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Preparative separation of isomeric 2-(2-quinolinyl)-1H-indene-1,3(2H)-dione monosulfonic acids of the color additive D&C Yellow No. 10 (Quinoline Yellow) by pH-zone-refining counter-current chromatography ☆

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Abstract

The main components of the color additive D&C Yellow No. 10 (Quinoline Yellow, Color Index No. 47005), 2-(2-quinolinyl)-1H-indene-1,3(2H)-dione-6′-sulfonic acid (6SA) and 2-(2-quinolinyl)-1H-indene-1,3(2H)-dione-8′-sulfonic acid (8SA), were isolated from the dye mixture by pH-zone-refining counter-current chromatography (CCC) in the ion-exchange mode. These positional isomers were separated from a portion of dye using sulfuric acid as the retainer acid and dodecylamine as the ligand (ion exchanger). The added ligand enhanced the partitioning of the hydrophilic components in the organic stationary phase of the two-phase solvent system that consisted of isoamyl alcohol–methyl *tert*.-butyl ether–acetonitrile–water (3:1:1:5). Thus, separation of 1.8 g of D&C Yellow No. 10 using the above method resulted in 0.6 g of 6SA and 0.18 g of 8SA of over 99% purity. The isolated compounds were characterized by mass spectrometry and proton nuclear magnetic resonance with correlated spectroscopy assignments. The study exemplifies a new field of applications for pH-zone-refining CCC, to the separation of positional isomers of strongly hydrophylic compounds containing sulfonic acid groups. Published by Elsevier Science B.V.

Keywords: Counter-current chromatography; Positional isomers; D&C Yellow No. 10; Quinoline Yellow; Monosulfonic acids; Sulfonic acids; Dyes

1. Introduction

D&C Yellow No. 10 (Quinoline Yellow, Color Index No. 47005) is a USA certified color additive listed for use in drugs and cosmetics. It consists primarily of a mixture of the sodium salts of the 6′- and 8′-monosulfonic acid positional isomers of 2-(2-quinolinyl)-1*H*-indene-1,3(2*H*)-dione (6SA and 8SA, respectively, in Fig. 1) with up to 15% of the

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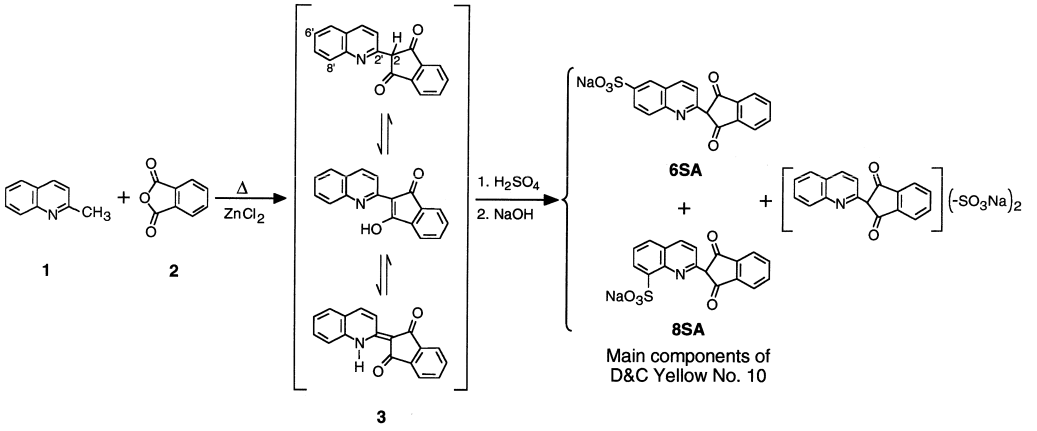

Fig. 1. Preparation of D&C Yellow No. 10 by condensing quinaldine, **1**, with phthalic anhydride, **2**, and sulfonating the condensation product **3**.

disodium salts of the disulfonated isomers [1]. By contrast, the counterpart of this color additive in Europe (E 104), Japan (Yellow No. 203) and other countries is a mixture that contains mostly the disulfonated components [2–5]. The primarily disulfonated Quinoline Yellow is not certifiable in the USA. D&C Yellow No. 10 is prepared currently [1] as was described more than a hundred years ago [6] by condensing 2-methylquinoline (quinaldine), **1**, with phthalic anhydride, **2**, using zinc chloride as the catalyst, at 190 to 210°C. The condensation product, 2-(2-quinolinyl)-1H-indene-1,3(2H)-dione, **3,** is then sulfonated and the products are isolated as sodium salts (Fig. 1). Purified 6SA and 8SA are used as reference materials for the development of thin-layer chromatographic (TLC) and high-performance liquid chromatographic (HPLC) methods to be used for US Food and Drug Administration (FDA) batch certification of D&C Yellow No. 10. These compounds are not commercially available. Previously, 6SA and 8SA were obtained (i) synthetically, by condensing the respective quinaldinesulfonic acid with phthalic anhydride [7] or by directly sulfonating 2-(2-quinolinyl)-1*H*-indene-1,3(2*H*)-dione [4]; or (ii) by separating them from commercial color additives. In the latter case, 200 mg of 8SA was separated by two recrystallization procedures and 40 mg of 6SA was separated by preparative TLC which was followed by ion-exchange chromatography [5]. The present study involves the use of the relatively recently-developed preparative-scale separation technique, pH-zone-refining counter-current chromatography (pH-zone-refining CCC) [8–11] for the separation of 6SA and 8SA from gram quantities of D&C Yellow No. 10. pH-Zone-refining CCC is a type of liquid–liquid partition chromatography that enables the separation of organic acids and bases into a succession of highly-concentrated rectangular peaks that elute according to their pK_a values and hydrophobicities. In previous studies, this technique was applied to the separation of dyes that contain carboxylic acid groups, such as hydroxyxanthene dyes [12–16]. Sulfonated dyes have not been amenable to separation by pH-zone-refining CCC because of their very low pK_a values which prevent their partitioning into the organic phase of a conventional two-phase solvent system. Recently, however, it was shown that the addition of a ligand (ion exchanger) to both the sample solution and the organic stationary phase enables separation of sulfonated dyes by pH-zone-refining CCC because the ligand enhances their partitioning into the organic stationary phase [17,11]. More recently other hydrophilic compounds (depolymerized fucans) were fractionated using a ligand (the anion exchanger Amberlite LA2) in the organic stationary phase [18]. In the present study, this new

approach is extended to the preparative separation of the monosulfonated positional isomers 6SA and 8SA from a batch of D&C Yellow No. 10.

2. Experimental

2.1. Materials

The D&C Yellow No. 10 used in this study originated from a commercial lot submitted to the US FDA for batch certification. Ammonium acetate (NH_4OAc), methanol, water and acetonitrile were of chromatography grade. Sulfuric acid (95.8%, Mallinckrodt) and isoamyl alcohol (98.8%, J.T. Baker, Phillipsburg, NJ, USA) were ACS reagent grade. Ammonium hydroxide (>25% NH_3 in water, Fluka, Buchs, Switzerland), *tert*.-butyl methyl ether (>99.5%, Fluka), dodecylamine (DA, >98%, Fluka) and [$^2H_6$]dimethylsulfoxide (DMSO-d_6, >99.8%, Aldrich, Milwaukee, WI, USA) were used as received.

2.2. pH-Zone-refining CCC

2.2.1. Instrumentation

The separation was performed with a commercial high-speed CCC system (Model CCC-1000, Pharma-Tech Research, Baltimore, MD, USA) that consisted of a column (three Ito multilayer-coils connected in series made of 1.6 mm I.D. Tefzel tubing with a total capacity of ~325 ml) mounted on a rotating frame, a speed controller and an LC pump. To facilitate data collection, several improvements were made to this basic system, including continuous pH monitoring and computerized data acquisition. The instrument used, along with the added improvements, was previously described in detail and depicted in a photograph [15].

2.2.2. Separation procedure

Solvent system: The two-phase solvent system used for the separation of the monosulfonated components in D&C Yellow No. 10 consisted of isoamyl alcohol–methyl *tert*.-butyl ether–acetonitrile–water (3:1:1:5, 600 ml:200 ml:200 ml:1000 ml). The solvent system was equilibrated in a separatory funnel, and the two phases were separated before use, resulting in 940 ml of upper organic phase (UP) and 1038 ml of lower aqueous phase (LP).

The amount of ligand and acid added to the stationary phase and the amount of ammonia added to the mobile phase were determined in preliminary experiments as described elsewhere [19].

Stationary phase: A combination of ligand-containing UP and ligand-free UP was used as the stationary phase as described below. The ligand-containing UP was prepared as follows: to 200 ml UP, 10.2 g DA (55 mmol) was added yielding a solution of ~5% (275 m*M*) DA. To this solution, ~2.1 ml sulfuric acid (~37.7 mmol) was added. The pH of the solution became ~1.9.

Mobile phase: To 1000 ml LP, 5.62 g ammonium hydroxide (8 ml, 80 mmol NH_3) was added yielding a solution of ~80 m*M* NH_3 with a pH of ~10.9.

Sample solution: 1.8 g (~4.22 mmol sodium salts of monosulfonated isomers and ~0.48 mmol disodium salts of disulfonated isomers) of D&C Yellow No. 10 was partly dissolved and partly suspended in 30 ml LP and 40 ml of stationary phase [which contained ligand (~11 mmol) and acid (~7.5 mmol)]. The pH of the mixture became ~2.0.

The separation was initiated by completely filling the column with ligand-free stationary phase (upper organic phase) by using the LC pump. Approximately 100 ml of ligand-containing stationary phase was pumped into the column displacing part of the column contents. The ligand-free stationary phase at the eluting end of the column prevents the contamination of the separated components in the collected fractions in case of low retention of the stationary phase or carryover of the ligand into the mobile phase. The sample solution was sonicated for 5 min and loaded into the column through the sample injection valve with pressurized nitrogen (70 p.s.i.; 1 p.s.i.=6894.76 Pa). The aqueous mobile phase was then pumped into the column at 3 ml/min while the column was rotated at 960 rpm. The column effluent was monitored (pH, UV-scanning from 220 to 450 nm) [15] and a fraction collector was used to obtain 6-ml fractions. The fractions collected were analyzed by analytical HPLC.

2.3. Analytical HPLC

The analytical reversed-phase (RP) HPLC analyses were performed with a Spectra-Physics system (San Jose, CA, USA) described previously [20]. The UV–Vis detector (Model 490, Waters, Milford, MA, USA) was set at 254 and 415 nm. The eluents were 0.1 *M* aqueous NH_4OAc and methanol. The column (Hypersil MOS-1 RPC-8, 5 μm particle size, 250× 4.6 mm I.D., Keystone Sci., Bellefonte, PA, USA) was eluted by using consecutive linear gradients of 25–90% methanol in 25 min, 90–100% methanol in 5 min, followed by 100% methanol for 10 min. The column was re-equilibrated with 25% methanol for 15 min. Other conditions included: injection volume, 20 μl; full-scale response, 0.128 absorbance units; and flow-rate, 1 ml/min.

An aliquot from the collected fractions obtained from the pH-zone-refining CCC separation was diluted with approximately 2 ml of water–methanol (75:25, v/v). For Figs. 2 and 3 the concentration of the solute was 0.1 mg/ml. The solution was filtered through a Uniprep 0.45-μm glass microfiber syringeless filter unit (Whatman, Clifton, NJ, USA) prior to chromatography.

2.4. Liquid chromatography–mass spectrometry (LC–MS)

The negative ion atmospheric pressure chemical ionization (APCI) mass spectra were obtained through use of a Finnigan TSQ 7000 mass spectrometer (ThermoQuest, San Jose, CA, USA) interfaced to an Alpha data system. The conditions for APCI-MS included sheath gas (N_2) set to 70 p.s.i., auxiliary gas (N_2) flow set to 1.85 l/min, vaporizer heated to 400°C, capillary heated to 150°C, and corona discharge set at 5 μA. For data acquisition, the instrument was scanned from *m/z* 110 to 610 in 1.0 s. LC was performed using the mobile phase and gradient described above at a flow-rate of 0.25 ml/min. A YMC Basic S5 (250×2 mm I.D., 5 μm particle size) column (YMC, Wilmington, NC, USA) was used for separations. The injection volume of the samples dissolved in water (6SA, 0.3 mg/ml; 8SA, 0.17 mg/ml) was 20 μl.

2.5. ^1H Nuclear magnetic resonance (NMR) spectrometry

The ^1H-NMR spectra and COSY (correlated spectroscopy) assignments of 6SA and 8SA were obtained on a Varian XL Fourier transform NMR spectrometer at 400 MHz. The purified compound, approximately 3 mg, was dissolved in 140 μl of DMSO-d_6. The following signals were obtained and assigned for each of the two isolated monosulfonated isomers: 6SA, [2-(2-quinolinyl)-1H-indene-1,3(2H)-dione-6′-sulfonic acid, (Fig. 4)], 8.53 ppm (d, 9.3; H-a), 8.51 ppm (d, 9.3; H-b), 8.15 ppm (d, 1.75;

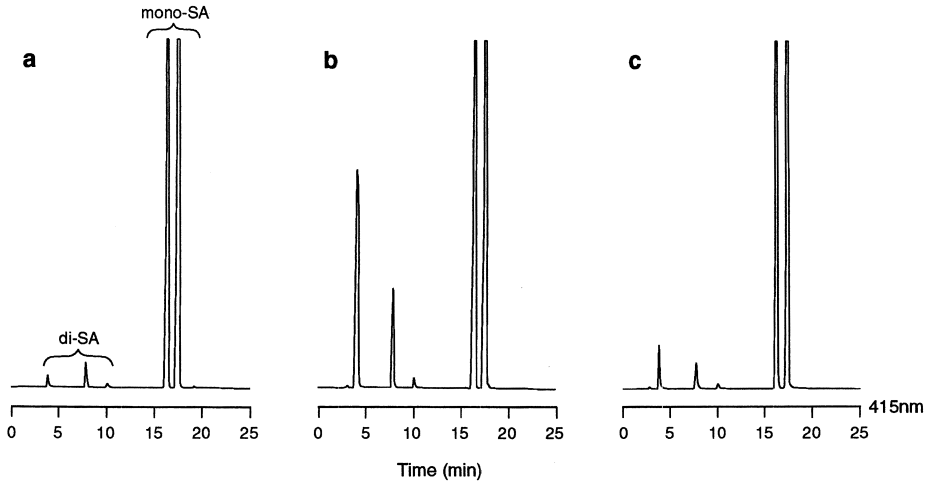

Fig. 2. Analytical RP-HPLC of samples of D&C Yellow No. 10 obtained from three different manufacturers.

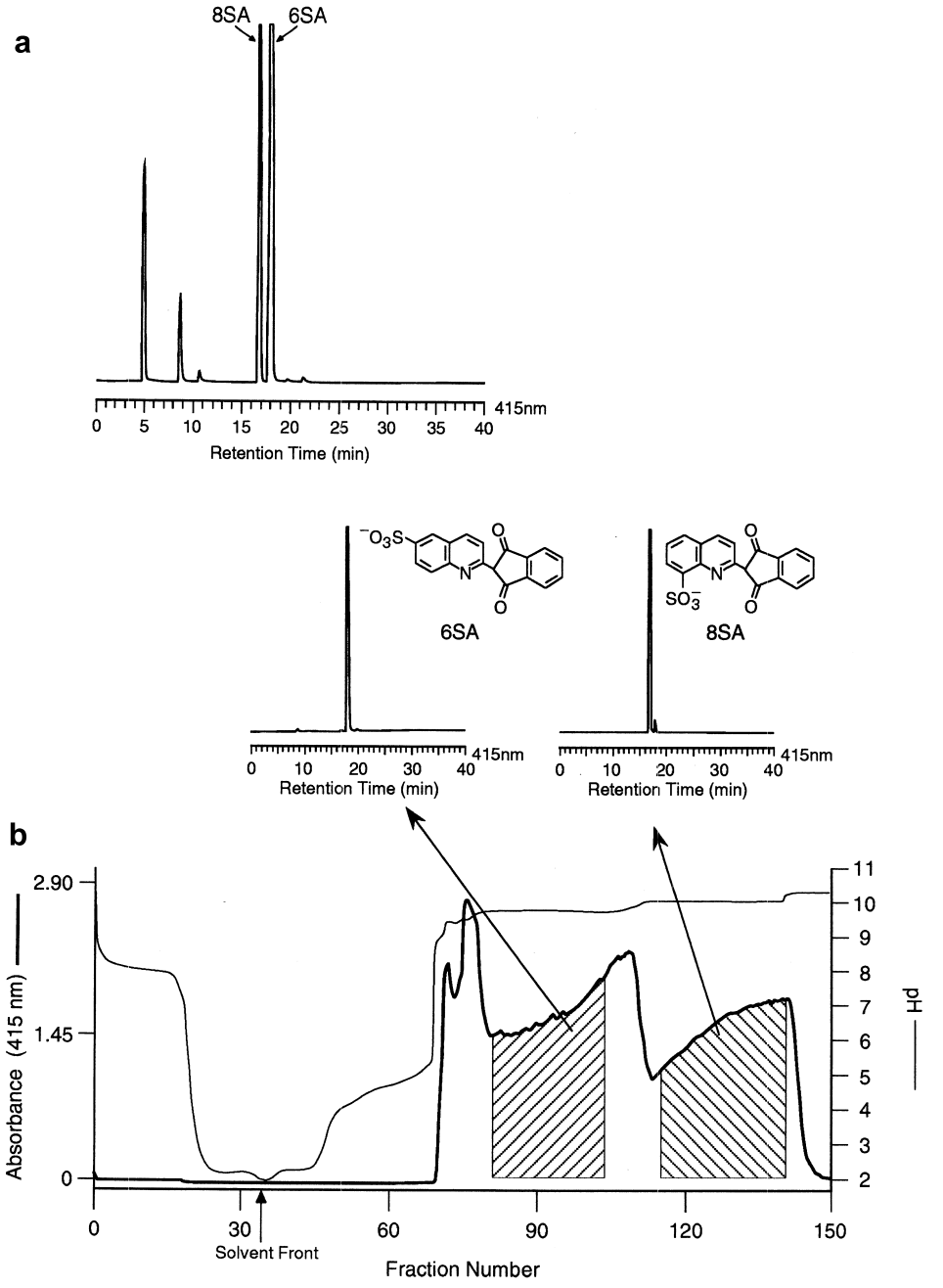

Fig. 3. Separation of 6SA and 8SA from a certified lot of D&C Yellow No. 10 by pH-zone-refining CCC. (a) HPLC analysis of the certified lot of color additive, (b) pH-zone-refining counter-current chromatogram of the separation of a 1.8-g portion of color additive and HPLC analyses of the separated components.

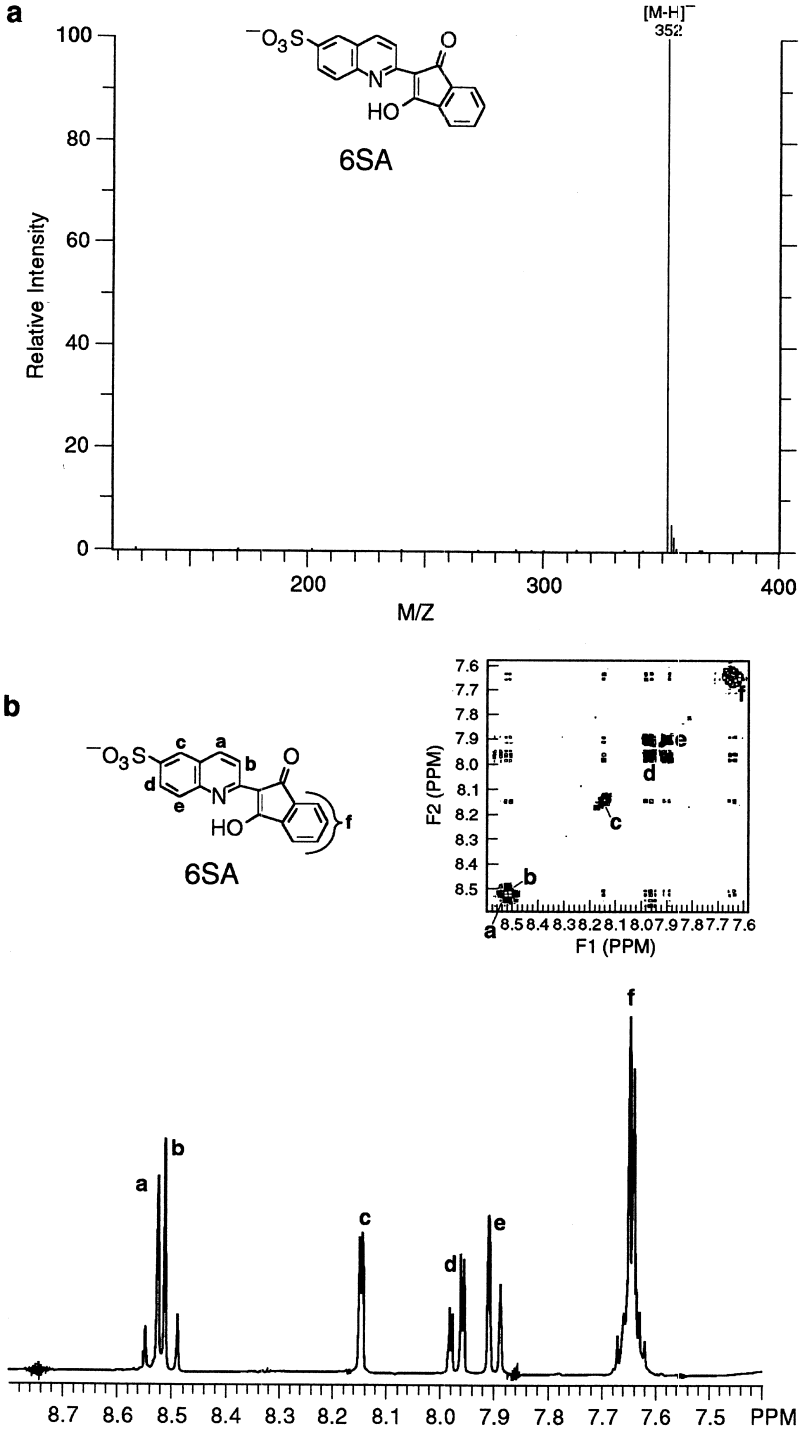

Fig. 4. Characterization of the compound contained in fractions 81–103 of the pH-zone-refining CCC separation shown in Fig. 3. (a) Negative ion APCI mass spectrum, (b) ^{1}H-NMR spectrum and COSY assignments.

H-c), 7.97 ppm (dd, 8.6, 1.75; H-d), 7.90 ppm (d, 8.6; H-e), and 7.65 ppm (m, 4H; f); 8SA, [2-(2-quinolinyl)-1H-indene-1,3(2H)-dione-8′-sulfonic acid, (Fig. 5)], 8.69 ppm (dd, 9.3, 1.6; H-a), 8.42 ppm (d, 9.3; H-b), 8.09 ppm (dd, 7.7, 1.3; H-c), 7.91 ppm (ddd, 7.7, 1.7, <1; H-d), 7.65 ppm (m, 4H; e), and 7.49 ppm (t, 7.7; H-f).

3. Results and discussion

The analytical RP-HPLC analysis of three typical commercial batches of D&C Yellow No. 10 are presented in Fig. 2. The two main components (6SA and 8SA) are accompanied by contaminants that vary in level among batches. The HPLC analysis of the batch of D&C Yellow No. 10 used in the present work is shown in Fig. 3a. The UV (415 nm) and pH profiles of the eluate obtained from the pH-zone-refining CCC separation of a 1.8 g test portion of this dye is shown in Fig. 3b.

The separation was completed in ~5 h. After the solvent front (first fraction containing mobile phase) emerged at fraction 34, a steady carryover of the stationary phase containing the ligand (dodecylamine) was observed until the pH of the effluent became basic when the elution of the ligand stopped at fraction 69 (Fig. 3b). The UV chromatogram obtained for this separation shows two characteristic rectangular-shaped peaks associated with two pH zones. The eluates corresponding to these peaks were collected in fractions 81–103 and 114–138. Fractions 81–103 (hatched area on the left in Fig. 3b) contained a single component whose RP-HPLC peak (Fig. 3b) corresponded to the retention time of 6SA in Fig. 3a. The 6SA isolated from these combined fractions (0.6 g) was over 99% pure (by HPLC at 415 and 254 nm, Fig. 3b). Fractions 114–138 (hatched area on the right in Fig. 3b) contained a single component whose RP-HPLC peak (Fig. 3b) corresponded to the retention time of 8SA in Fig. 3a. The 8SA isolated from these combined fractions (0.18 g) was over 99% pure (by HPLC at 415 and 254 nm, Fig. 3b). The isolated monosulfonated dyes were characterized by negative ion APCI-MS and ^{1}H-NMR spectra and COSY assignments. The soft ionization involved in the APCI process produced the quasi-molecular ions $[M-H]^-$, at m/z 352, that provided information about the molecular mass of the isomers (Figs. 4a and 5a). Certain assignments of the proton NMR signals of 6SA and 8SA were relatively straightforward; however, in 6SA, the specific assignment of the resonances at 8.53 ppm and 8.51 ppm to H-a and H-b, respectively, is not obvious (Fig. 4b). A COSY spectrum showed cross-peaks between the signal at 8.53 ppm and those at 8.15 ppm (H-c) and 7.90 ppm (H-e), characteristic of long-range, aromatic-proton couplings. These couplings established the 8.53-ppm signal as that of H-a. In 8SA, the signal assignments were somewhat more complex (Fig. 5b). A COSY spectrum demonstrated that the resonances at 8.09, 7.91, and 7.49 ppm belonged to the A-ring, three-spin system. Moreover, weak cross-peaks between the signal at 8.42 and that at 7.91 ppm established the former as that of H-b and the latter as due to H-d. The remaining resonance at 8.69 ppm is then assigned to H-a. These assignments are consistent with those of two previous investigations [2,3] and at variance with certain designations of another [5].

The retention of the stationary phase measured after the separation was 13.8%. Attempts to improve the retention of the stationary phase by both (a) increasing the quantity of retainer acid (H_2SO_4) in the sample solution and/or in the stationary phase and (b) by reducing the concentration of the eluter base (NH_3) in the mobile phase were not successful.

In order to enhance the partitioning of the strongly-acidic sulfonated dyes into the organic phase of the aqueous–organic biphasic solvent system used, it was necessary to add a basic ligand, dodecylamine (approximately 5%), in the organic phase. A higher concentration of dodecylamine was found to adversely affect the retention of the stationary phase in the column during separation. Preliminary experiments aimed at separating the monosulfonated dyes using a more hydrophobic basic ligand, tridodecylamine, were unsuccessful because of the high viscosity of the organic phase that resulted in minimal retention of the stationary phase during separation.

Several conditions must be met for conducting pH-zone-refining CCC. Two of them involve specifications for the chosen retainer acid: (i) the retainer acid should partition primarily into the organic stationary phase so that it can be gradually

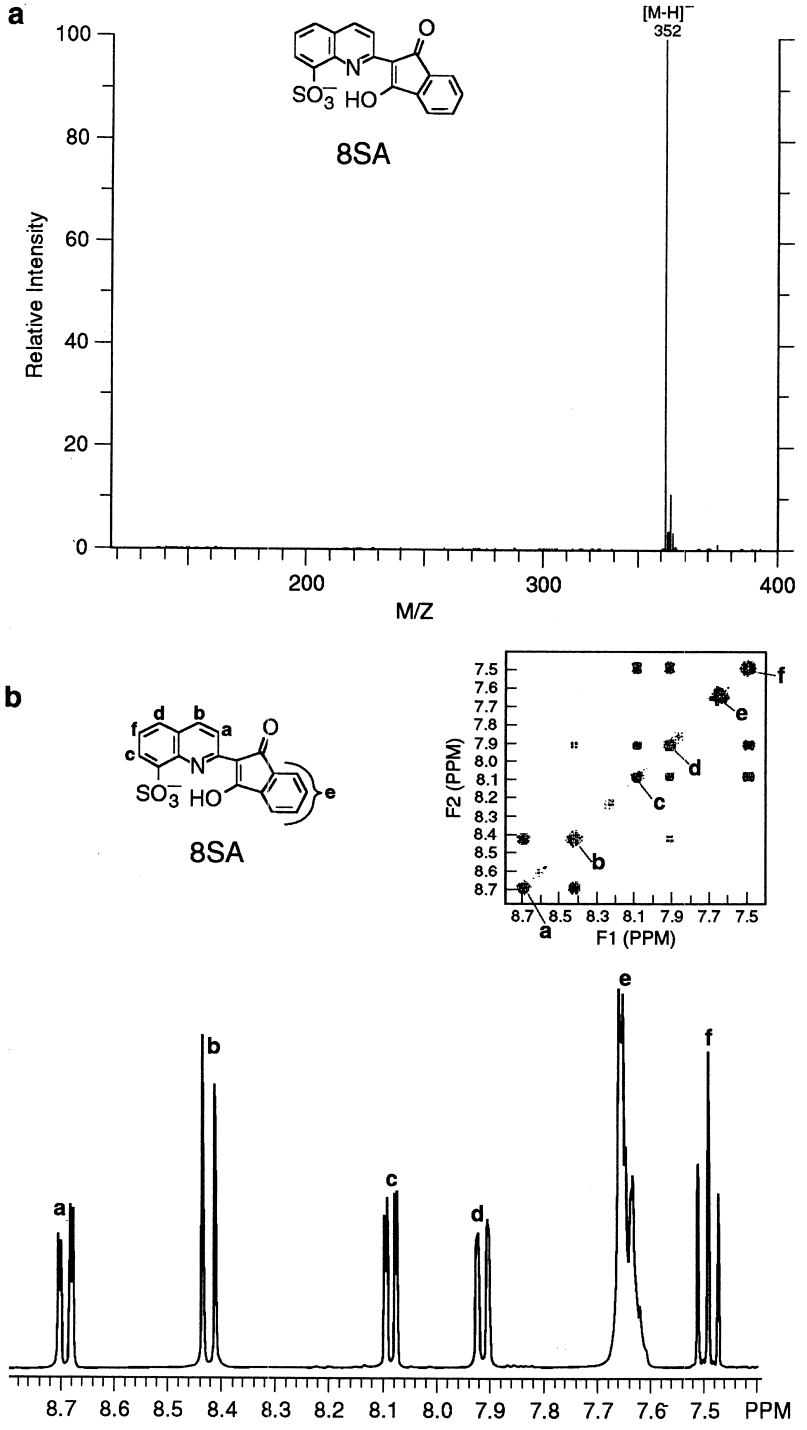

Fig. 5. Characterization of the compound contained in fractions 114–138 of the pH-zone-refining CCC separation shown in Fig. 3. (a) Negative ion APCI mass spectrum, (b) ^{1}H-NMR spectrum and COSY assignments.

eluted along with the flowing aqueous mobile phase, thus forming the sharp trailing border needed for separation [10]; and (ii) the pK_a value of the retainer acid should be lower than those of the acid analytes to be separated. Trifluoroacetic acid (TFA) meets the first specification, and with a pK_a value of ≈0.47 [21], it has been used as the retainer acid for protonating organic acids with relatively high pK_a values (between 3 and 4). Sulfonic acids, however, have much lower pK_a values (between −1.5 and −5.6 in water) [22] than that of TFA, and therefore, the latter is incapable of protonating the former and cannot be used as the retainer acid for the separation of the monosulfonated components of D&C Yellow No. 10. An alternative choice for the retainer acid might be an inorganic acid, whose pK_a value is lower, e.g., sulfuric acid whose pK_{a1}≈−3.1 [22], but that is also not possible since such an acid is found almost exclusively in the aqueous mobile phase of the two-phase solvent system and is quickly eluted out with the mobile phase. For the monosulfonated components of D&C Yellow No. 10, a different approach was necessary because they are both strong acids and partition primarily into the aqueous phase of a two-phase solvent system. Addition of a ligand (an ion exchanger) to the organic stationary phase enabled their separation. For the present study, DA was chosen as the ligand, since it is retained totally in the organic phase regardless of whether it is a free amine or in a form combined with the sulfonated analytes. Under those circumstances, the present CCC system becomes comparable to that of a conventional ion-exchange chromatographic system that uses solid support. Under the acidic conditions, the ligand becomes ionized and combines with the sulfonated dyes that are then retained in the organic stationary phase. When the mobile phase containing an eluter base (ammonia in this particular case) flows through the column, the dyes are gradually released from the ligand (according to their pK_a and hydrophobicity [10,11]) into the mobile phase where they serve as the counterion against NH_4^+, leaving the ligand as a free base in the organic phase. A schematic drawing of the separation process is shown in Fig. 6. This ion-exchange modification was effective for the separation of the D&C Yellow No. 10 monosulfonated positional isomers by pH-zone-refining CCC because the pK_a and hydrophobicity of these isomers seem to be significantly different. The same approach was previously used for the separation of the main component from the disulfonated azo dye FD&C Yellow No. 6 [17]. A similar approach (using an acid ligand, [di-(2-ethylhexyl)phosphoric acid], in the organic stationary phase and an eluter acid (HCl) in the mobile phase) was previously used for the separation of a 0.5 g mixture of catecholamines [23].

4. Conclusion

pH-Zone-refining CCC has been previously applied to the preparative separation of mixtures of compounds that contain carboxylic groups such as hydroxyxanthene dyes, amino acids and peptides, different stereoisomers and positional isomers [11]. This study expanded the applicability of pH-zone-refining CCC to a new field, the preparative sepa-

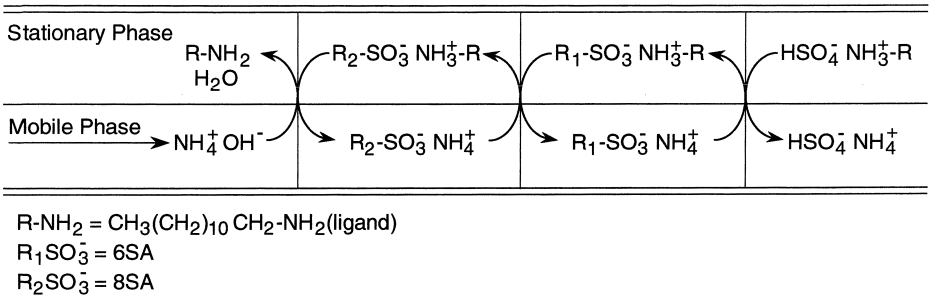

Fig. 6. Schematic chemohydrodynamic diagram of the reverse displacement mode of pH-zone-refining CCC in the separation of the two monosulfonic acid isomers of D&C Yellow No. 10 using an ion exchanger as the retainer ligand. The two middle fragments represent the processes that take place in the 6SA and 8SA separation zones.

ration of strongly hydrophilic compounds containing sulfonic acid groups.

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