Budapest University of Technology and Economics

PhD theses

Enantioseparation in supercritical carbon dioxide

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Introduction

In the last decades production of optically active compounds is a leading area of the modern chemical industry. Nowadays, application of chiral compounds as single enantiomeric drugs is dynamically increasing, and many drugs marketed previously in its racemic form is substituted by the single enantiomeric product. High percent of the single enantiomeric drugs is produced by resolution of the racemic form or a suitable synthesis intermedier. However, organic chemical syntheses and the resolution steps as well require huge amount of organic solvents, which are dangerous for the health and environment.

The specific properties of supercritical carbon dioxide ($scCO_2$) make it an interesting ‘green‘ replacement for organic solvents. At supercritical conditions the pressure (P) and temperature (T) of the given fluid are beyond the critical values (P_c, T_c). Critical conditions of carbon dioxide are 73.8 bar and 31.1 °C, which is only slightly higher than room temperature. Beside its environmental friendliness, $scCO_2$ offers easy pressure and temperature tuneable medium for chemical reactions and separations.

The pioneering work was done in the laboratories of Departments of Chemical Engineering and Organic Chemical Technology of Budapest University of Technology and Economics in the field of racemate purification (resolution) by supercritical fluid extraction (SFE). In the last decade efficient resolution and further purification of several chiral acids and bases were achieved with often higher enantioselectivities than those with conventional methods. The SFE method is based on partial diastereomeric salt formation and subsequent supercritical fluid extraction.

Aims of my PhD research work were the further studying of this process, deeper understanding of the influencing factors, application of the method for new compounds and its possible further development. In all cases O,O'-(2R,3R)-dibenzoyl-tartaric acid monohydrate (DBTA) was applied as resolving agent, since this compound is insoluble in $scCO_2$. Thus the formed diastereomeric salts and complexes do not pollute the extracts.

Methods

The racemate was reacted with less than one equivalent amount of DBTA in an appropriate solvent or in melt phase. Then achiral support was added to result a better extraction bed. The solvent (if present) was evaporated in vacuum and the solid material was dried. This sample was filled into the extractor vessel and supercritical fluid extraction was performed. Unreacted enantiomers were collected in the extract, and diastereomeric salts or complexes remained in the raffinate. These were then decomposed to recover the other enantiomeric mixture.

Supercritical fluid extraction (SFE) was performed with CO_2 (99.5 % purity) in a laboratory scale extraction unit, schematically presented in Fig. 1. Inner volumes of extractor and separator are 25 cm^3 each. Volume of atmospheric separator is 20 cm^3. Range of operation of extractor was $100<P<200$ bar and $T<95$ °C. Average mass flow rate was 0.9-1.1 g/s CO_2. Extracts were collected from the separators, material remaining in the extraction vessel was the raffinate.

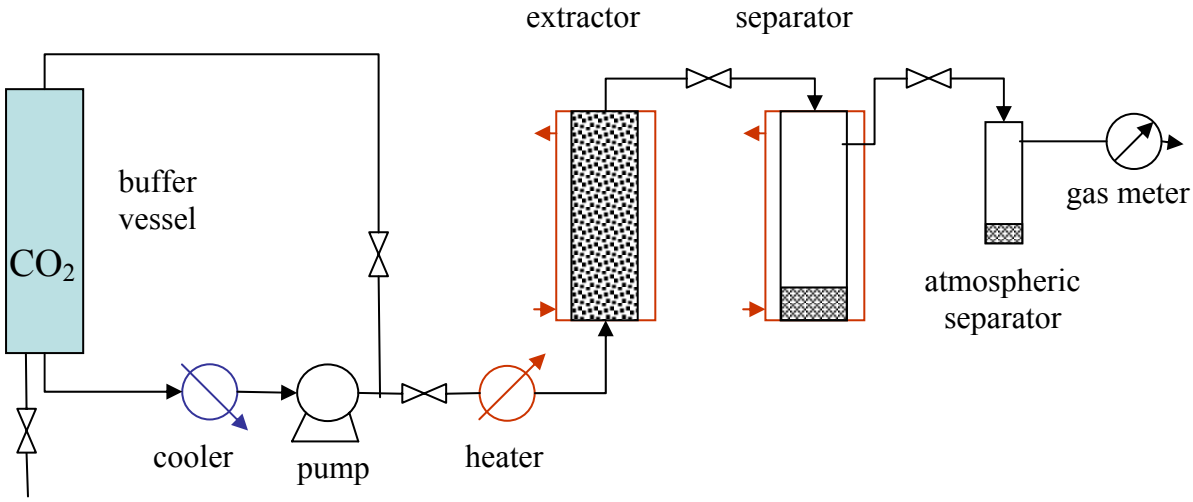

Fig. 1. Schematic draw of the laboratory scale extraction unit

Optical rotation (α) measurements were carried out on a Perkin-Elmer 241 polarimeter, and enantiomeric excess (ee[a]) values of extracts and raffinates were determined form α values using prior calibration.

The specific surface area and the total pore volume values of supports were determined from low temperature N_2-adsoption isotherm (Autosorb, Quantachrome, USA), calculation was performed with BET method.

Adsorption isotherms of tetramisole and DBTA were derived from the initial and equilibrium concentration of the liquid phase measured by a UV/VIS spectrophotometer (Uvikon Kontron, Zürich) at 223 nm.

TG and DSC measurements were carried out on TA Instruments 2050 TGA V5.3C and TA Instruments 2920 MDSC V2.4F, respectively. Argon and CO_2 atmosphere were used in a 0.01 m^3/h flow rate, 10 °C/min heating rate was applied. Mass of samples was 4-5 mg.

[a] $ee = \frac{R-S}{R+S}$, where R and S represent the enantiomers, and R>S.

Novel scientific results

1. On the example of the resolution of tetramisole it was proven that *the amount and quality of achiral support* may *significantly influence the resolution efficiency* (F^b). Beside SFE experiments, thermal and adsorbtion measurements were also carried out. Suprisingly, small amount of achiral support (both Perfil 100™ and activated carbon) highly increases the achieved F, while higher amount of support reduces it towards zero. The reason of these results is probably that it is a kinetic resolution process. Efficiency of resolution may be improved by the induced crystallisation caused by the support. There may be a competition for crystallisation places (bonding sites), thus if small amount of support is present, only the most stable diastereomeric salt will crystallise. If the surface given by the support is huge, all diasteromers present in the solution may crystallise at the same time (Fig. 2.).

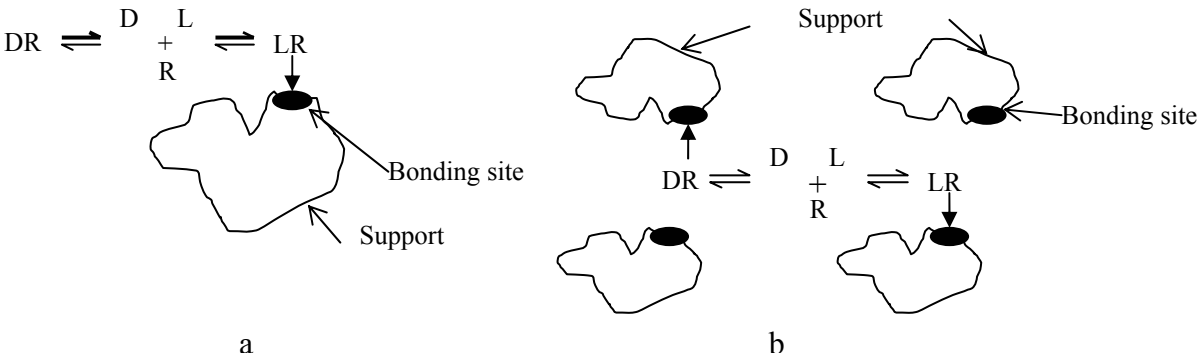

Fig. 2. Potential explanation of the effect of achiral support on resolution efficiency in cases af small (a) and huge (b) amount of support; kinetic resolution

This means that during the optimisation of a specific resolution task selection of achiral support is also an important point.

[b] $F = |ee_{E*}Y_E| + |ee_{R*}Y_R|$, where Y is the yield, subscripts E means extract, and R means raffinate

2. *Extraction curves of the resolution process can be well described with the generally used* $m = m_\infty(1-e^{-k \cdot m_{CO2}})$ *function*, where m is the amount of extract, m_{CO2} is the mass of carbon dioxide used, k and m_∞ are constants. With this function, after some experiments, the required amount of carbon dioxide can be easily calculated.

3. Not only diastereomeric salts, but also diastereomeric complexes may be stable enough in $scCO_2$ *to allow extracting the unreacted enantiomers.*

Resolutions of three chiral alcohols (*trans*-2-chloro-cyclohexanol, *trans*-2-bromo-cyclohexanol and *trans*-2-iodo-cyclohexanol) were performed by partial complexation with DBTA (Fig. 3.). DBTA formed diastereomeric complexes with all *S,S*-enantiomers stable enough to allow extracting the unreacted *R,R*-enantiomers with supercritical carbon dioxide. Thus not only racemic acids and bases, but also alcohols can be resolved by this method.

a. *Chiral differentiation increases with the size of the halogen substituent of the cyclohexanol.* If the achieved ee value is presented as function of molar ratio[c], straight lines going through the origin describe very well the experimental data. This is a very rare case. In one resolution step almost pure *R,R*-2-iodo-cylohexanol was produced at mr=0.74, with 39 % of the theoretical yield. On the other hand, at mr=0.09 ee=98 % was achieved for *S,S*-2-iodo-cylohexanol in the raffinate, but the yield was only 8 %.

[c] $mr = \dfrac{\text{amount of resolving agent (moles)}}{\text{amount of the racemic alcohol (moles)}}$

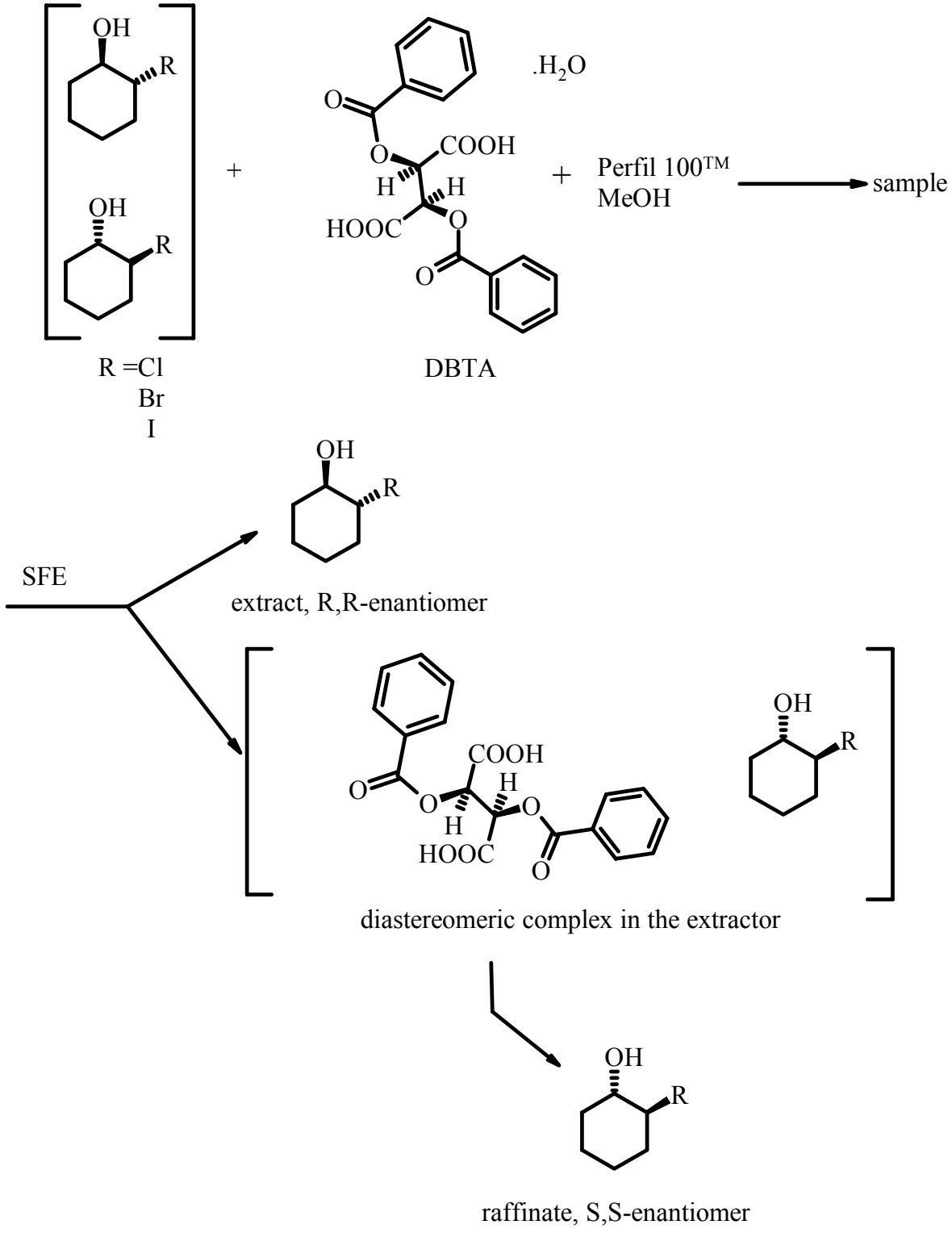

Fig. 3. Resolution of *trans*-2-halo-cyclohexanols by supercritical fluid extraction

b. *The optimal molar ratio (maximum of F) is determined by the structure of the diastereomeric salt or complex.* Optimal molar ratio of the resolution agent for a supercritical resolution process was determined by supercritical fluid extractions (SFE). Molar compositions of the complexes observed via thermal analysis (or other physicochemical method) are capable to estimate the half-an-equivalent molar ratios, which usually lead to the maximum resolution efficiency. The predicted optimum from thermal analysis and the measured optimum via SFE agree very well (*trans*-2-chloro-cyclohexanol: 0.58 and 0.55; *trans*-2-bromo-cyclohexanol: 0.71 and 0.66; *trans*-2-iodo-cyclohexanol: 0.77 and 0.68, respectively). The optimal amount of resolving agent increases with the size of the halogen substituent.

4. Enantioseparation by SFE has been being studied in our laboratories for more than 10 years. In many cases extraction pressure (P) and / or temperature (T) highly affected the efficiency of the process and the achieved ee values as well, but in a few cases these effects could not been observed. *On one hand the existence of the effects probably depends on the stability of the diastereomer in the supercritical medium and on the equilibrium conditions. On the other hand, since the process is an extraction, it should also depend on the contact time due to kinetic reasons.* Thus, usually, by compounds, which are relatively well soluble in the $scCO_2$ smaller effects are detected.

5. *A novel, organic solvent free procedure was developed for separation of enantiomers of a racemate.* Partial diastereomeric complex formation of *trans*-2-chloro-cyclohexan-1-ol and DBTA was performed in the melt of the alcohol. The highest resolution efficiency was achieved, when the unreacted alcohol (E1, *R,R*-enantiomer, ee=43.5 %, yield=45.3 %) was extracted with

supercritical carbon dioxide at mild conditions (100 bar, 33 °C). Then in a second step the diastereomeric complex was in situ decomposed in the extractor (200 bar, T>73 °C) and the *S,S*-enantiomer (E2, ee=61.6 %, yield=31.9 %) was also extracted. (Fig. 4.).

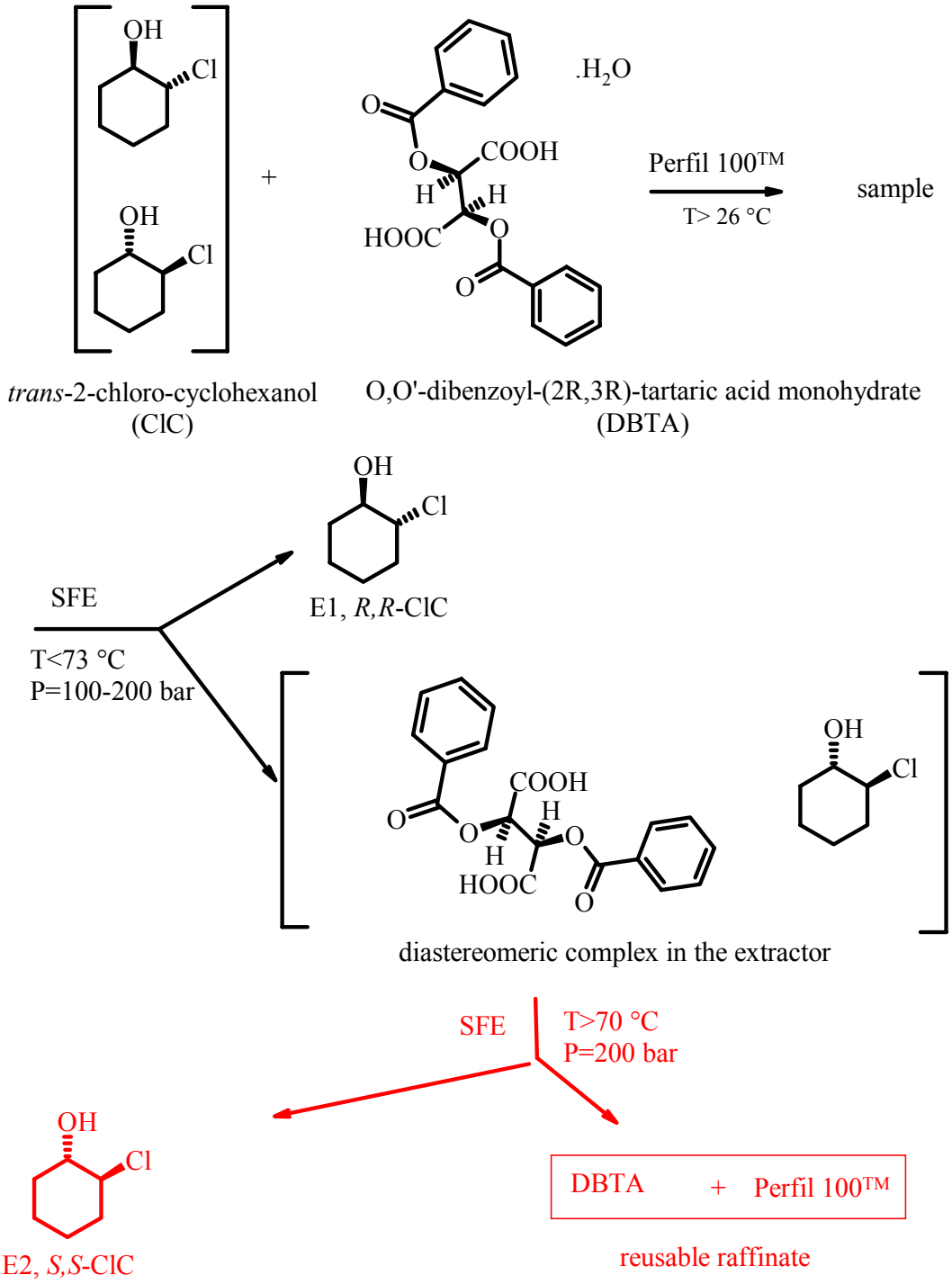

Fig. 4. Resolution of *trans*-2-chloro-cyclohexanol via fractionated supercritical fluid extraction

Interesting observation, that under atmospheric pressure, this complex starts to decompose only over 90 °C while in presence of $scCO_2$ 73 °C is enough for the decomposition of the complex and extraction of the alcohol. The raffinate is a physical mixture of the support and resolving agent, which can be instantly reused in another resolution cycle.

Fig. 5. is a typical extraction curve of this novel procedure with two separate extraction steps.

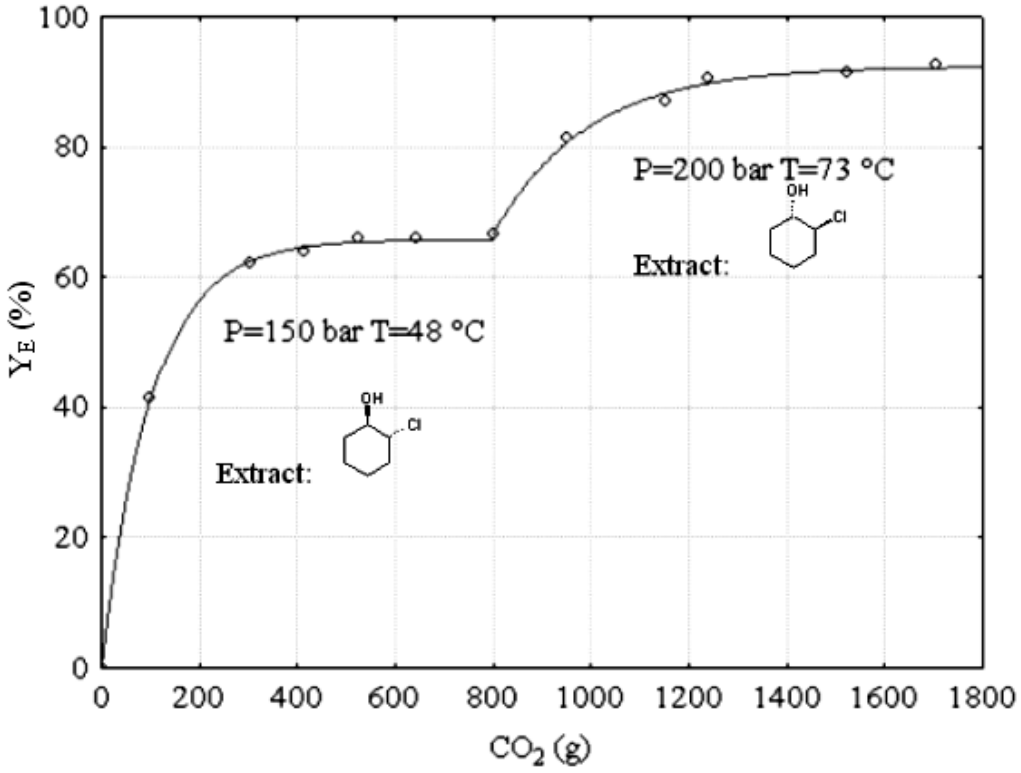

Fig. 5. Extraction curve of the fractionated supercritical fluid extraction

a. Effect of molar ratio was studied by this process as well. The optimal molar ratio (0.59) was almost the same as found by the previous methods (SFE: 0.55, TG: 0.58).

b. Effects of extraction pressure (100 - 200 bar) and temperature (33 - 63 °C) on F were studied. Linear effects of both factors were significant at 0.05. The lower pressure and temperature lead to higher F values.

Importance of results

Results of my PhD work highly improved the knowledge on influencing factors of enantioseparation via supercritical fluid extraction.

The application of this method combined with diasteromeric complex formation widened the possible application area of the SFE enantioseparation method to almost all chiral compound soluble in $scCO_2$.

For separation of enantiomers of a racemate a novel, organic solvent free procedure was developed. The method called enantioseparation via fractionated supercritical fluid extraction is a promising, real "green" technique.

Publications of the theses

Papers in international journals

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