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SUPERCRITICAL FLUID CHROMATOGRAPHY AS SUCCESSFUL SEPARATION 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 these 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 an excellent equipment 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 (column: 30 mm ID, 450 mm max. length) and a Simulated Moving Bed (SMB)-SFC (8 columns: each 30 mm ID, 190 mm max. length) are in operation. A simulation tool for process optimization is also available.

Different successful separations were performed experimentally and by simulations. A comparison between different chromatographic methods is carried out. It is shown that the supercritical fluid chromatographic technique is a suitable separation tool for industrial application.

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. Today most of the chromatographic separations are performed using liquid chromatography in elution mode on a single column. 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 depressurising 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 optimise the separation performance. Besides the batchwise chromatography in elution mode the Simulated Moving Bed (SMB) concept for

chromatography is used. This continuous countercurrent process leads to substantial lower solvent consumption.

There is an increasing number of references about large scale SFC. Lembke [1] described the isolation of ethyl esters from fish oil with preparative SFC using an aminopropyl stationary phase. The developed process 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. Clavier [4] described the separation of a synthetic mixture of γ-linoleic acid ethylester (GLA) and docosahexaen acid ethyl ester (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.

Successful separations e.g. of phytol isomers [4], ibuprofen and binaphthol enantiomers [5, 6, 7], and tocopherol homologues were performed experimentally and by simulations at TUHH. A comparison between different chromatographic methods is carried out.

EXPERIMENTAL

At 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 [8] and adsorption isotherms [5] 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, [9]) and a Simulated Moving Bed (SMB)-SFC (8 columns: each 30 mm ID, 190 mm max. length, [4, 10] have been built and put into operation. A simulation tool for process optimisation is available.

A schematic diagram of the preparative SFC for elution mode is shown in Figure 1. It consists of one separation column, two pumps for supplying the mobile phase, an injection system and four cyclones for collecting different fractions and is equipped with an UV detector.
The carbon dioxide is pumped by a continuous flow piston pump that pumps up to 25 kg/h. Modifier is added by an HPLC pump. The custom made SFC column with dynamic axial compression has an inner diameter of 30 mm and variable bed length. This column is designed for pressures up to 40 MPa and temperatures up to 200°C. The fractions are collected in high-pressure fluid cyclones with an inner diameter of 20 mm. The column and the cyclones are electrically heated. A more detailed description of the plant is given in [8].

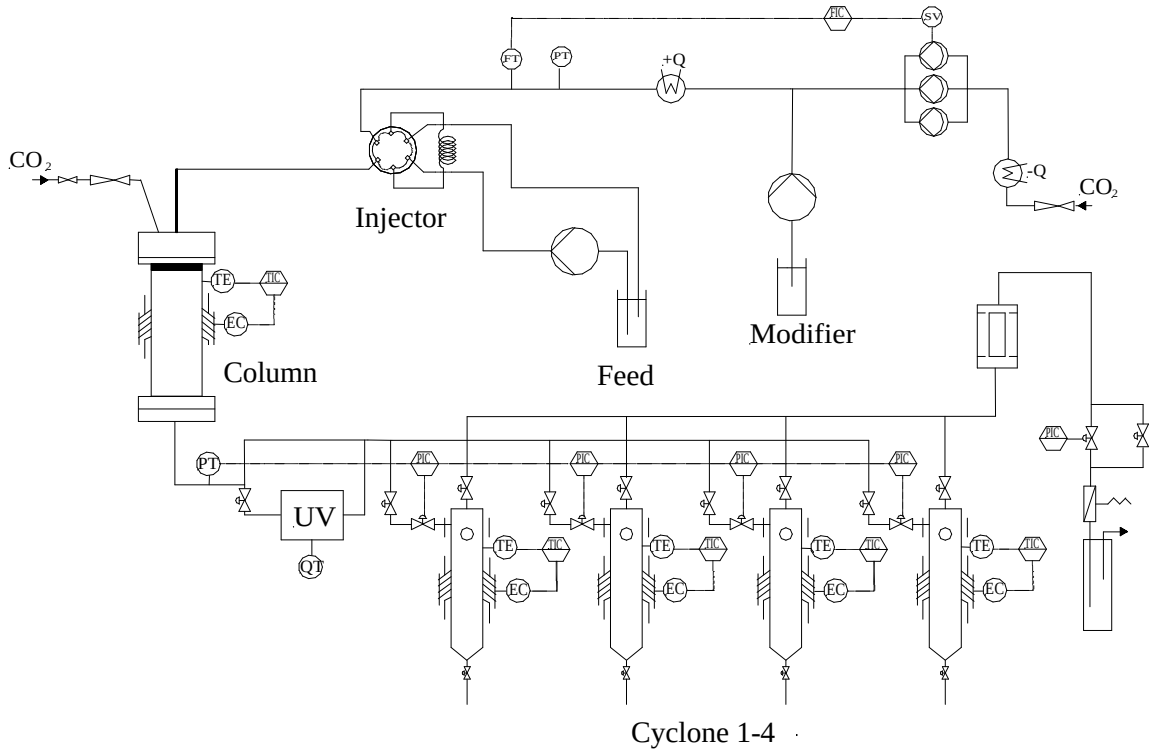

Figure 1: Preparative SFC at TUHH

PROCESS DEVELOPMENT

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

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.

RESULTS AND DISCUSSION

A parameter study on the SFC separation of α-/δ-tocopherol was carried out in analytical scale on Kromasil 60-10. Pressure, temperature and modifier content were varied within the maximal range as shown in Table 1. To start out from defined standard conditions the influence of the variables on the retention factor, separation factor and the peak resolution was determined. It could be seen that increasing the pressure results in decreasing the retention factors k and peak resolution R_S. There is nearly no influence of pressure on the separation factor α.

With increasing temperature the retention factor, separation factor and the peak resolution are increasing. However, the highest influence is given by the modifier content. An increase in modifier content results in decreases of retention factor, separation factor and peak resolution.

Table 1: Parameter study for the SFC separation of α-/δ-tocopherol on Kromasil 60-10with CO_2 and modifier isopropanol

Parameter	**Max. range**	**Standard condition**	**+10% of max. range**	**Dk_d [%]**	**Da [%]**	**DR_S [%]**
Pressure [MPa]	10,0-40,0	15,0	3,0	-15,8	+0,1	-7,9
Temperature [K]	253-353	313	10	+15,9	+7,9	+26,4
Modifier [Vol%]	0-20	5,0	2,0	-72,2	-15,5	-42,5

For optimal separation (high separation factor, high resolution and low retention factor) in this case one have to choose a suitable low modifier content, higher temperature and lower pressure. In Figure 2 a chromatogram of a mixture of tocochromanols under optimised conditions is shown.

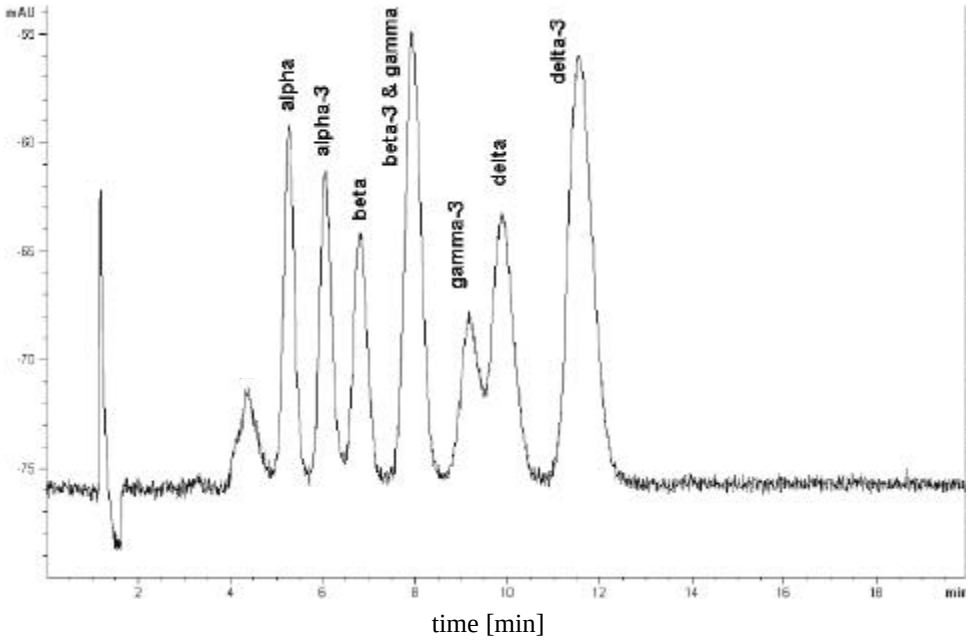

Figure 1: Chromatogram of tocochromanol mixture on Kromasil 60-10 (50°C, 18 MPa, CO_2 + 2% Isopropanol)

After the measurement of adsorption isotherms a scale up and an optimisation of the chromatographic process were carried out. A comparison of the SFC separation of a binary mixture of α-/δ-tocopherol on Kromasil 60-10 in different modes is shown in Table 2. Also shown are first experimental results of a SMB run with non optimised column length and

column configuration. By simulation it was found that the SFC in elution mode gives a two times higher productivity than the SFC-SMB. The solvent consumption in the SMB mode is lower. Some further optimisation of process parameter with regard to higher productivities are possible.

Table 2: Comparison of chromatographic separations of α-/δ-tocopherol on Kromasil 60-10

Method	**Column [L x ID (x n)] [cm x cm]**	**Productivity [mg/cm^{3}h]**	**Solvent consumption [g_{CO2}/g_{Feed}]**
SFC-SMB (exp.)	14,4 x 3,0 (x 8)	18,4	579
SFC-SMB (sim.)	6 x 3,0 (x 6)	58,3	495
SFC (sim.)	20 x 3,0	120,8	620

A comparison of different chromatographic separations of cis- and trans-phytol is shown in Table 3. The highest productivities were found for the SFC and the SFC-SMB process. The simulation of both modes leads to the same productivities. The solvent consumption for the SFC-SMB mode is substantial lower (factor about 5) compared to the SFC in elution mode.

Table 3: Comparison of chromatographic separations of phytol isomers

Method	**Column [L x ID (x n)] [cm x cm]**	**Productivity [mg/cm^{3}h]**	**Solvent consumption [g_{CO2}/g_{Feed}]**	Ref.
HPLC (sim.)	? x ?	177	?	[11]
HPLC-SMB	20 x 18 (x 8)	34	?	[12]
SFC	25 x 20	115	?	[11]
SFC (sim.)	**20 x 3,0**	**250**	**280**	**[13]**
SFC-SMB	11 x 3,0 (x 8)	54	60	[10]
SFC-SMB (sim.)	**5 x 3,0 (x 6)**	**250**	**69**	**[13]**

CONCLUSION

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 optimisation is available.

Successful separations of e.g. isomers, enantiomers, and natural mixtures were performed experimentally and by simulations. It could be shown that in SFC high productivities are possible. By using the SMB concept the solvent consumption can be reduced. It has been shown that the supercritical fluid chromatographic technique is ready for industrial application!

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