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HOST–GUEST COMPLEXATION IN CAPILLARY ISOTACHOPHORESIS

II[a]. DETERMINATION OF AMINOPHENOL AND DIAMINOBENZENE ISOMERS IN PERMANENT HAIR COLORANTS BY USING CAPILLARY ISOTACHOPHORESIS

SALVATORE FANALI
Istituto di Cromatografia del CNR, Area della Ricerca di Roma, C.P. 10, 00016 Monterotondo Stazione, Rome (Italy)

SUMMARY

The separation of 2-, 3- and 4-aminophenol and 1,2-, 1,3- and 1,4-diaminobenzene is studied by using capillary isotachophoresis. In order to obtain complete resolution of the six cations β-cyclodextrin was used. The analytical method was used for the determination of aminophenol and diaminobenzene isomers in permanent hair colorant creams.

INTRODUCTION

Oxidative hair colorants are permanent hair colourings usually used by mixing the dye precursor and hydrogen peroxide in an alkaline medium (ammonia and/or monoethanolamine). Hydrogen peroxide reacts with a primary intermediate (*e.g.*, 1,4-diaminobenzene, 4-aminophenol) to produce an imine, which in its turn reacts rapidly with a coupler (*e.g.*, 3-aminophenol, resorcinol) to produce the dye.

Allergic dermatitis due to 1,4-diaminobenzene and nefrotoxic effects due to 2-, 3-, 4-aminophenol have been reported[1,2]. The content and the tolerated concentrations of the chemical compounds in permanent hair colorants are listed in the Italian normative law No. 713, October 11th, 1986, in accord with European Economic Community (EEC) instruction No. 87/137.

Methods used so far for the determination of aminophenols and/or diaminobenzenes in hair colorants and other matrices are[3–8] gas–liquid, thin-layer (TLC) and high-performance liquid chromatography, titrimetry and spctrophotometry. The technique recommended by the Italian Gazette is TLC[9].

As capillary isotachophoresis is a promising method in pharmaceutical analysis[10–12], we decided to apply it to the analysis of cosmetics.

1,2-, 1,3- and 1,4-diaminobenzene and 2-, 3- and 4-aminophenol were complete-

[a] For Part I, see ref. 13.

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ly resolved by adding β-Cyclodextrin to the leading electrolyte. Samples were injected directly after dissolution and analysed with good results.

EXPERIMENTAL

Chemicals

Ethanol (95°), potassium hydroxide, acetic acid and 1,3-diaminobenzene dihydrochloride (*m*-BDA) were obtained from Carlo Erba (Milan, Italy), 2-pyridinecarboxylic acid (picolinic acid), 1,4-diaminobenzene (*p*-BDA), 2- (*o*-AP), 3- (*m*-AP) and 4-aminophenol (*p*-AP), α-cyclodextrin (α-CD) and vitamin C from Fluka (Buchs, Switzerland), poly(vinyl alcohol) 28/20 (PVA) from Serva (Heidelberg, F.R.G.), tetrahydrofuran and 1,2-diaminobenzene (*o*-BDA) from Merck (Darmstadt, F.R.G.) and β-cyclodextrin (β-CD) from Sigma (St. Louis, MO, U.S.A.). Samples of permanent hair colorants (designated A, B, C and D) were purchased from retail stores. Doubly distilled water was used to prepare the solutions.

All chemicals were of analytical-reagent grade and used as received, except for PVA, which was purified with a mixed-bed ion exchanger.

Apparatus

Experiments were performed by using a Tachophor 2127 apparatus (LKB, Bromma, Sweden) equipped with a conductivity detector. A polytetrafluoroethylene (PTFE) capillary tube (24 cm × 0.5 mm I.D.) was used. The detector cell was laboratory made as described previously[13]; the distance between the platinum sensing electrodes was 0.5 mm. The resistance and its derivative were recorded with an LKB 2210 line recorder at a chart speed of 50 mm/min.

The driving current used was 200 μA, reduced during the isotachophoresis. The zones were detected at 25 μA, unless specified otherwise. Samples and standards were injected with a 10-μl Hamilton (Bonaduz, Switzerland) microsyringe.

Electrolytes

Potassium hydroxide (10 m*M* adjusted to pH 5.4 with picolinic acid and acetic acid (5 m*M*) were used as the leading and terminating electrolytes, respectively. The leading electrolyte contained 0.4% (w/v) of PVA and the appropriate amount of α- and β-CD.

Preparation of standard solutions and samples

Standard solutions were prepared by dissolving the appropriate amount of aminophenol and diaminobenzene isomers in water containing 1 mg/ml of vitamin C. Calibration graphs were obtained with a mixture of *p*-BDA, *p*-AP and *o*-AP and 1 mg/ml of vitamin C. The final concentration of each standard was $1 \cdot 10^{-3}$ *M*. The solutions were stored in a refrigerator until their use.

Hair dyes were dissolved in water–ethanol–tetrahydrofuran (10:80:10, v/v/v) containing 1 mg/ml of vitamin C.

RESULTS AND DISCUSSION

In order to find the optimum conditions for the separation of aminophenol and diaminobenzene isomers, several electrolyte systems were tested. The cationic

electrolyte system recommended by Jokl *et al.*[14] with H^+ as terminator with a very low mobility allowed the analysis of *m*-AP, which otherwise is difficult. Under these conditions, *o*-BDA and *o*-AP were not separated from each other and it was therefore necessary to modify their effective mobilities. The use of uncharged complexing agents, *e.g.*, cyclodextrins is well known in capillary isotachophoresis for the separation of structural isomers, *e.g.*, benzoic acid derivatives[13,15] and aromatic quaternary ammonium ions[16]. In order to find the optimum separation conditions, different amounts of α- and β-cyclodextrins were added to the leading electrolyte and their effect was observed.

Fig. 1a and b show the relationship between the amount of α- and β-CD, respectively, added to the leading electrolyte and the RSH values of six aminophenol and diaminobenzene isomers, where RSH is the relative step height calculated using the equation

$$\mathrm{RSH} = \frac{h_x - h_L}{h_s - h_L} \tag{1}$$

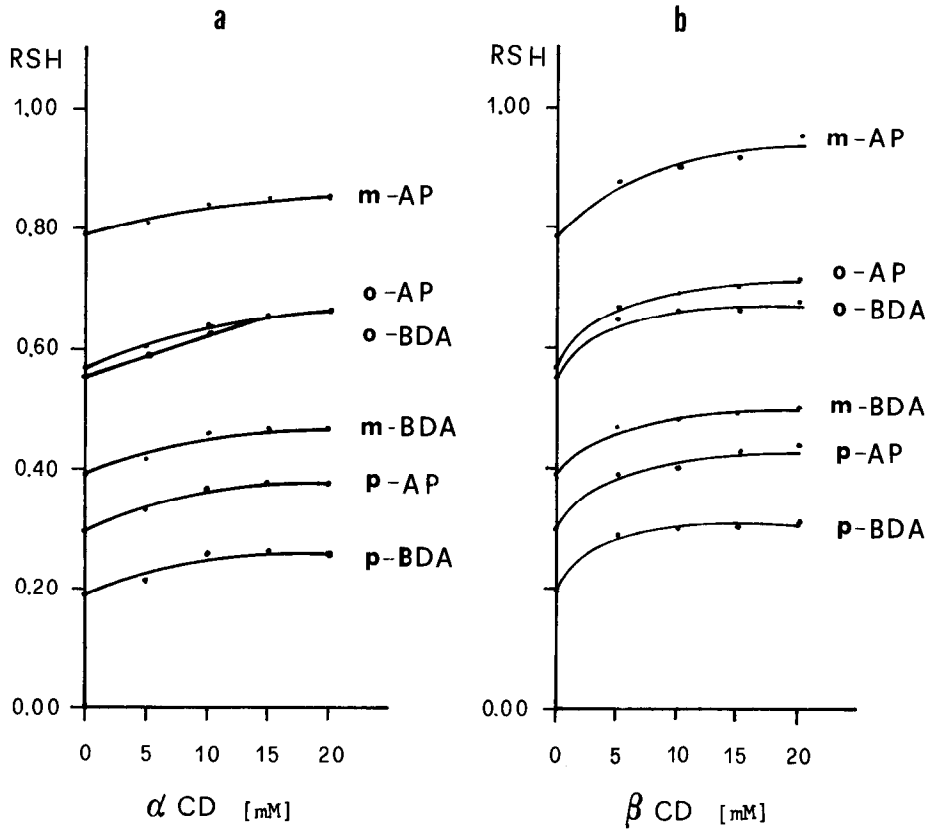

Fig. 1. Effect of the amount of cyclodextrins added to the leading electrolyte on the RSH values of 2-, 3- and 4-aminophenol (*o*-, *m*- and *p*-AP) and 1,2-, 1,3- and 1,4-diaminobenzene (*o*-, *m*- and *p*-BDA). (a) α-Cyclodextrin; (b) β-cyclodextrin.

Fig. 2. Isotachophoretic separation of aminophenol and diaminobenzene isomers. Sample: 6 μl of standard mixture, $1 \cdot 10^{-3}$ *M*; detection current, 50 μA. (a) Without cyclodextrin; (b) with 15 m*M* β-cyclodextrin.

where h_x, h_L and h_s are the step heights of the examined, leading and reference (H^+) cations, respectively.

By adding α-CD to the leading electrolyte, a reduction in effective mobility was achieved for all compounds, but an increase in the amount of α-CD did not result in resolution. The effective mobilities of the six examined compounds were reduced more effectively by using β-CD than α-CD. In fact, by increasing the amount of β-CD in the leading electrolyte all the RSHs increased and complete resolution was achieved with 15 m*M* β-CD.

Fig. 2a and b show the separation of *o*-, *m*- and *p*-aminophenols and *o*-, *m*- and *p*-diaminobenzenes without and with cyclodextrin, respectively. To verify the validity of the method, four hair colorant creams were examined. Qualitative analysis was performed by measuring the RSH and by injecting samples and standards together. Calibration graphs were drawn for *p*-BDA, *o*-AP and *p*-AP present in the samples. The lengths of the steps were plotted against concentration and the linearity was observed from $1 \cdot 10^{-9}$ to $15 \cdot 10^{-9}$ mol. The correlation coefficients were 0.9999, 0.9999 and 0.9997 for *p*-BDA, *p*-AP and *o*-AP, respectively. Relative standard deviations were obtained by injecting 7 μl of the standard solution (six measurements) and good results were achieved (0.6, 0.2 and 0.4%, respectively).

The recovery was determined by adding more of the analytes present in the samples and the results are presented in Table I. Fig. 3 shows the isotachopherogram of

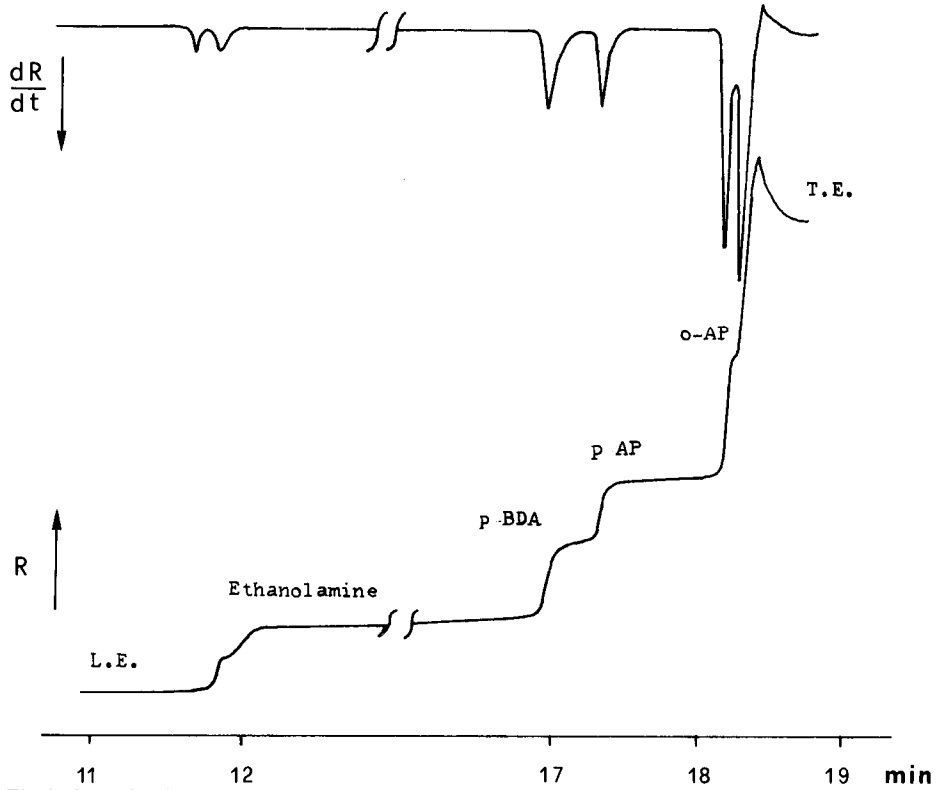

Fig. 3. Isorachopherogram of the analysis of a commercial hair colorant (D, Table I). Amount injected, 8 μl; detection current, 25 μA.

TABLE I

DETERMINATION OF 1,4-DIAMINOBENZENE, 2-AMINOPHENOL AND 4-AMINOPHENOL IN COMMERCIAL HAIR COLORANT CREAMS

Sample	*Compounds declared*[a]	*Prepared solution (g per 100 ml)*	*Amount found (%)*			*Recovery*					
			p-BDA	*p-AP*	*o-AP*	*p-BDA*		*p-AP*		*o-AP*	
						Added (mg)	*Recovered (%)*	*Added (mg)*	*Recovered (%)*	*Added (mg)*	*Recovered (%)*
A	*p*-BDA, RES, NH_3	0.403	1.92	–	–	11.6	98	–	–	–	–
B	*p*-BDA, *p*-AP, RES, NH_3	0.622	1.20	0.38	–	11.1	97	11.3	97	–	–
C	*p*-BDA, *o*-, *m*-, *p*-AP, RES, NH_3	1.549	0.24	0.36	–	10.0	92	13.1	100	–	–
D	*p*-BDA, RES, NH_3, *o*-, *m*-, *pp*-AP	2.840[b]	0.06	0.13	0.04	3.5	98	4.7	92	4.9	96

[a] RES = resorcinol.

[b] g per 50 ml.

one sample. The presence of ammonia and/or ethanolamine under these conditions does not interfere with the determinations.

From the results it can be concluded that capillary isotachophoresis is a rapid method for the qualitative analysis of aminophenol and diaminobenzene isomers present in cosmetics. The method can be used for routine control in order to reveal compounds that should not be included in the composition of the cosmetics, *e.g.*, 1,2-BDA[9], and to check if the allowed chemicals are or not in accord with the statutory requirements. Analysis can be performed without pre-treatment in spite of the complexity of the matrix.

Further studies will be made in order to determine other components, *e.g.*, derivatives such as aminotoluene, that could be present in hair colorants.

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