparation column, 50 μA in the analytical column.
[b] ε-Aminocaproic acid.
[c] Methylhydroxyethylcellulose.
[d] Morpholinoethanesulphonic acid.

TABLE II

CHARACTERISTIC CONSTANTS (r.s.h.) OF THE INDIVIDUAL STANDARDS OF SYNTHETIC COLORANTS IN ELECTROLYTIC SYSTEMS (ES) I–IV

Synthetic colorant	R.s.h.[a]			
	ES I	ES II	ES III	ES IV
Tartrazine	0.184	0.270	0.572	0.285
Sunset Yellow FCF	0.200	0.285	0.560	0.290
Cochineal red	0.196	0.272	0.565	0.283
Amaranth	0.191	0.266	0.554	0.262
Azorubine	0.268	0.309	0.756	0.423
Patent blue	0.320	0.434	0.825	0.464
Brilliant black	0.190	0.269	0.585	0.273
Indigotine	0.202	0.276	0.562	0.284

[a] R.s.h. = relative step height = $(h_i - h_L)/(h_T - h_L)$, where h_i = the line of the ith ion; h_L = the line of the leading electrolyte and h_T = the line of the terminating electrolyte.

(concentration 100 mg dm^{-3}) were used. This guarantees that the separation capacity of the column is not exceeded. Measurements were carried out in the preseparation column; this concentration precluded the possibility of the formation of a non-stationary zone [31]. Visual observation of the column was found to provide a suitable orientation for the evaluation of the separation of individual standards, assisting in the determination of the order of the dyes identified. By comparing the characteristic constants (r.s.h.) of the individual standards of synthetic colorants in the electrolyte systems I–IV as shown in Table II, it was found that only Patent blue and azorubine had sufficiently different r.s.h. values. For this reason, we investigated the possibility of the separation of the individual dyes into groups with two or three members in a mixture, aiming at a model mixture with the maximum number of dyes that could be separated in the individual electrolyte systems used. In combination with Indigotine we found that individual dyes were separated into independent zones but the detector failed to identity this owing to the minimal mobility differences between the individual component dyes. Also for this reason a selective visible spectrophotometric detector would be more suitable when analysing synthetic dyes. Our measurements demonstrated in electrolyte system I that up to four variously combined dyes could be separated as shown in Table III. This was better than the results obtained with

TABLE III

SEPARATED COMBINATIONS OF FOUR SYNTHETIC COLORANTS IN ELECTROLYTE SYSTEM I

No.	Synthetic colorants
1	Azorubine–Patent blue–Sunset Yellow FCF–tartrazine
2	Azorubine–Patent blue–Sunset Yellow FCF–cochineal red
3	Azorubine–Patent blue–Sunset Yellow FCF–brilliant black
4	Azorubine–Patent blue–Sunset Yellow FCF–amaranth
5	Azorubine blue–amaranth–indigotine
6	Indigotine–Patent blue–azorubine–brilliant black

electrolyte systems II–IV. In Table III, colorants are given according to the system and not according to their mobility.

The simplest and fastest method for the isolation of synthetic colorants from product mixtures is direct extraction using methanol–ammonic solution (95:5). This is only usable with solid samples as both methanol and ammonia mix well with water. The extracts thus obtained can be used directly for paper chromatography and capillary isotachophoresis. This method was used with powdered pudding samples.

When analysing liquid samples, water-immiscible extraction agents must be used, or the dyes can be adsorbed on a suitable adsorbent with reverse elution back into solution. In direct sampling of a liquid sample into the instrument, a record was needed from the analytical column; with sufficiently preconcentrated extracts, however, records from the isotachophoretic preseparation column were often adequate, resulting in a faster (15–20 min).

Extracts obtained by amyl alcohol extraction were suitable for paper chromatography but their further use for isotachophoretic analysis presented problems. In addition, in 10% hydrochloric acid one cannot analyse anything by isotachophoresis.

TABLE IV

RESULTS OF SAMPLE ANALYSES

No.	Sample	Composition of colour mix	Quantitative composition (mg kg^{-1})
1	Extra Tang (powdered beverage)	Tartrazine, Sunset Yellow FCF	405 ± 36 129 ± 17
2	Vita orange (powdered beverage)	Tartrazine	382 ± 22
3	Vita blackcurrant (powdered beverage)	Tartrazine	148 ± 19
4	Powdered pudding, "Golden ear" brand	Sunset Yellow FCF	64 ± 18
5	Powdered pudding, Orange flavour	Sunset Yellow FCF	197 ± 24
6	Raspberry drops (hard candy)	Azorubine	334 ± 43
7	Powdered pudding	Amaranth	79 ± 19
8	Powdered pudding, strawberry flavour	Amaranth, cochineal red	*ca.* 420 ± 16
9	Powdered pudding, raspberry flavour	Amaranth	269 ± 38
10	Powdered pudding, lemon flavour	Tartrazine	268 ± 25
11	Vita strawberry (powdered beverage)	Amaranth, cochineal red Azorubine	*ca.* 350 ± 20 195 ± 36
12	Amyl-vanilla (powdered pudding)	Tartrazine, Sunset Yellow FCF	202 ± 32 52 ± 13
13	Egg-shaped hard candy	Tartrazine	279 ± 43
14	Vita raspberry (powdered beverage)	Azorubine	583 ± 42
15	Orange lemonade	Sunset Yellow FCF	50 ± 8 (mg dm^{-3})

The method using adsorption on degreased wool fibre is time consuming; in addition, it was found experimentally that it also lacked quantitativeness.

Substantially better results were obtained when using granulated polyamide adsorption, with subsequent extraction into methanol–ammonia solution. The amount of dye adsorbed could be increased considerably by increasing the amount of adsorbing material.

Isolation of synthetic dyes from polyamide granues was carried out by extraction using 2% aqueous ammonia; ethanol–ammonia solution, (95:5), methanol–ammonia solution (95:5) or ethanol–ammonia solution (90:10), using various amounts and temperatures. None of the extraction mixtures offered complete isolation of the dye from the adsorbent. We decided to use methanol–ammonia solution (95:5) and determined the amount of dye remaining adsorbed, under standard conditions, per unit weight of the adsorbent. This amount was determined by isotachophoresis, based on the difference in the amount of dye before adsorption and after its reverse elution from the adsorbent. Laboratory temperatures were used throughout, with increasing amounts of the adsorbent. The amount of colorant not extracted from the polyamide granules was from 7.3 to 13.6%, depending on the colorant (tartrazine 7.3%, amaranth 13.6%).

The method described is suitable for model solutions of synthetic colorants as well as products samples containing only such colorants.

The results showed that electrolyte system I is suitable for analyses of food

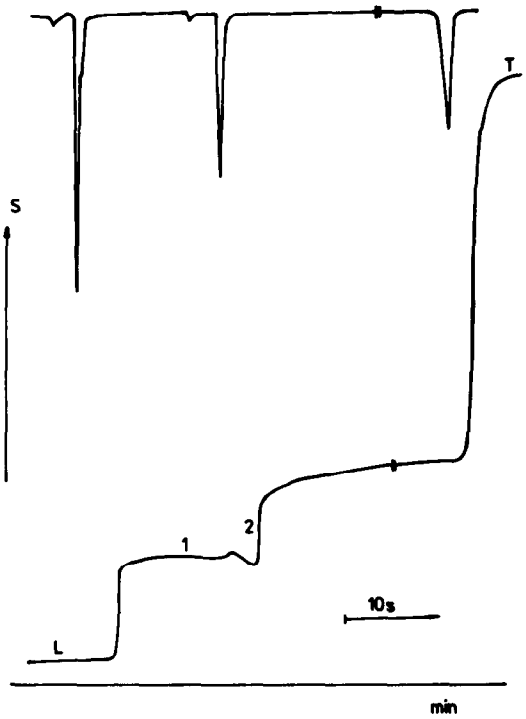

Fig. 1. Isotachopherogram of synthetic colorants in sample Extra Tang in electrolyte system I. S = Response of conductivity detector. 1 = Tartrazine; 2 = Sunset Yellow FCF; L = leading electrolyte; T = terminating electrolyte.

products for the determination of synthetic colorant contents, for which the qualitative composition of the samples is known in advance from paper chromatographic recordings [using *n*-butanol–acetone–water (1:1:1) as developing solvent]. The results were obtained by evaluating the isotachopherograms from the analytical column and by adjustment of the individual analytical times. Table IV shows some of the samples and mean values, obtained in those measurements, with the confidence interval of the mean shown at the 5% significance level ($\bar{x} \pm K_n R$), where $R = x_{max} - x_{min}$ and $K_n = 1.3$ [32]. Fig. 1 shows the record from the analysis of the sample Extra Tang.

Most of the samples analysed must be diluted with water at least 1:10 so that the amount of dye in the sample does not exceed the permitted limiting value of 100 mg kg^{-1}, with the exception of samples 6 and 13. Samples 8 and 11 also contained amaranth and cochineal red, but these have formed a mixed zone. However, as the difference in the b constants in the linear equations for amaranth is small [amaranth, $a = 0.237$; $b = 0.779$; $r = 0.9984$; cochineal red, $a = -0.589$; $b = 0.688$; $r = 0.9992$; a = section on y-axis (mm); b = gradient in mm/(mg dm^{-3})], a rough estimate of the dye contents in the mixture zone can be obtained by using the mean value, $b = 0.734$.

In conclusion, capillary isotachophoresis can be used in the determination of synthetic colorants in some food products. It is known that not all permitted colorants are simultaneously used for colouring products, but a maximum of six colorants are used in combination. The analysis of the products has shown that the artificial colourings used were mainly sunset yellow, tartrazine, azorubine, amaranth and cochineal red.

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