SUPERCRITICAL FLUID CHROMATOGRAPHY AS SUCCESSFUL TOOL IN CHEMICAL AND PHARMACEUTICAL INDUSTRY

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The increasing trend towards drugs of highest purity promotes preparative-scale chromatographic techniques. Most of the separations are performed using liquid chromatography. One important advantage of using supercritical fluids instead of liquids as solvents is the reduction of organic solvents, so that no or significant less expensive solvent recovery is necessary. A high productivity of the supercritical chromatographic process is possible because the low viscosity enables high flow rates at a moderate pressure drop and a high number of theoretical plates can be reached as a consequence of the high diffusion coefficients of supercritical fluids.

At our department a large number of experimental set-ups for chromatography with supercritical fluids (SFC) at different scales and for the determination of fundamental thermodynamic data like solubilities and adsorption isotherms is available. A preparative SFC for elution mode and a Simulated Moving Bed (SMB)-SFC have been built and put into operation. A simulation tool for process optimization is available. Successful separations e.g. of phytol isomers, ibuprofen enantiomers, and tocopherol homologues were performed experimentally and by simulations. It has been shown that the supercritical fluid chromatographic technique is now ready for industrial application.

1 INTRODUCTION

Due to a large number of different stationary and mobile phase combinations preparative chromatography is a high selective process, which is often used for the purification of high-value products in pharmaceutical industry, in the biotechnology area and in the production of fine chemicals. Most of the separations are performed using liquid chromatography in elution mode on a single column. However, the Simulated Moving Bed (SMB) concept for chromatography is a continuous countercurrent process. One important advantage of using supercritical fluids instead of liquids as solvents is the reduction of organic solvents, so that no or significant less expensive solvent recovery is necessary. The product recovery can easily be achieved by depressurizing the gas. A high productivity of the supercritical chromatographic (SFC) process is possible because the low viscosity enables high flow rates at a moderate pressure drop and a high number of theoretical plates can be reached as a consequence of the high diffusion coefficients of supercritical fluids. Another unique feature of the SFC process is the opportunity to change the elution strength of the mobile phase by density in order to optimize the separation performance.

Some references about large scale SFC were found. Lembke [1] described the isolation of ethylesters from fish oil with preparative SFC using an aminopropyl stationary phase. In 1998 the process developed was translated to production scale at KD-IQA (Tarragona, Spain) for

processing between 250 and 350 t fish oil per year [2]. Aaltonen et al. [3] replaced the traditional LC separation step for the separation of cyclosporin A from the fermentation broth by a preparative two step Batch-SFC. By using carbon dioxide as mobile phase the needed amount of toluene, hexene and methanol of more than one ton per kilogram product was reduced to several kilograms per kilogram product with the usage of ethanol and methanol as modifier. By this process up to 1000 kg cyclosporin A per year can be separated.

Up to now, besides our group, only one research group is known to have published results of SMB separation using supercritical carbon dioxide as mobile phase [4, 5]. Clavier [4] described the separation of a synthetic mixture of γ-linoleic acid ethylester (GLA) and docosahexaen acid ethylester (DHA) in a SMB plant using pure supercritical carbon dioxide as fluid phase. He worked with eight columns of 33 mm ID packed with C18 reversed phase silica. The obtained purities for raffinate (GLA) and extract (DHA) fractions were 97.7 and 97.8 % respectively. He reported a total productivity of 33.1 g per day in the isocratic mode. By the implementation of a pressure gradient in the system, a fourfold higher productivity was reached.

At our department a large number of experimental set-ups for chromatography with supercritical fluids (SFC) at different scales and for the determination of fundamental thermodynamic data like solubilities [6] and adsorption isotherms [7] is available. The apparatus for the determination of adsorption isotherms allows the measurement of isotherms for pure substances as well as for mixtures by frontal analysis, ECP and a perturbation method.
A preparative SFC for elution mode (column: 30 mm ID, 450 mm max. length, [8]) and a Simulated Moving Bed (SMB)-SFC (8 columns: each 30 mm ID, 190 mm max. length, [9, 10) have been built and put into operation. A simulation tool for process optimization is available. Successful SFC separations e.g. of phytol isomers, ibuprofen enantiomers, and tocopherol homologues were performed experimentally and by simulations (Table 1).

Table 1 : Examples of successful SFC separation development at TUHH

	Phytol	**Ibuprofen**	**Tocopherols**
Method development	Depta [10]	Johannsen [13]	Upnmoor [14]
Adsorption isotherms	Giese [11]	Peper et al. [7]	Upnmoor [14], Lübbert et al. [15], Lübbert et al. [16]
SFC in elution mode			Buß [8]
SMB-SFC	Depta [10], Johannsen et al. [12]	Peper et al. [7]	Johannsen et al. [17], Peper et al. [18]
Simulation	Giese [11]	Peper et al. [7]	Buß [8], Peper et al. [18]

2 PROCESS DEVELOPMENT

For process development different steps are needed: analytical method development, simulations, measurement of adsorption isotherms, experimental separations at low feed concentration and productivity optimization.

The development of a new chromatographic process is started with a screening of stationary phase and mobile phase combinations by analytical chromatography. The influence of pressure and temperature is studied. The most important thermodynamic information for a chromatographic process are the adsorption isotherms which are measured experimentally. Due to the large number of process parameters a simulation of the process is necessary in order to achieve optimal operating conditions. Separation experiments are performed at low concentration first. Afterwards the feed concentration is increased in order to increase the productivity.

3 RESULTS AND DISCUSSION

3.1 SFC SEPARATION OF PHYTOL ISOMERS

The SFC separation of the phytol isomers was studied in analytical scale first. Good separation was found on a silica gel stationary phase (LiChrospher Si 60, 15 µm) with 4.5 mass% isopropanol in CO_2 as mobile phase for a pressure of 24.0 MPa and a temperature of 313 K [10]. Next, experiments of the preparative separation of phytol isomers by SMB-SFC were performed at low concentration. By the pressure drop across the SMB columns a pressure gradient from 23.0 to 10.0 MPa was generated. The experimental results correspond to the model predictions based on the ´triangle theory´ [9].

The adsorption isotherms of the single isomers and of the isomer mixture were determined experimentally at a temperature of 313 K in the pressure range from 12.0 to 24.0 MPa and correlated with different models [11]. After further simulation the feed concentration was experimentally increased. This results in a specific productivity of the process up to 54 $g_{phytol}/l_{SP}h$ with a purity of 97 Area% trans- and 98 Area% cis-phytol [12].

Table 2 : Results of parameter studies of the SFC separation of phytol (by simulation), [11]

Column configuration	**Productivity [$g_{phytol}/l_{SP}h$]**	**CO_2-Consumption [g_{CO_2}/g_{phytol}]**
8 x 10 cm	46	59
8 x 5 cm	166	58
6 x 5 cm	251	69
1 x 20 cm	250	280

The results of parameter studies of column length and column configuration by simulation are shown in Table 2. A specific productivity of 250 $g_{phytol}/l_{SP}h$ is possible for a column configuration with 6 columns (5 cm each). The same productivity is also possible for the

elution mode on a single column, but the specific solvent consumption is significant higher [11].

3.2 SFC SEPARATION OF IBUPROFEN ENANTIOMERS

A packed column supercritical fluid chromatography method for the separation of the ibuprofen enantiomers on a chiral stationary phase and CO_2 with modifier as mobile phase has been developed at an analytical scale. Among 11 different stationary phases the Kromasil CHI-TBB phase showed by far the best separation properties. The influence of different modifiers, injection solvents, temperature, and pressure respectively density on the separation behavior have been studied. It was found that the separation behavior strongly depends on the type of modifier and the modifier content. Temperature and pressure are of less influence. Suitable conditions for the ibuprofen enantiomer separation on Kromasil CHI-TBB were found at 4.5 mass% isopropanol (IPA) in CO_2 for a pressure of 16.0 MPa and a temperature of 313 K [13].

Table 3 : SFC-SMB performance parameter of ibuprofen separation

	2/2/2/2 configuration, Experiment	**2/2/3/1 configuration, optimized**	**2/2/3/1 configuration, Experiment**
Feed injection [mg_{rac}/min]	20	140	140
Extract purity [area%]	> 99	> 99	> 95
Raffinate purity [area%]	> 99	> 99	> 99
Consumption CO_2 [g_{CO2}/g_{rac}]	590	267	280
Consumption IPA [g_{IPA}/g_{rac}]	27	12	13
Productivity [$g_{rac}/kg_{SP}h$]	3	21	21

Adsorption isotherms of the single enantiomers and of the racemate have been determined. The experimental data were correlated by means of a cubic adsorption isotherm [7]. The experimental SFC-SMB separation of the enantiomers was started in the linear mode with diluted feed. In the extract a concentration of the R(-)-enantiomers of >98 area% was found, the concentration of the S(+)-enantiomers in the raffinate was >98 area%, too. The specific productivity of this run was about 3 $g_{rac}/kg_{SP}h$. A good agreement between model predictions (triangle theory) and experimental results could be shown. In combination with the measured cubic adsorption isotherm the experimental SFC-SMB runs could also be described with the dynamic SMB model. After further optimization of the SMB-SFC process by simulation the specific productivity could be increased to 21 $g_{rac}/kg_{SP}h$ theoretically and also in experiment with a 2/2/3/1 column configuration (Table 3). The consumption of CO_2 and isopropanol referring to the injected racemate could be reduced [7]. However, up to now the system is not completely optimized. Only two different column configurations were investigated while column length, operating pressure, operating temperature and content of modifier were kept

constant. For example, a change of modifier might positively influence both the adsorption equilibrium and the solubility of the racemate in the mobile phase in terms of the productivity. For further optimization of the process, the influence of all parameter have to be investigated. Further productivity increasing by a factor of 5 is realistic.

3.3 SFC SEPARATION OF TOCOPHEROL HOMOLOGUES

The separation of tocopherol homologues with SFC at analytical conditions were studied on a silica gel stationary phase and CO_2 with alcohol as mobile phase . The elution order is α -, ß-, γ- and δ-tocopherol. Sufficient separation factors were found between α- and ß-tocopherol (α>1.4) even higher ones for γ- and δ-tocopherol (α>1.8). The separation factor between of ß- and γ-tocopherol is about 1.2 at best modifier condition [14].

Table 4 : Studies on adsorption behavior of tocopherol homologues on silica gels

Substances	**Modifier**	**Adsorbens**	**Temperature [K]**	**Pressure [MPa]**
α-Tocopherol [14]	methanol	LiChrosorb Si 60	303	15.0
α-Tocopherol [8]	ethanol	Zorbax Pro 10-60 CN	313	13.8
α-/δ-Tocopherol [15]	isopropanol	Nucleosil 100	31	16.0-26.0
α-/ δ-Tocopherol [16]	isopropanol	Kromasil 60	313	16.0-26.0

Extensive studies have been carried out for the adsorption behavior of tocopherol homologues on different silica gels systems (Table 4). Preparative separations have been carried out by SFC in elution mode [8] and by SMB-SFC [17, 18]. Several simulations have been carried out for comparison of elution and SMB-SFC [18].

4. CONCLUSION

Table 5 : Productivity of SFC separation experiments

Substances	**Separation Method**	**Adsorbens**	**Productivity [g/kg$_{SP}$h]**
DHA/DPA	SFC (semi)	Aluminiumoxide	1
cis-/trans-Phytol	SMB-SFC	LiChrospher Si 60	54 [$g_{phytol}/l_{SP}h$]
(R)-/(S)-Ibuprofen	SMB-SFC	Kromasil CHI-TBB	21
Tocopherols	SMB-SFC	Kromasil 60	8 (ongoing work)

For process development at the TUHH a large number of experimental set-ups for chromatography with supercritical fluids (SFC) at different scales and for the determination of fundamental thermodynamic data like solubilities and adsorption isotherms is available. A preparative SFC for elution mode and a Simulated Moving Bed (SMB)-SFC are in operation. A simulation tool for process optimization is available.

Successful separations of phytol isomers, ibuprofen enantiomers, and tocopherol homologues were performed experimentally and by simulations. The specific productivities of the systems are listed in Table 5. Further optimization of process parameter with regard to higher productivities are possible. Depending on the separation problem the solubility in the mobile phase or the maximum loading of the stationary phase are limiting the productivity of the process. It has been shown that the supercritical fluid chromatographic technique is now ready for industrial application!

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