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Scalable Enantioseparation of Amino Acid Derivatives Using Continuous Liquid−Liquid Extraction in a Cascade of Centrifugal Contactor Separators

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Abstract:

Using a cascade of six centrifugal contactor separators in a countercurrent liquid−liquid extraction mode allowed the separation of one of the enantiomers of 3,5-dinitrobenzoyl-leucine in 55% yield and 98% ee using a catalytic amount of a chiral host compound based on a cinchona alkaloid. This method allows the preparation of kilograms of enantiopure material in table-top-sized equipment that can be operated in a fume cupboard. The methodology can be easily scaled up to ton amounts as large-volume centrifugal contactor separators are commercially available as well.

Introduction

A growing demand for enantiopure compounds has stimulated research and development on efficient manufacture and separation technologies.[1] A well-known technique to obtain enantiopure compounds on industrial scale is resolution of the racemate through crystallization.[2−4] This technique is not always applicable, and alternatives have been explored. Examples are enantioseparation using simulated moving bed[5,6] related chromatographic techniques,[7] and the use of (liquid) membrane technology.[8−10] Liquid−liquid extraction is also considered a promising technology for chiral separation and examples may be found in the area of amino acid,[11−18] amine, and amino alcohol[19−22] separation en route to single enantiomer drugs.[23−25] An enantiopure host in catalytic amounts is used to preferentially transport one of the two enantiomers across a phase boundary. The potential of enantioselective liquid−liquid extraction lies in its versatility and ease of scale up.[26,27] However, the reported moderate selectivities for most systems would require the use of multistage processing to achieve high product ee's.[12,14−20,22−28] Remarkably, experimental studies on multistage chiral liquid−liquid extraction are scarce. We are aware of only two reports from Maier in which full separation of a racemate was achieved, one describing the use of a number of membranes in series and another one in which an analytical centrifugal partition chromatograph (CPC) was used.[7,10] Although these results are quite remarkable, the methods are not scalable. The productivity of the membrane systems is much too low, and the CPC only exists as an analytical instrument, although recently the construction of a scaled-up version with a volume of 18 L was reported.[29] Other than Chiral SMB, no scalable method exists for liquid phase enantioseparation.

In this paper we describe the use of centrifugal contactor separators (CCS) for multistage enantioselective liquid−liquid

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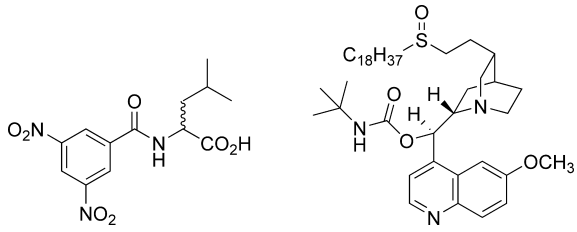
Scheme 1. **Structures of the guest (*R*,*S*)-DNB-Leu (l) and the host CA (r)**



extraction. These integrated devices combine the functions of fast mixing and separation and are thus an ideal instrument to achieve process intensification. We expected to achieve full enantioseparation with high productivities using a number of CCS's in series.

Recently, we demonstrated the potential of these CCS devices for combined reaction and separation.[30] We have also demonstrated the single-stage chiral separation of the amino acid 3,5-dinitrobenzoyl-(*R*),(*S*)-leucine [DNB-(*R*),(*S*)-Leu, Scheme 1] using the cinchona alkaloid *O*-(1-*tert*-butylcarbamoyl)-11-octadecylsulfinyl-10,11-dihydroquinine (CA, see Scheme 1)[31,32] a host−guest system developed by Lämmerhofer and Lindner.[11,13] Optimized single stage organic phase ee values of 34% were obtained for the (*S*)-enantiomer at a yield of 61%. From the previous work it had become clear that a cascade of CCS equipment operated in a countercurrent mode would be required to achieve full enantioseparation.

Results and Discussion

Here, we report the first experimental study on multistage chiral separation by liquid−liquid extraction. Similar to our previous studies with single staged extractions, DNB-(*R*),(*S*)-Leu is the model guest and CA the model host. A cascade of six CCS devices in series was used.

The cascade of six CCS devices of the type CINC V-02[33] (Figure 1, maximum throughput, 1.9 L/min) was applied in combination with a back-extraction step in a single CCS for recovery and recycling of the host. The optimum configuration of the cascade (e.g., feed input, concentrations, flow rates) was determined by mathematical modeling based on a previously developed equilibrium model. This model uses as input the mass balances for all components (DNB-*(R)*-Leu, DNB-*(S)*-Leu and CA) and the equilibrium relations for each stage.[34] The model predicts that 12 stages are required to obtain both enantiomers in high enantiomeric excess (>99% ee). With the experimental limitation of only six CCS devices available for the cascade and one for the back extraction unit, the model predicts that it is possible to obtain one of the enantiomers with an ee exceeding 99% in a yield up to 44%. To obtain the enantiopure (*S*)-enantiomer, the model predicts that the feed should be positioned at stage 5 (see Figure 2).

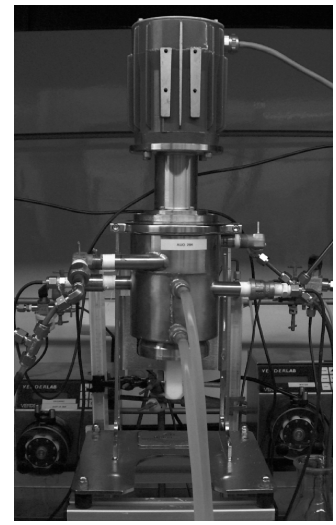


Figure 1. **Centrifugal contactor separator used in this study, real size from top to bottom is 65 cm.**

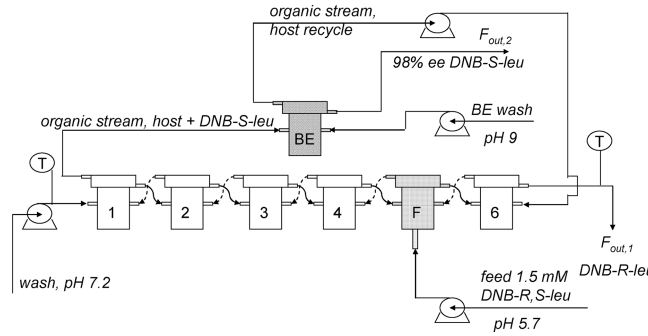


Figure 2. **Schematic representation of the cascade with six CCS devices in series with full recovery of the host in a single back-extraction stage (F = feed; BE = back extraction).**

In an experiment aimed to obtain enantiopure DNB-(*S*)-Leu, 1.35 L of host solution (4.3 mM) was recycled continuously through the seven CCSs with 100 mL/min, see Figure 2. The aqueous DNB-(*R*),(*S*)-Leu (1.5 mM, pH 5.7, $I = 0.07$ mol/L)[35] was fed with 42 mL/min at the fifth stage. At stage 1 an aqueous phosphate buffer (pH 7.2, $I = 0.08$ mol/L) entered the cascade with 60 mL/min to wash the undesired DNB-(*R*)-Leu out of the organic phase. The organic exit from stage 1, containing DNB-(*S*)-Leu complexed to the host in high purity was then back-extracted using 33 mL/min pH 9 phosphate buffer ($I =$ 0.12 mol/L) and the host was recycled to the cascade in stage 6. The DNB-(*R*),(*S*)-Leu concentrations were monitored in both aqueous exit streams ($F_{out,1}$ and $F_{out,2}$). Figure 3 shows the aqueous concentrations of the two enantiomers in the back-extraction outlet, $F_{out,2}$, Figure 4 shows the concentrations in $F_{out,1}$. The experiment was run for 10 h. The average concentrations in $F_{out,2}$ are 0.57 mM and 8.47 μM for DNB-*(S)*-Leu and DNB-(*R*)-Leu, respectively, corresponding with an average ee of 98% and a yield of 55% for the *S*-enantiomer in $F_{out,2}$. The average steady-state concentrations in $F_{out,1}$ were 0.11 and 0.28

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(35) We use ionic strength, *I*, instead of concentrations since there are ions with different valencies present in the aqueous solution. For further details see ref 34.

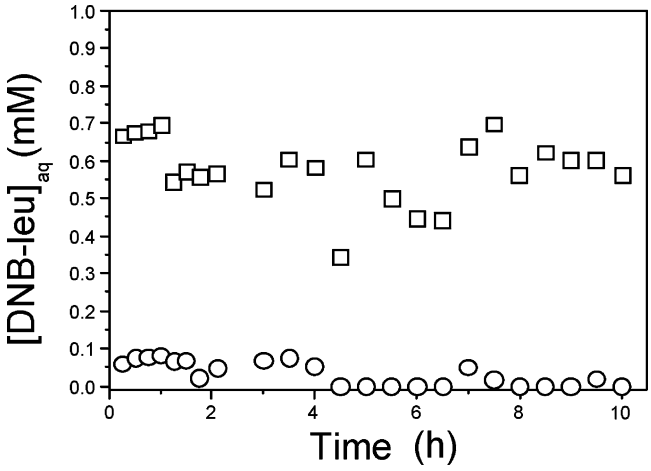

Figure 3. **Back-extraction aqueous outlet ($F_{out,2}$) DNB-Leu concentrations. □: DNB-(*S*)-Leu, ○: DNB-(*R*)-Leu.**

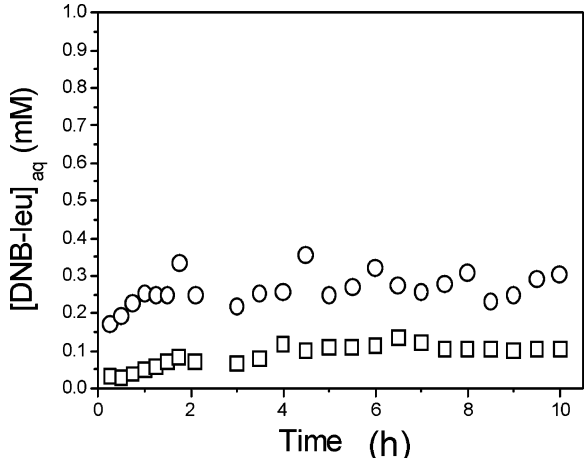

Figure 4. **Aqueous cascade outlet ($F_{out,1}$) DNB-Leu concentrations. □: DNB-(*S*)-Leu, ○: DNB-(*R*)-Leu.**

mM for DNB-*(S)*-Leu and DNB-(*R*)-Leu, respectively, corresponding with an ee of 42%. Mass balance calculation showed that the balance is closed with an experimental error of 8%. The model used to determine the optimum settings predicted 99% ee for DNB-(*S*)-Leu with a yield of 45%. The experimental values for the ee's are slightly lower than the model prediction. This is likely due to the solubility of DCE in water, leading to slightly higher host concentrations than intended. During the 10 h run, small portions of DCE (65 mL/h) were added to the system to compensate for this effect, but apparently this was not sufficient. The fluctuations in the DCE hold-up and concomitant in the host concentration are also likely the cause of the small fluctuations in concentrations in the steady-state profiles (Figures 3 and 4).

Erosion of the ee during the 10 h run time was not observed (Figures 3 and 4), and the host was recycled 50 times. Thus, the chemistry appears to be very robust, and the back-extraction step is very efficient. Dichloroethane (DCE) was used as solvent, as previous work had shown that use of this solvent leads to the highest selectivities.[31] It will be possible to use other solvents or mixtures of solvents, although this may necessitate the use of more CCSs to obtain full separation. No reaction between DCE and the host CA was observed over long periods of time.

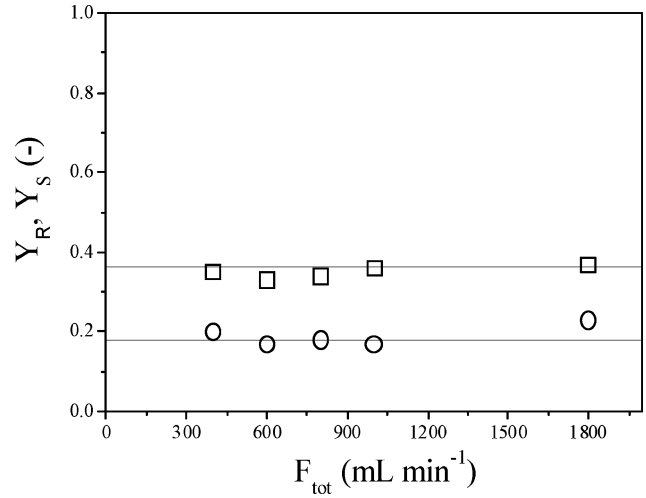

Figure 5. **Single CCS extraction yields versus total liquid throughput. □: DNB-(*S*)-Leu, ○: DNB-(*R*)-Leu. Lines: Single-stage model prediction.**

The experiments were carried out with relatively low reagent concentrations and flow rates, and this reduces the volumetric production rate considerably. To gain insights in the highest attainable production rates in the cascade, the solubility of the amino acid and also the maximum flow rate in the cascade were determined. The maximum solubility of DNB-(*R*),(*S*)-Leu$^-$Na$^+$ in water at ambient temperature was determined experimentally to be 29 ± 1 mM. Taking into account the margin needed to avoid precipitation, a maximum DNB-(*R*),(*S*)-Leu concentration of 15 mM may be fed to the cascade.

The maximum flow rate was determined by experiments in a single CCS. At otherwise constant conditions (1 mM aqueous DNB-(*R*),(*S*)-Leu, 0.27 mM organic CA, 50 Hz rotational frequency, and 294 K), the total flow rate was varied, and the effect on the outlet concentrations was studied. It was found that the outlet concentrations and hence the extraction yields were not a function of the flow rates over the entire operating range (Figure 5). This indicates that equilibrium is established even at the operating limit of the CCS of 1.8 L/min. With this information available, the maximum production rate in the cascade of six CCS devices may be calculated. The model predicts that, when applying a maximum total flow rate of 1.8 L/min for each CCS, the feed flow should be below 0.36 L/min. The maximum capacity when using this flow rate combined with the reagent concentrations set by the solubility limits is 5.4 mmol racemate/min, corresponding to 17.7 kg/week. For this production level only 60 g of the extractant is required. Using the largest CCS commercially available a production of 5−10 tons per week is feasible. In addition, it should be possible to achieve turnover numbers on host of 400−700 per week, which leads to perfectly acceptable economics. The host is a very stable compound and did not show any decomposition in our experiments. We believe that the method described in this paper has similar potential as chiral SMB for the ton-scale separation of racemates. We expect the cost of our method to be much lower as the CCSs are commercially available equipment that is used routinely in industry and hence are available at relatively low cost. In addition, the cost of the chiral

host compound is much lower than commercially available chiral stationary phases that are used in chiral SMB.

Experimental Section

***O-tert*-Butylcarbamoylquinine.**[13] Quinine (6.255 g, 19.3 mmol), *tert*-butylisocyanate (2.2 mL, 30.8 mmol), and dibutyltindilaurate (1 drop) were heated at 130 °C in toluene (30 mL) for 4 h. The solvent was then removed *in vacuo* and the product purified by column chromatography (silica/acetone) to give a light-yellow waxy powder. Yield 6.941 g (16.4 mmol) 85%. Spectral data were in accord with the literature.[13]

***O-tert*-Butylcarbamoyl-11-octadecylthio-10,11-dihydroquinine.**[13] *O-tert*-Butylcarbamoylquinine (6.941 g, 16.4 mmol), octadecan-1-thiol (8.755 g, 30.6 mmol), and AIBN (0.275 g, 1.7 mmol) in $CHCl_3$ (35 mL) were heated at reflux for 24 h. The solution was concentrated in vacuo and the product purified by column chromatography (silica/$CHCl_3$). The product was eluted as the second fraction with $CHCl_3$/MeOH (8:2) as the eluent. Yield 11.542 g (16.3 mmol) 99%. Spectral data were in accord with the literature.[13]

***O-tert*-Butylcarbamoyl-11-octadecylsulfinyl-10,11-dihydroquinine.**[13] *O-tert*-Butylcarbamoyl-11-octadecylthio-10,11-dihydroquinine (11.542 g, 16.3 mmol) and $NaIO_4$ (3.510 g, 16.4 mmol) were stirred at room temp in a mixture of MeOH and H_2O (400 mL, 9:1) for 3 h. H_2O (300 mL) was then added and the pH adjusted to 8.5 with a $NaHCO_3$/Na_2CO_3 mixture. The product was extracted into $CHCl_3$ (3 × 150 mL) and then purified by column chromatography (silica/EtOAc). The product was eluted with EtOAc/MeOH (9:1). Yield 9.562 g (13.2 mmol) 81%. Spectral data were in accord with the literature.[13]

Separation of DNB-Leu. Six centrifugal contactor separators of CINC, CINC V-02,[32] were used. The size of the weir depends on the solvent mixture used. For the dichloroethane/water mixture used here the size we used was 0.975 in. The six CCSs were connected with tubing as shown in Figure 2. Both liquids were transferred to the reactor using Verder VL1000 Control peristaltic tube pumps equipped with double pump heads (1.6 × 1.6 × 8R).

The experiment was initiated by switching on the rotors of all CCSs (40 Hz), opening the tap of the cooling water flow, cooling the CCSs 2, 4, and 6 in the cascade to maintain a constant temperature of 294 K, and starting the organic phase pump. After starting the organic phase flow (4.3 mM host CA in DCE, 100 mL/min), the CCSs were filled up in the order from 6 to 1 and finally the back-extraction CCS. After the recycle of the heavy organic phase was established, the flows of the light aqueous back-extraction (phosphate buffer pH 9.0, I = 0.12 mol/L, 33 mL/min) and wash streams pH 7.2, I = 0.08 mol/L, 60 mL/min) were started, and as soon as the wash flow had reached the feed stage, the feed flow was started (1.5 mM DNB-(*R*),(*S*)-Leu, pH 5.7, I = 0.07 mol/L, 42 mL/min). When the aqueous outlet started running, samples were taken every 15 min during the first 2 h. and afterward every 30 min from both the aqueous cascade outlet and the aqueous back-extraction outlet to determine the concentrations of DNB-(*R*),(*S*)-Leu using chiral HPLC (accuracy 3%). Every hour about 65 mL of DCE was added in small portions to the extract storage vessel to compensate for the losses due to solubility of DCE in water (0.8% vol). Every 2 h samples were taken from the aqueous flows in between the stages. The run time of the experiment was 10 h.

The concentrations of the DNB-Leu enantiomers in the aqueous phase were determined by HPLC using an Agilent LC 1100 series apparatus, equipped with an Astec Chirobiotic T column (now Supelco, Sigma-Aldrich). Detection was done using 270 nm UV light. The eluent was a 3:1 (v/v) mixture of acetonitrile and methanol, to which 0.25% (vol) triethylamine and 0.25% (vol) acetic acid were added. The flow rate was set at 1 mL/min. Before injecting the aqueous phase samples to the column, 0.10 mL of the samples was diluted with 1.0 mL eluens and filtered over a syringe filter with pore size 0.45 μm (Waters Chrom). Quantitative analysis was enabled using calibration curves.

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