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Preparative separation of isomeric sulfophthalic acids by conventional and pH-zone-refining counter-current chromatography ☆

Adrian Weisz[a,*], Eugene P. Mazzola[b], Constance M. Murphy[a], Yoichiro Ito[c]

[a]*Office of Cosmetics and Colors, Center for Food Safety and Applied Nutrition, Food and Drug Administration, 200 C Street SW, Washington, DC 20204, USA*

[b]*Office of Scientific Analysis and Support, Center for Food Safety and Applied Nutrition, Food and Drug Administration, 200 C Street SW, Washington, DC 20204, USA*

[c]*Laboratory of Biophysical Chemistry, NHLBI, National Institutes of Health, Bethesda, MD 20892, USA*

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Abstract

Two modes of high-speed counter-current chromatography (HSCCC) were applied to separate 3- and 4-sulfophthalic acid from a mixture. Conventional HSCCC was useful for the separation of up to several hundred milligram quantities of these positional isomers, while pH-zone-refining CCC was implemented successfully to separations at the multigram level. The conventional HSCCC separations were performed with a standard J-type HSCCC system that has a superior resolution but a lower level of retention of the stationary phase of the biphasic solvent system used (acidified *n*-butanol–water). The pH-zone-refining CCC separations were performed with an X-type HSCCC system (a cross-axis system) that has a higher capability for retention of the stationary phase. The purified positional isomers (over 99% pure as determined by HPLC) were characterized by ^{1}H NMR and negative ion electrospray ionization mass spectrometry.
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1. Introduction

D&C Yellow No. 10 [Quinoline Yellow (QY), Colour Index 47005] is a color additive permitted for use in drugs and cosmetics in the USA [1]. It is batch-certified by the US Food and Drug Administration (FDA) to ensure compliance with specifications required by the Code of Federal Regulations (CFR) [1]. D&C Yellow No. 10 is manufactured currently [1] as was described for the preparation of QY more than a hundred years ago [2]. Specifically, one condenses 2-methylquinoline, **1**, with phthalic anhydride, **2**, and the condensation product, 2-(2-quinolinyl)-1H-indene-1,3(2H)-dione, **3**, is then sulfonated. The resulting products are isolated as sodium salts (Fig. 1). D&C Yellow No. 10 consists primarily of a mixture of the sodium salts of monosulfonic acid isomers (mainly **4** and **5** in Fig. 1) with up to 15% of the disodium salts of the disulfonated isomers. A variant form of QY contains

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*Corresponding author. Tel.: +1-20-22-054-718; fax: +1-20-22-055-098.

E-mail address: aweisz@cfsan.fda.gov (A. Weisz).

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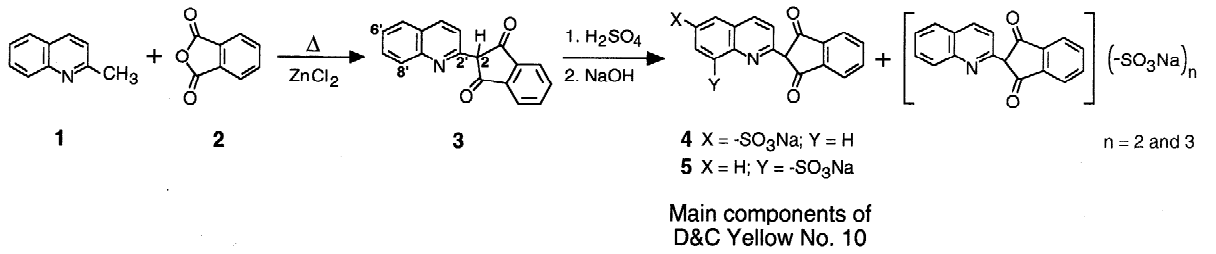

Fig. 1. Preparation of D&C Yellow No. 10 by condensing quinaldine, **1**, with phthalic anhydride, **2**, and sulfonating the condensation product **3**.

mostly di- and trisulfonated components and is not certifiable in the USA, but it is used for coloring foods in Europe (E-104) and drugs and cosmetics in Japan (Yellow 203) and other countries.

Among the CFR specifications enforced by the FDA are the permitted levels of sulfonated phthalic acids sodium salts (not more than 0.2%) present in D&C Yellow No. 10. These compounds [3-sulfophthalic acid (3SPA), 4-sulfophthalic acid (4SPA) and 3,5-disulfophthalic acid (3,5SPA)] may be produced as byproducts during the preparation of D&C Yellow No. 10. Also, their presence in the reaction mixture may result in the formation of adducts sulfonated in the indanedione moiety (e.g. **6** and **7** in Fig. 2).

For the development of analytical methods to be used for FDA batch-certification of D&C Yellow No. 10, purified mono-, di- and trisulfonated components of QY as well as purified sulfophthalic acids are required as reference materials. Most of these compounds are not commercially available; therefore, several of them were previously prepared in our laboratory [3,4]. Due to the nonspecific sulfonation of the phthalic acid, 3SPA and 4SPA are obtained as a mixture. This mixture is labeled on commercially-available lots as containing up to 25% 3SPA. While 4SPA may be purchased at a purity of ~97%, 3SPA is not commercially available. Besides its use as a reference material, purified 3SPA is needed as the starting material for the preparation of 3,5SPA [5], which is also not commercially available. Most of the literature related to these compounds is in patent format and pertains to their use as starting materials for the preparation of sulfonated phthalocyanine dyes or for other applications [6–8].

While two analytical methods for the separation of

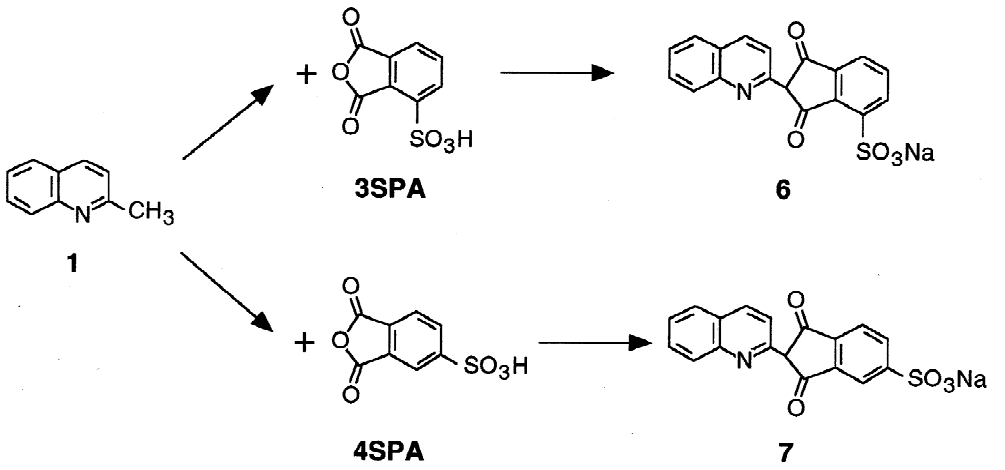

Fig. 2. Preparation of 2-(2-quinolinyl)-1H-indene-1,3(2H)-dione-4- or 5-sodium sulfonates (**6** or **7**, respectively) by condensing quinaldine, **1**, with 3- or 4-sulfophthalic anhydride, respectively.

3SPA from 4SPA have been published [9,10], no preparative separation method for these isomers has been reported. In the present study, high-speed counter-current chromatography (HSCCC) [11] was chosen to separate gram quantities of a mixture of 3SPA/4SPA that contained ~10% 3SPA. Counter-current chromatography is a liquid–liquid partition technique that does not involve use of a solid support. In conventional HSCCC, one of the liquid phases (stationary phase) is retained in an Ito multi-layered-coil column by centrifugal force while the other (immiscible) liquid phase is pumped through the column. The separation depends on the partition coefficient of the solute and the retention of the stationary phase. A variation of HSCCC was relatively recently developed and is known as pH-zone-refining CCC [12–15]. pH-Zone-refining CCC enables the separation of organic acids and bases according to their pK_a values and hydrophobicities. This technique was used previously for the separation of dyes and intermediates that contain carboxylic or sulfonic acid groups [3,4,16]. The separations are performed with various types of coil planet centrifuges (CPC) some of which were described earlier [11]. One system that is commercially available is the standard J-type HSCCC [17] system that provides excellent resolution. Another system that currently exists as a prototype is the X-type HSCCC system (a cross-axis system) [18] that provides higher retention of the stationary phase and is used for separations of peptides [19] and proteins [20,21].

In the present study, HSCCC in its two forms, conventional and pH-zone-refining CCC, was applied to the preparative separation of 3SPA and 4SPA from a commercial mixture using the J- and X-type coil planet centrifuges, respectively.

2. Experimental

2.1. Materials

The mixture of 3- and 4-sulfophthalic acids trisodium salts (labeled ''4-sulfophthalic acid, trisodium salt, tech., 75%. Remainder 3-sulfophthalic acid, trisodium salt'') used for CCC separations was purchased from Aldrich (Milwaukee, WI, USA). *n*-Butanol, water, hydrochloric acid (~37%), formic acid, ammonium hydroxide (28–30%) and acetonitrile were from J.T. Baker (Philipsburg, NJ, USA). Phosphoric acid (85%) was from Fisher Scientific (Fair Lawn, NJ, USA).

2.2. Conventional high-speed counter-current chromatography

2.2.1. Instrumentation

The conventional HSCCC separations were performed with a J-type 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 computerized data acquisition. The instrument used, along with the added improvements, was previously described [22].

2.2.2. Separation procedure

The conventional HSCCC separations were performed following the general directions described earlier [11]. The solvent system was chosen so that the value of the partition coefficient of the components, $K_{\text{Upper Phase/Lower Phase}}$, ($K_{\text{UP/LP}}$) would be in the vicinity of 1. The two-phase solvent system used, consisted of *n*-butanol–water (600 ml:600 ml). To this mixture was added 4.5 ml of HCl conc. and the pH became approximately 1.2. The sample in this solvent system had a $K_{\text{UP/LP}}$ of 0.7. The solvent system was equilibrated in a separatory funnel, and the two phases were separated before use, resulting in 650 ml of upper organic phase (UP) and 540 ml of lower aqueous phase (LP). The organic UP was used as the stationary phase and the aqueous LP was used as the mobile phase. The separation was initiated by filling the entire column with the stationary phase using the LC pump, and then loading the sample dissolved in a mixture of stationary and aqueous phases (in the ratio of 1:1, e.g. 5 ml:5 ml for a 230 mg sample portion). To the sample solution was added HCl conc. until the pH of the sample solution became 0.9. The mobile phase was then pumped into the column at 3 ml/min while the column was

rotated at 850 rev./min. The column effluent was monitored (UV scanning from 220 to 450 nm while the adjustable pathlength of the preparative flow cell was set to ~0.06 mm) and a fraction collector was used to obtain 3 ml fractions. The fractions collected were brought to dryness using a speed vac concentrator and were analyzed by high-performance liquid chromatography (HPLC).

2.3. pH-zone-refining counter-current chromatography

2.3.1. Instrumentation

The pH-zone-refining CCC separations were performed with a prototype of an X-type high-speed CCC system (cross-axis CPC) [18], that consisted of a column (two Ito multilayer-coils connected in series made of 2.6 mm I.D. PTFE tubing with a total capacity of ~575 ml) mounted on a rotating frame (with the axis of the column rotation perpendicular to the centrifuge axis), a speed controller and an LC pump. The instrument used was previously described and depicted in a photograph [18].

2.3.2. Separation procedure

The pH-zone-refining CCC separations followed previously established procedures [4]. The two-phase solvent system used consisted of *n*-butanol–water (1:1). The solvent system was equilibrated in a separatory funnel, and the two phases were separated before use. The organic UP was acidified with HCl conc. to pH ~0.5 (488 m*M* in HCl). The aqueous LP was rendered basic by addition of ammonium hydroxide resulting a ~105 m*M* solution in NH_3 (pH ~10.7). The acidic organic phase was used as the stationary phase and the basic LP was used as the mobile phase. The separation was initiated by filling the entire column with the stationary phase using the LC pump, and then loading the sample dissolved in a mixture of stationary and aqueous phases (in the ratio of 4:1, e.g. 40 ml:10 ml for a 5 g sample portion). To the sample solution was added HCl conc. until the pH of the sample solution became ~0.5. The mobile phase was then pumped into the column at 2 ml/min while the column was rotated at 715 rev./min in the combined head to tail elution mode P_1–H–O [11,18]. The absorbance of the eluate was continuously monitored at 206 nm and 4-ml fractions were collected. The pH of each eluted fraction was measured with a pH meter. The fractions collected were brought to dryness using a speed vac concentrator and were analyzed by HPLC.

2.4. High-performance liquid chromatography

Analytical reversed-phase HPLC were performed with a Waters Alliance 2690 Separation Module (Waters, Milford, MA, USA). The eluent was 10 m*M* phosphoric acid (pH ~2.4). The column [Prodigy ODS (2), 5 μm particle size, 100×1.0 mm I.D., Phenomenex, Torrance, CA, USA] was eluted isocratically at 0.1 ml/min. The effluent was monitored with a Waters 996 photodiode array detector set at 254 nm. Injection volume was 5 μl.

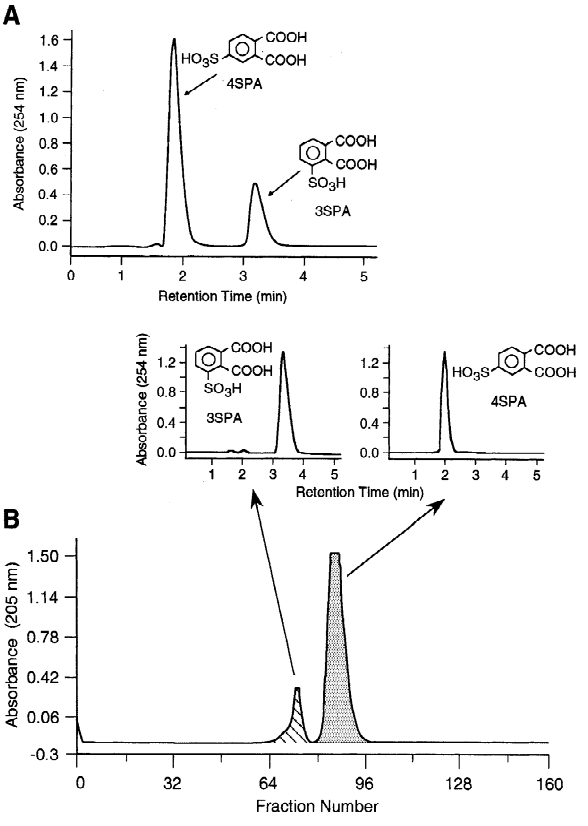

Fig. 3. Separation of a mixture of 3SPA and 4SPA by conventional HSCCC. (A) HPLC analysis of the original mixture, (B) high-speed countercurrent chromatogram of the separation of a 230 mg portion of the mixture and HPLC analyses of the separated components.

A 10 μl aliquot from the CCC collected fractions was diluted with HPLC eluent (0.5 ml) and filtered through a Mini-UniPrep 0.45-μm pore size PTFE syringeless filter device (Whatman, Clifton, NJ, USA) prior to chromatography. For the 5 and 10 g separations where the collected fractions were more concentrated, 50 μl from the above solution was diluted further by a factor of 10 prior to filtration and injection.

2.5. Mass spectrometry

The mass spectra were acquired with a LCQ ion trap mass spectrometer (Finnigan Mat, ThermoQuest, San Jose, CA, USA). The instrument was fitted with an electrospray (ESI) source. All samples were dissolved in acetonitrile–water (1:1) to which was added 0.1% formic acid, and infused at a rate of 3 μl/min. Lens voltages were optimized in negative ion mode by turning on the ion of interest. The data was acquired and processed using the Xcalibur software v. 1.0. The negative ion ESI parameters were: sheath gas 60 arbitrary units, spray voltage 4.5 kV, capillary temperature 200 °C, capillary voltage 26 V.

2.6. ^{1}H nuclear magnetic resonance spectrometry

The ^{1}H-nuclear magnetic resonance (NMR) spec-

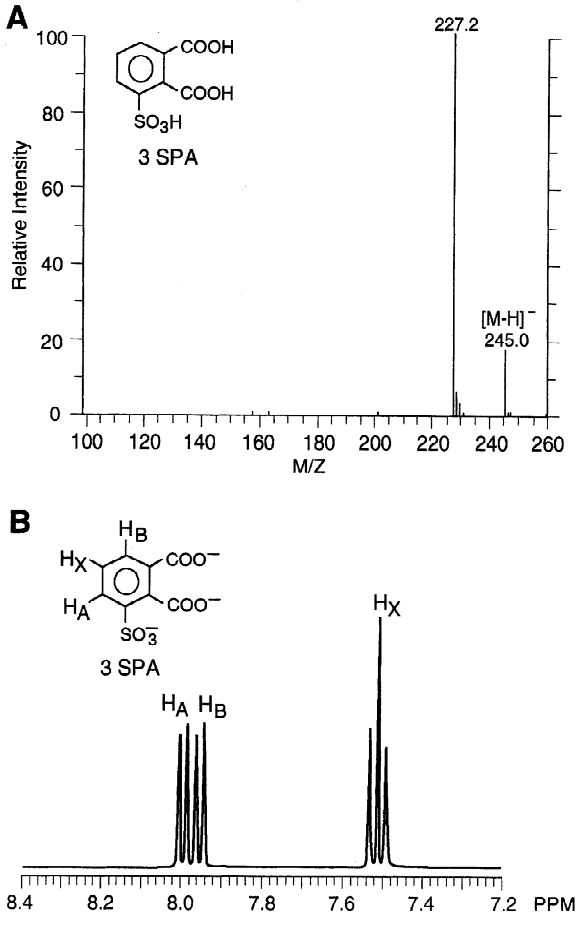

Fig. 4. Characterization of the compound contained in fractions 68–76 of the HSCCC separation in Fig. 3. (A) Negative ion ESI mass spectrum (B) ^{1}H NMR spectrum (in $2H_2O$, 400 MHz).

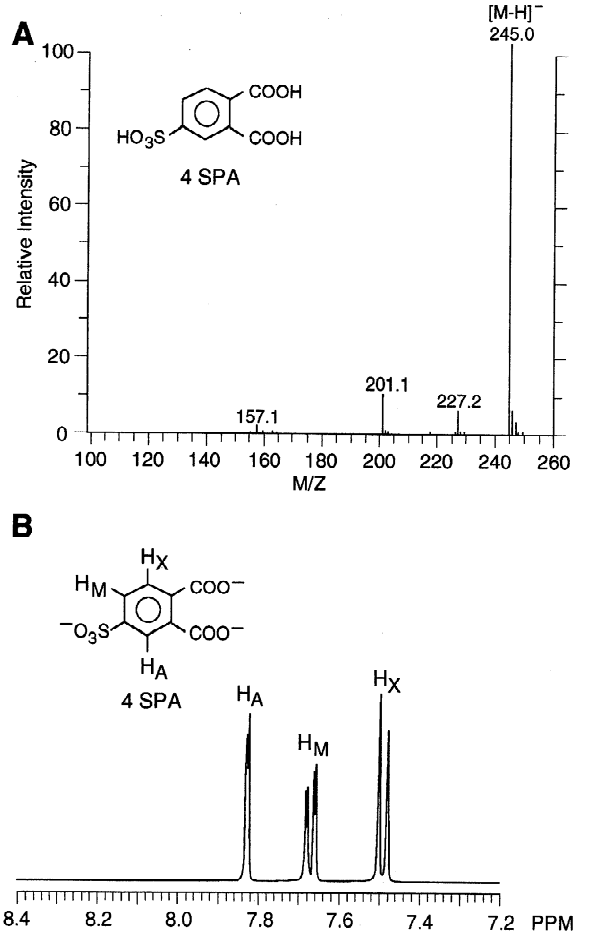

Fig. 5. Characterization of the compound contained in fractions 81–91 of the HSCCC separation in Fig. 3. (A) Negative ion ESI mass spectrum (B) ^{1}H NMR spectrum (in $2H_2O$, 400 MHz).

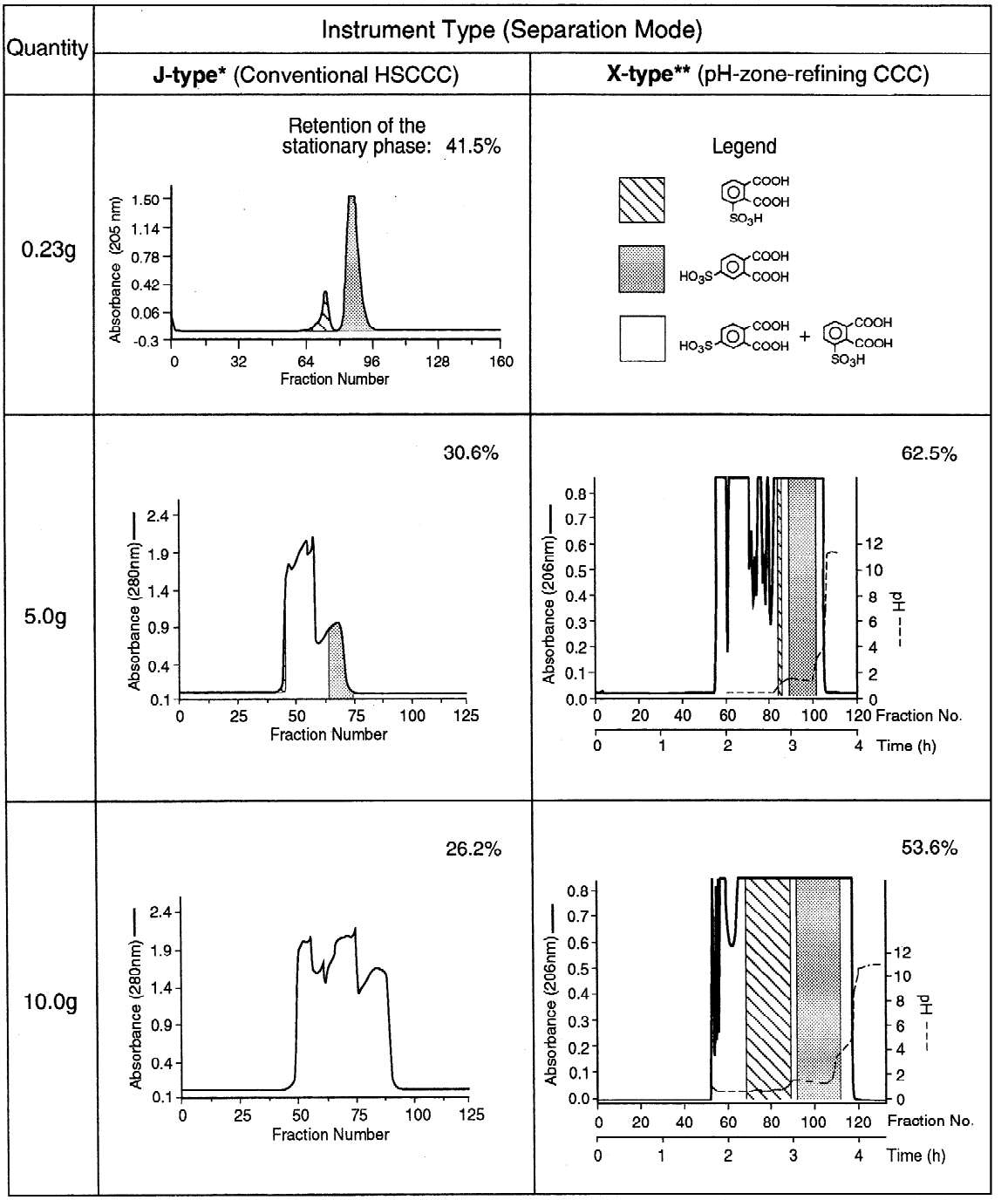

Fig. 6. Separation of 3SPA and 4SPA by conventional and pH-zone-refining HSCCC using the J- and X-type coil planet centrifuges, respectively.

tra of 3SPA and 4SPA were obtained on a Varian VXR-400S spectrometer operating at 400 MHz. Approximately 10 mg each of the purified compounds were dissolved in 140 μl of $^{2}H_{2}O$ and the spectra recorded with standard 10-ppm spectral widths and acquisition parameters. The following signals were obtained and assigned for each of the two isolated sulfophthalic isomers: **3SPA**, (3-sulfophthalic acid, Fig. 4), 8.00 ppm (dd, 8,1 Hz; H_A), 7.96 ppm (dd, 8,1 Hz; H_B) and 7.51 ppm (t, 8 Hz; H_X); **4SPA**, (4-sulfophthalic acid, Fig. 5), 7.83 ppm (d, 1.7 Hz; H_A), 7.67 ppm (dd, 8, 1.7 Hz; H_M) and 7.49 ppm (d, 8 Hz; H_X).

3. Results and discussion

The HPLC analysis of the commercial mixture of 3- and 4SPA trisodium salt (labeled ''4-sulfophthalic acid, trisodium salt, tech., 75%. Remainder 3-sulfophthalic acid, trisodium salt'') is shown in Fig. 3A. The mixture was found by HPLC (206 nm) to contain ~10% 3SPA. Fig. 3B shows the countercurrent chromatogram obtained for the separation of 230 mg of this mixture by conventional HSCCC using a commercially-available J-type CPC. The solvent front (first fraction containing mobile phase) emerged at fraction 58 and the retention of the stationary phase, measured after the separation, was 41.5% of the total column volume. The chromatogram consisted of two peaks. The fractions that corresponded to these peaks (68–76 and 81–91) contained single components (as shown by the associated HPLC chromatograms in Fig. 3B) which were isolated and were identified by negative ion ESI–MS and ^{1}H NMR as 3SPA (Fig. 4) and 4SPA (Fig. 5), respectively.

Attempts to separate larger quantities of this mixture, 5 and 10 g portions, by conventional HSCCC failed mainly due to the poor retention of the stationary phase. The retention of the stationary phase (which is a measure for the quality of the separation) dropped to 30.6 and 26.2%, respectively. The CCC chromatograms for these separations are shown in Fig. 6. The experiment that involved 5 g of sample, resulted in partial separation of the 4SPA while the experiment that involved 10 g of sample, resulted only in fractions with various degrees of mixture.

To separate larger quantities of these isomers, a different approach was necessary. The samples (5 and 10 g) were subjected to pH-zone-refining CCC (a relatively new HSCCC technique for the preparative-scale separation of ionizable compounds) [12–15] using an X-type (cross-axis) CPC [18]. The X-type CPC has a higher capability for retention of the stationary phase than the J-type instrument. This capability was demonstrated when stationary phase retention of 62.5 and 53.6% were obtained for the 5 and 10 g separations, respectively. The pH-zone-refining CC chromatograms for these separations are shown in Fig. 6. The chromatograms have the broad rectangular shape characteristic of pH-zone-refining CCC [12–15]. The two broad absorbance plateaus (hatched area, more evident in the 10 g separation) correspond to the two pH plateaus (dotted line). Each plateau represents elution of a pure compound. For the 10 g separation, the eluates collected in fractions 76–88 contained ~440 mg of over 99% pure 3SPA (by HPLC) and the eluates collected in fractions 98–107 contained ~4.73 g of over 99% pure 4SPA (by HPLC).

4. Conclusion

It has been shown that HSCCC chromatography, in both its conventional and pH-zone-refining modes, is an effective method for the separation of 3- and 4-sulfophthalic acid mixtures. Conventional HSCCC is useful for separating up to several hundred milligram quantities of these positional isomers, while pH-zone-refining CCC, using an X-type (cross-axis) CPC, results in excellent separations at the multigram level.

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