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Continuous fractionation of enantiomer pairs in free solution using an electrophoretic analog of simulated moving bed chromatography

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Abstract

Continuous fractionation of the left and right enantiomers of Piperoxan was performed in free solution in a vortex-stabilized electrophoresis apparatus. Sulfated β-cyclodextrin was used as the chiral selector. A capillary electrophoresis (CE) study of the separation of Piperoxan enantiomers was carried out in order to find the buffer conditions that produce the maximum peak separation time between the two enantiomers and the optimal chiral selector concentration. These peak separation times were then used to calculate the electrophoretic mobilities of the enantiomer–ligand complexes. The difference in electrophoretic mobilities, when used in a preliminary model of the enantiomer separation, indicated that, by imposing a fluid flow opposite the direction of electromigration, it would be possible to force the fast and slow enantiomers to move in opposite directions within the vortex-stabilized apparatus. Using the predictions of the preliminary separation model, the vortex stabilized electrophoresis apparatus was configured with a feed port at the center of the chamber axis and offtake ports near the cathode and anode. This allowed for continuous operation of the apparatus. Continuous fractionations were completed at throughputs of 1.5 and 4.0 mg/h with both offtakes showing greater than 99% enantiomeric purity at 4.0 mg/h using CE. Fractionation was achieved at a throughput of 10 mg/h, but while the slow enantiomer was recovered with greater than 99% purity, only 96% enantiomeric purity of the fast stereoisomer was achieved. The loss of resolution at higher volumetric throughputs supports our hypothesis that a mobility-dependent ''window'' of operation exists in which two solutes can be completely separated.

Keywords: Enantiomer separation; Simulated moving bed chromatography; Preparative electrophoresis; Pharmaceutical analysis; Piperoxan; Cyclodextrins

1. Introduction

In the year 2000, chiral drug sales accounted for one third of all pharmaceutical products sold worldwide [1]. While most of these drugs are synthesized stereospecifically, the advent of powerful analytical and process scale separation techniques gives downstream process developers more options when the drugs are in the research pipeline. These options allow drug companies to evaluate a greater number of potentially less expensive synthesis methods and can present significant cost savings in the overall drug discovery process [2].

Electrophoretic separations of enantiomer pairs have been conducted using capillary electrophoresis (CE) since the early 1990s but, unlike analytical chiral chromatography, there is no useful technique that scales directly to production levels by simply

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increasing the capillary diameter. Because of its high resolution, CE has the advantage of being able to separate enantiomers even when the selectivity is prohibitively low for a chromatographic separation [3]. Another advantage of chiral electrophoresis is the wide variety of enantioselective ligands available. Most of these ligands are based on either micelles of small molecules, cyclodextrins or the 18-crown-6 ethers which both allow anionic, cationic and neutral functional groups to be attached, increasing their utility as chiral selectors [4]. In fact, more than 30 distinct functionalized classes of electrophoretic chiral selectors exist based on cyclodextrins alone [5], and kits are commercially available which allow rapid screening of the many types of selectors [6].

1.1. Current enantiomer separation technologies in production

Usually the cheapest available method for enantiomer separation is crystallization as energy consumption is low and only enantiomerically pure seed crystals are required [7]. It is commonly used in the generic drug market where manufacturing cost pressures are important and large quantities of stereoisomers are being processed [8].

Though enantioselective chromatography is usually expensive, it takes less time to develop than crystallization and is readily scaleable to production levels from analytical data. In the production scale separation of enantiomers, two main techniques are generally used, batchwise and simulated moving bed (SMB) chromatography [3]. The advantage of batchwise chromatography is that it is directly scalable from an analytical separation, but it has several disadvantages. Batchwise chromatography usually generates large amounts of organic eluent waste, significantly dilutes the products and long elution times are required to recover the pure products from the column [9].

SMB chromatography was developed in order to decrease the amount of solvent waste generated and increase process throughput per gram of resin by eluting multiple columns simultaneously and recycling the eluent. Since the enantioselective resins are a significant portion of the cost of chromatography systems, the increased throughput of SMB provides an operating cost advantage over that of batchwise chromatography [3]. SMB also lends itself to production scale better than batchwise chromatography because of its continuous operation, but it requires more development time and is sensitive to dead volume effects which must be accurately modeled [9]. Despite the disadvantages of SMB, several contract manufacturers and pharmaceutical companies such as Aerojet Fine Chemicals, Novasep, UPT and Merck have invested heavily in SMB technology, making it a cost effective production scale enantiomer separation technique [1–3,9].

1.2. Preparative scale electrophoretic enantiomer separations

Though there is no simple technique that directly scales from CE, several groups have actively been attempting to conduct electrophoretic enantiomer separations at the preparative scale. Stalcup et al. have performed both batch [10] and continuous elution [11] separations in gel media containing a sulfated β-cyclodextrin chiral selector. The continuous elution separation was carried out in a Bio-Rad Mini Prep Cell and was reported to resolve a 0.5 mg loading of racemic Piperoxan in 220 min [11]. Stalcup and co-workers have also reported separations of 0.45 mg/h racemic Piperoxan using a sulfated β-cyclodextrin running buffer in a thick free flow electrophoresis chamber cooled with PTFE capillaries [12,13]. Thormann and co-workers [14,15] have utilized continuous flow zone electrophoresis in the Octopus apparatus to process up to 15 mg/h feeds of both racemic chlorpheniramine and methadone. Between 700 and 800 μg/h of each chlorpheniramine enantiomer was recovered at greater than 97% optical purity. For methadone, up to 3.8 mg/h of the *R*-enantiomer and 1.1 mg/h of the *S*-enantiomer were recovered at 97% purity [14]. Vigh and co-workers have successfully implemented isoelectric focusing of enantiomer pairs using the Octopus apparatus which allowed a separation of 1.3 mg/h of racemic dansylated amino acids [16,17]. Glukhovskiy and Vigh have also reported that certain analyte–selector systems, when used at the proper concentrations, can provide the different enantiomers with equal and opposite mobilities. This allowed them to separate up to 2.8 mg/h of racemic ter-

butaline in the Octopus apparatus with a purity of 97% and a recovery of 50% [18,19].

The Octopus apparatus used by Thormann and co-workers and Vigh and co-workers is analogous to rotating annular chromatography as described by Canon et al. [20]. The disadvantage of this process is that all the offtake fractions are removed from the unit regardless of whether sample is contained, which increases solvent waste. An SMB separation has a single offtake upstream of the feed and a single offtake downstream of the feed reducing the amount of solvent used. Until now, there has never been a continuous electrophoretic chiral separation comparable to SMB chromatography that can be adapted for use with any analyte–selector system.

The present work demonstrates a continuous chiral separation which is analogous to SMB chromatography, at the preparative scale in a vortex-stabilized electrophoresis chamber in free solution, i.e., without packings or gels. CE was used to pre-optimize the separation buffer conditions for maximum peak separation prior to the preparative scale separations and the andrenergic blocker Piperoxan, shown in Fig. 1, was chosen as the candidate enantiomer as it has been previously shown to work in CE with sulfated-β-cyclodextrin as the chiral selector [21].

2. Theory

The basic principle of electrophoresis is that charged particles move in an electric field. It is useful as a separation technique because different compounds move at different speeds, depending on their electrophoretic mobilities. The velocity of a compound in an electric field is:

$$u = \mu E \tag{1}$$

where E is the electric field strength, u is the electromigration velocity and μ is the electrophoretic mobility. Since stereoisomers have the same charge and the molecules are the same size, the electrophoretic mobilities of both enantiomers are expected to be the same. In order to resolve the stereoisomers, an enantioselective ligand is attached to the enantiomers and the resulting enantiomer–selector complexes have different electrophoretic mobilities [22].

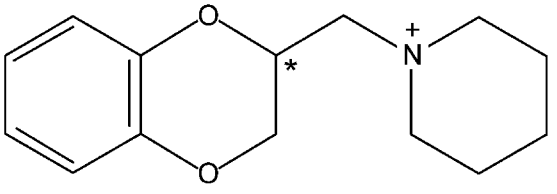


Fig. 1. Piperoxan under neutral pH. The asterisk denotes the chiral center.

Culbertson and Jorgenson [23] have shown that the net electromigration can be halted by a counteracting hydrodynamic flow in capillary electrophoresis. The development of a continuous countercurrent enantiomer separation builds from this idea. The velocity difference between the enantiomer complexes (Δu) can be expressed as:

$$\Delta u = (\mu_F - \mu_S)E \tag{2}$$

where μ_F and μ_S are the mobilities of the fast and slow enantiomer complexes, respectively. In order to allow continuous separation, a fluid flow is imposed in the direction opposite of electromigration at the arithmetic average velocity of the fast and slow enantiomer–ligand complexes, defined as:

$$\left\langle u \right\rangle = \frac{\mu_F + \mu_S}{2} \cdot E \tag{3}$$

Combining the electrophoretic and hydrodynamic migration components gives the total velocity of each enantiomer complex:

$$u_F = \mu_F E - \left\langle u \right\rangle = \frac{\mu_F - \mu_S}{2} \cdot E > 0 \tag{4}$$

$$u_S = \mu_S E - \left\langle u \right\rangle = \frac{\mu_S - \mu_F}{2} \cdot E < 0 \tag{5}$$

which demonstrates that the imposed hydrodynamic counterflow forces the fast and slow enantiomer–ligand complexes to move in opposite directions. Fig. 2 illustrates this principle with a theoretical continuous separation. Here, the racemic enantiomer is continuously fed into the middle of the electrophoresis chamber, halfway between the cathode and the anode, and products are continuously drawn off near the cathode (slow enantiomer complex) and anode (fast enantiomer complex) of the apparatus.

With this method, unlike traditional thin-film preparative electrophoresis, the residence time of the enantiomers is not limited by the fluid flow in the

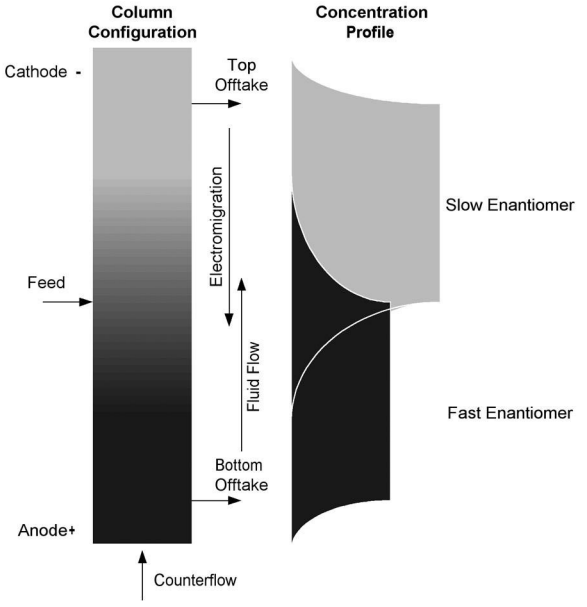


Fig. 2. A schematic depiction of a moving-bed electrophoretic enantiomer separation. A racemic feed is injected into the middle of the apparatus and the fast and slow products are removed near the ends. A counterflow of a solution of the chiral selector is pumped in from the bottom such that the chamber velocity is greater than the slow enantiomer–ligand complex velocity and less than the fast enantiomer–ligand complex velocity, resulting in the concentration profile shown on the right.

chamber. The stereoisomers do not leave the column until they reach the top and bottom offtake ports which in turn allows differing amounts of each enantiomer to accumulate within the column before the apparatus reaches steady-state. Changing the counterflow velocity within the column affects the amounts of the fast and slow enantiomers present at steady-state. As the counterflow velocity is increased within the apparatus, the fast enantiomer is retained longer as its total velocity decreases. Conversely, the slow enantiomer retention time is decreased as the counterflow velocity is increased, since its total velocity increases. The opposite happens when the counterflow is decreased but in all cases the rate of each enantiomer removed from the column must equal the amount fed into the column to satisfy a mass balance at steady state.

SMB chromatography and moving bed electrophoresis are similar in that they both require control of four distinct regions and produce pure products at the proper eluent or counterflow velocities, respectively, but there are several distinct advantages of the electrophoretic moving bed technique over SMB. First, no packing is required and true continuous operation is possible without using any valve or column switching. Juza et al. have previously defined this type of separation in chromatography as a true moving bed (TMB) separation [2]. Second, since the pharmaceutical and fine chemicals industries are predominantly based upon batch processes, it is important to segregate production lots and avoid cross-contamination. The use of a soluble chiral selector allows the unit to be completely cleaned and sanitized in place between each lot since the soluble selector ligand can be easily flushed from the apparatus. The economics of chromatographic separations necessitate the reuse of the stationary phases, so cleaning the column between lots has to be done delicately to avoid damaging the expensive resins.

3. Experimental

3.1. Chemicals and buffer preparations

All chemicals used were of analytical or research grade. β-Cyclodextrin, sulfated (degree of substitution 7–11), sodium salt (Na-SCD) was obtained from Aldrich (Milwaukee, WI, USA). Racemic Piperoxan hydrochloride, Trizma base (Tris), and Bis–Tris, were obtained from Sigma (St. Louis, MO, USA). Glacial acetic acid was obtained from Fisher Scientific (Fair Lawn, NJ, USA), and sodium hydroxide pellets, sodium chloride, 86% phosphoric acid, and 36.5–38% hydrochloric acid were obtained from J.T. Baker (Phillipsburg, NJ, USA). Nanopure water was obtained from a Barnstead Thermolyne (Dubuque, IA, USA) Nanopure Infinity UV/UF system.

Buffers were prepared by measuring the weight of base, adding it to the quantity of water desired and adjusting the pH with the concentrated acid component. All pH values were measured with the chiral selector in solution. All solutions were prepared the night before their use and stored in the refrigerator.

3.2. Capillary electrophoresis system

A HP^{3D} capillary electrophoresis system (Hewlett-Packard, Waldbronn, Germany) with a 51.5 cm×48

μm I.D. bare fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) was used. The detector was set to 214 nm, the capillary cartridge thermostat was set to 24 °C and sample injection was accomplished using a pressure of 50 mbar for 5 s. Capillary preconditioning consisted of flushing with 0.1 *M* sodium hydroxide for 1 min, 0.1 *M* phosphoric acid for 1 min, nanopure water for 1 min and running buffer for 2 min. Unless otherwise stated the running buffer was 1% (w/v) Na-SCD, 10 m*M* Tris–acetate at pH 4.80.

3.3. Vortex-stabilized electrophoresis chamber

The apparatus used for the continuous enantiomer separation was a vortex-stabilized electrophoresis device designed by Ivory and Gobie [24]. This apparatus consists of a 1 in. diameter grooved, hollow, boron nitride rotor and complimentarily grooved Plexiglas stator, a two-tank electrode buffer recirculation system and a motor with speed control circuitry to spin the rotor (1 in. = 2.54 cm). A photograph of the apparatus is shown in Fig. 3a with an exploded view of the rotor and stator shown in Fig. 3b and a schematic of the system with peripheral components is shown in Fig. 3c.

The 0.2 mm grooves in the rotor and stator create a vortex pattern in the annular fluid which is similar in structure to, but distinct in nature from, Taylor vortices. Taylor vortices are the end result of a centrifugal instability which occurs when a smooth cylinder is rotated above a critical angular velocity inside of a concentric smooth stator, as shown in Fig. 3b. The stable vortex flows in this chamber are driven and shaped by the complementary grooves machined into the rotor and stator. The axis of rotation of these vortices is perpendicular to the surface of the cylinders and form pairs that rotate in opposite directions within the annulus.

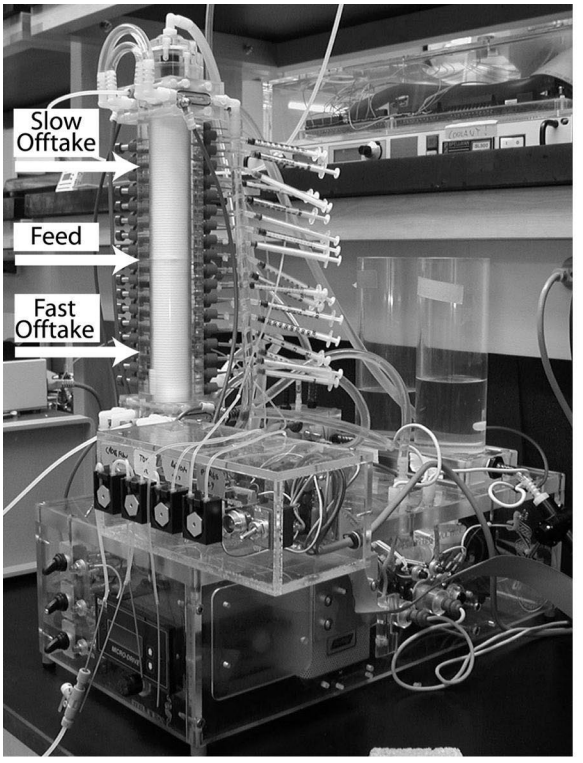
These vortices drive radial mixing which allows for better heat and mass transfer between the rotor and stator and are also used to stop natural convection from mixing the fluid axially. The Taylor-like vortices stabilize the axial concentration profile, preventing natural convection from disturbing the concentration profile which results from the electrophoretic separation. These vortices also provide for nearly an order of magnitude higher available power dissipation within the apparatus as compared to running without vortex formation.

A Syltherm XLT coolant oil (Dow, Midland, MI, USA) was recirculated through the inside of the rotor to dissipate the Joule heat generated from electrophoresis. This oil was cooled using a VWR 1140 recirculating cooler (VWR, S. Plainfield, NJ, USA), and a static mixer (Omega, Stamford, CT, USA) was used inside the rotor to promote internal heat transfer.

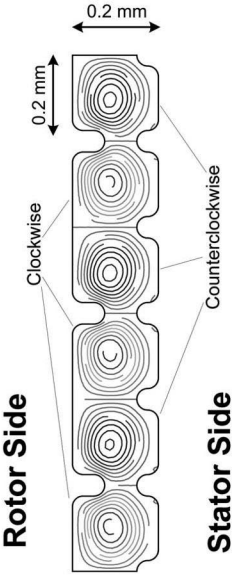
The ends of the stator on the electrophoresis chamber are machined to accept Plexiglas electrode compartments which are partitioned from the separation chamber with dialysis membranes. For the enantiomer separation, there are four electrode chambers in which buffer is recirculated and four inner buffer chambers which segregate the electrode compartments from the separation chamber. The outer electrode and inner buffer chambers are partitioned by 6000 MWCO dialysis membranes (Spectrum Labs., Houston, TX, USA) and the inner buffer and separation chambers are separated by Spectra/Por CE 500 MWCO dialysis membranes (Spectrum Labs.) in order to prevent cyclodextrin from diffusing into the buffer chamber. The dual buffer chamber system was installed in the apparatus to remove up to 250 ml/h of electrolysis gases that accumulate during an enantiomer separation.

The electrode buffers are housed in two tanks at the back of the apparatus. These cylindrical tanks hold approximately 0.5 l and have a Plexiglas weir which allows the electrolysis gases to escape from the fluid. Two March 1A-MD-1 pumps (March, Glenview, IL, USA) are housed beneath the buffer tanks. The outer electrode buffer was run through a 2 in. diameter cylinder, 4 in. long packed debubbler filled with 200 mesh glass beads. This debubbler consolidated small bubbles that formed within the electrode chamber, and allowed them to escape from the top via a 6 ft. long section of 1/4 in. I.D. Tygon tubing suspended from the ceiling (1 ft. = 30.48 cm). In order to overcome the added pressure drop, the outer electrode buffer system was fitted with another March 1A-MD-1 pump external to the Chamber immediately in front of the debubbler apparatus.

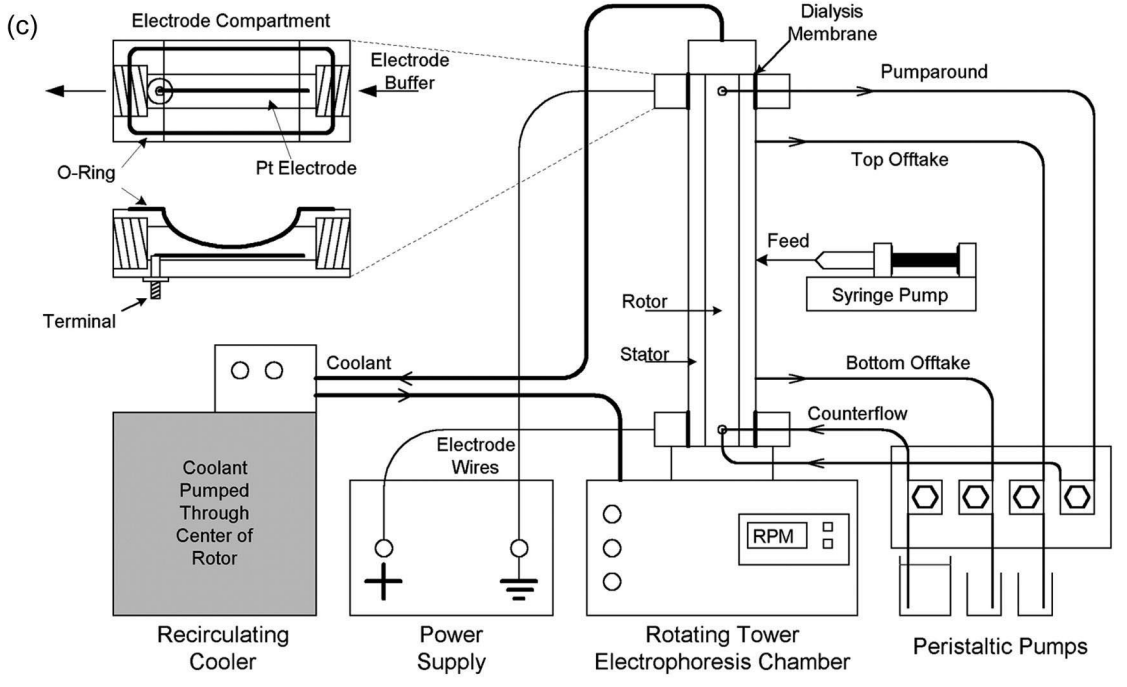
A custom pump box was designed to house four Instech P625/275 pumps (Instech Labs., Plymouth Meeting, PA, USA) as can be seen in Fig. 3a. These peristaltic pumps were controlled via a personal


Slow Offtake
Feed
Fast Offtake

(a)


0.2 mm
0.2 mm
Clockwise
Counterclockwise
Rotor Side
Stator Side

(b)


(c)
Electrode Compartment
Electrode Buffer
O-Ring
Pt Electrode
Terminal
Dialysis Membrane
Pumparound
Top Offtake
Feed
Syringe Pump
Rotor
Stator
Coolant
Bottom Offtake
Counterflow
Electrode Wires
Coolant Pumped Through Center of Rotor
RPM
Recirculating Cooler
Power Supply
Rotating Tower Electrophoresis Chamber
Peristaltic Pumps

computer running LabVIEW with a Computer Boards DAC08 D/A board (Computer Boards, Middleboro, MA, USA). These four pumps allowed continuous variation of the counterflow, top and bottom offtakes, and the purge streams during the continuous separations. The feed was introduced into the column using a Cole Parmer 74900 series syringe pump (Cole Parmer, Vernon Hills, IL, USA).

3.4. Analysis of top and bottom products and final column fractions

All samples taken from the Chamber were analyzed using CE as detailed above. A calibration curve for Piperoxan was developed with 16 concentrations from 1.49 μ*M* to 0.05 *M*. It was found that the useable range of the calibration curve was parabolic, with the fast enantiomer equation being $C = -6.56 \cdot 10^{-10} \cdot A^2 + 7.51 \cdot 10^{-6} \cdot A \pm 9.55\%$ and the slow enantiomer equation as $C = -6.03 \cdot 10^{-10} \cdot A^2 + 6.65 \cdot 10^{-6} \cdot A \pm 9.10\%$, where A is the peak area in mAU min, and C is the molar concentration. The detection limit was determined to be 6 μ*M* which, at the levels of detection used in the continuous runs, allows the determination of up to 99.2% enantiomeric purity.

3.5. Ion-exchange chromatography

In order to reduce the conductivity of the sulfated cyclodextrin running buffer, ion-exchange chromatography was employed to remove sodium ions from the sulfate groups. A Perseptive Biosystems BioCAD 700E HPLC system was used with a Pharmacia SR 10/50 column packed with 34 cm of Dowex-50W strongly acidic cation-exchange resin. A 2% solution of Na-SCD in nanopure water was exchanged to the hydrogen state, H-SCD, then diluted to 1% with nanopure water and titrated with Bis–Tris and acetic acid forming 1% Bis–Tris–SCD (BT-SCD) in a 5 m*M* Bis–Tris–acetate buffer at pH 4.80. This lowered the conductivity of the overall buffer system to 1.5 mS, which represents a 50% reduction from the 3.0 mS, 1% Na-SCD 10% Tris–acetate buffer used in the initial preparative scale experiments. A CE electropherogram of a Piperoxan separation in the BT-SCD buffer is shown in Fig. 4.

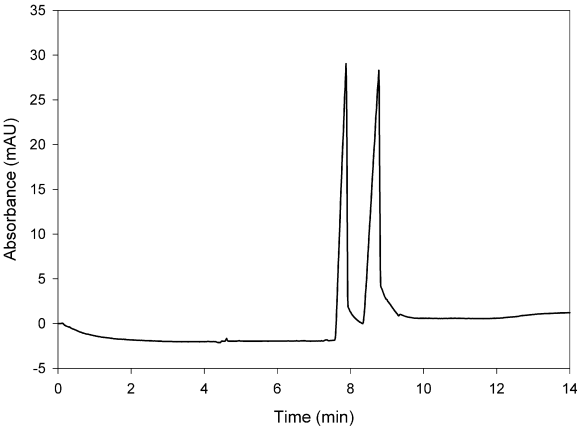


Fig. 4. CE electropherogram of 1 mg/ml Piperoxan in a 1% BT-SCD, 5 m*M* Bis–Tris–acetate buffer at pH 4.80. The 48 μm I.D. capillary was 51.5 cm long, with an applied voltage of 15 kV.

4. Results and discussion

4.1. CE separation of Piperoxan

As a starting point, 2% Na-SCD in a 10 m*M* phosphate buffer at pH 3.8 was used as these were the conditions originally used by Stalcup and Gahm [21]. This resulted in a good baseline separation, but it was desired to maximize the peak separation to allow for faster processing at preparative scale. Fig.

Fig. 3. (a) A picture of the vortex-stabilized apparatus. The two cylinders in the rear are the buffer tanks for the inner and outer electrode buffer compartments. The separation chamber is a 30 cm long vertical cylinder, and is shown in the front. Syringes are placed on the back side to allow for sampling of the column fractions. A custom pump box is shown at the front of the apparatus which controls the top and bottom offtakes, the crossflow and the purge/pumparound streams. (b) An exploded view of a numerical simulation of the vortices formed in the annulus. The rotation between the complimentary grooved rotor and stator creates pairs of convective rolls which revolve in opposite directions. These rolls can be used to stabilize the natural convection that would otherwise destroy an electrophoretic separation [23]. The 0.2 mm grooves in the rotor and stator form an average annular gap of 0.18 mm within the apparatus. (c) A schematic diagram of the complete apparatus used for continuous enantiomer separation.

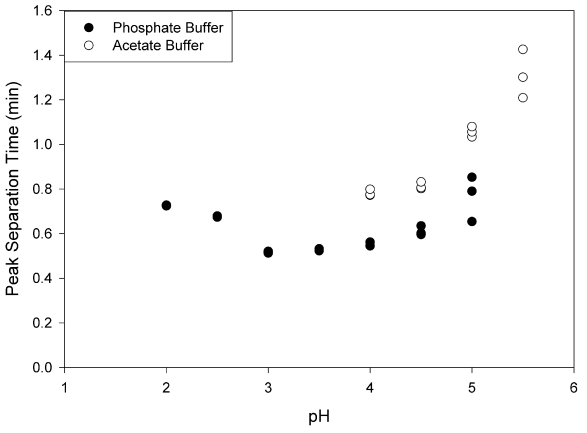


Fig. 5. Piperoxan CE peak separation times as a function of pH in 10 m*M* sodium phosphate and 10 m*M* Tris–acetate buffers. The peak separation time is the difference in elution time between the fast and slow enantiomers. The data scatter at high pH for both buffer systems is due to the low buffering capacities of the 10 m*M* phosphate and 10 m*M* acetate which affected the pH after each run.

5 shows the separation times between the enantiomer peaks as a function of pH in 10 m*M* sodium phosphate or 10 m*M* Tris–acetate buffers. For the phosphate system, the largest separation time measured within the buffering range was at a pH of 2.0. This solution however, was too conductive to use in a preparative scale apparatus because the electrical resistance of a column of fluid is inversely proportional to the conductivity and the cross sectional area and this area increases by five orders of magnitude as it is scaled from CE to the preparative apparatus. Fig. 5 showed an increase in peak separation time with rising pH, but phosphate has insufficient buffering capacity between pH 4 and 5 so a 10 m*M* Tris–acetate buffer was used in place of the phosphate buffer system. The best peak separation time occurred at a pH of 5.5, but this was again outside the buffering range of the system, and a large variance in the electrophoretic mobility was observed. It was decided to use a pH of 4.8, as this provided a reproducible peak separation time of 0.9 min.

Since Na-SCD was the most conductive salt in the running buffer solution, it had the greatest affect on the overall buffer conductivity, and it was necessary to minimize its concentration. Solutions containing 0.1 to 2% Na-SCD were evaluated for use as chiral selectors in the CE buffer and a plot of the normalized Piperoxan peak widths versus Na-SCD concentration is shown in Fig. 6. At Na-SCD concentrations below 0.5%, dispersion began to have a significant broadening effect on the peaks due to the low enantiomer binding capacity of the SCD at these concentrations. It was decided to use 1% Na-SCD in the preparative scale apparatus, as it had a conductivity of 3.0 mS, provided low CE peak dispersion and its enantiomer binding capacity was suitable for preparative scale separations.

4.2. Preparative scale batch Piperoxan separations

A set of batch experiments were performed by injecting a 0.5 ml bolus of 0.1 *M* Piperoxan into the center of the separation chamber and running a counterflow injected at the anode to oppose electromigration. These experiments were performed in order to demonstrate that Piperoxan enantiomers could be separated in the apparatus and to approximate the counterflow velocity required for the Piperoxan fractionation. On the rear of the stator there are 17, 1 ml syringes which are spaced evenly along the axis of the unit between the anode and cathode. These syringes were used to sample the enantiomeric profile across the apparatus after running for 3 h at 550 V (0.16 A/cm^2). The 17 samples

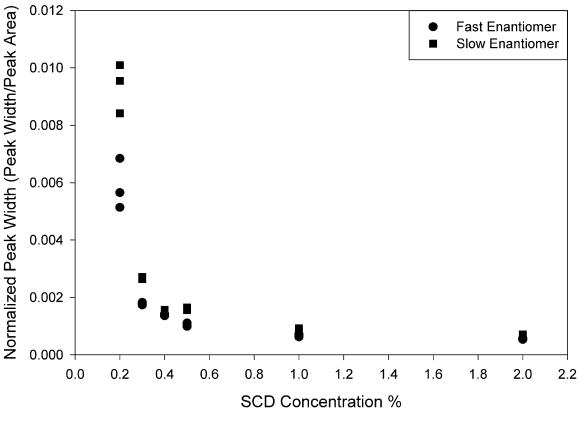


Fig. 6. Normalized peak width vs. chiral selector concentration. As the chiral selector concentration drops below 0.5%, the dispersion becomes large rather quickly.

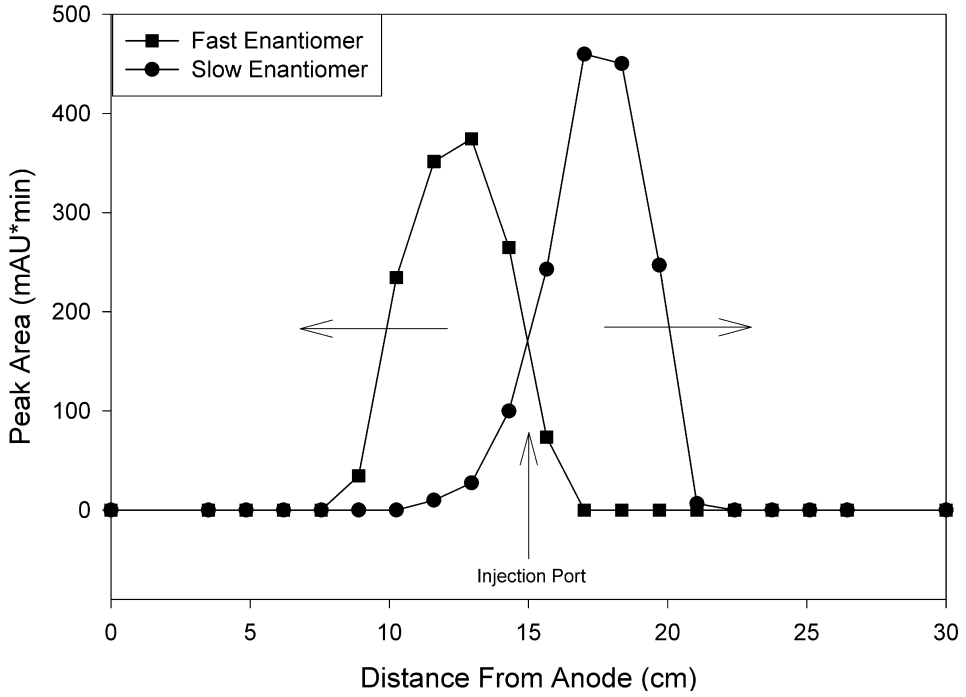


Fig. 7. A batch separation of Piperoxan in the preparative chamber. A bolus of 0.3 ml, 0.1 *M* Piperoxan was injected into the center of the separation chamber and the chamber was run at 550 V for 3 h. A counterflow of 0.102 ml/min was pumped away from the anode.

were analyzed using CE in order to determine the enantiomer composition profile within the chamber.

Fig. 7 shows the enantiomer profile in the chamber after a 3 h batch run. The running buffer used was 1% Na-SCD, 10 m*M* Tris–acetate at pH 4.80 and the electrode buffer was 10 m*M* Tris–acetate at pH 4.80 adjusted to a conductivity of 3.0 mS with sodium chloride to match the conductivity of the running buffer. With the power supply set at 550 V (0.16 A/cm^2) and using a counterflow of 0.102 ml/min, it was observed that the slow and fast enantiomers migrated in opposite directions within the chamber. This batch separation provided a solid starting point for a continuous separation by predicting an approximate counterflow rate and demonstrating that the enantiomer bands could, in fact, be forced to move in opposite directions by adjusting the counterflow.

4.3. Continuous preparative scale Piperoxan separations

The first successful continuous enantiomer separation in the chamber was conducted using a 0.2 ml/h feed injection of 0.025 *M* Piperoxan. This resulted in a separation of 1.5 mg/h of racemic Piperoxan. The buffer system used was 1% BT-SCD in a 5 m*M* Bis–Tris–acetate buffer and the preparative chamber was configured with 0.075 ml/min offtakes 3.5 cm from the top (cathode) and bottom (anode) of the separation chamber. The crossflow was run at 0.192 ml/min, and the power supply was set at 950 V (0.14 A/cm^2) during the course of the separation. When the enantiomeric concentrations at the offtake ports did not change significantly for 3 h, the separation was considered to be at steady state. After 18 h the pumps and the power supply were turned off and the axial Piperoxan enantiomer profile in the chamber was generated by removing 0.3 ml samples from the annulus using the 17 syringes along the rear of the separation chamber.

Fig. 8a shows the final axial Piperoxan enantiomer profile after 18 h in the preparative chamber. The electropherogram of the bottom offtake product, Fig. 8b shows greater than 99% purity of fast enantiomer.

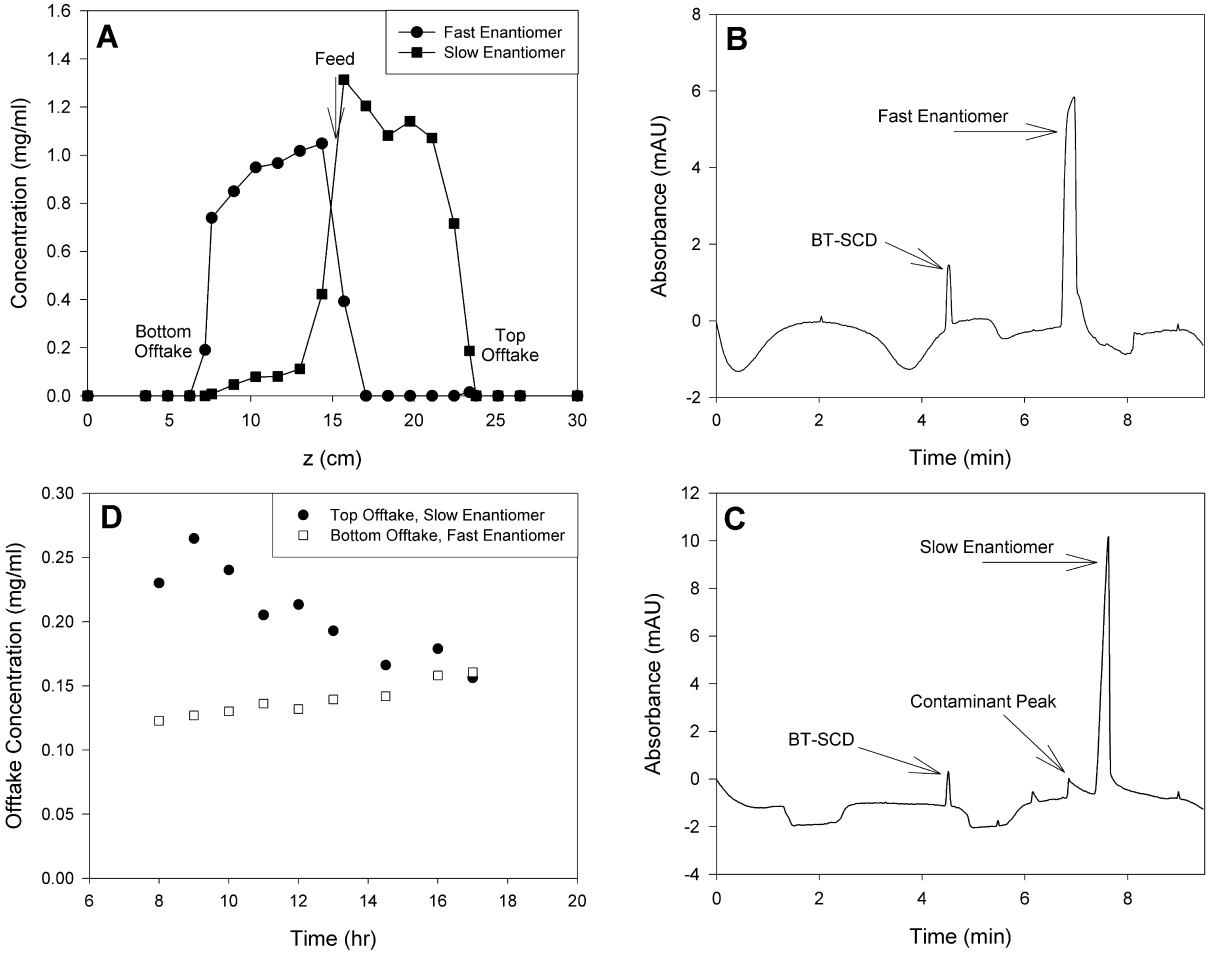


Fig. 8. (A) The Piperoxan enantiomer profile within the preparative chamber after 18 h at 950 V (0.14 A/cm^2) and a throughput of 1.5 mg/h Piperoxan. The anode is $z=0$ cm. (B) An electropherogram of the bottom offtake ($z=7.2$ cm) product showing the pure fast enantiomer. (C) An electropherogram of the top offtake ($z=23.4$ cm) product showing the pure slow enantiomer. There is a small contaminant peak which eluted at the time as the fast enantiomer, but since the profile shows that there is no contamination in the region near the top offtake this was assumed to be some other contaminant. (D) The top and bottom offtake fractions as a function of time showing the approach to steady state.

The electropherogram for the top offtake fraction, Fig. 8c appears to have a small contamination peak near the retention time for the fast enantiomer. As can be seen in the steady-state profile in Fig. 8a, there are no peaks for the fast enantiomer in the vicinity of the top offtake, which makes it unlikely that the small peak is actually a Piperoxan peak. Looking at the electropherogram in Fig. 8c, the baseline has variations of 1 mAU, casting suspicion on any peak smaller than 1 mAU. The 1 mAU contaminant peak that eluted at the same time as the fast enantiomer is not shaped like a Piperoxan peak of similar size, and there are several other 0.5 mAU contaminant peaks in that region of the electropherogram, which leads to the conclusion that this peak is not related to Piperoxan. It is believed that the top offtake was actually greater than 99% pure slow enantiomer, but the contaminant peak makes it impossible to say for certain that the top offtake was greater than 95% pure slow enantiomer.

The approach to steady state during this 1.5 mg/h throughput separation is shown in Fig. 8d. The Piperoxan enantiomer concentrations are plotted as a function of time to demonstrate that the separation had reached steady state within the chamber. It is interesting to note that the slow enantiomer concentration at the top offtake rises to a value above the steady state concentration and then gradually lowers itself. Since the slow enantiomer is migrating against the counterflow, but cannot overtake it, this front becomes self-sharpening as it moves from the middle to the top of the chamber. After the slow enantiomer front reaches the top offtake, the concentration recedes to the steady state value, allowing the mass balance to close.

The reason for the long ''tail'' of slow enantiomer in Fig. 8a is not understood at this time. In the CE electropherograms, Figs. 8b and c, the tailing tends to point towards the cathode, but in the preparative chamber the tailing points toward the anode. It is possible that this is due to a conductivity mismatch between the buffer system and the feed injection

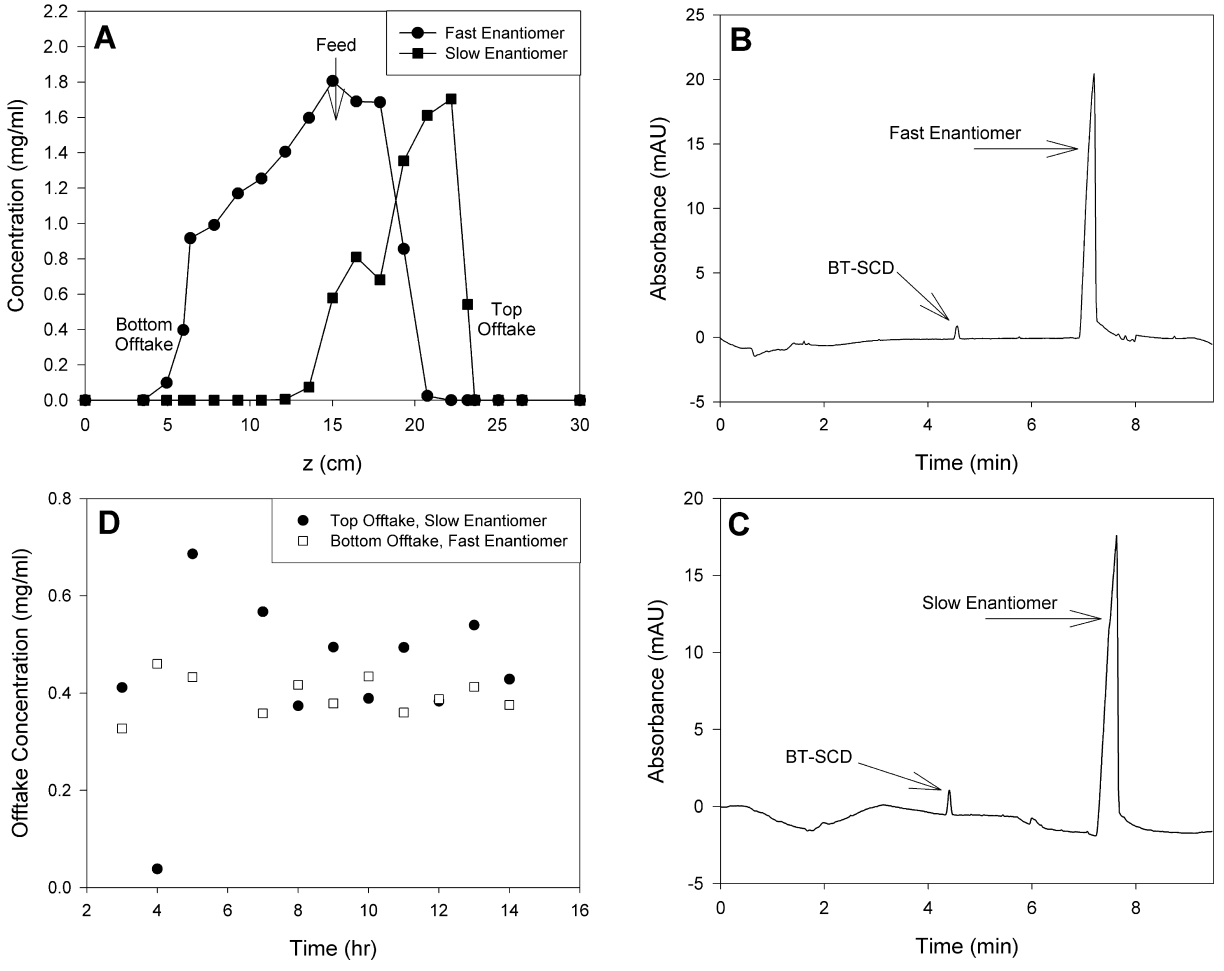


Fig. 9. (A) Separation profile after 14 h at 950 V (0.14 A/cm^2) in the preparative chamber at a throughput of 4.0 mg/h Piperoxan. The anode is $z=0$ cm. (B) An electropherogram of the bottom offtake ($z=7.2$ cm) product showing the pure fast enantiomer. (C) An electropherogram of the top offtake ($z=23.4$ cm) product showing the pure slow enantiomer. (D) The top and bottom offtake fractions as a function of time showing the approach to steady state.

[25], but there were no experiments done to confirm this as minimizing the conductivity is paramount to a successful preparative electrophoretic separation.

With the demonstration of a successful enantioseparation at 1.5 mg/h, it was desired to find the maximum Piperoxan throughput possible using this chamber. In the next set of experiments, the feed concentration was increased to 0.05 *M* and the feedrate was increased to 0.3 ml/h giving a throughput of 4.0 mg/h of racemic Piperoxan. The axial concentration profile, steady state approach, top and bottom offtake electropherograms for a 4.0 mg/h separation are shown in Fig. 9.

For the 4.0 mg/h separation, the counterflow had to be adjusted to 0.298 ml/min, which is significantly greater than the 0.192 ml/min required for the 1.5 mg/h separation. The underlying reason for the discrepancy between these numbers is not understood at this time, but preliminary experiments in the CE have shown that the peak separation time between the enantiomers increases with increasing sample concentration. This phenomenon conflicts

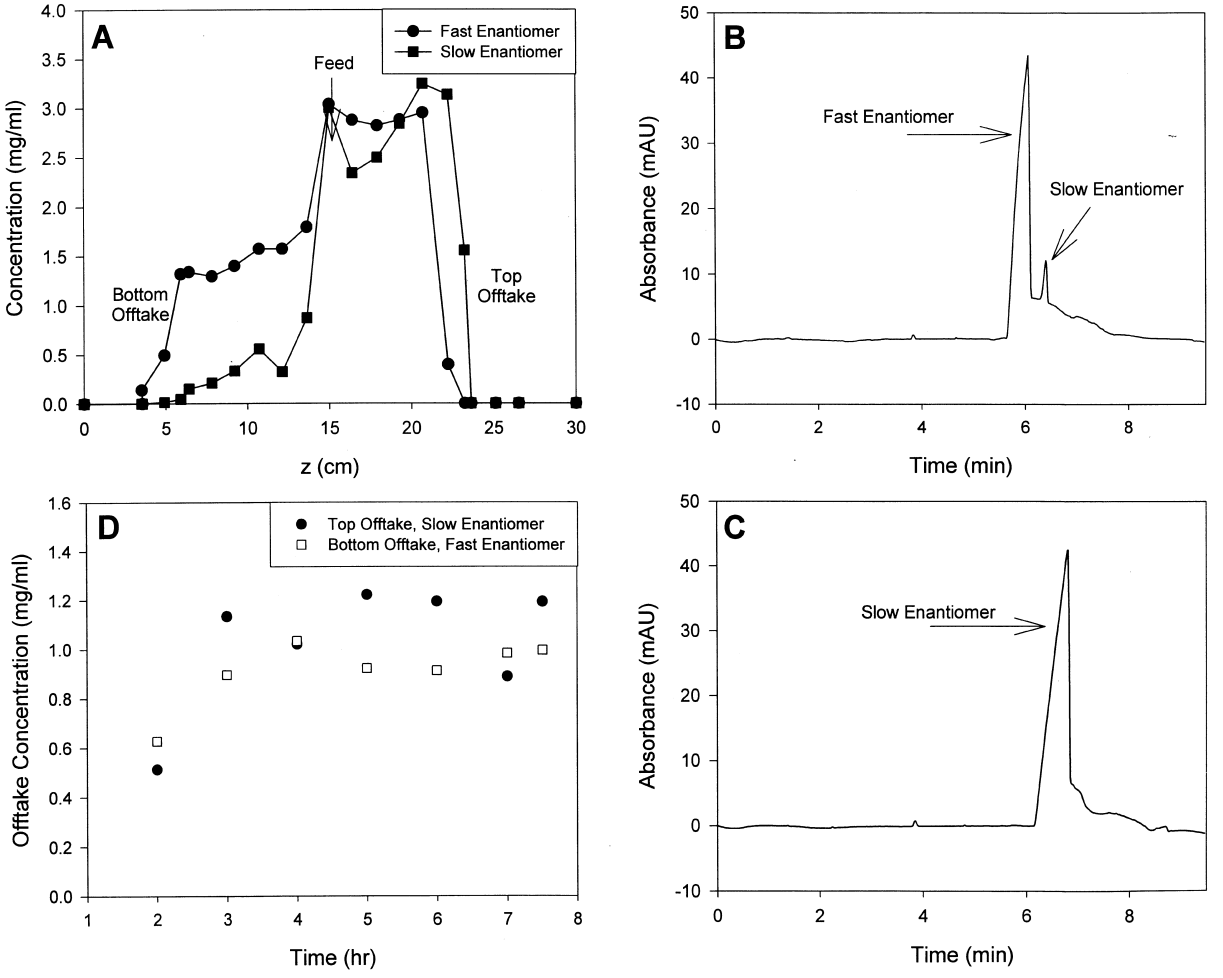


Fig. 10. (A) Separation profile after 7.5 h at 950 V (0.14 A/cm^2) in the preparative chamber at a throughput of 10.0 mg/h Piperoxan. The anode is $z=0$ cm. (B) An electropherogram of the bottom offtake ($z=7.2$ cm) product showing 96% fast enantiomer and 4% slow enantiomer. (C) An electropherogram of the top offtake ($z=23.4$ cm) product showing the pure slow enantiomer. (D) The top and bottom offtake fractions as a function of time showing the approach to steady state.

with the traditional chromatographic observation that resolution decreases due to the larger plate heights with higher loading [26]. The CE qualitatively predicts the changes observed in the chamber separations but it is unable to quantitatively forecast the proper counterflow for successful operation.

It is believed that the larger quantities of Piperoxan HCl being injected into the preparative chamber during the 4.0 mg/h separation caused accumulations of chloride ions near the cathode which increased the conductivity of the separation medium near the electrode and, in turn, lowered the electric field throughout the rest of the separation chamber. It was also observed that a pH gradient formed during the separation with the anode at pH 3.0 and the cathode at pH 4.8. Since sulfated cyclodextrin has a pK_a of approximately 2.0, this was most likely due to these ions accumulating near the anode. Gratz et al. [13] have used dyes that selectively bind to the sulfated cyclodextrin anion and have shown that cyclodextrin does in fact accumulate at the anode during the course of an enantiomer separation. To remedy this situation, the separation chamber was modified so that buffer was pumped from the anode end of the column to the cathode instead of injecting fresh running buffer at the cathode end of the chamber. This pumparound stream decreased the pH gradient effect and stabilized the electric field within the chamber.

The final experiments conducted used 0.05 *M* Piperoxan fed at 0.75 ml/h which corresponds to a throughput of 10 mg/h. The buffer system was changed to 1% BT-SCD in a 10 m*M* Bis–Tris–acetate buffer at pH 4.80, as it was determined by CE that this buffer system had a larger peak separation time than the 5 m*M* Bis–Tris–acetate buffer used previously. With a counterflow of 0.194 ml/min, the separation appeared to reach steady state after 4 h, and the steady state enantiomer profile was sampled at 7.5 h as shown in Fig. 10. As can be seen from the Figure, under these conditions the top offtake product has no detectable impurities but the bottom offtake product has 3.8% cross-contamination of slow enantiomer.

During normal operation, the rotating electrophoresis apparatus has a step change in velocity at the feed port. This is due to the feed flow-rate affecting the counterflow velocity in the upper half of the instrument. As the apparatus is run at higher throughputs, the feed flow-rate increases and the velocity difference between the top and bottom halves of the column becomes larger. If this change in velocity between the regions above and below the feed is greater than the difference in the electrophoretic migration velocities of the enantiomer–ligand complexes, the separation can no longer deliver individual enantiomers in the outlet fractions at both the top and bottom simultaneously. Several experiments were performed in order to find the proper counterflow for the 10 mg/h enantiomer separation. It was determined that 99% purity could not be achieved in both offtakes simultaneously, thus the 0.75 ml/h feedrate was above the maximum feedrate. After this maximum feed flow-rate is reached, the only way to increase the enantiomer throughput is by increasing the feed concentration but, above 0.05 *M*, Piperoxan solutions become cloudy due to solubility limitations.

5. Conclusions

A continuous separation of enantiomer pairs in free solution using an electrophoresis method analogous to SMB chromatography has been described here. Four steady state separations were performed at throughputs of 1.5 and 4 mg/h of racemic Piperoxan, and greater than 99% purity in the outlet fractions was observed at 4 mg/h. In addition, two separations of 10 mg/h racemic Piperoxan resulted in purities greater than 96% at both product offtakes. This work also demonstrated the use of CE as a tool for predicting the scale up of continuous separations. Although the CE analysis was able to find optimum peak separation conditions, it was unable to quantitatively predict the actual operating conditions within the chamber.

As it stands, the enantiomer separation chamber presented here could be used at laboratory scale for the separation of up to 10 mg/h of racemic pharmaceutical compounds with similar enantiomeric resolution. We believe that the vortex-stabilized chamber is scaleable to about 1 g/h although an apparatus larger than our preparative chamber, i.e., a larger rotor and stator, would have to be used. To go beyond 1 g/h by chiral electrophoresis it will be

necessary to use a fundamentally different chamber design due to the large cost of the materials associated with scaling the vortex stabilized apparatus. While there are several approaches described in the literature [27–29] and a few instruments have been pushed beyond 1 g/h with proteins, going to process throughputs in excess of 100 g/h of enantiomer presents a serious challenge to electrophoresis. The challenge is to maintain a suitably high resolution of key components at high power densities without suffering the various detrimental effects of overheating, natural convection and electrohydrodynamic flows, both stable and unstable [30,31].

In the context of the pharmaceutical industry, chiral electrophoresis at scale would provide synthetic chemists with an alternative way to fractionate enantiomers of either a reaction intermediate or a final product. This would allow more options in the quest for the least expensive way to manufacture a compound of interest.

When compared to SMB chromatography, the method of enantiomer fractionation presented in this paper has several advantages. The wide availability of soluble chiral selectors allows the separation of a greater number of stereoisomers, the electrophoretic separation does not require any stationary phase, the unit can be completely cleaned in place preventing lot cross-contamination and in addition, only one chamber is required with no valving, making the process simpler. The major disadvantage of chiral electrophoresis is that no chamber currently exists that will separate more than 1 g/h so it is not currently feasible for use in drug production. SMB chromatography has been used at racemic throughputs greater than 150 g/h [1], which makes SMB the current production method of choice despite its disadvantages. In the future, non-aqueous solvents will be evaluated for chiral electrophoresis as they have been shown to provide higher solubilities and lower conductivities which should allow higher throughputs to be obtained. It is believed that chiral electrophoresis can be scaled to between 10 and 100 g/h using a properly designed thin film electrophoresis chamber.

When considering the process cost of both SMB chromatography and chiral electrophoresis, variables such as labor, solvents and chiral selectors contribute nearly equally to the total expenditure of both systems. The capital cost for a large scale electrophoresis system is expected to be less because the pumps required for operation are less expensive than the high pressure pumps required for SMB, although no instrument has ever been made for comparison. The dominant factor in making an chiral electrophoretic separation economically viable is the successful reprocessing of the solvent and the enantioselective ligand such that they can be reused multiple times. This is similar to SMB, where the resins are reused to lower the operational cost.

References

[1] S.C. Stinson, C&E News 79 (No. 20) (2001) 45.
[2] M. Juza, M. Mazzotti, M. Morbidelli, Trends Biotechnol. 18 (2000) 108.
[3] E.R. Francotte, J. Chromatogr. A 906 (2001) 379.
[4] M. Novotny, H. Soini, M. Stefansson, Anal. Chem. 66 (1994) 648A.
[5] G. Gübitz, M. Schmid, Electrophoresis 21 (2000) 4112.
[6] N. Roos, K. Ganzler, J. Szemán, S. Fanali, J. Chromatogr. A 782 (1997) 257.
[7] V.M. Profir, M. Matsuoka, Colloids Surfaces 164 (2000) 315.
[8] M. McCoy, C&E News 79 (No. 21) (2001) 37.
[9] M. Schulte, J. Strube, J. Chromatogr. A 906 (2001) 399.
[10] A.M. Stalcup, K.H. Gahm, S.R. Gratz, R.M.C. Sutton, Anal. Chem. 70 (1998) 144.
[11] R.M.C. Sutton, S.R. Gratz, A.M. Stalcup, Analyst 123 (1998) 1477.
[12] A.M. Stalcup, R.M.C. Sutton, P. Painuly, J.V. Rodrigo, S.R. Gratz, E.G. Yanes, Analyst 125 (2000) 1719.
[13] S.R. Gratz, E. Schneiderman, T.R. Mertens, A.M. Stalcup, Anal. Chem. 73 (2001) 3999.
[14] M. Wind, P. Hoffmann, H. Wagner, W. Thormann, J. Chromatogr. A 895 (2000) 51.
[15] P. Hoffmann, H. Wagner, G. Weber, M. Lanz, J. Caslavska, W. Thormann, Anal. Chem. 71 (1999) 1840.
[16] P. Glukhovskiy, G. Vigh, Anal. Chem. 71 (1999) 3814.
[17] P. Glukhovskiy, T.A. Landers, G. Vigh, Electrophoresis 21 (2000) 762.
[18] P. Glukhovskiy, G. Vigh, Electrophoresis 21 (2000) 2010.
[19] P. Glukhovskiy, G. Vigh, Electrophoresis 22 (2001) 2639.
[20] R.M. Canon, J.M. Begovich, W.G. Sisson, Sep. Sci. Technol. 15 (1980) 655.
[21] A.M. Stalcup, K.H. Gahm, Anal. Chem. 68 (1996) 1360.
[22] A.S. Cohen, S. Terabe, J.A. Smith, B.L. Karger, Anal. Chem. 59 (1987) 1021.
[23] C.T. Culbertson, J.W. Jorgenson, Anal. Chem. 66 (1994) 955.
[24] C.F. Ivory, W.A. Gobie, US Pat. 5 298 143, March 29, 1994 and 5 200 050, April 6, 1993.

[25] R.L. Williams, B. Childs, E.V. Dose, G. Guiochon, G. Vigh, Anal. Chem. 69 (1997) 1347.
[26] U.D. Neue, in: HPLC Columns, Theory, Technology, and Practice, Wiley, New York, 1997, p. 6.
[27] M. Bier, N.B. Egen et al., in: C.J. King, J.D. Navratil (Eds.), Chemical Separations, Vol. 1, Litarvan Literature, Denver, CO, 1986, p. 133.
[28] C.F. Ivory, Separation and Science Technology 23 (1988) 875.
[29] M. Poggel, T. Melin, Electrophoresis 22 (2001) 1008.
[30] C.F. Ivory, in: J. Asenjo (Ed.), Downstream Processing in Biotechnology, Marcel Dekker, New York, 1990, p. 517.
[31] C.F. Ivory, in: M.C. Flickinger, S.W. Drew (Eds.), Encyclopedia of Bioprocess Technology: Fermentation, Biocatalysis and Bioseparation, Wiley, New York, 1999, p. 910.