CHAPTER 5

Enantioselective liquid-liquid extraction studies on (D,L)-tryptophan using a cationic Pd-XylBINAP complex as chiral host in 1-octanol

Abstract

The use of a chiral cationic Pd( PF$_6$)$_2$(S)-XylBINAP) complex as host for the enantioselective liquid-liquid extraction (ELLE) of racemic tryptophan (Trp) was investigated at 6°C in a modified Lewis-cell. The racemic amino acid was present in the water phase whereas the metal complex was dissolved in 1-octanol and in situ prepared by complexation of PdCl$_2$(CH$_3$CN)$_2$ with (S)-XylBINAP and subsequent anion exchange with AgPF$_6$. The reactive extraction process was shown to be relatively slow and time to reach equilibrium takes typically more than 6 h. The maximum operational selectivity was 1.8 when using racemic tryptophan. Remarkably, and unprecedented, separate experiments with the pure enantiomers of tryptophan gave the same extraction results in terms of kinetics and equilibrium compositions as for the racemic mixture. A number of additional experiments were conducted to rationalize this finding.
5.1. Introduction

Enantioselective liquid-liquid extraction (ELLE) has received high attention in the last decade and is considered a promising technology for the separation of chiral compounds. Advantages of ELLE are its high flexibility, for instance several classes of racemates may be separated using the same host, it does not involve solids handling and is relatively easy to scale-up. ELLE involves contacting a racemic mixture, often dissolved in water, with an organic solution containing a chiral host. The differences in complexation constants of the two enantiomers and the host result in enantioselectivity. Therefore, the design and synthesis of highly selective hosts is an important activity in developing efficient ELLE systems.

Numerous hosts have been reported for the extraction of various classes of substrates. Chiral crown ether-based systems are among the most selective hosts known and operational selectivities as high as 31 have been reported. In case of lower selectivities, multistage extraction is required to obtain the desired enantioseparation. For instance, with an operational selectivity of 3, about ten equilibrium extractions are required.

Recently, it has been demonstrated that organometallic complexes with chiral hosts also have potentials for ELLE. Particularly, (substituted)-BINAP-metal complexes have shown high selectivity for several classes of substrates. For instance, the use of palladium BINAP complexes for ELLE of tryptophan and phenylalanine analogues was reported by Verkuijl et al. High enantioselectivities ($\alpha_{op}$ = 3.2-7.0) were obtained using PdCl$_2$(S)-XylBINAP in dichloromethane. Recently, it was reported that PdCl$_2$(S)-BINAP in 1,2-dichloroethane gives also a high operational selectivity for (D,L)-$\alpha$-methyl phenylglycine amide (7.4).

However, (substituted)-BINAP-metal-based ELLE systems have mainly been applied for equilibrium studies. In depth kinetic studies on the use of (substituted)-BINAP-metal-based ELLE are to the best of our knowledge not reported. Knowledge of the extraction kinetics is important for a better understanding of the underlying complexation chemistry, as well as for optimization and scale-up of the process.

Here we report on the use of Pd(PF$_6$)$_2$(S)-xylBINAP as a chiral host for ELLE of (D,L)-Trp (Figure 5.1) using 1-octanol as the organic solvent. The use of Pd(PF$_6$)$_2$(S)-xylBINAP as a chiral host for ELLE has not been reported in the literature.

Experiments were performed in 1-octanol instead of the conventionally applied chlorinated solvents such as 1,2-dichloroethane. Long chain alcohols are considered as green solvents and recommended by a number of solvent selection guides.

Initial extraction studies were performed using PdCl$_2$(S)-XylBINAP based on earlier research carried out in our group in chlorinated solvents. However, when using 1-octanol instead of chlorinated solvents,
it was shown that the operational selectivity was a function of the host concentration. This unexpected finding is likely caused by the formation of dimeric or oligomeric Pd complexes with bridging chlorides. To avoid this complication, the use of cationic Pd complexes with poor- or non-coordinating anions like PF₆⁻ was envisaged. It is well known that the counter ion has an effect on the ELLE performance. For instance, the use of PdBr₂ as a metal precursor for the ELLE of Trp with Pd-BINAP complexes gave a higher enantioselectivity (αₒₚ of 2.7) compared to PdCl₂ (αₒₚ = 2.2) in chlorinated solvents.

The ELLE experiments were carried out using racemic Trp as well as the individual pure enantiomers. In addition, the reversibility of the system was investigated to gain insights in the extraction mechanism.

### 5.2. Experimental section

#### 5.2.1. Materials

(D,L)-tryptophan (≥ 99%), (D)-tryptophan (≥ 98%), and (L)-tryptophan (≥ 98%), 1-octanol (≥ 99%), AgPF₆ (≥ 98%), and HClO₄ (70%) were obtained from Sigma-Aldrich. The metal precursor PdCl₂(CH₃CN)₂ (≥ 99%) and (S)-xylBINAP (≥ 99%) were purchased from Strem Chemicals. Prior to use in the ELLE experiments, the 1-octanol was contacted with double distilled water for a number of hours to saturate it with water. All experiments were performed using double distilled water.

**In situ preparation of Pd(PF₆)₂((S)-xylBINAP)**

Pd(PF₆)₂((S)-xylBINAP) was prepared *in situ* under nitrogen using PdCl₂(CH₃CN)₂, (S)-xylBINAP and AgPF₆ in 1-octanol. Typically, a stock
solution of PdCl$_2$((S)-XylBINAP) in 1-octanol (5 mM) was prepared by adding PdCl$_2$(CH$_3$CN)$_2$ (259.4 mg, 1 mmol) and (S)-XylBINAP (845.1 mg, 1.15 mmol, 15% molar excess with respect to Pd) to 1-octanol saturated with water (150 mL). The mixture was stirred overnight under a nitrogen atmosphere at room temperature to give a yellow solution. The solution was diluted by adding 50 ml of 1-octanol. Subsequently, AgPF$_6$ (510.4 mg, 2 mmol) was added and the contents were stirred for 48 h, giving a red solution with a white precipitate. Before each experiment, the solution was diluted with 1-octanol (saturated with water) to obtain the pre-set concentration.

5.2.2. Enantioselective liquid-liquid extraction of DL-Tryptophan with Pd(PF$_6$)$_2$((S)-XylBINAP)

5.2.2.1. Extraction set-up
The ELLE experiments were carried out in a modified Lewis-cell as shown in Figure 5.2. The cell was equipped with a sample port closed with a septum and was double walled to allow for cooling by water. For this purpose, a thermostat Julabo F25 water bath (set at 6°C) was used. The stirrer was set at 2200 rpm to ensure vigorous mixing of the two liquid phases.

![Figure 5.2. Scheme of the modified Lewis cell used in this study.](image)

5.2.2.2. Experimental procedures
The organic solution containing the host (150 mL) was added to the Lewis cell and cooled to 6°C. Separately, the aqueous phase (2 mM tryptophan, racemate or pure enantiomer) in water (unbuffered)) was cooled to 6°C.
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The extraction experiment was started by adding the aqueous phase to the Lewis cell. A sample (2 mL) was periodically collected and the two liquid phases were rapidly separated by centrifugation at ~13000 rpm for 30 s. The composition of the aqueous phase was subsequently determined by HPLC (vide infra). The organic phase was characterized using orbitrap mass spectroscopy (vide infra).

5.2.3. Analytical procedures
The concentrations of (D)- and (L)-tryptophan in the aqueous phase were determined by RP-HPLC using a Shimadzu SIL-20A (with a CTO-20AC column oven and LC-20AD pumps), equipped with a Crownpak CR(+) chiral column (Daicel, Japan) and a UV detector. Typically, 10 μL of sample was injected. Aqueous perchloric acid (pH = 1.5) was used as the mobile phase at a flow rate of 1.3 mL/min. Calibration curves using standard solution of both enantiomers of tryptophan were prepared and used to determine the concentrations in the sample.

High resolution mass spectra (HRMS) were recorded on a LTQ Orbitrap XL (ESI+) (Thermo Fisher Scientific) mass spectrometer. The samples (organic phase) were injected directly into the mass spectrometer without pretreatment. Full scan positive mode FTMS spectra were measured between m/z 300-3000.

5.3. Theory and definitions

The concentrations of both enantiomers in the water phase were determined experimentally (HPLC). The concentrations in the organic phase were calculated using the overall mass balances (Eq 5.1-5.2).

\[
V_{aq}[D]_{aq,all,0} = V_{aq}[D]_{aq,all,t} + V_{aq}[D]_{org,all,t} \quad (5.1)
\]

\[
V_{aq}[L]_{aq,all,0} = V_{aq}[L]_{aq,all,t} + V_{aq}[L]_{org,all,t} \quad (5.2)
\]

Here, [D] and [L] represent the concentrations of the (D)- and (L)-enantiomer, respectively, and V is the volume of a phase. Subscript \(aq\), \(org\), \(all\), \(0\) and \(t\) represent the aqueous phase, the organic phase, total, initial and at certain time, respectively.

The operational selectivity (\(\alpha_{aq}\)) is an important performance parameter for ELLE and is defined in Eq. 5.3.
The experimental study of the enantioselective liquid-liquid extraction (ELLE) of racemic Trp in water (2 mM) with host Pd(PF$_6$)$_2$((S)-XylBINAP) in 1-octanol (2 mM) was performed in a modified Lewis cell at 6°C using a one to one phase ratio (Figure 5.1). The concentrations of (D)- and (L)-Trp in the aqueous phase were measured using HPLC from 0.5-1300 min and the results are given in Figure 5.3a.

As shown in the inset in Figure 5.3a, the concentrations of enantiomers in the aqueous phase are reduced in time due to extraction to the organic phase and ultimately reach a close to constant (equilibrium) value. The time to achieve equilibrium is higher than 1000 min, indicating that the complexation reaction is relatively slow. Furthermore, the concentration of the (D)-enantiomer is lower than the (L)-enantiomer, revealing that the host has a higher affinity for the (D)-enantiomer.

Of interest is the observation of a very fast decline in the Trp concentrations in the first minute of contacting of the two liquid phases, followed by a more gradual decrease, ultimately to the equilibrium value. In the initial stage, the extraction is non-selective, and both enantiomers are extracted to the same extent. This rapid decrease could be due to physical adsorption of the enantiomers to the organic phase, the rate being a function of the physical mass transfer characteristics of the system. To determine the validity of this hypothesis, separate experiments were performed at similar conditions, the only difference being that the Pd-source was not added. Details are given in Appendix 5A. The physical distribution coefficients of both Trp enantiomers (m) could be established in this way and were shown to be 0.133 ± 0.003 (mol/mol), implying that Trp is indeed better soluble in the water phase, though also present in

$$\alpha_{op} = \frac{K_D}{K_L}, \text{if } K_D > K_L \quad (5.3)$$

Here, $K$ is the distribution of the enantiomers between the two liquid phases, which are defined in Eq. 5.4 and 5.5.

$$K_D = \frac{[D]_{org,all}}{[D]_{aq,all}} \quad (5.4)$$

$$K_L = \frac{[L]_{org,all}}{[L]_{aq,all}} \quad (5.5)$$

### 5.4. Results and discussions

The experimental study of the enantioselective liquid-liquid extraction (ELLE) of racemic Trp in water (2 mM) with host Pd(PF$_6$)$_2$((S)-XylBINAP) in 1-octanol (2 mM) was performed in a modified Lewis cell at 6°C using a one to one phase ratio (Figure 5.1). The concentrations of (D)- and (L)-Trp in the aqueous phase were measured using HPLC from 0.5-1300 min and the results are given in Figure 5.3a.

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the organic phase. When performing an extraction experiment with 1 mM of an enantiomer and using an equal phase ratio, it is expected that the aqueous phase reaches a Trp concentration of 0.88 mM at physical equilibrium. However, as is evident from Figure 3a, the concentration is by far lower (< 0.7 mM), which cannot be explained by the establishment of a rapid physical extraction equilibrium. We assume that the initial drop is due to the presence of Pd nanoparticles in the solution, which are known to have an affinity for Trp. Thus, a reactive extraction process is likely to occur between Trp and Pd-nanoparticles. These nanoparticles may be formed by the reaction of the Pd(PF$_6$)$_2$((S)-XylBINAP) with residual oxygen, which was not removed rigorously when preparing the solutions and during an extraction experiment. Alternatively, the nanoparticles may also be formed already during the in-situ synthesis of the Pd complex. It is well known that Pd nanoparticles may be formed from Pd$^{2+}$ precursors, particularly in the presence of alcohols.
The operational selectivity ($\alpha_{op}$), as defined in Eq. 5.3, versus time is shown in Figure 5.3b. The operational selectivity increases gradually in time and remains constant after about 400 min extraction. The maximum operational selectivity was of 1.8. The initial non-selective extraction is likely due to complexation of Trp with Pd-nanoparticles, followed by a slow complexation with the actual chiral Pd(PF$_6$)$_2$((S)-XylBINAP) compound with a certain preference of the host for the (D)-enantiomer.

To gain more insight in the complexation process, subsequent experiments were performed with different enantiomer-host molar ratios.

**ELLE of racemic Trp using different concentrations of the Pd(PF$_6$)$_2$((S)-XylBINAP) complex.**

A number of experiments were carried out with 2 mM racemic tryptophan (Trp) at variable host concentrations (1 to 3 mM). Higher host concentration proved not possible due to experimental issues (a.o. high liquid viscosities leading to stirring issues). All experiments were performed at 6°C and a phase ratio of 1. The aqueous concentration of both Trp enantiomers versus extraction time for the three experiments at different host concentrations are shown in Figure 5.4. Qualitatively, the shape of the concentration versus time curves are rather similar, showing a rapid drop in concentration in the initial stage followed by a more gradual decrease at longer times, though the actual equilibrium concentrations after long extraction times differ.

The concentration of both Trp enantiomers in the aqueous phase is lower at higher host concentration, indicating that the amount of Trp transferred from the aqueous to the organic phase is higher with increasing host concentration, in line with expectations. The effect of host concentration on performance is clearly seen in the distribution of the enantiomers over the two phases (Figure 5.5a and 5.5b). However, the

![Figure 5.4. Concentration of tryptophan enantiomers in aqueous phase at a). 1 mM host, b). 2 mM host, and c) 3 mM host.](image-url)
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α_{op} values for the three experiments are similar, as shown in Figure 5.4c, revealing that the α_{op} is independent of the host-substrate molar ratio.

**Reactive extraction of enantiopure tryptophan**

The ELLE experiments discussed so-far were performed with racemic Trp. To explore possible interactions between the enantiomers and the organometallic host, extractions using both enantiopure Trp forms were carried out in the Lewis cell at 6°C using either 1 or 2 mM Trp at a constant host concentration (2 mM) with a phase ratio of 1. The results for the individual experiments with enantiopure Trp are depicted in Figure 5.6. Remarkably and unprecedented, the concentration versus time profiles for Trp in the aqueous phase for both individual enantiomers are similar. This reveals that there is no preference for complexation of the (D)- or (L)-enantiomer with the chiral Pd host, in contrast to the experiments performed with racemic Trp. Also, the total amount of Trp transferred to the organic phase for the extractions with enantiopure Trp (2 mM) is similar to that when using racemic Trp (2 mM, Figure 5.7).

So far, we do not have a sound explanation for this remarkable and unprecedented behavior. It suggests that enantio-discrimination is only possible when both enantiomers are present in the organic phase and have interaction with the chiral Pd complex. This is not possible when simply assuming two independents 1 to 1 complexation reactions, one of the (D)-enantiomer and one of the (L)-enantiomer with the chiral host. A possible explanation is the involvement of not only 1 to 1 but also 2 to 1 complexes between Trp and the chiral Pd complex (Figure 5.8). In this case, the complexation constants of the possible 2 to 1 complexes may differ and lead to enantioselectivity. We are currently performing extensive equilibrium modeling studies to identify whether the findings can be rationalized using this scheme.

**Figure 5.5.** Distribution of (D)-enantiomer (a), (L)-enantiomer, and (C) operational selectivity at different host concentrations. Conditions: fixed Trp concentration of 2 mM, phase ratio of 1, 6°C.
In addition, experiments were performed to determine whether such 2 to 1 species are present in solution. It involved ELLE of racemic Trp with the host (2 mM, 2 mM host, 2200 rpm, 6°C). Periodically, samples were taken and the organic phase was analysed by orbitrap mass spectroscopy. All spectra show two main peaks (i.e. at m/z 767 and 1043), see Figure 5.9 for details. The peak at m/z 1043 corresponds to a 1 to 1 PdXylBINAP-trp complex, clear peaks for 2 to 1 complexes were absent. The peak at m/z of 767 is in agreement with the presence of the oxidized form of XylBINAP, either formed during in situ preparation of the Pd complex or during extraction or analyses.

The complexation reactions as depicted in Figure 5.8, are expected to be reversible. To determine whether the equilibria involved in the system
under study are indeed reversible and do not involve irreversible reactions, reversibility was checked by performing additional experiments involving sequential extractions. It involved a two-step extraction procedure starting with the extraction of enantiopure (D)-tryptophan (Trp) with the chiral host followed by the addition of enantiopure (L)-Trp in the second step. In the case of full reversibility of the system, enantioselectivity is expected with an operational selectivity similar to an individual experiment with racemic Trp.

In the first step, the extraction was carried out using (D)-Trp in water (2 mM) and host in 1-octanol (2 mM) at a phase ratio (V_{org}:V_{aq}) of 2 until the system reached equilibrium. In the second step, a certain amount of an aqueous solution of (L)-Trp (2 mM) was added to set the phase ratio to 1. The aqueous Trp concentration versus the extraction time was determined for both steps. The results for the first extraction step using an extraction time of 1400 min are depicted in Figure 5.10a and show the expected trend. Subsequently, a certain amount of (L)-Trp was added and the results are given in Figure 5.10b. It is clear that the concentration of the (D) and (L)-enantiomer in the aqueous phase is changing upon the addition of the (L)-enantiomer and a new equilibrium composition is established. This is already an indication that the complexation chemistry is reversible. Full reversibility and the absence of irreversible reactions can further be concluded from the observation that the operational selectivity

Figure 5.8. Reaction network involving possible 2 to 1 complexes for the reaction between Trp (D and L) and the chiral Pd complex (H).

Figure 5.9. Orbitrap mass spectrrum of the organic phase for ELLE of (D,L)-Trp (2 mM) with Pd(PF_6)_2XylBINAP (2 mM) after an extraction time of about 1300 min.
procedure starting with the extraction of enantiopure \((D)\)-tryptophan
(Trp) with the chiral host followed by the addition of enantiopure \((L)\)-
Trp in the second step. In the case of full reversibility of the system, en-
antioselectivity is expected with an operational selectivity similar to an
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(2 mM) and host in 1-octanol (2 mM) at a phase ratio \((V_{\text{org}}:V_{\text{aq}})\) of 2 until
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an aqueous solution of \((L)\)-Trp (2 mM) was added to set the phase ratio
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tablished. This is already an indication that the complexation chemistry
is reversible. Full reversibility and the absence of irreversible reactions
can further be concluded from the observation that the operational se-
lectivity (ca. 2) for the two-step experiment at long extraction time is
comparable with the selectivity for ELLE using the racemate in a single
extraction step \((\alpha_{\text{op}} = 1.8)\).

### 5.5. Conclusions

The enantioselective liquid-liquid extraction (ELLE) of racemic and
enantiopure Trp with Pd(PF₆)₂(\((S)\)-XylBINAP) as the host in 1-octanol
was investigated experimentally. The kinetic profiles show that the
complexation reaction between the host and Trp is slow and the time to reach equilibrium typically exceeds 6 h. In addition, the kinetic profiles are complex and actually show two regimes, a fast process in the timescale of minutes and a slower process ultimately reaching equilibrium. The fast initial stage is attributed to the reactive extraction of Trp by Pd nanoparticles, which act as (non enantio-selective) hosts, the second stage involves the chiral reactive extraction of Trp with the chiral organometallic complex. The operational selectivity was independent of the host-substrate molar ratio with a maximum selectivity of 1.8. Surprisingly and unprecedented, separate experiments with enantiopure Trp did not results in enantio-separation, indicating that the presence of both Trp enantiomers is required for the ELLE process. Possibly the formation of 2 to 1 complexes plays a role, though this could not be substantiated by MS studies. Irreversible reactions also do not play a role as was shown by sequential extraction experiments. Additional investigations (a.o kinetic and equilibrium modelling) are required to draw hard conclusions on the complexation chemistry and factors that determine enantioselectivity in these organometallic host systems for ELLE.

References


14) Tang, K.; Fu, T.; Zhang, P.; Yang, C. Modeling and Experimental Evaluation of


Appendix 5A

The distribution coefficient for the individual Trp enantiomers in water/1-octanol in the presence Xyl-(S)-BINAP and AgPF$_6$ was investigated using a solution of 2 mM (D,L)-tryptophan in water and at two concentrations of Xyl-(S)-BINAP and AgPF$_6$ (1 mM and 3 mM) in 1-octanol. Each measurement was performed in duplicate.

The distribution coefficient defined as:

$$m = \left( \frac{[D]_{aq}}{[D]_{org}} \right)_{equilibrium} = \left( \frac{[L]_{aq}}{[L]_{org}} \right)_{equilibrium}$$  \hspace{1cm} (5A.1)

was found to be 0.133 ± 0.003 for both enantiomers, see Figure 5A.1. for details.

![Figure 5A.1](image)

**Figure 5A.1.** Distribution coefficients for D and L-Trp in water/1-octanol in the presence of Xyl-(S)-BINAP and AgPF$_6$ (no Pd source)