Equilibrium Studies on Enantioselective Liquid−Liquid Amino Acid Extraction Using a Cinchona Alkaloid Extractant

3 AUTHORS, INCLUDING:

H.J. Heeres
University of Groningen
214 PUBLICATIONS **5,051** CITATIONS

SEE PROFILE

Jos Winkelman
University of Groningen
28 PUBLICATIONS **815** CITATIONS

SEE PROFILE

Equilibrium Studies on Enantioselective Liquid−Liquid Amino Acid Extraction Using a Cinchona Alkaloid Extractant

Boelo Schuur, Jozef G. M. Winkelman, and Hero J. Heeres*

University of Groningen, Department of Chemical Engineering, Nijenborgh 4, 9747 AG Groningen, Netherlands

The enantioselective extraction of aqueous 3,5-dinitrobenzoyl-*R*,*S*-leucine ($A_{R,S}$) by a cinchona alkaloid extractant (C) in 1,2-dichloroethane was studied at room temperature (294 K) in a batch system for a range of intake concentrations (10^{-4}–10^{-3} mol/L) and pH values (3.8–6.6). The experimental data were described by a reactive extraction model with a homogeneous organic phase reaction of $A_{R,S}$ with C. Important parameters of this model were determined experimentally. The acid dissociation constant, K_a, of $A_{R,S}$ was $(1.92 \pm 0.07) \times 10^{-4}$ mol/L. The physical distribution coefficient of $A_{R,S}$ between the organic and aqueous phase was 8.04 ± 0.39. The equilibrium constants of the organic phase complexation reaction were $(9.31 \pm 0.76) \times 10^4$ L/mol and $(2.71 \pm 0.76) \times 10^4$ L/mol for the S- and R-enantiomers, respectively. With these parameters an optimum performance factor, PF, of 0.19 was predicted. The PF was independent of the pH provided that pH $\gg$ pK_a. The model was verified experimentally with excellent results ($\pm 7.9\%$).

1. Introduction

The demand for enantiopure compounds is growing rapidly.[1] Especially in the fragrance, pharmaceutical, and food industries a clear tendency toward the production of enantiopure compounds exists as both enantiomers often show different bioactivity in the human body.[2] The most common technique for obtaining enantiopure compounds on a commercial scale is classical resolution by crystallization.[3] This technique is not always applicable, and interest in other methods such as enantioselective synthesis or racemic synthesis followed by enantioseparation is growing. Among other techniques, such as racemic synthesis followed by separation using liquid membranes[4] or chromatographic techniques,[5] racemic synthesis and subsequent separation of enantiomers by liquid−liquid extraction is considered a very promising technique.[6−10] However, to the best of our knowledge, processes making use of this technique have not been commercialized. Compared to other methods, such as chiral liquid chromatography[11] and chiral capillary electrophoresis,[12] liquid−liquid extraction is expected to be cheaper and easier to scale up to commercial scale. The process requires an enantioselective extractant dissolved in the extract phase which reacts with the solute in the feed. Although ample literature is available for enantioselective extraction, only a few studies provide fundamental insights in the reaction engineering mechanisms.[13−18]

Chiral cinchona alkaloid extractants, patented by Lindner and Lämmerhofer,[19] are considered ideal extractants for enantiopurification of amino acids and amino acid derivatives. Research on the molecular aspects of the enantioselective extractions by cinchona alkaloids has been reported by Kellner et al.[20] This has provided valuable information on the complexation mechanism between cinchona alkaloids and 3,5-dinitrobenzoyl-*R*,*S*-leucine ($A_{R,S}$, Figure 1, left) on a molecular level.[21] However, process studies combining experimental studies and mathematical modeling to predict and optimize the extraction performance of this system have not been reported.

The aims of this study are to determine the effects of process conditions, such as the concentrations, volume ratio, and aqueous pH, on the enantioselective extraction of $A_{R,S}$ and to optimize
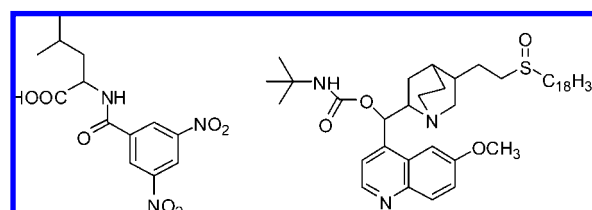

Figure 1. Chemical structures of the racemic mixture of solute A_R and A_S abbreviated as $A_{R,S}$ (left) and of the chiral extractant C (right).

the extraction process by equilibrium modeling. This information is essential input for further development of this system, especially for the design of a continuous extraction process in dedicated equipment such as integrated mixer−settler devices. A cinchona alkaloid extractant was chosen as this family of compounds is known to be very versatile for (substituted) amino acids.[20] *O*-(1-*tert*-Butylcarbamoyl)-11-octadecylsulfinyl-10,11-dihydroquinine (further referred to as C, Figure 1, right) was selected as the model extractant for its favorable selectivity compared to other cinchona alkaloids.[22] 1,2-Dichloroethane was selected as the solvent of choice as it gives high enantioselectivity compared to other solvents.

2. Experimental Section

2.1. Chemicals. Purified water was obtained by reverse osmosis followed by distillation. 1,2-Dichloroethane (99.8%) was obtained from Sigma-Aldrich; potassium dihydrogen phosphate (p.a.), disodium hydrogen phosphate dodecahydrate (p.a.), and triethylamine (99%) were obtained from Merck; glacial acetic acid was obtained from Acros; methanol (AR) and acetonitrile were obtained from Labscan. *O*-(1-*tert*-Butylcarbamoyl)-11-octadecylsulfinyl-10,11-dihydroquinine (C), 3,5-dinitrobenzoyl-*R*,*S*-leucine ($A_{R,S}$), 3,5-dinitrobenzoyl-*R*-leucine (A_R), and 3,5-dinitrobenzoyl-L-leucine (A_S) were kindly provided by DSM Research.

2.2. Procedures. All experiments in this study were performed in batch at a temperature of 294 K.

2.2.1. Acid Dissociation Constant of $A_{R,S}$. The acid dissociation constant of $A_{R,S}$ was determined by measuring the pH of aqueous solutions with $A_{R,S}$ concentrations in the range $(0.1–3.0) \times 10^{-4}$ mol/L. The solutions were obtained by

* To whom correspondence should be addressed. Fax: +31 50 363 4479. E-mail: h.j.heeres@rug.nl.

dilution of a stock solution with water in 100 mL flasks. The pH of each solution was measured after stirring several minutes to ensure homogeneity.

2.2.2. Distribution Coefficient of $A_{R,S}$. Experiments to determine the distribution coefficient of $A_{R,S}$ over the aqueous and 1,2-dichloroethane phases were carried out in 150 mL flasks. To 100 mL of aqueous $A_{R,S}$ solutions with a concentrations in the range $(1.0-3.4) \times 10^{-4}$ mol/L, typically $10-15$ mL 1,2-dichloroethane was added. The biphasic systems were stirred vigorously for 12 h, after which the phases were allowed to settle. The pH of the aqueous phase was measured and its composition was analyzed by HPLC. The organic phase concentration of $A_{R,S}$ was determined from a mass balance for $A_{R,S}$ over both phases. A similar series of experiments with buffered solutions was performed to investigate the influence of the pH on the distribution.

2.2.3. Reactive Liquid−Liquid Extraction of A_R, A_S, and $A_{R,S}$. Reactive extraction experiments were carried out with the pure enantiomers, A_R and A_S, to obtain the equilibrium constants of the organic phase complexation reactions. A subsequent series with racemic mixtures, $A_{R,S}$, was performed to verify the extraction mechanism and the proposed model with its parameters. In all experiments, phosphate buffers were used to set the desired pH.

In a typical reactive extraction experiment for parameter estimation, about 5 mL of a pH-buffered 4×10^{-4} mol/L A_R or A_S solution was mixed in a 20 mL flask for 2 h with $1-10$ mL of a $(1.0-4.0) \times 10^{-4}$ mol/L solution of C in 1,2-dichloroethane. After 2 h the phases were allowed to settle, after which the pH of the aqueous phase was measured and its composition was analyzed by HPLC.

In experiments with the racemate, $A_{R,S}$, the aqueous phase pH was buffered at 6.58 and an aqueous racemate concentration of 1×10^{-3} mol/L was applied. To 5 mL of the aqueous solution, 10 mL of a $(0.2-1.0) \times 10^{-3}$ mol/L solution of C in 1,2-dichloroethane was added. The composition of the aqueous phase was determined as described above.

2.3. Analytical Procedures. The concentrations of the enantiomers, A_R and A_S, 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). An UV detector operated at 270 nm was applied. 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 into the column, 0.10 mL of the samples was diluted with 1.0 mL of eluent and filtered over a syringe filter with pore size 0.45 μm (Waters Chrom). Quantitative analysis ($\pm$3%) was enabled by using calibration curves. The pH of the aqueous phase was measured using an Inolab pH 730 pH meter equipped with a SenTix 81 probe (both probe and meter from WTW, Germany).

2.4. Modeling Software and Optimization. Parameter fitting for all parameters was done using a nonlinear-least-squares method (lsqnonlin) provided by the software package Matlab (Mathworks). The reported confidence intervals of the parameter values are 95% confidence limits.

3. Theory and Reactive Extraction Modeling

3.1. Theory of Enantioselective Extraction. For optimization of a reactive extraction process, knowledge of the extraction mechanism is required. In aqueous−organic biphasic extraction systems, the reaction may take place in either the organic phase, the aqueous phase or at the interface. For metal extractions[23,24]
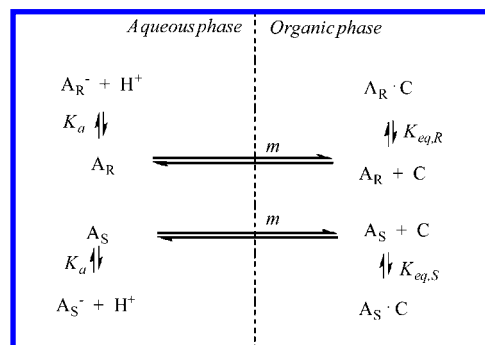

Figure 2. Homogeneous organic phase ligand addition extraction mechanism of $A_{R,S}$.
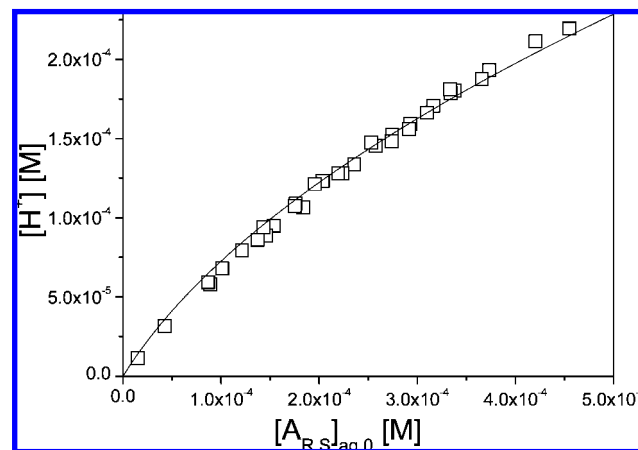

Figure 3. $[H^+]$ vs $[A_{R,S}]_0$. Symbols: experimental data. Line: calculated with $K_a = 1.92 \times 10^{-4}$ mol/L.

the locus of the reaction is usually assumed to be the interface. This is rationalized by the often poor solubility of the polar solutes in the organic phase and the poor solubility of the extractant in the aqueous phase. This extraction mechanism, also known as interfacial complexation, was also reported for the enantioselective solvent extraction of the ligand exchange type.[13,14]

The extraction of $A_{R,S}$ by C is of the ligand addition type,[25] where C reacts with the neutral forms of A_R or A_S. In general, such reactions occur either at the interface or in one of the phases. However, in this case the extractant, C, is highly hydrophobic, which excludes the possibility that the reaction takes place in the aqueous phase. Depending on the solubility of $A_{R,S}$ in the organic phase, the complexation reaction will either be limited to the interface or may take place in the organic phase. The more hydrophobic amino acids and derivatives are known to distribute over the aqueous and organic phases.[17,25] Therefore we have applied the homogeneous organic phase ligand addition mechanism here. Further in this paper we will validate this mechanism for the system under study. The model is analogous to the one developed by Steensma et al.,[25] and is also commonly used in organic acid extractions.[26−28] The homogeneous organic phase ligand addition mechanism is depicted in Figure 2.

3.2. Model Equations. The extraction system displayed in Figure 2 may be modeled by a series of coupled equilibrium relations and component balances, as follows.

aqueous phase acid dissociation equilibria:

$$K_a = \left(\frac{(\gamma_{H^+})(\gamma_{A_R^-})}{\gamma_{A_R}} \frac{[H^+][A_R^-]}{[A_R]} \right)_{aq} \tag{1a}$$

$$K_a = \left(\frac{(\gamma_{H^+})(\gamma_{A_S^-})}{\gamma_{A_S}} \frac{[H^+][A_S^-]}{[A_S]} \right)_{aq} \tag{1b}$$

distribution equilibria between the aqueous and organic phases:

$$m = \frac{[A_R]_{org}}{[A_R]_{aq}} \tag{2a}$$

$$m = \frac{[A_S]_{org}}{[A_S]_{aq}} \tag{2b}$$

organic phase complexation equilibria:

$$K_{eq,R} = \left(\frac{[A_R C]}{[A_R][C]} \right)_{org} \tag{3}$$

$$K_{eq,S} = \left(\frac{[A_S C]}{[A_S][C]} \right)_{org} \tag{4}$$

component balances for the enantiomers, A_R and A_S, and the extractant, C: component balances for the enantiomers, A_R and A_S, and the extractant, C

$$V_{aq}[A_R]_{aq,0} = V_{aq}([A_R]_{aq} + [A_R^-]_{aq}) + V_{org}([A_R]_{org} + [A_R C]_{org}) \tag{5}$$

$$V_{aq}[A_S]_{aq,0} = V_{aq}([A_S]_{aq} + [A_S^-]_{aq}) + V_{org}([A_S]_{org} + [A_S C]_{org}) \tag{6}$$

$$V_{org}[C]_{org,0} = V_{org}([C]_{org} + [A_R C]_{org} + [A_S C]_{org}) \tag{7}$$

The concentration of the undissociated $A_{R,S}$ enantiomers is very low in our studies, and therefore their activity coefficients in eqs 1a and 1b are assumed to be 1. For the ionic species, nonideality was taken into account because buffered solutions are used to control the pH. The ionic activities were obtained from the Debye−Hückel law.[29,30]

$$\log(\gamma_i) = \frac{-A z_i^2 I^{1/2}}{1 + B I^{1/2}} \tag{8}$$

The values of the constants A and B for aqueous sodium chloride solutions at 25 °C[30] have been used as an approximation: $A = 0.5115$ and $B = 1.316$. The ionic strength in eq 8 is calculated according to[30]

$$I = \frac{1}{2} \sum_i z_i^2 c_i \tag{9}$$

4. Results and Discussion

To model the extraction process with homogeneous organic phase complexation, the values of important physical parameters are needed. These were determined experimentally, and the results are provided in the following subsections. Next, we will discuss the validation of the model presented above, and the optimization of the extraction process.

4.1. Acid Dissociation Constant K_a. Due to the low solubility of $A_{R,S}$ the calculation of K_a from titration curves gave large errors Therefore, the K_a of $A_{R,S}$ was determined by measuring the pH of dilute unbuffered solutions. The $A_{R,S}$ concentration was always small, $(0.1-3.0) \times 10^{-4}$ mol/L; therefore, ion activity corrections could be neglected. The acid dissociation constant was calculated as $(1.92 \pm 0.07) \times 10^{-4}$ mol/L by minimizing the differences between the experimental and calculated $[H^+]$ data; see Figure 3. The estimated K_a of $A_{R,S}$ is in good agreement with the reported acidities of organic acids with comparable chemical structures.[31]
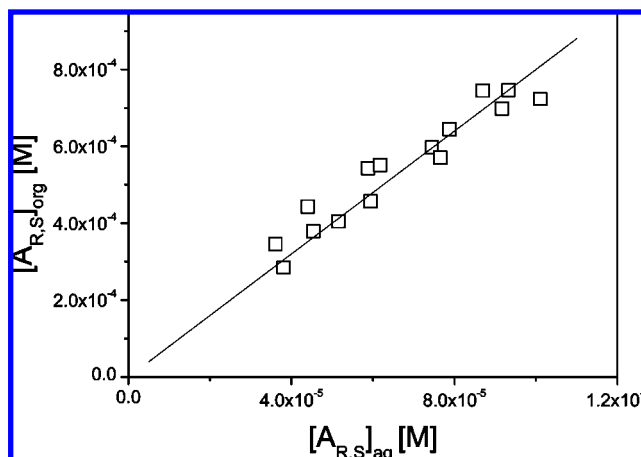

Figure 4. Equilibrium concentrations $[A_{R,S}]_{org}$ vs $[A_{R,S}]_{aq}$. Symbols: experimental data. Line: linear correlation with $m = 8.04$.

4.2. Distribution Coefficient m. The distribution coefficient m was determined using physical extraction experiments as described in section 2.2.2. In the absence of a pH buffer, and for pH <5, $[A_{R,S}^-] = [H^+] = 10^{-pH}$. The total aqueous phase amino acid concentration was obtained from the HPLC measurements. These data, together with the $A_{R,S}$ intake concentration, allowed the determination of the undissociated amounts of amino acid in both phases, $[A_{R,S}]_{aq}$ and $[A_{R,S}]_{org}$. The results are shown in Figure 4. Apparently, within the concentration range applied here, $[A_{R,S}]_{org}$ is proportional to $[A_{R,S}]_{aq}$ with a constant distribution coefficient. From linear regression, the distribution coefficient m, according to eqs 2a and 2b, was obtained as 8.04 ± 0.39. The amino acid is considerably better soluble in the organic phase than the aqueous phase, which is not surprising considering the presence of the highly hydrophobic aromatic ring and the C_4 carbon group in the compound; see Figure 1.

4.3. Complexation Equilibrium Constants $K_{eq,R}$ and $K_{eq,S}$. Several methods, i.e., ^1H NMR, UV−vis spectrometry, and IR spectrometry, were investigated for direct, independent measurement of the organic phase complexation equilibria. However, in all measurements the extractant−enantiomer complexes, $A_{R,S}C$, could not be distinguished clearly from the uncomplexed compound, C. The equilibrium constants were therefore determined using reactive extraction experiments with the pure enantiomers, A_R and A_S, as described in section 2.2.3.

For A_R, an optimum value of $K_{eq,R}$ was obtained by minimizing the differences between the experimental and calculated values of the total aqueous phase A_R concentration, $[A_R]_{aq} + [A_R^-]_{aq}$, over all experiments. The experimental values of $[A_R]_{aq} + [A_R^-]_{aq}$ were obtained from HPLC analysis, while the calculated values were obtained from simultaneously solving the model equations 1a−9. In this way, an optimum value for $K_{eq,R}$ was found of $(2.71 \pm 0.76) \times 10^4$ L/mol. A parity plot of the total aqueous phase A_R concentration, $[A_R]_{aq} + [A_R^-]_{aq}$, is shown in Figure 5.

A similar procedure using the experiments with the pure S-enantiomer resulted in a $K_{eq,S}$ value of $(9.31 \pm 0.76) \times 10^4$ L/mol. For this case, the calculated and experimental values of the total aqueous concentration of A_S, $[A_S]_{aq} + [A_S^-]_{aq}$, are shown in Figure 6.

For a number of reactive extractions known to proceed according to the homogeneous complexation model, Steensma et al.[15] reported equilibrium complexation constants ranging from a few hundred to 1.5×10^5 L/mol. The equilibrium constants obtained here for the $A_{R,S}$ extraction by C are at the
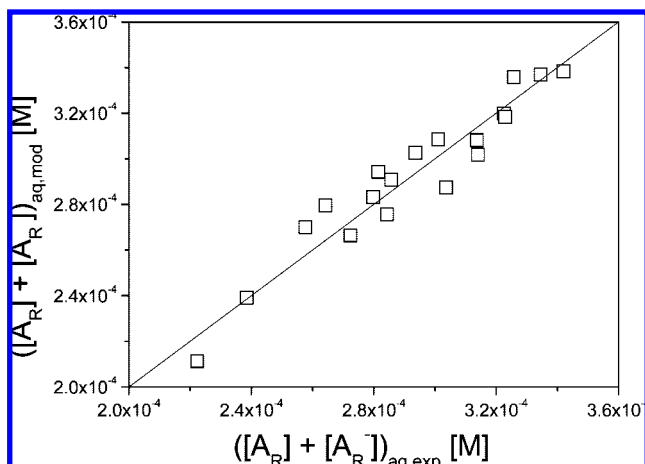
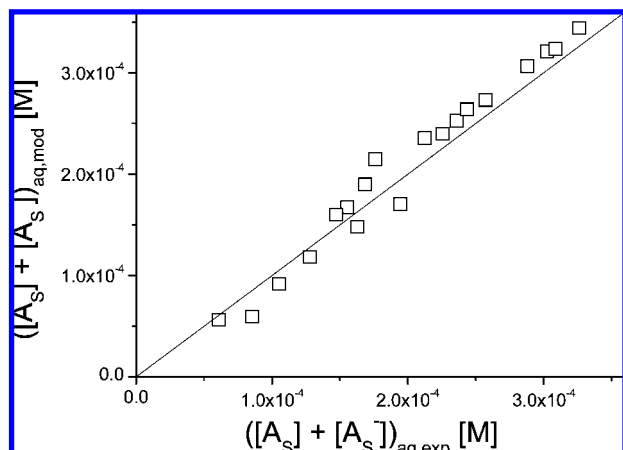**Figure 5.** Parity plot for reactive extraction with enantiopure A_R.

Figure 6. Parity plot for reactive extraction with enantiopure A_S.

high end of this range. The intrinsic selectivity of the system, defined as the ratio of the equilibrium complexation constants, is obtained here as $K_{eq,S}/K_{eq,R} = 3.43$. This value is well in line with those reported by Maximini et al.[4] for a comparable system (DNB-R,S-leu with a related cinchona alkaloid extractant) and at the high end of the values reported in the literature for other systems.[13,15,18,32] Based on predictions using the Fenske equation for total reflux conditions,[33] with this selectivity about nine theoretical equilibrium stages are sufficient to fully separate the two enantiomers with enantiomeric excess of at least 99% in both phases. Here, the Fenske equation for a countercurrent separation of DNB-R,S-leu is defined as

$$N_{min} = \frac{\ln\left[\dfrac{x_{S,e}/(1-x_{S,e})}{x_{S,r}/(1-x_{S,r})}\right]}{\ln(\alpha)} \qquad (10)$$

where x_e is the fraction of the feed that ends up in the extract, x_r is the fraction that ends up in the raffinate, and α is the selectivity ($K_{eq,S}/K_{eq,R}$). With this equation, an indication of the minimum number of stages required for any given separation may be predicted, provided that the selectivity and desired purity are known.

4.4. Model Validation. In cases where the extractant is insoluble in the aqueous phase, two important reactive extraction models have been reported in the literature: the interfacial reaction model and the homogeneous organic phase reaction model.[17] The main difference between both models is the locus of the chemical reaction between substrate and extractant and
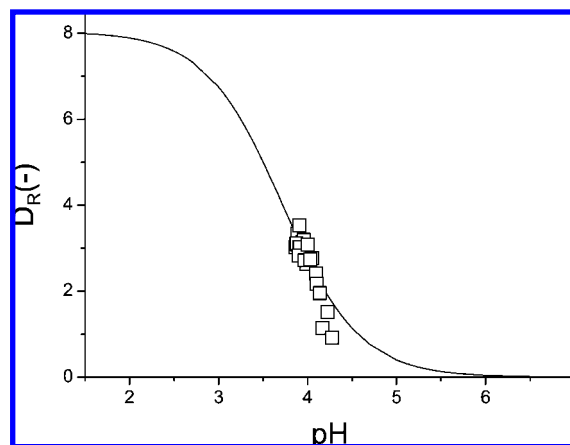

Figure 7. Overall distribution D_R vs pH. Symbols: experimental data. Line: model prediction.

is among other factors determined by the charge of the substrate. In the current system, either the undissociated $A_{R,S}$ or its anion may be involved in the reaction. In case the anion is the reacting species, the complexation reaction is expected to take place solely at the interface, because ion transfer from the aqueous phase into the organic phase is not facile. If the undissociated forms of $A_{R,S}$ are the reactive species, the location of the reaction is either the organic or the aqueous phase, depending on the partitioning of $A_{R,S}$ between both phases.

To discriminate between both models, experimental studies on the effect of the pH on the distribution of $A_{R,S}$ over the aqueous and organic phases in the presence and absence of the extractant were performed. First, a number of physical extraction experiments with A_R were performed at different pH values. The overall distribution, D_R, was determined for each experiment. Here D_R is defined as the ratio of the total amounts of A_R in the organic and aqueous phases:

$$D_R = \frac{[A_R]_{org} + [A_R C]_{org}}{[A_R]_{aq} + [A_R^-]_{aq}} \qquad (11)$$

A comparison of the experimental values with the model predictions of D_R is shown in Figure 7. Evidently, D_R is a function of the pH, with low pH values leading to higher values for D_R. To understand the results, the Henderson–Hasselbalch equation[34] is illustrative:

$$pH = pK_a + \log\frac{[A_R^-]_{aq}}{[A_R]_{aq}} \qquad (12)$$

This equation predicts that for pH $\ll$ pK_a the predominant compound in solution is the undissociated form A_R. This species is expected to be better soluble in the organic phase than the aqueous phase (vide infra), leading to higher values of D_R.

A second series of reactive experiments was carried out with A_R using extractant C. The effect of the $[C]_{org,0}$ on D_R is shown in Figure 8. The slope of the plot of the D_R versus the $[C]_{org,0}$ is a clear function of the aqueous phase pH. At higher pH values, the slope is considerably reduced. Thus, the effect of the extractant concentration on the D_R is reduced at higher pH values. This clearly indicates that the undissociated form of A_R is the reactive species and not the anion. If the latter were involved, larger effects of the extractant concentration on the distribution of A_R would be expected at high pH values. Similar observations were also observed for experiments with the pure S-enantiomer. All experimental observations are in line with
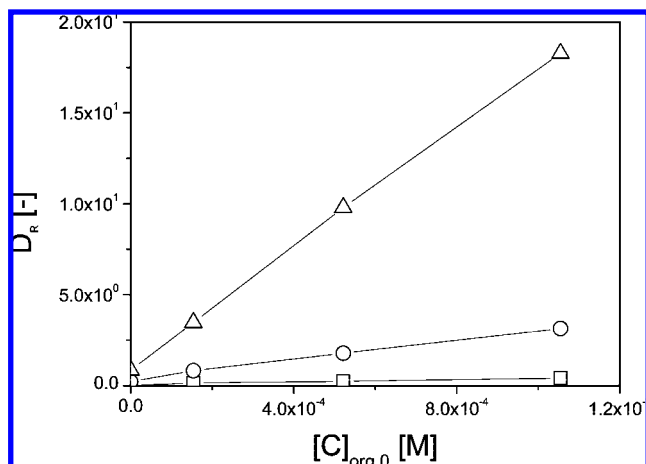
Figure 8. Overall distribution D_R vs $[C]_{org,0}$. Symbols, experimental data: $-\triangle-$, pH 3.8; $-\bigcirc-$, pH 4.9; $-\square-$, pH 6.1.
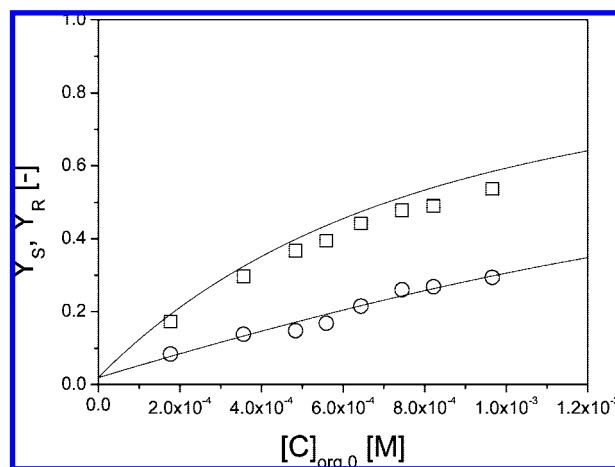

Figure 9. Extraction yields of both enantiomers vs $[C]_{org,0}$ with racemic $A_{R,S}$ intake. Symbols, experimental data: $\bigcirc$, Y_R; $\square$, Y_S. Lines: model predictions.

the homogeneous organic phase ligand addition mechanism as depicted in Figure 2.

4.5. Experimental Validation of the Homogeneous Extraction Model. The extraction model for the reactive extraction of $A_{R,S}$ with C was tested experimentally by performing eight reactive extraction experiments with racemic $A_{R,S}$ mixtures and various extractant concentrations as described in section 2.2.3. The results of these experiments are compared graphically with the model predictions in Figure 9, where the yields are shown. Here, the yield of an enantiomer is defined as the fraction of the aqueous feed that ends up in the organic extract phase:

$$Y_R = \frac{[A_R]_{org} + [A_R C]_{org}}{[A_R]_{aq,0}} \frac{V_{org}}{V_{aq}} \tag{13a}$$

$$Y_S = \frac{[A_S]_{org} + [A_S C]_{org}}{[A_S]_{aq,0}} \frac{V_{org}}{V_{aq}} \tag{13b}$$

The agreement between the modeled and experimental data is good, as shown by a mean absolute relative error of 7.9%. Thus, it may be concluded that the extraction model developed in this paper is applicable to predict the performance of the reactive extractions of racemic $A_{R,S}$ with C.

4.6. Optimization of the Reactive Extraction of $A_{R,S}$ with C Using the Performance Factor. In chiral chemistry and engineering, the enantiomeric excess (ee) is used as a
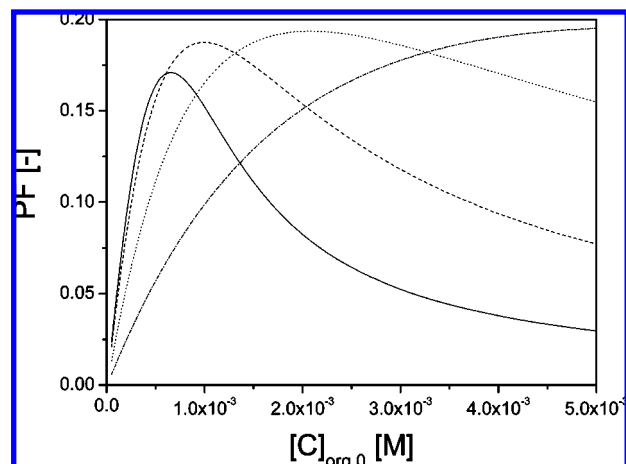

Figure 10. Calculated PF values vs $[C]_{org,0}$. Conditions: $[A_{R,S}]_{aq,0} = 1 \times 10^{-3}$ mol/L, $V_{org}/V_{aq} = 1$, and $\gamma_+ = \gamma_- = 0.757$. Lines: —, pH 5.5; ---, pH 6.0; $\cdots$, pH 6.5; $- \cdot -$, pH 7.0.

measure of the enantioselectivity of a process. For the system described here, the reaction takes place in the organic phase and the extractant C is selective toward the S-enantiomer. Therefore, the ee in the organic phase is defined as the excess of A_S in that phase. Similarly, an ee in the aqueous phase was defined:

$$ee_{org} = \frac{[A_S]_{org,all\,forms} - [A_R]_{org,all\,forms}}{[A_S]_{org,all\,forms} + [A_R]_{org,all\,forms}} \tag{14a}$$

$$ee_{aq} = \frac{[A_R]_{aq,all\,forms} - [A_S]_{aq,all\,forms}}{[A_R]_{aq,all\,forms} + [A_S]_{aq,all\,forms}} \tag{14b}$$

Requirements for a good enantioselective extraction process are not only a high ee of the desired enantiomer but also a high yield. Koska and Haynes[13] combined the yield and ee in the performance factor PF. The PF is a very useful tool to optimize an enantioselective extraction process and is defined as

$$PF = ee_{org} Y_S \tag{15}$$

The model described in section 3 with the parameters obtained in section 4 is used to optimize the enantioselective reactive extraction process in terms of the PF. In Figure 10, the PF is plotted as function of the extractant intake concentration for several pH values. The volumetric phase ratio, the ion activity, and the amino acid intake concentration are equal for all cases.

The PF for each pH exhibits a clear maximum. Both the maximum value of the PF and the position of the maximum are dependent on the pH. The observation of a maximum PF is the result of two opposing effects, i.e., the yield and the ee. At very low extractant concentrations, the yield is very low, resulting in a low PF. An increase in the extractant concentration will increase the yield and PF. However, at some point, the extractant is present in excess with respect to the desired enantiomer and the undesired enantiomer will also be extracted in considerable amounts. This will lead to a significant drop in the ee and a reduction of the PF. An illustration of the effects of ee and yield as a function of the intake concentration of the extractant is depicted in Figure 11 for an aqueous phase pH 6.5.

Figure 10 furthermore illustrates that, at pH >6, the maximum PF becomes nearly independent of the pH. Thus, under conditions where pH $\gg$ pK_a the maximum value of PF is independent of the pH and has a value of 0.19.

Figure 12 shows the PF as function of $[C]_{org,0}$ (mol/L) for different values of $[A_{R,S}]_{aq,0}$. The figure illustrates that, at least
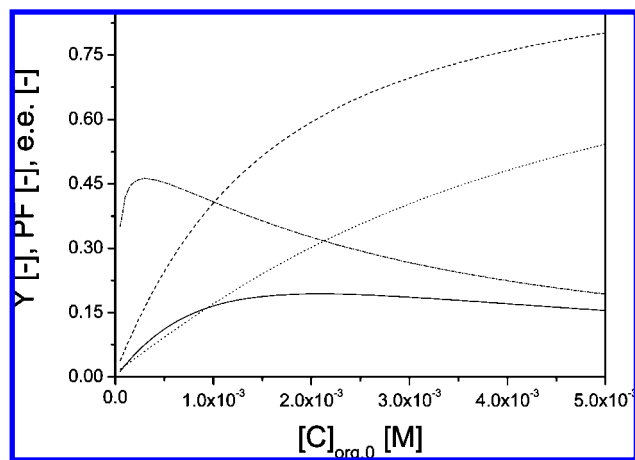
Figure 11. Calculated results vs $[C]_{org,0}$. Conditions: pH 6.5, $[A_{R,S}]_{aq,0} = 1 \times 10^{-3}$ mol/L, $V_{org}/V_{aq} = 1$, and $\gamma_+ = \gamma_- = 0.757$. Lines: —, PF; ---, Y_S; ···, Y_R; − · −, ee_{org}.

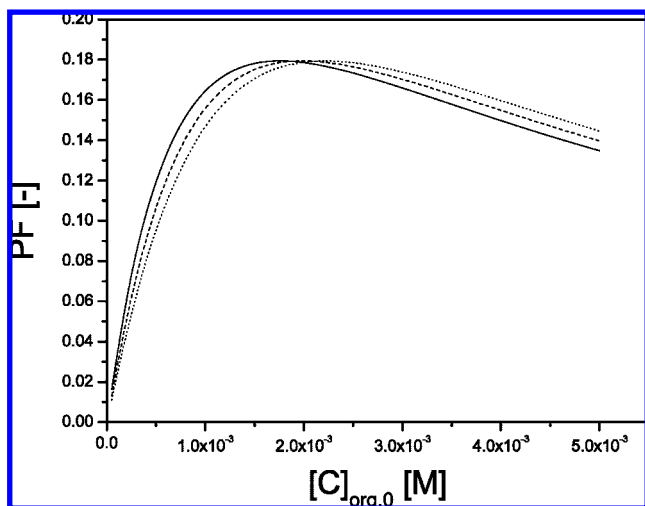**Figure 12.** Calculated PF values vs $[C]_{org,0}$. Conditions: pH 6.5, $V_{org}/V_{aq} = 1$, and $\gamma_+ = \gamma_- = 0.757$. Lines: —, $[A_{R,S}]_{aq,0} = 5 \times 10^{-4}$ mol/L; ---, $[A_{R,S}]_{aq,0} = 1.0 \times 10^{-3}$ mol/L; ···, $[A_{R,S}]_{aq,0} = 1.5 \times 10^{-3}$ mol/L.

for pH 6.5 ($\gg pK_a$), the optimum value of the PF is independent of the concentration of $A_{R,S}$. This is due to the low concentration of undissociated $A_{R,S}$ species at these high pH values, almost completely excluding physical phase transfer of $A_{R,S}$. At the observed maxima, the ratio of species present in the organic phase is constant; thus for higher intakes of $A_{R,S}$, the required amount of extractant at the maximum PF is also higher.

5. Conclusions

The enantioselective reactive extraction of $A_{R,S}$ by a cinchona alkaloid extractant, C, has been investigated. Experimental data indicate that the reactive extraction process proceeds according to a homogeneous complexation model and involves reaction of the undissociated form of the amino acid derivative and the extractant in the organic phase. The experimental data were modeled according to this extraction model, and excellent agreement between the model and experimental data ($\pm 7.9\%$) was observed.

The performance of the extraction process was evaluated using the performance factor, PF. The model predicts a maximum value for PF of 0.19, in line with experimental values (0.20). This indicates that a high ee in combination with a high yield is not possible in a single equilibrium step and that

multistage extraction will be required for full separation of the racemate $A_{R,S}$.

Nomenclature

a = activity (mol L^{-1})
A = constant in Debye−Hückel law (L$^{1/2}$ mol$^{-1/2}$)
$A_{R,S}$ = 3,5-dinitrobenzoyl-R,S-leucine (solute)
B = constant in Debye−Hückel law (L$^{1/2}$ mol$^{-1/2}$)
C = cinchona alkaloid extractant
D = overall distribution
DNB-R,S-leu = 3,5-dinitrobenzoyl-R,S-leucine (solute)
ee = enantiomeric excess
I = ionic strength (mol L^{-1})
K_a = acid dissociation constant of $A_{R,S}$ (mol/L)
K_{eq} = equilibrium constant of the organic phase reaction between $A_{R,S}$ and C (L/mol)
m = distribution coefficient of undissociated $A_{R,S}$
N = number of equilibrium stages
PF = performance factor
T = temperature (K)
V = volume (L)
Y = yield
z = ion valence
[ ] = concentration (mol/L)

Greek Symbols

α = (enantio)selectivity
γ = activity coefficient

Subscripts

0 = initial or feed
a = acidity
aq = aqueous
eq = equilibrium
min = minimum
org = organic
R = R-enantiomer
S = S-enantiomer

Literature Cited

(1) Rouhi, A. M. Chiral Business. *Chem. Eng. News* **2003**, *81*, 45.

(2) Sheldon, R. A. *Chirotechnology; industrial synthesis of optically active compounds*; Marcel Dekker Inc.: New York, 1993.

(3) Vries, T.; Wynberg, H.; van Echten, E.; Koek, J.; ten Hoeve, W.; Kellogg, R. M.; Broxterman, Q. B.; Minnaard, A.; Kaptein, B.; van der Sluis, S.; Hulshof, L.; Kooistra, J. The Family Approach to the Resolution of Racemates. *Angew. Chem., Int. Ed.* **1998**, *37*, 2349.

(4) Maximini, A.; Chmiel, H.; Holdik, H.; Maier, N. W. Development of a Supported Liquid Membrane Process for Separating Enantiomers of N-Protected Amino Acid Derivatives. *J. Membr. Sci.* **2006**, *276*, 221.

(5) Gavioli, E.; Maier, N. M.; Minguillon, C.; Lindner, W. Preparative Enantiomer Separation of Dichlorprop With a Cinchona-Derived Chiral Selector Employing Centrifugal Partition Chromatography and High-Performance Liquid Chromatography: A Comparative Study. *Anal. Chem.* **2004**, *76*, 5837.

(6) Abe, Y.; Shoji, T.; Fukui, S.; Sasamoto, M.; Nishizawa, H. Enantioseparation by Dual-Flow Countercurrent Extraction: Its Application to the Enantioseparation of (±)-Propranolol. *Chem. Pharm. Bull.* **1996**, *44*, 1521.

(7) Lacour, J.; Goujon-Ginglinger, C.; Torche-Haldimann, S.; Jodry, J. J. Efficient Enantioselective Extraction of Tris(Diimine)Ruthenium(II) Complexes by Chiral, Lipophilic TRISPHAT Anions. *Angew. Chem., Int. Ed.* **2000**, *39*, 3695.

(8) Prelog, V.; Stojanac, Z.; Kovacevic, K. Separation of Enantiomers by Partition Between Liquid-Phases. *Helv. Chim. Acta* **1982**, *65*, 377.

(9) Tsukube, H.; Shinoda, S.; Uenishi, J.; Kanatani, T.; Itoh, H.; Shiode, M.; Iwachido, T.; Yonemitsu, O. Molecular Recognition With Lanthanide(III) Tris(Beta-Diketonate) Complexes: Extraction, Transport, and Chiral Recognition of Unprotected Amino Acids. *Inorg. Chem.* **1998**, *37*, 1585.

(10) Ohki, A.; Miyashita, R.; Naka, K.; Maeda, S. Enantioselective Extraction of Di-O-Benzoyltartrate Anion by Ion-Pair Extractant Having Binaphthyl-Unit. *Bull. Chem. Soc. Jpn.* **1991**, *64*, 2714.

(11) Davankov, V. A. Chiral Selectors With Chelating Properties in Liquid-Chromatography—Fundamental Reflections and Selective Review of Recent Developments. *J. Chromatogr., A* **1994**, *666*, 55.

(12) Gubitz, G.; Schmid, M. G. Chiral Separation Principles in Capillary Electrophoresis. *J. Chromatogr., A* **1997**, *792*, 179.

(13) Koska, J.; Haynes, C. A. Modelling Multiple Chemical Equilbria in Chiral Partition Systems. *Chem. Eng. Sci.* **2001**, *56*, 5853.

(14) Pickering, P. J.; Chaudhuri, J. B. Equilibrium and Kinetic Studies of the Enantioselective Complexation of (D/L)-Phenylalanine With Copper (II) N-Decyl-(L)-Hydroxyproline. *Chem. Eng. Sci.* **1997**, *52*, 377.

(15) Steensma, M.; Kuipers, N. J.; de Haan, A. B.; Kwant, G. Influence of Process Parameters on Extraction Equilibria for the Chiral Separation of Amines and Amino-Alcohols With a Chiral Crown Ether. *J. Chem. Technol. Biotechnol.* **2006**, *81*, 588.

(16) Takeuchi, T.; Horikawa, R.; Tanimura, T. Enantioselective Solvent-Extraction of Neutral DL-Amino Acids in 2-Phase Systems Containing N-N-Alkyl-L-Proline Derivatives and Copper(II) Ion. *Anal. Chem.* **1984**, *56*, 1152.

(17) Tan, B.; Luo, G. S.; Qi, X.; Wang, J. D. Enantio Selective Extraction of D,L-Tryptophan by a New Chiral Selector: Complex Formation With Di(2-Ethylhexyl)Phosphoric Acid and O,O′-Dibenzoyl-(2R,3R)-Tartaric Acid. *Sep. Purif. Technol.* **2006**, *49*, 186.

(18) Viegas, R. M. C.; Afonso, C. A. M.; Crespo, J. G.; Coelhoso, I. M. Modelling of the Enantio-Selective Extraction of Propranolol in a Biphasic System. *Sep. Purif. Technol.* **2007**, *53*, 224.

(19) Lindner, W.; Lämmerhofer, M. Eur. Patent 96 109 072.7, 1996.

(20) Kellner, K. H.; Blasch, A.; Chmiel, H.; Lämmerhofer, M; Lindner, W. Enantioseparation of N-Protected Alpha-Amino Acid Derivatives by Liquid-Liquid Extraction Technique Employing Stereoselective Ion-Pair Formation With a Carbamoylated Quinine Derivative. *Chirality* **1997**, *9*, 268.

(21) Maier, N. M.; Schefzick, S.; Lombardo, G. M.; Feliz, M.; Rissanen, K.; Lindner, W.; Lipkowitz, K. B. Elucidation of the Chiral Recognition Mechanism of Cinchona Alkaloid Carbamate-Type Receptors for 3,5-Dinitrobenzoyl Amino Acids. *J. Am. Chem. Soc.* **2002**, *124*, 8611.

(22) Hallett, A. J.; Kwant, G.; de Vries, J. G. Cinchona alkaloids as chiral hosts for the continuous enantioselective separation of *N*-derivatised amino acids using centrifugal contact separators. Submitted for publication in *J. Org. Chem.*

(23) Cox, M. In *Science and Practice of Liquid-Liquid Extraction*; Thornton, J., Ed.; Clarendon: Oxford, 1992; pp 1−101.

(24) Bart, H. J.; *Reactive Extraction 18*; Springer Verlag: Berlin, 2001.

(25) Steensma, M.; Kuipers, N. J. M.; de Haan, A. B.; Kwant, G. Modelling and Experimental Evaluation of Reaction Kinetics in Reactive Extraction for Chiral Separation of Amines, Amino Acids and Amino-Alcohols. *Chem. Eng. Sci.* **2007**, *62*, 1395.

(26) Prochazka, J.; Heyberger, A.; Bizek, V.; Kousova, M.; Volaufova, E. Amine Extraction of Hydroxycarboxylic Acids. 2. Comparison of Equilibria for Lactic, Malic, and Citric Acids. *Ind. Eng. Chem. Res.* **1994**, *33*, 1565.

(27) Wasewar, K. L.; Heesink, A. B. M.; Versteeg, G. F.; Pangarkar, V. G. Reactive Extraction of Lactic Acid Using Alamine 336 in MIBK: Equilibria and Kinetics. *J. Biotechnol.* **2002**, *97*, 59.

(28) Ziegenfuss, H.; Maurer, G. Distribution of Acetic-Acid Between Water and Organic Solutions of Tri-N-Octylamine. *Fluid Phase Equilib.* **1994**, *102*, 211.

(29) Atkins, P. W. *Physical Chemistry*, 5th ed.; Oxford University Press: Oxford, 1994.

(30) Robinson, R. A.; Stokes, R. H. *Electrolyte Solutions* Dover: New York, 2002.

(31) Kortüm, G.; Vogel, W.; Andrussow, K. *Dissociation constants of organic acids in aqueous solution*; Butterworths: London, 1961.

(32) Abe, Y.; Shoji, T.; Kobayashi, M.; Qing, W.; Asai, N.; Nishizawa, H. Enantioselective Distribution of Amino-Alcohols in A Liquid-Liquid 2-Phase System Containing Dialkyl L-Tartrate and Boric-Acid. *Chem. Pharm. Bull.* **1995**, *43*, 262.

(33) Pratt, H. R. C. *Countercurrent Separation Processes*; Elsevier: Amsterdam, 1967.

(34) Stryer, L. *Biochemistry*, 4th ed.; Freeman: New York, 1995.