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High-Pressure Technology

Supercritical Fluid Extraction (SFE)

High-pressure pilot unit for the extraction of solids using supercritical gases as solvents (500 bar, 120 °C, 30 kg/h CO2 capacity, 2 litre extractor with 1.2 litre basket insert, fluid cyclone as intermediate separator, 1.2 litre final separator).

Features and advantages

Low temperature separation
A highly pure unaltered extract obtained, without solvent residues and without degradation of quality by high temperature or oxidation.

Non-toxic solvents
such as Carbon Dioxide, leave no harmful residue in the extract.

Solubility variation
Solvent characteristics, such as: viscosity – coefficient of diffusion – dielectric constant – and density (capacity) of a gas in the supercritical state, can be varied by simply increasing or lowering extraction pressure and temperature.

Fractionated extraction and separation
can process both solids in batch mode and fluids in continuous and batch mode.

Inexpensive solvents
Units can be adapted for various gases and for gas mixtures, or for the preparation of gases with co-solvent (e.g. acetone, alcohol, etc.). Recommended gas for extraction is CO2.

Low energy process
Electricity is the only form of energy required because there is no solvent distillation and no steam purging of the residue.

Fast diffusion
accomplished because of the low viscosity of CO2.

Chemically inert solvent
Oxygen is completely excluded from the process because of the closed system design.

No risk to the environment
by the use of the inexpensive extraction agent – CO2.

No explosion or fire hazards

High selectivity for the extraction of non-polar compounds.

Applications

The following listing represents only a portion of the possible applications for CO_2:

- Production and transfer of flavours
- Production of natural extracts
- Production of active ingredients for pharmaceuticals and cosmetics
- Extraction of undesired components from liquid and solid products
- Higher efficiency of protein recovery from vegetable and animal materials
- Recovery of mineral oils from rock, sand and slate
- Separation of asphalt from tar
- Blowing of tobacco and extraction of tobacco flavours
- Regeneration of molecular sieves
- Degreasing of catalysts
- Production of essential oils from blossoms, leaves and roots
- Non-alcoholic wine
- Fractionated separation of products
- Fractionated extraction
- Extraction with entrainers
- Extraction with different gases

Main products

Essential oils	e.g.	oil of mint, oil of galbanum, oil of orris root
Essential oils and resins	e.g.	from hops
Essential oils and active ingredients	e.g.	from camomile, garlic , onions, valerian
Active ingredients	e.g.	caffeine
Oleoresins	e.g.	from pepper, nutmeg, cloves
Flavours	e.g.	from fruits, vegetables, fruit juices
Natural colours	e.g.	carotene, bixine
Vegetable oils and fats	e.g.	from soya beans, germinated wheat, germinated maize
Degreasing	e.g.	of starch, lecithin, pancreas, animal proteins
Deodoration	e.g.	of fats and oils (extraction of free fatty acids)
Regenaration	e.g.	of filters, catalysts, activated charcoal
Fractionation of mixtures	e.g.	tar, mineral oil

Natural products flavours extracted with CO2

chillies
curcuma
fennel
cloves
ginger
bay
mace
nutmeg
pepper
thyme
vanilla
juniper berries
cinnamon
onion

valerian
hay
camomile
mint
wormwood
leaves
pineapple
apple juice
apple rape
pear juice
blackcurrant juice
pine
strawberries
currants

hazel nuts
hops
cheese
orange peel
roasted coffee
tobacco
vine
cherry juice
angelica root
galbanum
orris root
garlic
raspberries

Related applications

Drying of aerogels

Water and waste water cleaning

Conservation of paper

Treatment of leather and paper

Multipurpose extraction pilot unit for the treatment of solid and liquid products (300 bar, 200°C, 16 kg/h CO2 capacity, 2 m column ID 38 mm, 2 litre extractor with 1.2 litre basket insert, intermediate separator (fluid cyclone), 1.2 litre final separator).

Multipurpose high-pressure extraction pilot unit (technical description)

This turn-key **multipurpose pilot unit** is assembled in a mobile frame and is built for the

- continuous extraction of **liquid products** in a counter current column B3 combined with the possibility of
- batch-wise extraction of **solids products** in an extraction vessel B1

The Pilot Unit may therefore be operated continuously, extracting solutes from liquid material or in the batch mode extracting solutes from solids.

The liquid product is pumped into the column B3 through an upper connector from where it drops to the bottom in counter flow with the up streaming supercritical solvent fluid. The residue is collected at the bottom of the column B3 and is automatically discharged at a rate controlled by the level controller. The extract on the other hand is carried over to the separator B2 by the supercritical gas. The liquid product capacity is adjustable.

The high capacity diaphragm metering pump P1 delivers contaminant free supercritical fluid. Its capacity is also adjustable from 0 – 100 %. The fluid has already reached extraction conditions when entering the column, wherein the temperature is kept constant up to the outlet by the vessel heating.

Before entering the separation vessel B2 the pressure is reduced by a control valve C1 lowering the solvent power of the carrier gas to practically zero. There are three distinct phases entering the separation vessel B2: Liquid CO2, gaseous CO2, and the extract. The extract drops to the bottom of the separation vessel B2 from where it is collected and removed through a hand valve. In the separation vessel B2 a liquid level is maintained to improve the separation of the extract. The entering liquid CO2 is evaporated continuously at moderate temperature.

In the condenser W3 the gas is condensed and in the following metering pump P1 the supercritical solvent is again brought up to the chosen extraction pressure. In the downstream heat exchanger W1 the solvent fluid is heated up to the required extraction temperature which means that the solvent fluid has already reached the extraction conditions when entering the column B3 through the bottom connector.

Flowing up the column in counter flow with the injected liquid product from the top, additional extract is dissolved continuously and the closed process cycle continues.

To extract solutes from **solid material** the pilot unit is operated in the batch mode. The solid product is introduced into the extractor B1 in a cylindrical basket with filter elements on both ends. These filter elements retain the product but are permeable for the solvent fluid and the dissolved extract. As in the process with liquid products, the solvent fluid dissolves the solute as it flows upstream through the solid product.

The further steps of the process are the same as with liquid products e.g. pressure reduction, separation, evaporation, condensing, pumping and heating, thus closing the process cycle again.

The spray nozzle, solution and dissolution may as an option be transmitted on TV-screens or Videos through the installed standard optical windows. In the separator B2 the phase boundaries can be observed and the liquid level is controlled automatically.

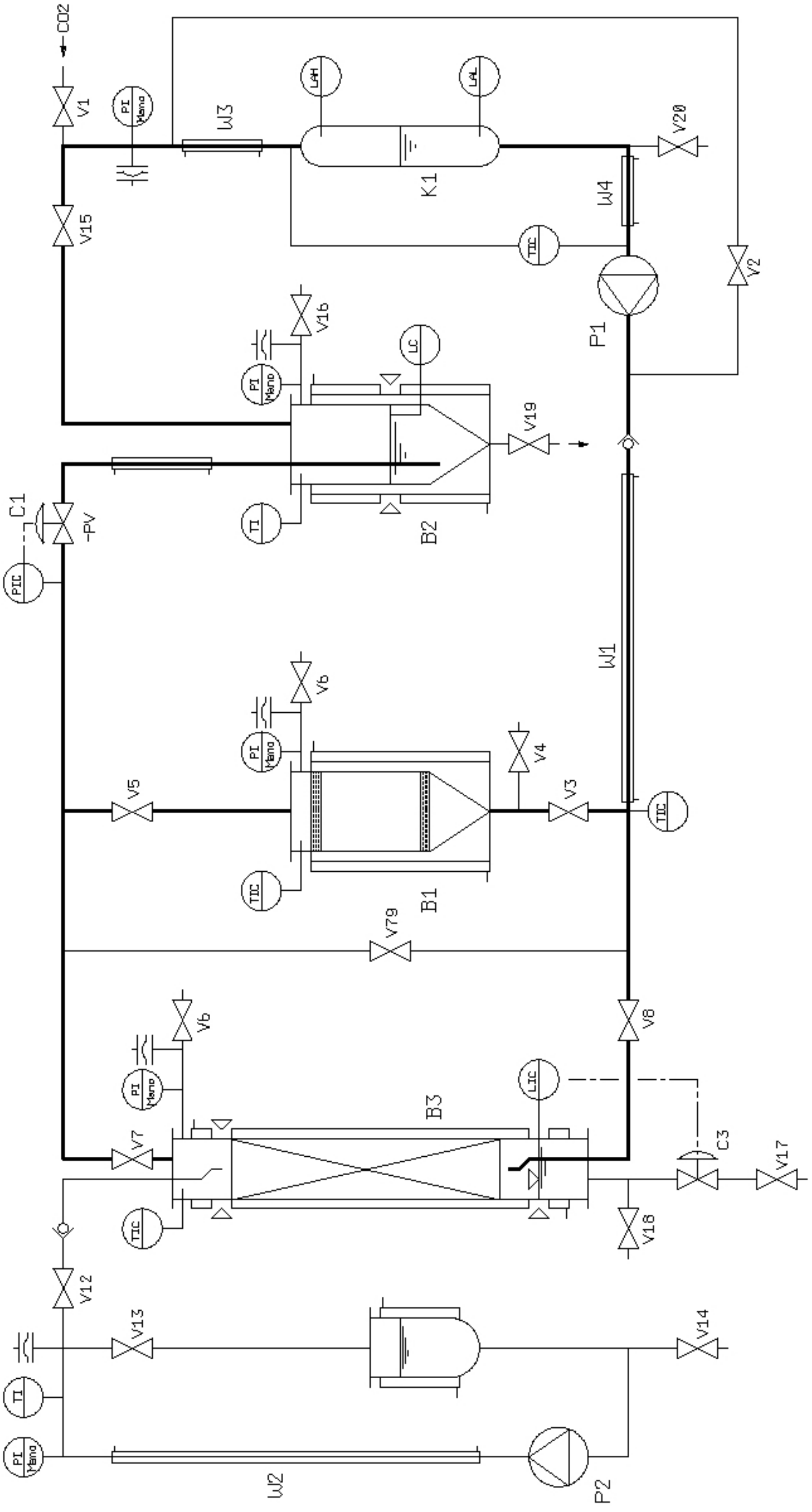
The vessels are easily accessible and the closures are hand operated. The extract from the separator B2 is subtracted manually and the residue from the bottom of the column continuously.

The temperatures are controlled by separate heaters; the extraction pressure by a triggered valve system which eliminates the clogging risk by the extract in the control valve seat.

For reliable scale-up and economical reasons the design of the system is based on pumping the supercritical fluid rather than compressing it in the non-relevant and costly gaseous state. Built for preparative work, the unit can also be used for screening and analytical research. For certain industries such as pharmaceuticals it can even be used as a full production unit. The capacity of this extraction unit is large enough for the production of batches for market testing.

All relevant data are indicated on digital displays on the front panel flow sheet and, as an option may be brought up to a Personal Computer using the SITEC data acquisition system.

Various options are available, such as larger vessel capacities of 2 litre, 4 litre, 10 litre and 20 litre; longer columns; higher pressures, temperatures and mass flows, several vessels in parallel, in series or in the carousel mode.


CO2
V1
PI Mano
W3
LAH
LAL
K1
V20
W4
TIC
V2
P1
V16
PI Mano
LC
V19
TI
B2
C1
PV
PIC
W1
V6
PI Mano
V4
V5
V3
TIC
TIC
B1
V79
V8
V6
PI Mano
LIC
B3
C3
V7
V17
TIC
V18
V12
V13
V14
TI
PI Mano
W2
P2

Questionnaire for multipurpose supercritical extraction pilot unit for liquid and solid raw material

Operating pressure max.:		**300 bar**	500 bar	(700 bar)
Fluid (CO2) pump capacity max.:	10 l/h	☐	☐	☐
	18 l/h	☐	☐	☐
	30 l/h	☐	☐	
	50 l/h	☐	☐	
	100 l/h	☐		

Operating temperature max.: ☐ **80 °C** ☐ 120 °C ☐ 150 °C ☐ 200 °C

☐ ……………………………………………………………………………………

Supercritical solvent: ☐ **carbon dioxide (CO2)** ☐ ……………………………………………………

Extraction vessel capacities:

☐ **1 litre with 600 ml basket insert**
☐ 2 litre with 1.2 litre basket insert
☐ 4 litre with 2.4 litre basket insert
☐ 6 litre with 3.9 litre basket insert
☐ 10 litre with 7 litre basket insert
☐ 20 litre with 14 litre basket insert

☐ ……………………………………………………………………………………

Number of extraction vessels: ☐ **1** ☐ 2 ☐ 3 ☐ ……………………

Extraction column diameter:

☐ **∅ 38 mm**
☐ ∅ 50 mm ☐ ∅ 65 mm ☐ ∅ 90 mm

☐ ……………………………………………………………………………………

Extraction column length:

☐ **2 m**
☐ 3 m ☐ 4 m ☐ 5 m

☐ ……………………………………………………………………………………

Liquid (raw material) capacity:

☐ **2 l/h**
☐ 4 l/h ☐ 10 l/h ☐ 18 l/h ☐ ……………………

Options:

☐ Mass-Flowmeter for ☐ carbon dioxide (recommended)
☐ liquid product
☐ co-solvent

☐ Intermediate separation system(s) ☐ 1 ☐ 2 ☐ 3
☐ Co-solvent system
☐ Data acquisition system by PC
☐ PLC control with integrated batch documentation
☐ Continuous recovery of ☐ extract ☐ co-solvent
☐ Preparation of rack for RETROFIT of a(n) ☐ extractor ☐ column ☐ separator

Contact Details

Last name: ....

First name: ....

Title/gender: ....

Company: ....

Department: ....

Street: ....

P.O. Box: ....

Zip code: ....

Town / city: ....

Country: ....

Phone: ....

Fax: ....

E-mail: ....

www. ....

Please fill in this form and return it to SITEC-Sieber Engineering AG.

Data acquisition and online visualisation

As a useful addition to the SITEC high-pressure pilot units, we are able to offer you a simple and also a very flexible data acquisition program. This program allows to visualise your process data online during the experiments and to save it on your hard disk for a later interpretation.

The data acquisition and visualisation program will be completely integrated in your high pressure pilot unit and is configured for a specific application. It is also possible to upgrade an existing high pressure pilot unit, but with a bigger expenditure.

Based on the SCADA Software SpecView from EUROTHERM (see overview hereafter) SITEC provides several windows specifically programmed for a certain application.

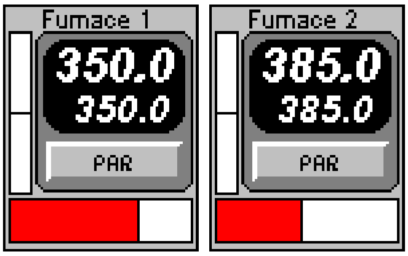
One window shows all the controller symbols and allows set-point adjustment or also alteration of control parameters. In addition, all monitoring signals are shown. On an additional screens all the data are graphically and digitally displayed.

All the data are automatically stored always the data acquisition program is started. On request a certain section (time interval) can be extracted and exported to Microsoft Excel.

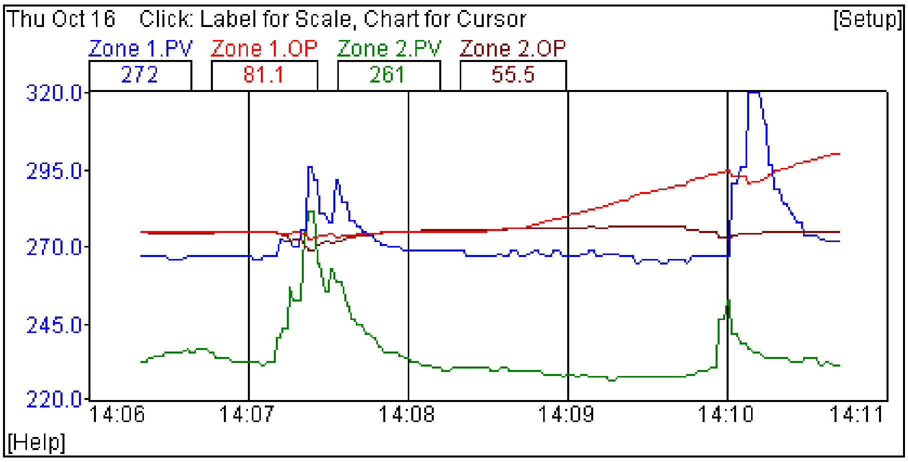
The full development package of SpecView which is also supplied allows to change an existing or to create a new user interface using easy to handle “drag and drop” methods. On request, we will gladly send you a more detailed description of this development package.

The communication PC <-> Pilot Unit is made via USB. The controllers are interconnected by a RS485 interface.

The system requirements for the installation of SpecView: Windows PC with Windows 95, 98, Me, NT, 2000, XP or 2003 Server operating system, 64MB RAM minimum (128MB recommended), USB interface.


Furnace 1
350.0
350.0

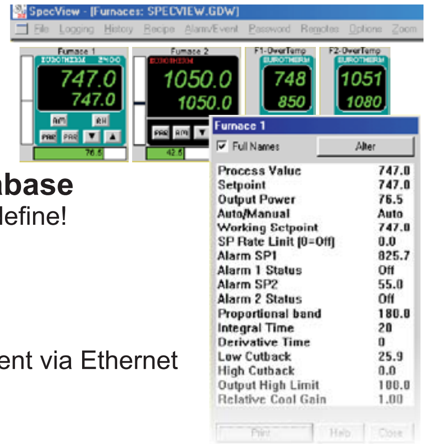
Furnace 2
385.0
385.0


Thu Oct 16 Click: Label for Scale, Chart for Cursor

Zone 1.PV	Zone 1.OP	Zone 2.PV	Zone 2.OP
272	81.1	261	55.5

CONNECT to Control and Measuring Instruments

Simple Display and Logging Applications setup in minutes!


SpecView - [Furnaces: SPECVIEW.GDW]

Furnace 1

☑ Full Names — Alter

Process Value	747.0
Setpoint	747.0
Output Power	76.5
Auto/Manual	Auto
Working Setpoint	747.0
SP Rate Limit (0=Off)	0.0
Alarm SP1	825.7
Alarm 1 Status	Off
Alarm SP2	55.0
Alarm 2 Status	Off
Proportional band	180.0
Integral Time	20
Derivative Time	0
Low Cutback	25.9
High Cutback	0.0
Output High Limit	100.0
Relative Cool Gain	1.00

Auto-Detection of Connected Instruments

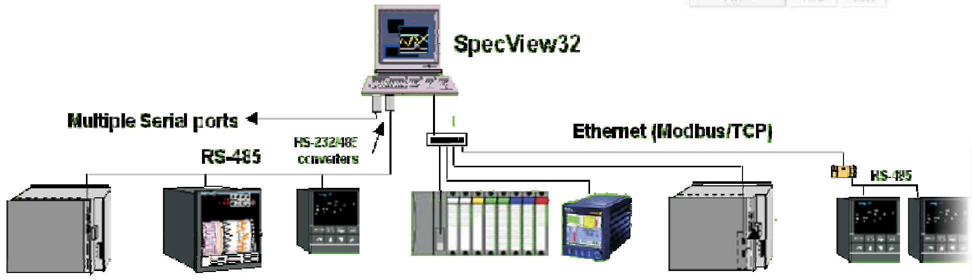
Pre-built Instrument View Database
Over 1000 instruments - no tags to define!
Access all instrument parameters

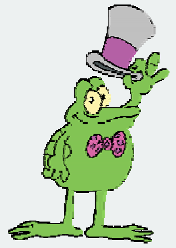
Concurrent Connections
RS232/RS485 (up to 40 ports)
TCP/IP, connect any RS485 instrument via Ethernet
OPC

Temperature
Pressure
Flow
Level
Vacuum
Speed
Status
Power

Controls
Systems
Meters
Indicators
Remote I/O
Data Acquisition
PLCs

RS232 / RS485
Ethernet
Internet
OPC
Radio





SpecView

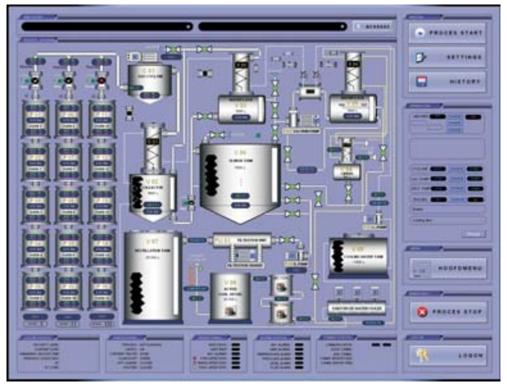
SCADA software for
people with other jobs!

OPERATE

Operator Interface (HMI)

Create easy to use HMI
Unambiguous screens ensure error free operation

Visualize your process at a glance

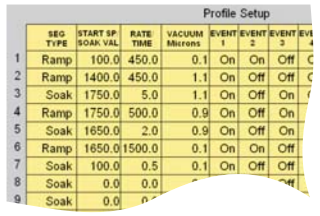


Touch Screen Compatible

Passwords
Multiple password levels
Restrict access to specified items
Record user login

Events & Alarms
All operator actions recorded
Pop-up alarm window
Remote notification: pager / E-mail / SMS

Recipes
Multiple recipe screens
Process snapshot
Flexibility:
  Full Access: select / review / edit / send
  Read-Only: select / review / send
  Single button send
Bar code recipe selection


Profile Setup

	SEG TYPE	START SP/ SOAK VAL	RATE/ TIME	VACUUM Microns	EVENT 1	EVENT 2	EVENT 3
1	Ramp	100.0	450.0	0.1	On	On	Off
2	Ramp	1400.0	450.0	1.1	On	Off	Off
3	Soak	1750.0	5.0	1.1	On	Off	On
4	Ramp	1750.0	500.0	0.9	On	Off	On
5	Soak	1650.0	2.0	0.9	On	Off	On
6	Ramp	1650.0	1500.0	0.1	On	On	Off
7	Soak	100.0	0.5	0.1	On	Off	Off
8	Soak	0.0	0.0				
9	Soak	0.0					

NETWORK

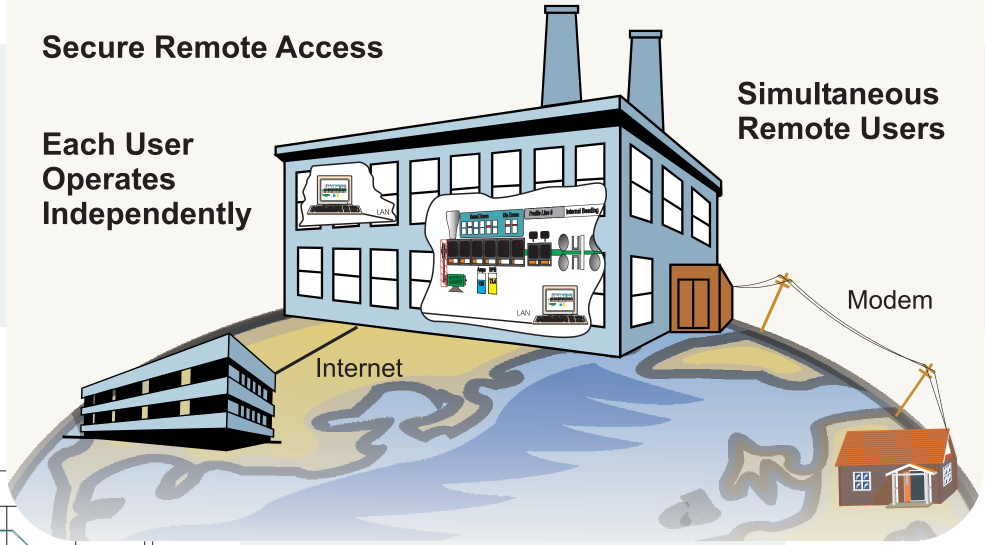
Use SpecView from Office or Home

Multiple Operator Stations for Large Machines

Secure Remote Access

Each User Operates Independently

Simultaneous Remote Users



SpecView is unique. Many of my customers configure the program unaided. Some choose to have some assistance and for those that want a turn-key installation SpecView gives me the tools I need.

Rick Sabo, AQI Engineering

Loy Instrument, Inc. has installed close to one hundred copies of SpecView. I have found that SpecView is unmatched by anything else on the market. Its versatility has allowed us to use it in everything from straight data logging to full scale SCADA systems. For the money, no other software on the market can touch it.

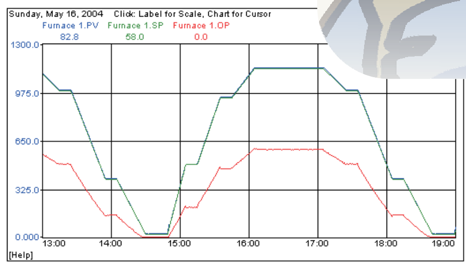
Mark McDaniel, Loy Instrument Inc.

RECORD

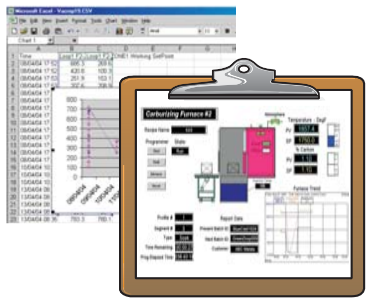
Data Management

Logging
Record any item
By time or event

Trends
Unlimited pens
Unlimited charts
Multiple time axes
Automatic printing

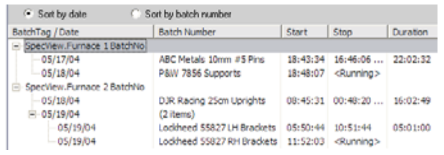


User Variables
Batch data, comments etc
Integrate test data



Reports
Log reports for spreadsheet analysis
Batch “Clipboard” report
Automatic process / shift reports

Batch Records
Find data quickly and easily
Multiple parallel batches


● Sort by date ○ Sort by batch number

BatchTag / Date	Batch Number	Start	Stop	Duration
SpecView.Furnace 1 BatchNo				
05/17/04	ABC Metals 10mm #5 Pins	18:43:34	16:46:06 ...	22:02:32
05/18/04	P&W 7856 Supports	18:48:07	<Running>	
SpecView.Furnace 2 BatchNo				
05/18/04	DJR Racing 25cm Uprights	08:45:31	00:48:20 ...	16:02:49
05/19/04	(2 items)			
05/19/04	Lockheed 55827 LH Brackets	05:50:44	10:51:44	05:01:00
05/19/04	Lockheed 55827 RH Brackets	11:52:03	<Running>	

Alarm / Event
Start, acknowledge & clear timestamps
Alarm history
All operator actions recorded

Historical Replay
‘Video’ replay of whole screen
Review your process in ‘Fast Forward’

I received SpecView as a CD and manual. In a short time I had a configured the program to provide both data logging and an operator interface to a number of different control devices. I had no previous experience with this kind of software. SpecView made it easy.

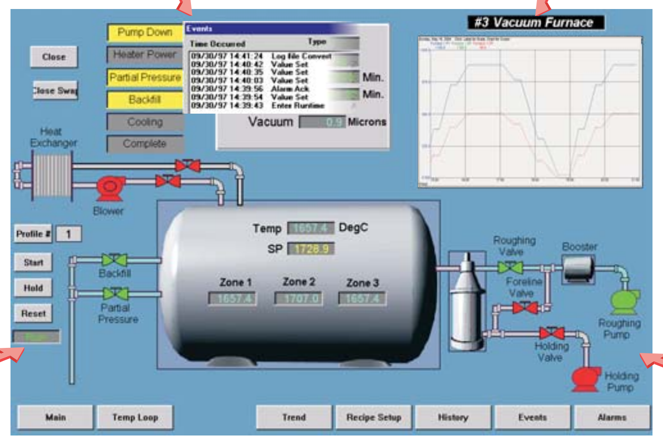
Lisa Reep, Corning

ANALYSE

Explore and Troubleshoot

Event Window
What did the operator do?
...and when?

Integrate
Test measurements
Operator comments
Peripheral data



Events

Time Occurred	Type
09/30/97 14:41:24	Log file Convert
09/30/97 14:40:42	Value Set
09/30/97 14:40:35	Value Set
09/30/97 14:40:03	Value Set
09/30/97 14:39:56	Alarm Ack
09/30/97 14:39:54	Value Set
09/30/97 14:39:43	Enter Runtime

Visual Feedback
“I wish I had been standing here when...”
“Did the purge valve open correctly?”

Performance
How many parts?
Percentage downtime
Correlate run-to-run data

Process control with integrated batch documentation

This control allows you to master your processes by combing display, regulation, control and even batch documentation – all in one.

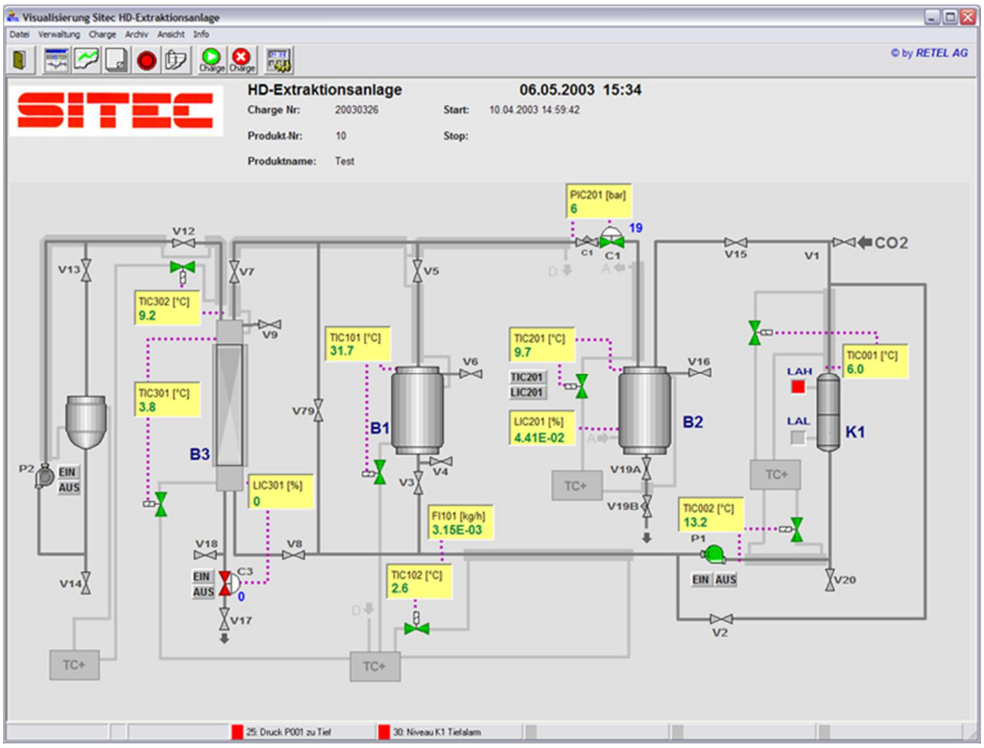
Advantages:

- **complete batch documentation**
- **trend display of process flow**
- **dynamic overview of the installation**
- **easy input of nominal values**
- **exportable into standard formats**
- **protocols and diagrams accessible over network**

The user-friendliness of the system operation is achieved by clearly arranged displays. This control system includes functions which otherwise are integrated in process control systems only.

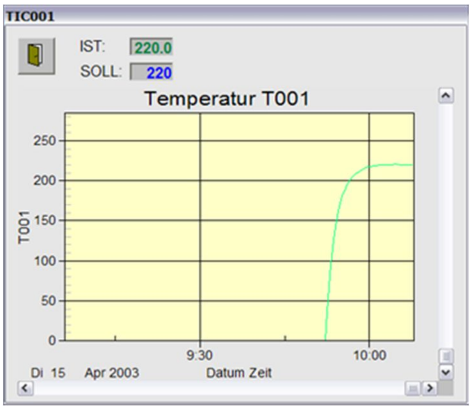
The control system allows manual and – as an option – automatic control of the installation. All process parameters can be displayed.

The data of the current batch as well as the system overview is displayed on the main window. The operator can control the process over the display picture.

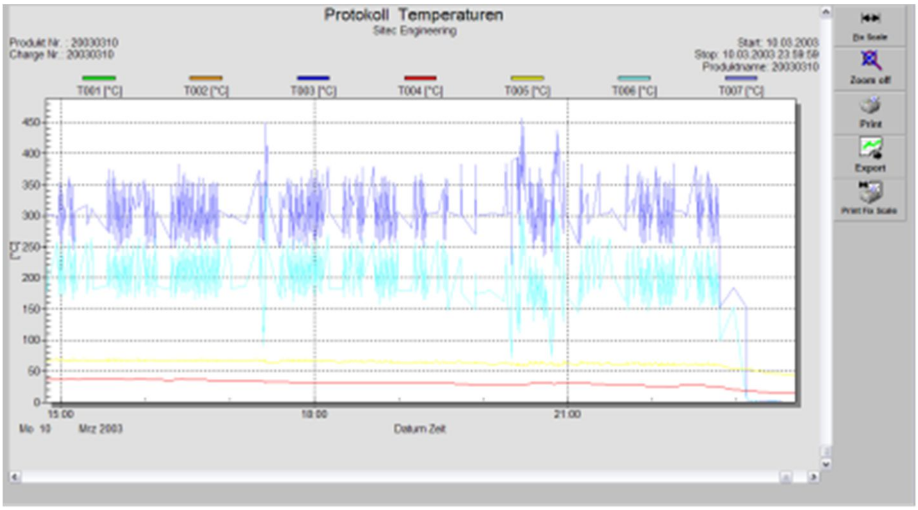


HD-Extraktionsanlage
06.05.2003 15:34
Charge Nr: 20030326
Start: 10.04.2003 14:59:42
Produkt-Nr: 10
Stop:
Produktname: Test

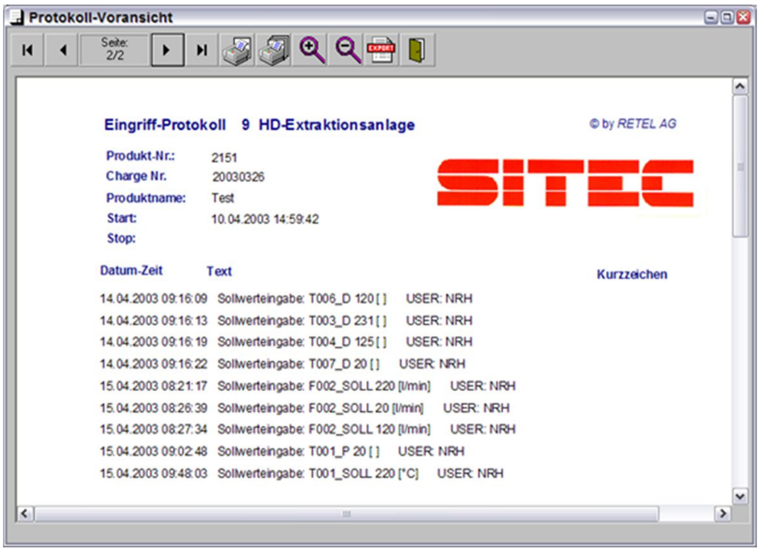
25: Druck P001 zu Tief
30: Niveau K1 Tiefalarm



The automatic control circuits have special display windows with integrated diagrams and nominal value input.



Important process parameters are shown in a trend display. The trend display has a zoom and Fix Scale function and can be printed or exported into bmp, wmf, or jpg graphic formats.



Eingriff-Protokoll 9 HD-Extraktionsanlage

© by RETEL AG

Produkt-Nr.: 2151
Charge Nr.: 20030326
Produktname: Test
Start: 10.04.2003 14:59:42
Stop:

Datum-Zeit	Text	Kurzzeichen
14.04.2003 09:16:09	Sollwerteingabe: T006_D 120 [ ] USER: NRH	
14.04.2003 09:16:13	Sollwerteingabe: T003_D 231 [ ] USER: NRH	
14.04.2003 09:16:19	Sollwerteingabe: T004_D 125 [ ] USER: NRH	
14.04.2003 09:16:22	Sollwerteingabe: T007_D 20 [ ] USER: NRH	
15.04.2003 08:21:17	Sollwerteingabe: F002_SOLL 220 [l/min] USER: NRH	
15.04.2003 08:26:39	Sollwerteingabe: F002_SOLL 20 [l/min] USER: NRH	
15.04.2003 08:27:34	Sollwerteingabe: F002_SOLL 120 [l/min] USER: NRH	
15.04.2003 09:02:48	Sollwerteingabe: T001_P 20 [ ] USER: NRH	
15.04.2003 09:48:03	Sollwerteingabe: T001_SOLL 220 [°C] USER: NRH	

With the help of the integrated logging program the process can be documented completely. All protocols are exportable in pdf, html, or txt formats.

- data protocol
- error protocol
- event protocol
- comment protocol

The batches recorded can be retrieved from the archive. All protocols and diagrams can be exported and re-printed as desired.

Reference List for Pilot Plants

Rhône Poulenc, France	Chemical
University of Delft, Holland	Process engineering
University Wageningen, Holland	Agricultural research
BASF Ludwigshafen, Germany	Chemical
Salzgitter, Germany	Chemical
Hüls Chemie, Germany	Chemical
ENI, Italy	Petrochemical
Givaudan, Switzerland	Flavours and fragrances
Research Centre Karlsruhe	Environmental
Reemtsma, Hamburg, Germany	Tobacco
CNRS, France	Food research
TUBITAK, Turkey	Food research
SASOL, South Africa	Waxes
DEGUSSA-SKW, Trostberg, Germany	Hops, spices
Guinness, Ireland	Brewery research
English Hop Products, Great Britain	Hops
Fraunhofer Institute Pfinzthal, Germany	Process engineering
University of Bremerhaven, Germany	Food research
Novartis, Switzerland	Chemical
Firmenich, Switzerland	Flavours and fragrances
Haarmann & Reimer, Germany	Flavours and fragrances
University of Messina, Italy	Chemical engineering
MERCK, Germany	Chemical
University of Bari, Italy	Research
LIPI, Indonesia	Natural products
F.Hoffmann-La Roche, Switzerland	Reactions
University of Tübingen, Germany	Pharmaceutical research
National Technical University of Athens, Greece	Research
Inst. for “Nichtklassische Chemie”, Leipzig, Germany	Research
University of Halle-Wittenberg, Germany	Research
Janssen Pharmaceutica, Beerse, Belgium	Drug delivery research
MAINELAB, Angers, France	Drug delivery research
Semnan University, Semnan, Iran	Research
JSC “Interbridge”, Moscow, Russia	Research
Ecole des Mines d’Albi, Albi, France	Research
KRAFT Foods, UK	Coffee
Hochschule Niederrhein, Germany	Research
Solvay Solexis, Italy	Research (polymers)
EPFL, Switzerland	Research
University of Alicante, Spain	Research
King Fahd University of Petroleum, Saudi Arabia	Research
Inst. Nawozow Sztucznych Pulawy, Poland	Research
TU Bergakademie Freiberg, Germany	Research
3M, Seefeld, Germany	Research
FAPEX, Salvador de Bahia, Brazil	Research
University of Copenhagen, Frederiksberg, Denmark	Research
University Duisburg-Essen, Essen, Germany	Research
C. Illies, Hamburg, Germany (for China)	Research
AiFame GmbH, Wald-Schönengrund, Switzerland	Natural products

Additional units in following countries:

Denmark
Turkey
Belgium
Italy
Canada
Greece
Spain
Brazil
Germany
Holland
Switzerland
India
Bulgaria
Iran
Saudi Arabia
China
South Africa
France
Great Britain
Ireland
Indonesia
Russia
Poland

Activities:

Flavours and Fragrances
Biotechnology
Chemical Industry
Coal Industry
Food Industry
Pharmaceutical Industry
Oil/Gas Industry

CO_2 in solvent extraction

Papers presented at a meeting of the SCI Food Group
Food Engineering Panel, held in London on 4 February 1982

CO_2 as a solvent: its properties and applications

Heidi Brogle

Many applications for the use of carbon dioxide as a solvent are discussed in the literature; there are also a number of patents granted or pending. Depending on the application and on the installation, the carbon dioxide is used in the liquid, supercritical or gaseous state, which means at pressures and temperatures which differ considerably. An understanding of the complex solvent character of carbon dioxide is needed to understand the known applications and to predict new ones, and in order to direct experimental work in this area.

Liquid CO_2: a non-polar solvent

The first extractions carried out using CO_2 as a solvent, used liquid CO_2. Subcritical liquid CO_2 exists from −55°C to +31°C and from 5bar to 74bar. Provided liquid CO_2 is kept under constant pressure, it can be handled like an ordinary liquid. This means that on raising its temperature it will reach its boiling point and eventually will evaporate. The vapour that exists at higher temperatures can be reliquefied by cooling. As would be expected from any vapour phase of a liquid solvent, the solvent power of the CO_2 vapour is minimal. This means that solvent and solute can be separated by evaporation.

Liquid CO_2 is miscible with all common liquid solvents from methanol to pentane and with pure hydrocarbon monoterpenes, aldehydic and ketonic monoterpenes and all liquid carbonic acids.[1] Water is soluble in liquid CO_2 with a solubility of 0.1 per cent. A closer look at the saturation concentrations of many other compounds, leads to the conclusion that CO_2 is a very non-polar solvent, ranging in polarity near hexane and pentane.[1]

The solvent power of liquid CO_2 is not high compared with ordinary liquid solvents. For these ordinary non-polar solvents an increase in temperature usually leads to an increase in solvent power. This rule is not always valid for CO_2, however, even if at higher temperatures the pressure is also increased to guarantee a liquid phase.

Liquid supercritical and gaseous CO_2 – solvent character from the PT diagram

A closer look at the pressure-temperature (PT) diagram of CO_2 can give an answer to this ambiguity and a clue to the overall complex solvent character of CO_2. Figure 1 shows the PT diagram with boiling line, melting line and sublimation line which separate the areas of liquid, gaseous and solid CO_2. Subcritical liquid CO_2 is found in the triangle formed by the boiling line, the melting line and the line of critical pressure. From anywhere in this triangle the boiling line can be reached by heating at constant pressure or by decreasing the pressure at constant temperature, which means that the CO_2 can be evaporated.

Once in the supercritical region, separation of solute and solvent by simple evaporation is no longer possible. Apart from this, there is no principal difference between the liquid and the supercritical phase. Supercritical CO_2 can therefore also be used as a solvent. Its solvent power is highly dependent on its pressure and temperature, as shown in Fig 2; at low pressures the solvent power of CO_2 sinks with rising temperatures, at higher pressures it increases. If we replace the parameter pressure by the parameter density the relationships become much more simple (Fig 3).

The graphs in Figs 2 and 3 demonstrate that it is the density which has the primary effect on the solvent power and not the pressure. Figure 3 shows tendencies which have been found to be valid as general rules for supercritical solvents:

- Solvent power of a supercritical solvent increases with density at a given temperature.
- Solvent power of a supercritical solvent increases with temperature at a given density.

Many measurements in the region of up to 1000bar and 100°C have proved the validity of these rules. The PT diagram (Fig 1) shows that there is no phase transition either between the supercritical and the liquid phase or between the supercritical and the gaseous phase, as there are no phase transition lines like the boiling line or the melting line. This means that no physical property of the phase changes suddenly with a move from the supercritical to the liquid or gaseous state. A general rule like the one given above for the solvent power of a supercritical solvent must therefore be valid for the liquid or gaseous solvent also, as long as it is not applied too far from the region where it has been measured and found correct.

As density has been shown to be an important parameter, it is helpful to superimpose it as a third dimension onto the PT diagram, (Fig 4). The lines of equal density happen to be near straight lines and the graph can therefore be compared with a very tight 180° turn of a staircase. It starts at densities of less than 100g/litre in the gaseous state and moves parallel to the boiling line, around the critical point and back parallel to the boiling line to higher densities. The further away from boiling line and critical point this move is carried out, the less steep is the slope. The boiling line represents a real step in density which closes up towards the critical point. The effective density at the critical point is 468g/litre.

If in Fig 4, every line of constant density is imagined not as a horizontal line on a given level above the PT plane, but as a sloping curve going upwards towards higher temperatures (like the curves in Fig 3b), the three-dimensional diagram that then results represents the solvent power of CO_2 as a function of pressure and temperature. A real three-dimensional diagram can be compiled for any pair of solvent and solute if the appropriate saturation concentrations are measured. The tendencies in all these diagrams will be the same and sum up in the above mentioned imaginary diagram – imaginary because there is no measure for the solvent power. This imaginary diagram, however, provides much general information about CO_2 as a solvent. For the subcritical liquid CO_2, the diagram shows medium solvent power owing to comparatively high density and low temperatures (for extractions liquid CO_2 is mostly used between 0 and 20°C).

For improved solvent power, the pressure must be increased for higher density which will lead into the high pressure liquid area. For further improvement, the pressure and temperature must be increased leading into the supercritical area. Contrary to popular belief, maximum solvent power is not a property of the supercritical area as a whole. Conditions vary according to the exact place in the supercritical area. If one also realises that the graph in Fig 4 ends at 400bar and 100°C, which is not the end of the natural system at all, then one realises that the supercritical area spans the widest range of pressure and temperature and therefore the widest range of solvent power. The solvent power reaches very high levels at high temperatures and high pressures, but there is very low solvent power in the neighbourhood of the critical point, an area which is interesting for process development because of its high density gradient.

The gaseous state is characterised by very low density and solvent power nearing zero. Solubility in the subcritical gaseous state is negligible but moving to higher temperatures into the high temperature gaseous state, it must be remembered that even if CO_2 itself at these low densities can no longer act as a solvent, a measurable amount of solute (owing to its vapour pressure) may still remain in this phase. The phase then represents a mixture of vapours and not a proper solution. Nevertheless, it is possible to extract with high temperature gaseous CO_2. Such an extraction, however, is more comparable with steam distillation than with solvent extraction. At supercritical temperature, a continuous transition from extraction (at elevated pressure) to distillation (at low pressure) is possible.

Fig 1 PT diagram of CO_2

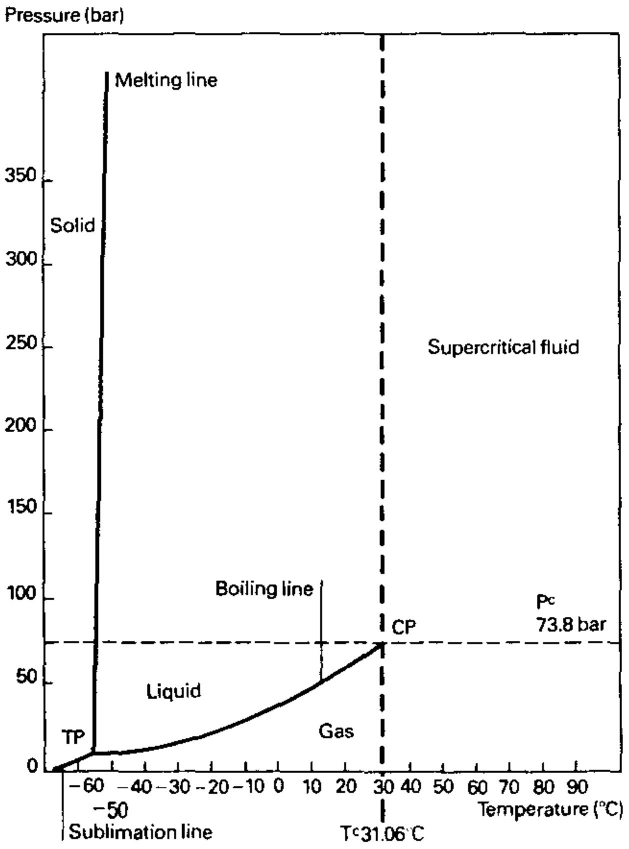
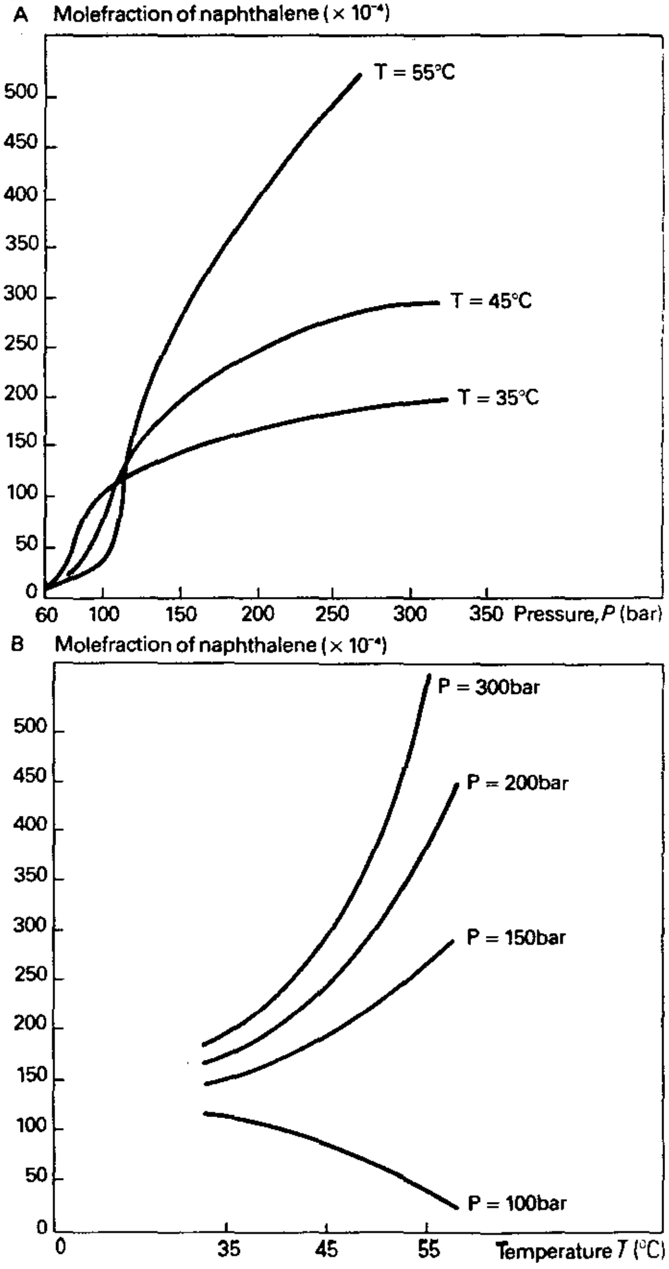


Fig 2 Solubility of naphthalene in CO_2 as a function of pressure at constant temperatures and as a function of temperature at constant pressures



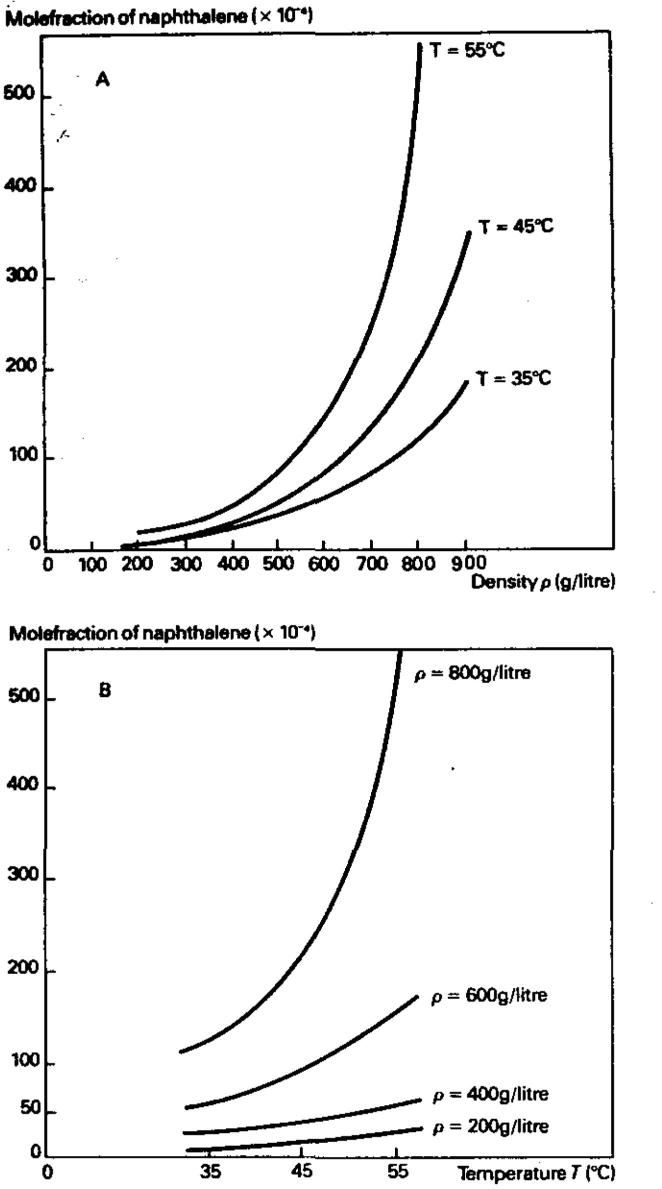


Fig 3 Solubility of naphthalene in CO_2 as a function of density at constant temperatures and as a function of temperature at constant densities

Figure 4 is used to describe the relative solvent power of the liquid, supercritical and gaseous phases. It can, however, give only general information and further details must be determined by experiments as shown in the following example. Moving along the boiling line on its liquid side towards the critical point, solvent power should increase as temperature increases. At the same time, density decreases which means decreasing solvent power. There is no way of predicting which influence will dominate. We can be certain, however, that towards the upper end of the boiling line the influence of the density will increase, as its gradient increases drastically. For the upper end of the boiling line one can therefore expect decreasing solvent power. For some compounds in hop extract, the saturation concentration in liquid CO_2 along the boiling line has been measured [2] and a maximum has been found at 7°C for one and at 20°C for another, which confirms the prediction of falling solvent power at the upper end of the boiling line.

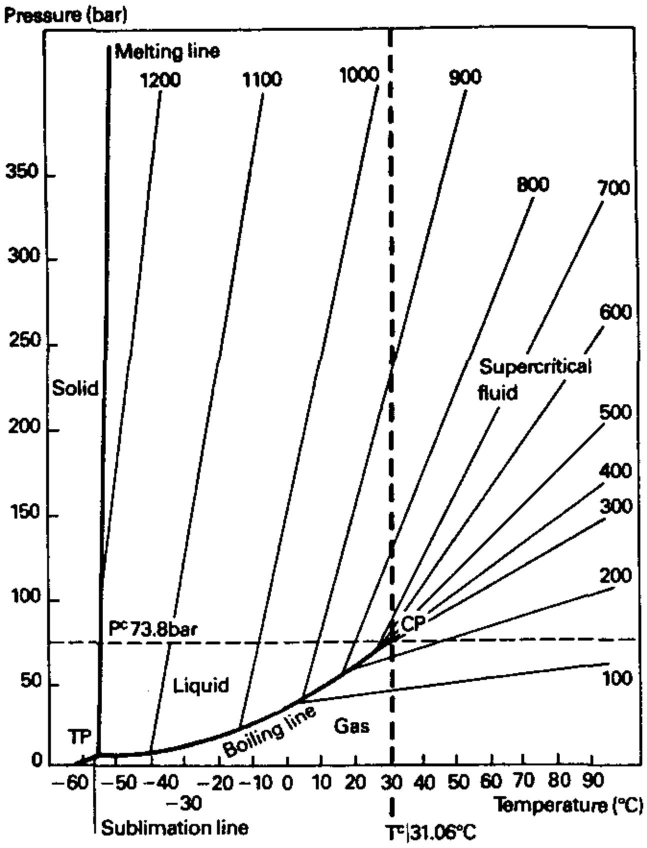


Fig 4 PT diagram of CO_2 with the density as third dimension. Densities given from 100 to 1200g/litre

With the help of the tendencies and rules discussed here, the known applications of CO_2 as a solvent can be understood and new ones can be visualised. A further help is the knowledge that there is no principal difference between CO_2 and any other non-polar liquid solvent and that rules like the following therefore apply to CO_2 also.

- The more physically and chemically similar the solvent and the solute, the higher the solubility.
- Given a range of compounds with comparble polarity, volatility decreases with rising molecular weight. Solubility in a given solvent also decreases.
- Any increase in solvent power increases not only the solubility of a given solute but also the number of compounds soluble at a measurable quantity. In other words, a solvent will dissolve more compounds of a given mixture when used with high solvent power than in the case of its use with low solvent power. Low solvent power therefore means high selectivity, high solvent power means low selectivity.

Extraction using CO_2 as solvent

'Simple' extraction

The simplest case of an extraction is the extraction of a single compound from an insoluble matrix. For a non-polar solvent such as CO_2 this insoluble matrix may consist of cellulose, starch, organic or inorganic high molecular weight polymers, sugars, glycosidic compounds, proteins, metals or metal salts. For such an extraction there is no need for selectivity so CO_2 should be used at highest solvent power to ensure an optimal process. Highest solvent power means highest pressure and temperature possible. The limit to the temperature is mostly given by the temperature sensitivity of the product, whereas the limit to the pressure has to be found as a compromise between process optimum, running costs and acceptable costs for the installation.

For such 'simple' extractions any ordinary liquid solvent

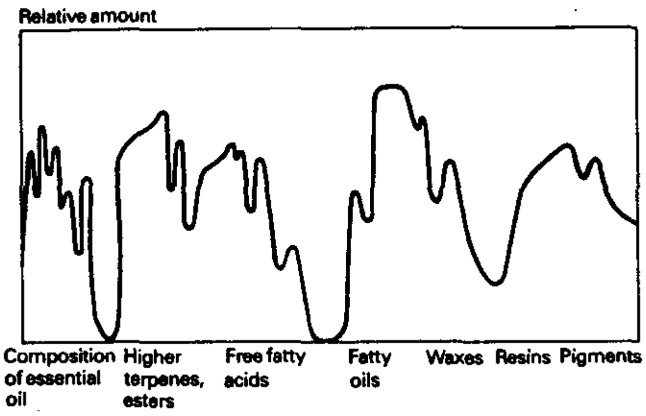


Fig 5 Apolar compounds of a model natural product

would serve just as well. However, the additional expenditure for the installation needed for high pressure extraction can be justified by some advantages that mark out CO_2 among all other non-polar solvents.

- CO_2 can be separated from any solute more easily and more completely because of its extreme volatility.
- CO_2 has low viscosity and high diffusivity.
- CO_2 is absolutely non-toxic.
- CO_2 causes no environmental problems.
- CO_2 is not inflammable.
- CO_2 is cheap.

Practical examples of 'simple' extractions using CO_2 as a solvent are:

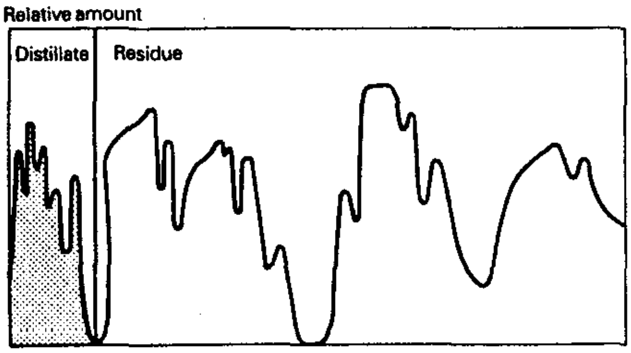
- Regeneration of catalysts;
- Regeneration of activated charcoal;
- Extraction of solvents from reaction products;
- Extraction of solvent left in a residue by an extraction with liquid solvent.

Extraction of natural products

Natural products usually contain large numbers of different chemical compounds which in a given solvent can be anything between highly soluble and not soluble at all. Extracts of the same product made with different solvents are therefore never quite the same. How and why the composition of an extract varies according to the extraction process used is a complex question. With the aid of the following model, a comparison of solvent extraction, steam distillation and CO_2 extraction is simplified.

Figure 5 shows all apolar compounds of a model natural product. The ordinate represents their amount, the abscissa a synthetic parameter made up of volatility, molecular weight, polarity, chemical character etc, comparable with the retention time in a column for gas chromatography. On the left hand side of the graph there are the very volatile compounds, such as the components of essential oils. More to the right come heavier terpenes followed by fatty oils and finally waxes, resins and pigments.

Fig 5a Steam distillation: model of extract and residue composition



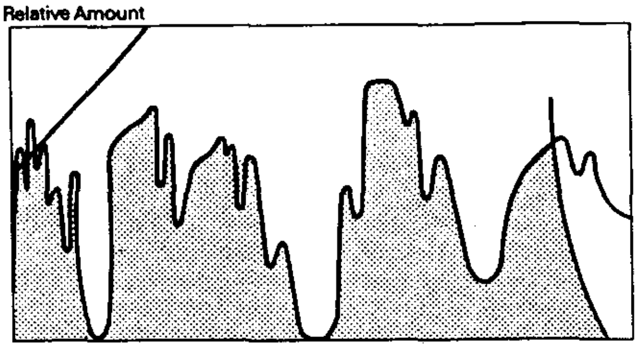


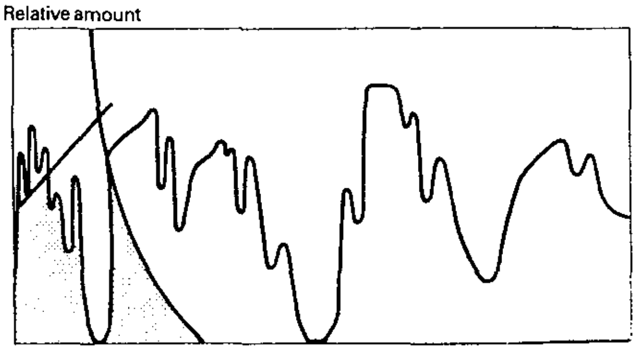
Fig 5b Extraction with methylenechloride: the hatched area shows the extract, right (not hatched) part stays in the residue; top left is lost with evaporation of solvent

Steam distillation selects the extractable compounds strictly by volatility. It cuts the graph (Fig 5a) with a vertical line. The compounds on the left make up the extract, compounds on the right stay in the residue. Furthermore, the distillate is characterised by compounds which did not exist in the original product but have been formed in the special environment of the steam distillation.

Extraction with a good non-polar solvent like methylenechloride extracts everything except the very heavy polymers. Separation of extract and solvent will result in a partial loss of very volatile compounds (Fig 5b). Extraction with a polar solvent (for instance a water-ethanol mixture) extracts the compounds on the far left only. The extract also contains polar substances not included in the graph such as sugars, glycosides, tannins, salts and so on. The loss of volatiles with the evaporation of the solvent is even more severe (Fig 5c). The composition of an extract depends not only on the extraction solvent but also on the degree of exhaustion to which it has been carried. Extractions broken off before exhaustion would have much flatter separation lines in Figs 5b and 5c.

With maximum solvent power, CO_2 is expected to produce an extract similar to the one made with methylenechloride. Experience shows that CO_2 used at 300bar and 60°C (density 830g/litre) extracts always slightly less than methylenechloride (Fig 5d). Evaporation of the CO_2 causes only a negligible loss of volatiles. Any reduction in solvent power makes CO_2 less effective and therefore the corresponding separation lines on Fig 5d are moved to the

Fig 5c Extraction with ethanol-water mixture: compounds of the hatched area are extracted; compounds of right hand not hatched area stay in the residue; compounds of the top left are lost with evaporation of solvent



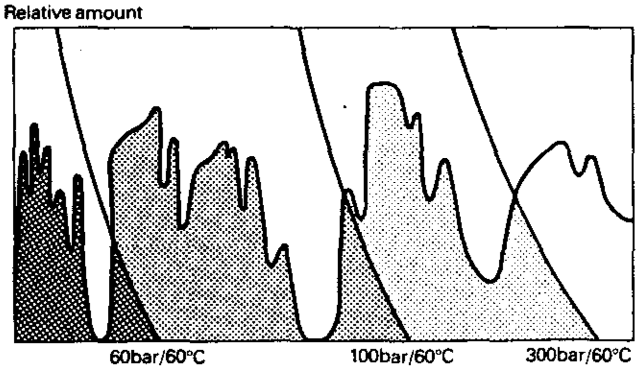


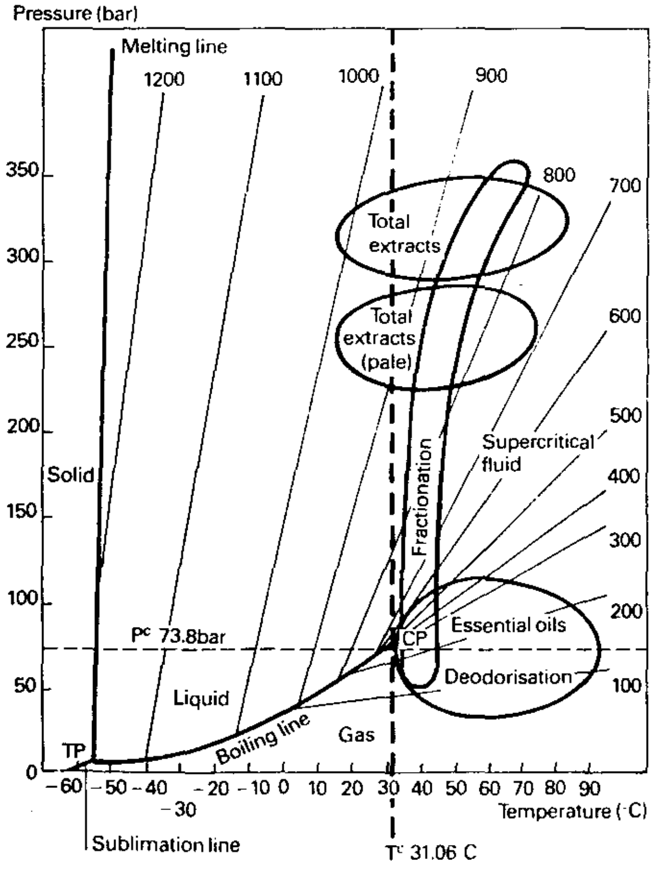
Fig 5d Extraction with CO_2: the differently hatched areas show extracts produced with CO_2 of varying solvent power

left. For CO_2 extraction also, a less exhaustive extraction results in a flatter separation line. Figure 5d shows that many different extracts can be produced with CO_2, but none of them is exactly the same as an extract produced with solvent extraction or steam distillation.

All the separation lines in Fig 5a to 5d are sloping. This shows that compounds situated close beside each other on the graphs can never be separated completely by extraction. Complete separations are only possible for products that show gaps of a reasonable width on their graphs. In order to produce a CO_2 extract as close as possible to a steam distillate, the CO_2 has to be used at very low solvent power. From Fig 4 it is known that this can be found in the supercritical area near the critical point or in the gas area (Fig 6). The same CO_2 (very low solvent power) is used for deodorisation of natural products.

In order to produce a total extract from a natural product, CO_2 has to be used at maximum solvent power (Fig 6). This type of extraction resembles the above mentioned 'simple' extraction and follows the same rules. CO_2 is used in the high pressure liquid state or in the supercritical state at elevated pressures and temperatures. Total extracts produced in such a way are mostly dark in colour; if the pigments are not desired in the extract, CO_2 of slightly lower solvent power must be used. It has been shown that hop extract produced with CO_2 of 300bar is green, whereas that produced with CO_2 of 140bar is yellow.[3] The same is found with extraction of olive oil. Pyrethrum extracts vary from very dark to nearly colourless according to the extraction parameters.[4]

Fig 6 Areas chosen for optimal results in some applications of extractions using CO_2 with varying solvent power



Practical examples for total extracts from natural products are:

- Spice extracts;
- Hop extracts;
- Fatty oils.

Fractionated extraction

Figure 5d shows that a stepwise increase in solvent power of the CO_2 used for an extraction, produces portions of extracts of different quality and therefore allows a fractionated extraction. A search for good parameters for such a fractionated extraction leads back to Fig 4. In the neighbourhood of the critical point in the gaseous and supercritical state, the gradient in density is the highest found on the graph. In that area a wide variation in solvent power is possible in a relatively small range of pressure and temperature. A fractionated extraction is best started in the gaseous state near the critical point and carried up to higher pressures and temperatures according to the solubility of the least soluble compounds that should be extracted (Fig 6).

Practical examples for fractionated extractions are:

- Fractionation of spice extraction into flavour fraction (essential oil) and taste fraction (e.g. piperine fraction for pepper);
- Fractionation of fatty oils into a first fraction rich in flavour and free fatty acids, a second fraction of mostly glycerides and a third fraction rich in waxes and pigments;
- Fractionation of fatty oils into fractions rich in mono-di- or triglycerides respectively.

As mentioned above it is not possible to separate closely related compounds completely by extraction. Improved separation can be achieved with a combination of extraction and rectification. The saturated solution of extract in CO_2 is passed through a rectification column in which a gradient in solvent power causes a counter current. The separation of oleic and stearic acid has been achieved using such a process.[5]

Liquid-liquid and liquid-supercritical extraction

Work on liquid-liquid extraction using the system water-liquid CO_2 has been carried out by Schultz.[6] Some distribution coefficients are given in his publications, but there is no published knowledge on their dependence upon pressure and temperature to give a guide line for liquid-supercritical extraction.

Practical applications for liquid-liquid or liquid-supercritical extraction are:

- Extraction of scents and flavours from liquid materials (e.g. fruit juices);
- Deodorisation of any waterbased substrate;
- Extraction of non-polar impurities from water;
- Extraction of any suitable compound from biochemical reaction mixtures (e.g. ethanol from fermentation solution).

Other processes using CO_2 as solvent

Besides extraction, any other process which uses a non-polar solvent can be carried out using CO_2 as the solvent. Its use can be justified by the same advantages which apply to the use of CO_2 in 'simple' extractions. There is also the added advantage that its solvent power can be varied in a large range.

Examples for such processes include:

- Recrystallisation;
- Organic synthesis;
- Chromatography; column chromatography using supercritical CO_2 as the mobile phase has produced some astonishing results in the separation of polymers.[7]

References

1 Francis, A. W., *J. Phys. Chem.*, 1954, **58**, 1099-144
2 McRae, J. B., & Wheldon, A. G., *J. Inst. Brew.*, 1980, **86**, 296-8
3 Krüger, E., *Mon. F. Brauerei*, 28.3.80, 104-10
4 Stahl, E. & Schütz, E., *Planta Medica*, 1980, **40**, 12-21
5 Peter, S., *DOS*, 1975, 2 340 566
6 Schultz, W. G., & Randall, J. M., *Food Technol.*, 1970, **24**, 1282-6
7 Klesper, E., *Angew. Chem. (Int. Edn.)*, 1978, **90**, 785-93

Supercritical fluid extraction

Recent developments and equipment for laboratory and pilot plant work

R. Sieber *

The aim of an increasing number of research centres at university level as well as in industry at supercritical fluid extraction is not a surprise to the insiders of this relatively new process. A steadily increasing number of important laboratories and industrial firms are studying new fields of application and are developing new processes. From the number of laboratory apparatus and pilot plants built by SITEC, we can estimate that today 100 – 200 units are in operation.

Samenvatting

In dit artikel staan recente ontwikkelingen bij en apparatuur voor laboratorium- en pilot plant onderzoek op het gebied van superkritische vloeistofextractie centraal. Om te kunnen profiteren van de nieuwe mogelijkheden die superkritische vloeistofextractie biedt – ook op het gebied van biotechnologie – dient een laboratorium unit voor het betreffende onderzoek niet alleen te bestaan uit een 'multi purpose' pilot plant maar ook uit een on-line superkritisch vloeistofchromatografiesysteem en een 'fasenevenwicht'-meetcel. Data-opslag en terugkoppeling van analyseresultaten zijn supplementair. Naast superkritische extractie zijn ook andere processen onder superkritische condities van belang voor de voedingsmiddelenindustrie.

Summary

In order to take advantage of the completely new perspectives offered by supercritical fluid extraction, a laboratory unit for studying this process should include on line supercritical fluid chromatography and phase equilibria equipment besides the standard polyvalent pilot plant. Data compilation and analytical feed back are supplementary. Besides supercritical extraction, which is a separation process of interest in biotechnological systems, other processes under supercritical conditions are of interest for the food industry too.

This is due to the outstanding advantages this method has over conventional seperation processes.
Major advantages are:

- a highly pure unadulterated extract is obtained, without solvent residues and without degradation of quality by high temperature or oxidation;
- considerable energy saving (no solvent distillation, no steam purging of the residue, etc.);
- electricity is the only form of energy required;
- fractionation or refining can be carried out against new criteria;
- no adverse environmental effects; inexpensive extraction agent (CO^2).

The main applications in the field of supercritical fluid extraction are:

- production and transfer of flavours;
- production of natural extracts;
- production of active ingredients for pharmaceutics and cosmetics;
- extraction of undesired components from liquid and solid products;
- recovery of proteines from vegetable and animal materials;
- extraction of tobacco flavours;
- regeneration of molecular sieves;
- production of active ingredients from drugs;
- production of essential oils from blossoms, leaves and roots;
- non alcoholic wine;
- fractionated separation of products;
- extraction with entrainers;
- extraction with different gas mixtures.

These applications show that supercritical fluid extraction has promising potentials as a seperation technique in biotechnological processes.

Complete and versatile laboratory unit for supercritical extraction

For a complete laboratory unit for supercritical fluid extraction it is recommended to include in the investment plans also the

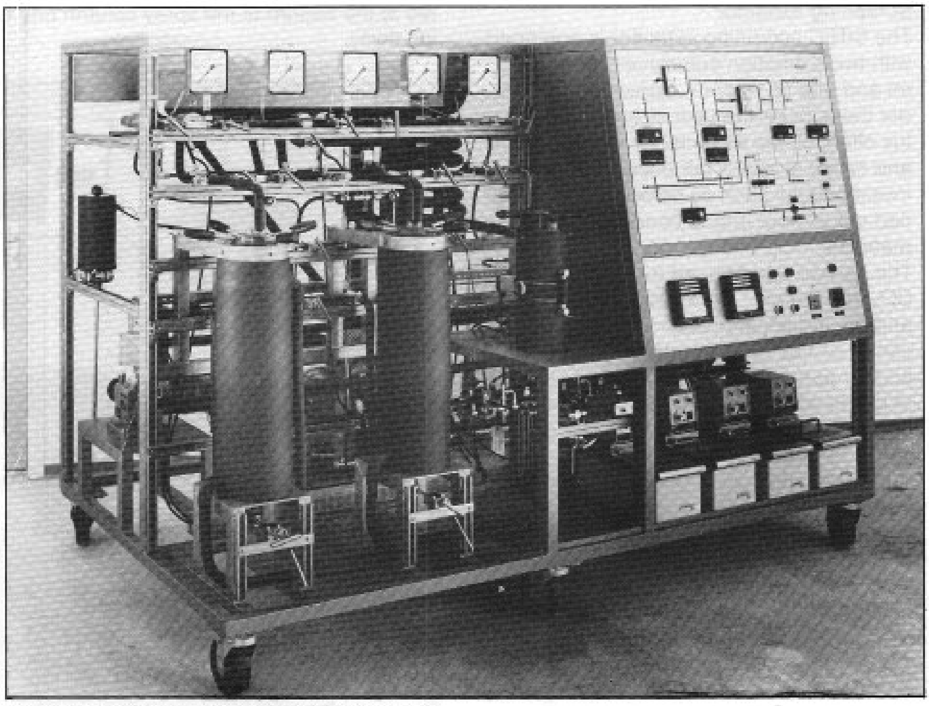


Fig. 1 Complete and versatile laboratory unit

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back-up equipment like phase equilibria apparatus, supercritical fluid chromatography, data compilation and the analytical feedback for process control, if required (figure 1).

The main research and development work is, nevertheless, accomplished with the appropiate pilot plant. The importance of pilot plant work for process development is emphasized because it generates the fastest results. In addition, product specific characteristics are also considered and may be optimized in addition to the basic thermodynamic properties of the pure substances. Results from application oriented test-runs on the pilot plant can be applied directly in the scale-up if the product specific parameters are included in the search for the most economic process. Greatest attention should be paid to the preparation of the product before it enters the extractor. Overlooking this step may have quite an adverse effect on the test results and even set back a project where it could be a success. Product preparation can include such simple measures as preheating the product before it enters the extraction vessel. Other preparation methods, which effect diffusion and absorption properties, are:

- grinding to optimum partical size (too small particles lead to channeling);
- soaking in water or an entrainer accelerates the extraction;
- steaming breaks bonds between solute and matrix;
- premixing with the solvent gas and/or entrainers;
- dilute the product in order to be able to pump it as a slurry;
- cell cracking which opens the cells and has a conversation effect by killing bacteria and germs.

Screening extractor

The SITEC screening extractor is equipped with two extraction autoclaves, one of 400 ml and one of 1000 ml capacity. We consider these to be the best sizes. The apparatus offers all the advantages of automation and safety at a very fair price. It allows quick investigations such as screening to be carried out without undue consumption of raw materials, CO_2, energy, etc.

The gas we recommend for extraction is CO_2, but the pilot extractor, the pilot units and the industrial plants mentioned below can of course be adapted for other gases and for gas mixtures, or for the preparation of gases with entrainers (e.g. acetone, alcohol, etc.).

Polyvalent pilot extractor for fluids and solids

This SITEC unit (figure 2) can be used for the continious extraction of liquid products in a column combined with the possibility of batch-wise extraction of solids in an extraction vessel.

In the case of *liquids or viscous products* the apparatus can be used in two ways:

— Continuous extraction of fluids. For this purpose, SITEC has developed a research extraction column, which is incorporated in the latest pilot plants. Up to 15 sight glasses at 5 column levels can be provided as a standard. The column is connected simply by opening and closing the relevant valves. The column can be equipped with perforated plates (for low-viscosity fluids) or with Raschig rings, mixer packings, etc., as dictated by the nature of the product. The level in the head of the column is kept constant by a suitable controller.

— The fluid to be extracted is sprayed into the upper part of the column and meets the supercritical CO_2, which is injected at the bottom, in counterflow. The residue is collected at the bottom of the spray column and is led away.

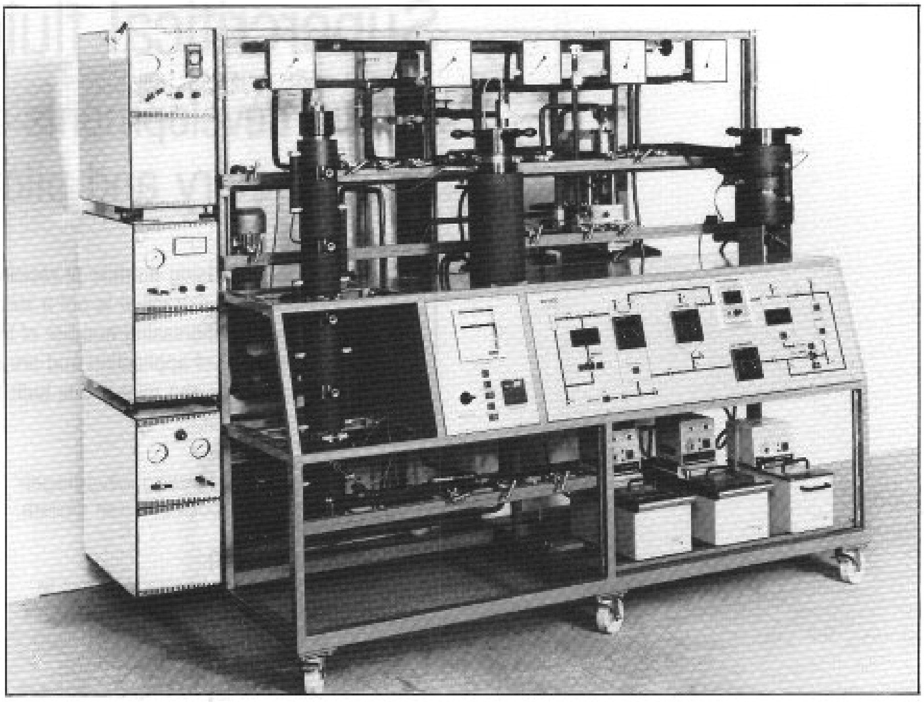

Fig. 2 Polyvalent pilot extractor

The apparatus can be used in the same way either where the residue is the desired product (refining) or where the extract is the desired product.

For certain fluids, which require lengthier contact with the supercritical CO_2 (so that optimum phase equilibrium is achieved and thermodynamic saturation is approached at the same time), it is better to use the filled-column method.

To extract solutes from *solid material* the pilot extractor is operated in the batch mode. The solid product is introduced into the extractor in a cylindrical basket which has filter elements at both ends. These filter ele-

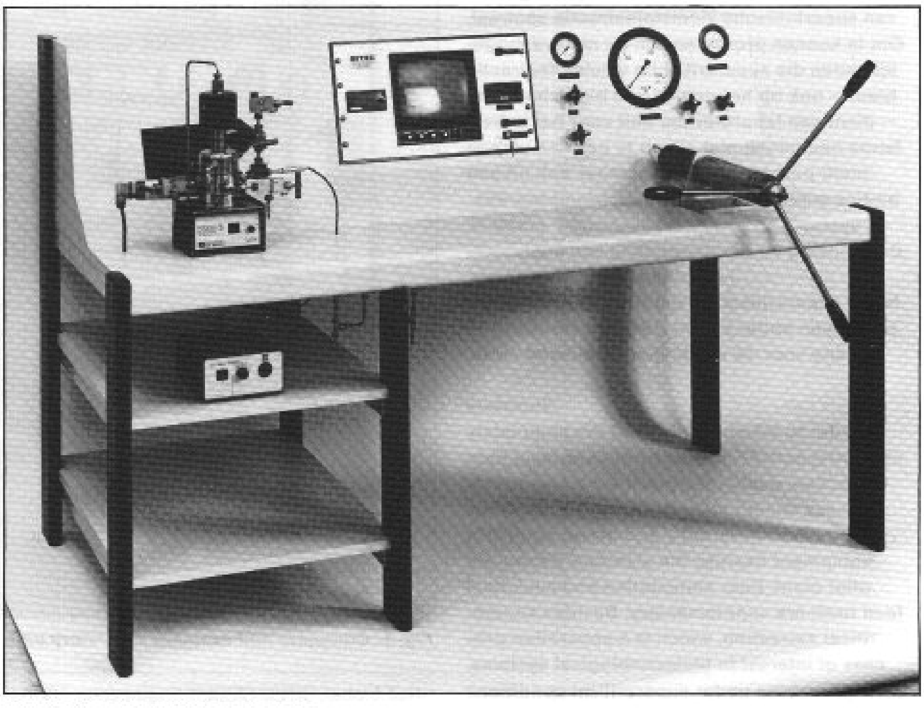

Fig. 3 Phase equilibria apparatus

ments retain the product but are permeable for the solvent fluid with the dissolved solute. As in the process with liquid products, the solvent fluid dissolves the solute as it flows upstream through the solid product.

Options which ought to be available as a standard (if required) are:

- processing of liquids and/or solids;
- large capacity fluid pumps for short test runs;
- fractionated seperation;
- pressure ratings: 320, 500, 700, 1000 bar;
- temperature ratings: 90, 120, 150, 200°C;
- multi-vessel design for semicontinuous operation with solid products.

Phase equilibria apparatus

Phase equilibria units (figure 3) are built for measurements and detection of phase equilibria and phase transitions by optical and analytical means. The picture from the optical cell is transmitted through the sapphire window by the directly connected camera system. It is displayed on the monitor in the front panel. The mixture of solute and solvent gas is agitated by the magnetic stirrer. Whenever samples are drawn from the top or bottom connection in the cell, the directly connected counterbalance piston moves towards the center of the cell, thus keeping the pressure in the measuring cell constant even during sampling operations.

The solvent gas pressure is generated by a pressure generator which allows a very fine adjustment of the required cell pressure. For final adjustment the counterbalance piston can be used.

The temperature in the optical cell is controlled by a thermocouple which acts on a superimposed control cycle.

Supercritical fluid chromatography

Supercritical fluid chromatography is best suited to complement supercritical extraction since it can be operated on line employing the same mobile phase. The highly compressed gas from the extraction cycle is directly used to dissolve and feed the solute through the SFC column (figure 4).

The on line application of SFC in connection with supercritical extraction has many advantages:

- quickly establish the vital parametres for running the pilot plant;
- the analytical results may appear as quickly as within 15 seconds and detection can be made in the IR- and the UV-area, in the latter case at wavelengths down to 180 nm. As a result SFC can in many cases replace analytical extractions with hexane, etc.;
- analytical feedback for process control and automatic count-down process control with the same results as described above;
- a first step to preparative chromatography as the results can be transferred correctly.

In addition the following favourable factors have to be emphasized:

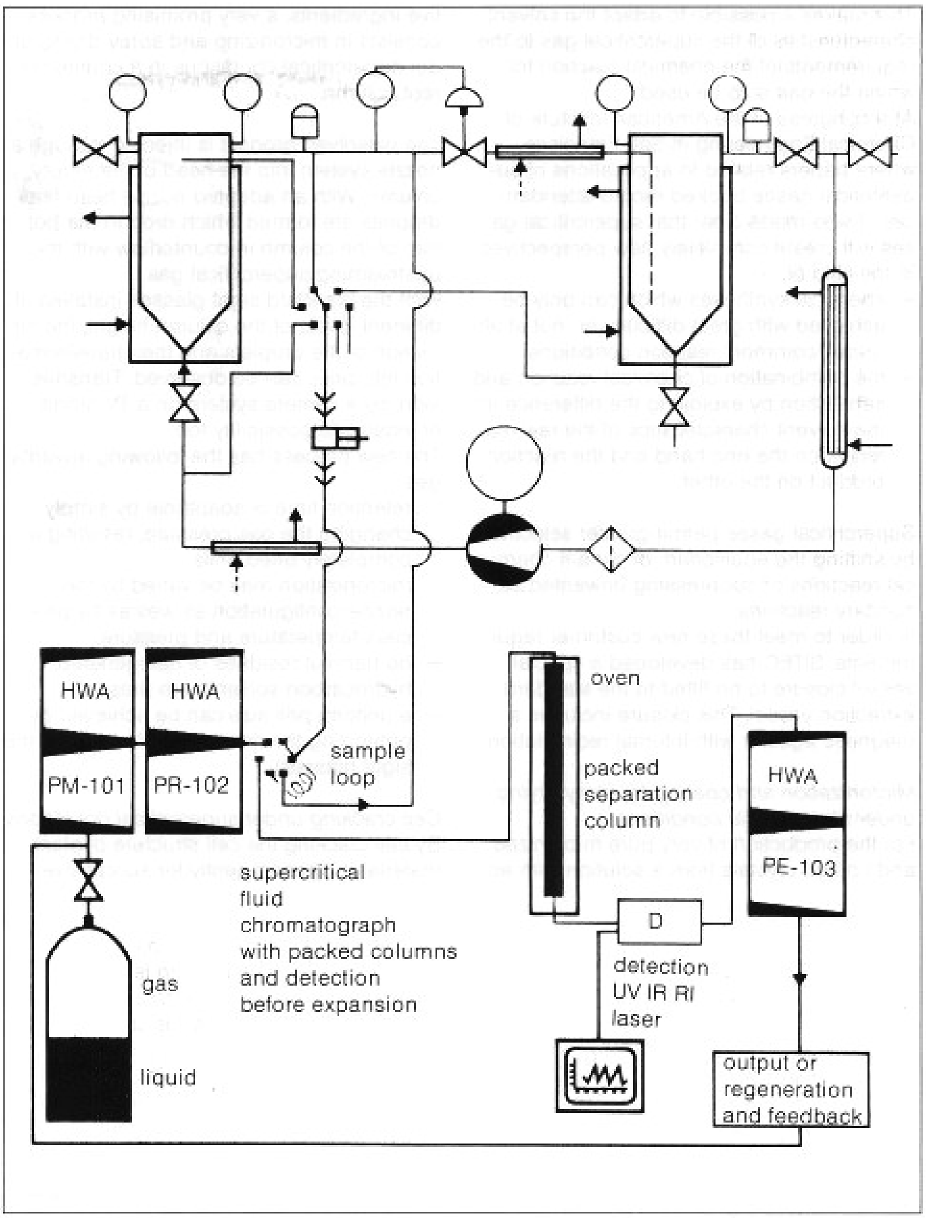

Fig. 4 SITEC extraction with on line π/SFC

- low temperature processing with the separation being achieved by pressure variation;
- no damage to the substances at these low temperatures;
- high solubility and diffusion coefficients in combination with a low viscosity of the mobile phase;
- no halogenated hydrocarbons are used, which is favourable from an environmental point of view.

Data compilation and computer assisted process control

Process data may be converted and sent to a common terminal board for computer compilation. Also pictures from the column, the separator and the phase equilibria cell may be recorded for later reference.

A further step in the direction of an overall process control is the analytical feed-back. This creates the possibility of an immediate reaction on process parameters based on a programmed procedure of analytical requirements. An example to be mentioned is the automatic count-down which stops the process once a predetermined depletion of the raw product is achieved.

New fields of application for supercritical fluids supplementing supercritical extraction

Reactions under supercritical conditions

For a number of years intense development work has been done on studies with regard to chemical reactions, in which gases under supercritical conditions were involved. In order to understand the principles of this new application, we must be aware of the fact that gases in the supercritical state are a solvent with variable solvent characteristics. The following characteristics:

- viscosity;
- coefficient of diffusion;
- dielectric constant and
- density, i.e. solvent capacity

of a gas in the supercritical state can be varied by varying the pressure and temperature.

This makes it possible to adapt the solvent characteristics of the supercritical gas to the requirements of the chemical reaction for which the gas is to be used.
At a congress of the American Institute of Chemical Engineering in San Francisco, where papers related to applications of supercritical gases booked record attendances, it was made clear that supercritical gases will create completely new perspectives in the field of:

- chemical syntheses which can only be achieved with great difficulty or not at all under 'common' reaction conditions;
- the combination of chemical reaction and separation by exploiting the difference in the solvent characteristics of the raw materials on the one hand and the reaction product on the other.

Supercritical gases permit greater selectivity by shifting the equilibrium of certain chemical reactions or suppressing unwanted secondary reactions.
In order to meet these new customer requirements, SITEC has developed a special vessel closure to be fitted to the standard extraction vessel. This closure includes a magnetic agitator with internal recirculation.

Micronization and coating by spray drying under supercritical conditions
For the production of very pure micronized and coated crystals from a solution with active ingredients, a very promising process consists in micronizing and spray drying under supercritical conditions in a countercurrent column.

The dissolved product is injected through a nozzle system into the head of the empty column. With an adapted nozzle head fine droplets are formed which drop to the bottom of the column in counterflow with the upstreaming supercritical gas.
With the standard sight glasses installed at different levels of the column height, the formation of the droplets and their transformation into prills can be observed. Transmission, by a camera system on a TV-monitor or video is a possibility too.
The new process has the following advantages:

- retention time is adaptable by simply changing the gas pressure, resulting in completely dried prills;
- micronization may be varied by the nozzle configuration as well as by process temperature and pressure;
- no harmful residues of halogenated hydrocarbon solvents are present;
- a uniform prill size can be achieved by optimizing the nozzle (visible through the sight glasses).

Cell cracking under supercritical conditions
By cell cracking the cell structure of plant material is opened gently for successive extraction. Due to the low operating temperature and the use of CO_2 as driving gas, deterioration of the product by heat or oxidation is prevented. In addition to the improved yield, this process has a sterilizing effect on the products.

Cell cracking is recommended with spices, oil seeds, kernels and other vegetable raw material for the food and pharmaceutical industry.

The individual cells are cracked open from the inside by expanding CO_2. Simultaneously the product is also cooled by the expansion of the gas, thereby preventing local overheating on the surface of the grains (as often occurs in conventional grinding).

Recovery of greaves from fats and offal under supercritical conditions
At moderate temperatures fats and greaves may be separated by simply exposing the raw fat material to supercritical CO_2.

The recovered de-greased greaves consist of valuable, fully active proteins which may be used for human nutrition. The bacteria count is reduced drastically by the exposure to the supercritical CO_2 and the fat fractions may be separated in a combined extraction cycle.
The refined fat is recovered in a novel way as fine, solid particels.

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Pharmaceutical applications of supercritical carbon dioxide

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The appearance of a supercritical state was already observed at the beginning of the 19th century. Nevertheless, the industrial extraction of plant and other natural materials started about twenty years ago with the decaffeination of coffee. Today carbon dioxide is the most common gas for supercritical fluid extraction in food and pharmaceutical industry. Since pure supercritical carbon dioxide is a lipophilic solvent, mixtures with organic solvents, especially alcohols, are used to increase the polarity of the extraction fluid; more polar compounds can be extracted in this way. The main fields of interest are the extraction of vegetable oils from plant material in analytical and preparative scale, the preparation of essential oils for food and cosmetic industry and the isolation of substances of pharmaceutical relevance. Progress in research was made by the precise measurement of phase equilibria data by means of different methods. Apart from extraction, supercritical fluid chromatography was introduced in the field of analytics, as well as micro- and nanoparticle formation using supercritical fluids as solvent or antisolvent. This review presents pharmaceutical relevant literature of the last twenty years with special emphasis on extraction of natural materials.

1. Introduction

Extraction of natural material using supercritical carbon dioxide is a separation method known since about 30 years. Compared with traditional extraction methods like solvent extraction it shows several advantages. The use of chlorinated hydrocarbons or other organic solvents in industrial extraction processes is intended to be reduced in order to avoid health hazards and to minimize cost of energy for solvent removal and recycling. Steam distillation of volatile compounds e.g. essential oils is as well an energy-intensive separation technique and high temperatures may cause thermal degradation of valuable constituents. Therefore supercritical carbon dioxide is a selective solvent for the production of solvent-free extracts and is gaining increasing importance in pharmaceutical industry, whereas in food technology it is already established. Beside supercritical fluid extraction (SFE) other applications have been developed. Supercritical fluid chromatography (SFC) has been derived from conventional liquid chromatography methods, mainly HPLC as a powerful and universal separation method in both analytical and preparative scale. During the last decade so called nanoparticles gained more and more interest due to their very small particle size, their high surface area and consequently their enhanced dissolution rate and bioavailability. For that purpose particle design by means of supercritical fluids offers a promising alternative. The authors want to give a comprehensive literature survey of supercritical fluid applications relevant for the pharmaceutical and nutrition sector. Special attention is turned to supercritical fluid extraction of plant material, particularly with carbon dioxide. Chromatography and particle design are summarized in some review articles.

2. Historical review

At the beginning of the 19th century Cagniard de la Tour observed the appearance of the supercritical state for the first time [1]. Hannay and Hogarth proved the solvent power of supercritical fluids in 1879 by dissolving inorganic salts in supercritical ethanol [2]. Since the 1930s separations with supercritical fluids were proposed and applied to the refinement of crude oil and the fractionation and purification of natural and synthetic oils. The extraction of plant material or other natural sources in industrial scale started about 20 years ago with the decaffeination of crude coffee beans and the extraction of hops. The production of flavours, fragrances and spices are as well realized in commercial scale by supercritical fluid extraction. A detailed review in SFE applications until the middle of the eighties is given by Stahl et al. [3]. In the last fifteen years the supercritical fluid extraction techniques have been rapidly extended to many different plant species, which results in a broad spectrum of data available in the literature.

3. Supercritical fluids

3.1. *The supercritical state*

The supercritical state is defined by temperature and pressure of a pure component that lie above the critical values ($T > T_c$; $p > p_c$) of the regarded component. In a p-T-diagram the critical point marks the end of the vapour-pressure line. Above the critical temperature a gas cannot be liquefied by increasing pressure. Physical properties (e. g. density, dielectric constant) of the component vary continuously from gaseous or liquid condition to supercritical condition, thus no phase boundary is observed (Figs. 1, 2). Supercritical fluids have properties similar to those of liquids or gases. The densities are in the order of magnitude of liquid solvents, whereas the viscosities are in the same range as those of gases. The low viscosity and comparatively high diffusion coefficients improve mass transfer from drug matrix to the solvent and accelerate extraction processes. The solvent capacity of a supercritical fluid is decisively determined by its density. It can be varied by changing temperature and pressure, with a much stronger influence of the latter one, particularly in direct vicinity of the critical point, the so called near-critical region (Fig. 2). The density increases with increasing pressure and decreasing temperature. Thus the solvent power of supercritical fluids can be easily adjusted by changing these two parameters. Brunner [4] gives an extensive overview of properties of supercritical fluids. Based on supercritical

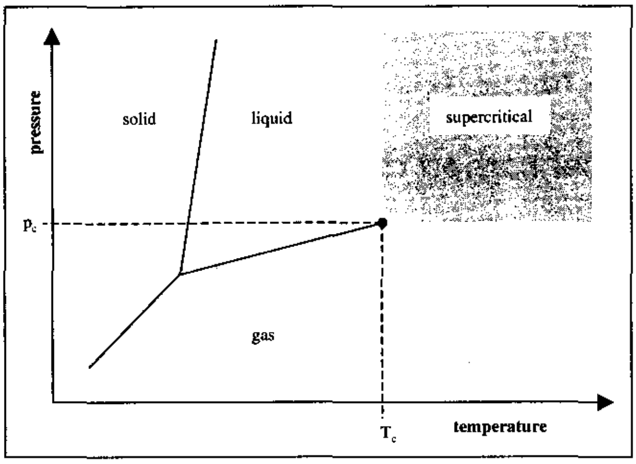

Fig. 1: p,T-Diagramm

fluid chromatography Smith has proposed definitions, as well as nomenclature and abbreviations according to IUPAC recommendations.

3.2. *Supercritical carbon dioxide*

Carbon dioxide is the most common solvent in supercritical fluid extraction, because of its superior properties compared to other gases or liquids. The critical temperature (31 °C) is rather low and the critical pressure (73.8 bar) is easy to realize as well. Further advantages are that carbon dioxide is not flammable or explosive, bacteriostatic, physiologically harmless, environmentally benign and easily available. The carbon dioxide molecule is totally symmetric so there is no dipole moment; this is the reason for its solvent power mainly for nonpolar or slightly polar substances.

3.3. *Modifier effects – binary and ternary systems*

To improve the solubility of polar solutes in the lipophilic solvent carbon dioxide, additional components may be applied. These more polar solvents are called modifier or entrainer and they are added in order to enhance the solvent power of the supercritical gas or to reduce the extraction pressure because of an increased solubility. Traditional solvents like alcohols (especially ethanol and methanol), chlorinated hydrocarbons, hexane, water or acetone are frequently used. These modifiers have volatilities between that of the solute and the supercritical fluid and boiling points between 20 and 100 °C. Phase equilibrium data for binary or ternary mixtures including carbon dioxide-methanol-(water) [6–10] and carbon dioxide-ethanol-(water) [11–13] are available. Mixtures of carbon dioxide with other modifiers (e.g. isopropanol, acetone) are as well described in the literature [15–18]. A general review about modified carbon dioxide including phase equilibria data is given by Page et al. [19].

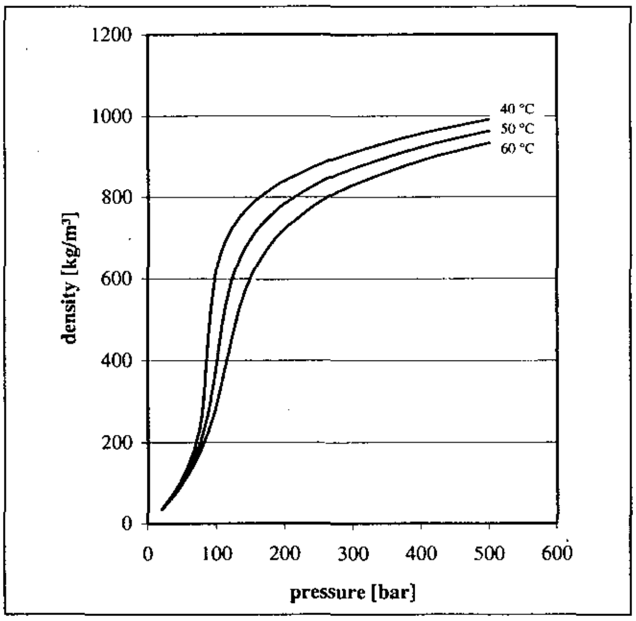

Fig. 2: Density of CO_2 as a function of pressure and temperature

4. Supercritical fluid extraction from plant material

Available literature about SFE covers a wide range of plant species, so different constituents have to be considered. Having a large survey of literature about supercritical fluid extraction within the last fifteen years, one can distinguish three main groups of constituents as targets for an extraction process. On a first level the three groups vegetable oil and related compounds, essential oil and other pharmacologically active compounds as well are divided. In a second step the scale of the extraction process is taken into account, analytical and preparative extraction processes are differentiated. The boundary was set in the range from 30 to 50 ml volume of the extraction vessel.

Analytical SFE is used predominantly as an alternative method for sample preparation in botanical and chemical analysis. Mostly the results are compared to conventional methods like soxhlet extraction or steam distillation. The intention of preparative scale SFE is to produce a commercial – ideally solvent free – extract for either food industry or as a basis for high valuable pharmaceutical formulations of medicinal plants. Until today such formulations usually contain solvent based extracts. If a technical- or pilot-scale SFE should be established first of all the feasibility of SFE for a specified separation problem has to be proven, considering also economic aspects. For countercurrent multistage separation processes Machado and Brunner describe a detailed process design methodology [20, 21]. For packed bed SFE from plant material it is done in a similar way by Lack and Marr [22]. Smith et al. and Reverchon and Osseo made a general energy analysis of supercritical fluid extraction processes [23, 24]. The latter examine three different flow schemes of process plants for the extraction of vegetable oil from soybeans considering particularly energy consumption.

4.1. *Vegetable oils*

4.1.1. Vegetable oils – analytical scale

Analytical scale supercritical fluid extraction is used as an alternative to conventional solvent extraction (e.g. soxhlet). Pure carbon dioxide is the most common extraction solvent but sometimes modifiers like hexane, ethanol, methanol or isopropanol are applied additionally. Various plants as well as algae, mushrooms and fish material are examined for their vegetable oil content by supercritical fluid extraction. A comparison to other methods for determination of vegetable oil content is given for *Brassica napus* [25, 26], *Glycine maximus* [26], *Helianthus annuus* [26] and *Lupinus luteus* [25]. Oils obtained by supercritical fluid extraction show the highest tocopherol but also the highest free fatty acids content. Fatty acids composition was determined in vegetable oils from *Gossypium spp.* [27], *Olea europaea* [28], *Oryza sativa* [29], *Rosa canina* [30], *Silybum marianum* [31] and *Solanum lycopersicum* [32], from the algae *Hypnea charoide* [33] and *Sargassum hemiphyllum* [34], the mushrooms *Pythium irregulare* [35] and *Saprolegnia parasitica* [36] and from the fish *Sardina pilchardus* [37]. Special attention was turned to the content of polyunsaturated fatty acids. *Rosa canina* [30] and *Silybum marianum* [31] were extracted with pure carbon dioxide, pure propane and a mixture of both solvents. Extractions from special matrices were carried out for the fractionation of vegetable oil from corn bran by carbon dioxide-ethanol mixtures [38] and for the extraction of phospholipids from egg yolk [39]. Enrichment and fractionation of polyunsaturated fatty acid esters from esterified fish oil was achieved by in line coupled supercritical fluid extraction and chromatography using ethanol modified carbon dioxide [40]. The selective removal of free fatty acids from seeds of *Nigella sativa* [41] and the modelling of oil recovery from the mackerel species *Scomber scombrus* [42] are further applications of analytical scale supercritical fluid extraction. Some models

Table 1: Vegetable oils – preparative scale

Plant	Targets	Gas	Conditions	Year	Ref.
Anacardium occidentale (cashew nut), shells	Composition of shell liquid, phenolic lipids, unsaturated fatty acids	CO_2	250 bar 40 °C	1991	[46]
Astrocaryum vulgare (tucuma palm), pulp	Extraction of carotenoid rich vegetable oil	CO_2	200–300 bar 40–70 °C	1999	[47]
Brassica napus (canola), oil seeds *Brassica campestris* (canola), flakes and meal	Recovery of vegetable oil; content of phospholipid, lecithin, tocopherol, sterols; solubility of canola seed oil	CO_2 + ethanol	208–668 bar 40–75 °C	1995 1998 1992	[48] [49] [50]
Carya illinoensis (pecan), halves	Palmitic, stearic, oleic, linoleic, linolenic acid in pecan oil	CO_2	413–668 bar 45–75 °C	1997	[51]
Corn, germ	Content of phospholipids; properties of protein residue	CO_2 + ethanol	300 bar 42 °C	1998	[52]
Egg yolk	Extraction and fractionation of lipids; fatty acids composition; kinetic studies and modelling; solubility data	CO_2 + methanol CO_2 + ethanol	150–360 bar 35–75 °C	1992 2001	[53] [54]
Elaeis guineensis (palm), fibers	Extraction of carotenoid and PUFA-rich oil	CO_2	200–300 bar 45/55 °C	1997	[55]
Fish oil ethyl esters	Fractionation of fatty acids ethyl esters	CO_2	145–195 bar 60–80 °C	1998	[56]
Glyceride mixtures (mono-, di-, triacylglycerol)	Fractionation	CO_2	172–344 bar 65–95 °C	1997	[57]
Glycine maximus (soy), seeds	Extraction of phospholipids, lecithins	CO_2 + ethanol	166–689 bar 60–80 °C	1999	[58]
Guilielma speciosa (pupunha), fruits	Fatty acids composition; modelling and kinetic studies	CO_2	250/300 bar 45/50 °C	2000	[59]
Helianthus annuus (sunflower), seeds	Extraction of vegetable oil; modelling and kinetic studies	CO_2	280 bar 40 °C	1997	[60]
Myristica fragrans (nutmeg), seeds	Simultaneous extraction of essential and vegetable oil	CO_2	90 bar 23 °C	1999	[61]
Oenothera biennis (evening primrose), seeds	Fractionation of triglycerides; fatty acids (PUFA) composition; solubility of vegetable oil	CO_2	200–700 bar 40–60 °C	1991 1998	[62] [63]
Olea europaea (olive), oil	Refining of olive oil: deacidification; content of stigmasta-3,5-diene	CO_2	260/310 bar 80/86 °C	1998	[64]
Panicum miliaceum (millet), bran	Fractional separation of vegetable oil kinetic studies	CO_2	300–500 bar 40–60 °C	2000	[65]
Prunus dulcis (almond), seeds	Extraction of vegetable oil; calory reduction of almonds; modelling and kinetic studies	CO_2	350/483 bar 40/60 °C	1993 1998	[66] [67]
Rosa rubiginosa (rose hip), seeds	Colour of vegetable oil; fatty acids composition; kinetic studies	CO_2	300–700 bar 40–80 °C	2000 2000	[68] [69]
Rubus chamaemorus (cloudberry), seed oil	Enrichment of carotenoids, tocopherols; recovery of PUFA-rich oil	CO_2	90–300 bar 40/60 °C	1997	[70]
Triticum vulgare (wheat), germ/gluten	Extraction/removal of vegetable oil; fatty acids composition; tocopherol content	CO_2	100–500 bar 10–65 °C	2000 2000	[71] [69]

Table 2: Essential oils – analytical scale

Plant	Targets	Gas	Conditions	Year	Ref.
Allium cepa (onion)	Concentration of sulphur in oleoresins	CO_2	150–450 bar 35–65 °C	1998	[72]
Archangelica officinalis (angelica), fruits	Angelica oil compounds (phellandrene, spathulenol); furanocoumarins	CO_2	80–140 bar 40–100 °C	1996	[73]
Baccharis dracunifolia (vassoura)	Vassoura oil composition (nerolidiol, spathulenol)	CO_2	90–120 bar 40–60 °C	2000	[74]
Boswellia thurifera (frankincense)	Oil composition in Chinese herbal medicines	CO_2	60–200 bar 50 °C	1991	[75]
Carum carvi (caraway), fruits	Caraway oil content (carvon, limonene) in consecutive extractions	CO_2	97 bar 50 °C	1994	[76]
Cellulose, spiked (model matrix)	Limonene, eugenol, caryophyllene, carvon, santonin	CO_2	50–250 bar −10–80 °C	1992	[77]
Chamomilla recutita (camomile), flowers	Composition of camomile oil	CO_2	250 bar 45 °C	1994	[78]
Cinnamomum cassia (cassia) *Cinnamomum ceylanicum* (cinnamon), bark	Cinnamon flavour compounds (coumarins, cinnamyl aldehyde)	CO_2 + acetonitrile CO_2 + tetrahydrofuran CO_2 + ethyl acetate CO_2 + methylbutylether	300 bar 70 °C static + dynamic	1995	[79]
Commiphora molmol (myrrh)	Oil composition in Chinese herbal medicines	CO_2	60–200 bar 50 °C	1991	[75]
Crocus sativus (saffron), stigmas	Determination of safranal content as well as its precursors	CO_2	100–300 bar 40–120 °C	2000	[80]
Curcuma longa (tumeric), rhizomes	Tumeric oil (turmerone, curcumene, citronellal); kinetic studies	CO_2	200–400 bar 40–60 °C	2000	[81]
Curcuma zedoaria	Oil composition (camphor, zederone)	CO_2	80–200 bar 50–100 °C	1995	[82]
Dragophalum moldavica (dragonhead)	Composition of peppermint oil (menthol, menthone); content of n-alkanes	CO_2 + acetone CO_2 + hexane CO_2 + dichlormethane	400 bar 70 °C	1993	[83]
Evodia rutaecarpaa	Oil composition in Chinese herbal medicines	CO_2	60–200 bar 50 °C	1991	[75]
Ferula galbaniflua (galbanum), latex	Galbanum oil (α-pinene, α-thujene)	CO_2 + methanol	90 bar 45 °C	1998	[84]
Ferulago nodosa	Volatile fraction (pinene, myrcene)	CO_2	91 bar 70 °C	1999	[85]
Humulus lupulus (hops), flowers	Enrichment of bitter principles (humulones, lupulones) and separation from essential oil	CO_2	0,2–0,9 g/ml 50 °C	1992	[86]
Illicum verum (star anise), fruits	Selective isolation of anethole	CO_2 + methanol	120 bar 80 °C	1996	[87]
Laurus nobilis (laurel), leaves	Composition of laurel leaf oil (cineole, terpinylacetate)	CO_2	80–150 bar 40/50 °C	2000	[88]
Lavandula angustifolia (lavender), flowers	Lavender oil (pinene, linalool); modelling and kinetic studies	CO_2	345 bar/50 °C 303 bar/50 °C	1994	[89]
Lavandula stoechas (Turkish lavender), flowers	Lavender oil (campher, fenchon)	CO_2	80–140 bar 35–50 °C	2000	[90]
Lemon oil-cyclodextrin complex powder	Determination of lemon oil (limonene, pinene)	CO_2	147–245 bar 30–60 °C	2000	[91]
Levisticum officinale (lovage), root, seeds, stems, leaves model plant matrix	Lovage oil (caryophyllene, limonene); mass transfer rate and coefficient; solubility data	CO_2	80–350 bar 10–55 °C	1998 1999	[92] [93]
Mentha piperita (peppermint), leaves	Composition peppermint oil (menthol, menthone, eucalyptol); content of n-alkanes/cuticular waxes	CO_2 (+ acetone) CO_2 + hexane CO_2 + dichlormethane	65–400 bar 25–70 °C	1993 1996 1999	[83] [94] [95]
Mentha pulegium (pennyroyal), blossoms and leaves	Aroma composition of pennyroyal (pulegeone, isopulegol)	CO_2	90–200 bar 40–50 °C	1998	[96]
Mentha spicata (spearmint), leaves	Spearmint oil (carvon, limonene)	CO_2 + ethanol	69–103 bar 39/49 °C	2000	[97]

Table 2 (continued)

Plant	Targets	Gas	Conditions	Year	Ref.
Origanum vulgare (wild marjoram), leaves *Pimpinella anisum* (anise), grain	Composition of marjoram oil (thymol, carvacrol); composition of anise oil (anethole)	CO_2	167 bar 55 °C	1990	[98]
Picea abies (spruce), needles *Pinus sylvestris* (pine), needles	Main constituents: spruce (pinene) and pine (cineol, camphor)	CO_2 + ethanol CO_2 + dichlormethane	300 bar 45 °C	1998	[99]
Piper nigrum (black pepper), seeds	Essential oil (caryophyllene, limonene, caren)	CO_2	150–300 bar 30–50 °C	1999	[100]
Rosmarinus officinalis (rosemary), leaves	Rosemary oil: pinene, camphor, limonene; modelling and kinetic studies	CO_2	100–345 bar 35–50 °C	1994 1997	[89] [101]
Sassafras albidum (sassafras), root bark	Safrole and related allylbenzenes (eugenol)	CO_2 + methanol	276–690 bar 50/80 °C	1994	[102]
Satureja hortensis (savory), leaves	Composition/fractionation of savory oil (carvacrol, terpinene, χ-terpinene); content of n-alkanes; study of antioxidant activity	CO_2 + acetone CO_2 + hexane CO_2 + dichlormethane	120–400 bar 40 °C–70 °C	1993 1999	[83] [103]
Syzygium aromaticum (clove), buds	Oil of cloves (eugenol, eugenylacetate, caryophyllene)	CO_2	200 bar 55 °C	1999	[104]
Tamarindus indica (tamarind), fruit pulp	Tamarind oil (aromadendrene, furfural, humulene)	CO_2	207 bar 50 °C	1994	[105]
Tanacetum parthenium (feverfew), flowers	Composition of feverfew oil (camphor, parthenolide)	CO_2	250 bar 45 °C	1994	[78]
Tanacetum vulgare (tansy), flowers	Composition of tansy oil (thujone)	CO_2	250 bar 45 °C	1994	[78]
Terpene mixture	Fractionation on silanized silica; modelling and phase equilibria data of various terpenes	CO_2	110–210 bar 37–57 °C	1998	[106]
Thymus zygis (thyme), blossoms and leaves	Aroma composition of thyme (thymol, geranyl acetate)	CO_2	90–200 bar 40–50 °C	1998	[96]
Zingiber officinalis (ginger), rhizome	Ginger oil (neral, geranial, zingiberene, bisabolene ...); kinetic studies and modelling	CO_2	0,68–0,94 g/ml 245 bar 40 °C	1994 1996	[107] [108]

for the extraction of vegetable oils from plant matrices are described as an example for *Hippochae rhamnoides* [43], *Rosa canina* [44] and *Vitis vinifera* [45]. For further details about extraction models see chapter 6.

4.1.2. Vegetable oils – preparative scale

Lipids represent a complex chemical mixture consisting of mono-, di- and triglycerides as well as free fatty acids and some minor constituents. They are obtained from different groups of organisms like vegetables, fish, fungal or algae material. The recovery of high valuable vegetable oils has gained importance in the last few years. The pharmaceutical industry is mainly interested in polyunsaturated fatty acids (PUFAs) because of their beneficial pharmaceutical effects. Nutrition enriched in ω-3-fatty acids show antihypertensive and cardioprotective as well as anti-inflammatory effects. To improve the quality of those lipids a lot of refining steps are needed and supercritical fluid extraction is an alternative production method that is able to combine several steps under mild conditions. Supercritical fluid extraction with pure or modified carbon dioxide is not only applied for the recovery of oils but also for separation and fractionation of free fatty acids, for deodorization of vegetable oils and for delipidation of food products. The literature available on this topic is summarized in Table 1.

4.2. Essential oils

Essential oils for pharmaceutical use are volatile, strong smelling mixtures of lipophilic pure or oxygenated hydrocarbons; they are recovered from plant material. The conventional preparation methods are steam distillation, solvent extraction, enfleurage, maceration or cold expression, but SFE has proven to be most efficient. In contrast to steam distillation extracts produced by solvent or supercritical fluid extraction contain not only volatile but also a lot of other lipophilic constituents like sterols, waxes or colorants; those extracts are called oleoresins. Therefore sometimes refining procedures, fractional extraction or special separation techniques are necessary. Distinct essential oils mainly from *Citrus* species contain terpenes that do not contribute to the flavour or pharmacological effect but suffer from rapid oxidation or polymerisation. These are also reasons for fractional extraction or an additional deterpenation step using supercritical carbon dioxide.

Essential oils show cleansing, preservative, flavouring and mood elevating effects in cosmetics and perfumery products. They are pharmacologically used because of gastrointestinal effects as stomachics (*Citrus aurantium*, *Humulus lupulus*), as cholagogues (*Peumus boldus*, *Curcuma longa*) or carminatives (e.g. *Pimpinella anisum*, *Foeniculum vulgare*, *Carum carvi*). For the respiratory system extracts for example from *Pinus* species, *Picea* species, *Eucalyptus globulus*, *Thymus vulgaris* or *Salvia officinalis*

Table 3: Essential oils – preparative scale

Matrix	Targets	Gas	Conditions	Year	Ref.
Allium cepa (onion), bulbs	Onion oleoresin; sulphur content; fractional extraction	CO_2	100/300 bar 45/65 °C	1998	[109]
Angelica archangelica (angelica), root	Angelica root oil (pinene, phellandrene, cymene); content of coumarins	CO_2	80–400 bar 40 °C	1991 1998	[110] [111]
Anthriscus cerefolium (chervil), herb	Chervil oil (methylchavicol, allyldimethoxybenzene)	CO_2	300 bar 40 °C	1996	[112]
Apium graveolens (celery), herb and seeds	Oleoresins (triglycerides, essential oil, water) essential oil composition	CO_2	70–450 bar 20–55 °C	1996 1994	[113] [114]
Carum carvi (caraway), seeds	Caraway oil (carvone, limonene); modelling and kinetic studies	CO_2	75–300 bar 23–75 °C	1994 1999	[115] [116]
Carum opticum (ajowan), seeds	Essential oil composition of indian spices (pinene, limonene, thymol)	CO_2	80–450 bar 35–55 °C	1994	[114]
Chamomilla recutita (camomile), flowers, ligulate flowers	Camomile oil (apigenin, bisabolol, bisabolol-oxides, chamazulene); fractional extraction; modelling and kinetic studies; scale-up	CO_2 CO_2 + ethanol CO_2 + methanol CO_2 + propylene glycol	80–260 bar 40–80 °C	1990 2000 1994 1994 1995 1995 1997 1999	[117] [118] [119] [120] [121] [122] [123] [124]
Citrus aurantifolia (lime), peel oil	Fractional desorption from silica gel; deterpenation	CO_2	75–120 bar 40 °C	1997	[125]
Citrus aurantium (bitter orange), concrete	Orange oil; fractional separation; modelling	CO_2	80–100 bar 40 °C	1999	[126]
Citrus bergamia (bergamot), peel, peel oil	Bergamot oil (limonene, linalyl acetate) bergapten content; modelling and kinetic studies; fractional desorption from silica gel; deterpenation; phase equilibria data	CO_2	78–200 bar 40–80 °C	1999 1997 2000	[127] [128] [129]
Citrus medica (citrus), oil and peel + model mixture of limonene and linalool	Fractionation by pressure swing adsorption on silica gel; deterpenation of citrus oil; modelling, phase equilibria data of CO_2-limonene-linalool	CO_2	80–250 bar 40 °C–80 °C	1998 1996 1998	[130] [131] [132]
Citrus paradisi (grapefruit), flavedo	Grapefruit oil (limonene, cymene)	CO_2	80/250 bar 40 °C	1998	[133]
Citrus reticulata (mandarin), peel oil	Fractional desorption from silica gel; deterpenation	CO_2	75–120 bar 40 °C	1997	[125]
Citrus sinensis (orange), oil, peel, peel oil	Deterpenation of citrus oil; phase equilibria data of CO_2-limonene linalool; modelling and kinetic studies fractionated extraction	CO_2 CO_2 + hexane CO_2 + ethyl ether CO_2 + acetone	10–280 bar 20–75 °C	1997 1998 1996 1998 1999 2000 1999	[134] [135] [136] [137] [138] [139] [140]
Coleus aromaticus, leaves	Essential oil (terpinene, carvacrol)	CO_2	276 bar 60 °C	1996	[141]
Coriander sativum (coriander), fruits, seeds	Coriander oil (linalol, campher); cuticular waxes (n-alkanes), lipids; fractional separation	CO_2	150/250 bar 40/50 °C	1997 1996	[142] [113]
Cuminum cyminum (cumin), seeds	Essential oil composition of indian spices (pinene, limonene, thymol); cumin oil (pinene, terpinene)	CO_2	80–450 bar 35–55 °C	1994 1999	[113] [143]
Curcuma longa (tumeric), rhizomes	Tumeric oil (atlantona, tumerone, zingiberene); modelling and kinetic studies	CO_2 CO_2 + ethanol	250–300 bar 40/45 °C	2000 2000	[144] [145]
Cymbopogon citratus (lemon grass), leaves	Lemon grass oil; fractional extraction	CO_2 + hexane CO_2 + acetone CO_2 + methanol	81 bar 75 °C	1997	[146]

Table 3 (continued)

Matrix	Targets	Gas	Conditions	Year	Ref.
Eletaria cardamomum (cardamom), seeds	Cardamom oil (cineol, terpinylacetate)	CO_2	100–600 bar 40–60 °C	1991	[147]
Eucalyptus globulus (eucalyptus), leaves	Eucalyptus oil (pinene, cineole); fractional separation	CO_2	90 bar 50 °C	1999	[148]
Eugenia caryophyllata (clove), buds	Essential oil composition: eugenol, caryophyllene, eugenylacetat fractional separation	CO_2	80–120 bar 35–50 °C	1997 1998	[149] [150]
Eureka limon (lemon), peel oil	Fractional desorption from silica gel; deterpenation; elimination of psoralens, coumarins	CO_2	75–115 bar 40 °C	1994	[151]
Foeniculum vulgare (fennel), seeds	Fractional extraction of essential oil (fenchone, anethole) **and** vegetable oil; modelling and kinetic studies	CO_2	81–300 bar 40/50 °C	1999 1999	[152] [153]
Humulus lupulus (hop), cones and leaves	Hop oil (α-, β-acids, myrcene, caryophyllene, humulene)	CO_2	125–275 bar 40/60 °C	1990	[154]
Illicum verum (star anise), fruits	Essential oil (caryophyllene, eugenol, eugenylacetate)	CO_2	90 bar 50 °C	1998	[150]
Jasminum grandiflorum (jasmine), concrete and flowers	Jasmine oil (benzyl acetate, benzyl benzoate, linalool) fractional extraction and separation; effect of polar modifiers on extraction; modelling	CO_2 CO_2 + methanol CO_2 + acetone CO_2 + dimethylsulfoxide	80–200 bar 40 °C	1995 1992	[155] [156]
Lavandula angustifolia (lavender), flowers	Lavender oil (cineole, linalole, linalyl acetate); fractional separation; modelling	CO_2	90 bar 48 °C	1995	[157]
Lavandula intermedia (lavender), flowers	Lavender oil (linalol, linalylacetate); fractional extraction and separation	CO_2	80/300 bar 40 °C	1996	[158]
Lavandula officinalis (lavender) flowers	Essential oil (composition); fractional separation	CO_2	80–120 bar 35–50 °C	1997	[149]
Lavandula stoechas (lavender) flowers	Lavender oil (fenchone, campher)	CO_2	70–110 bar 30–50 °C	1994	[159]
Lippia alba, leaves	Essential oil (limonene, carvone)	CO_2	276 bar 60 °C	1997	[160]
Mentha pulegium (pennyroyal), flowers, leaves	Pennyroyal oil; modelling and kinetic studies; fractional separation	CO_2	100 bar 50 °C	2000 1999	[161] [162]
Mentha spicata (spearmint), leaves	Spearmint oil (piperitenone oxide, cineole)	CO_2	276 bar 60 °C	1999	[163]
Mentha x piperita (peppermint), leaves, stems	Peppermint oil (menthol, menthone, carvon, limonen); fractional separation	CO_2	60–180 bar 24–50 °C	1992 1994	[164] [165]
Milk fat	Flavouring compounds (lactones); modelling	CO_2	550–900 kg/m^3 45–75 °C	1990	[166]
Myristica fragrans (nutmeg), seeds	Simultaneous extraction of essential and vegetable oil	CO_2	90 bar 23 °C	1999	[61]
Ocimum basilicum (basil), leaves	Basil oil (linalool, methyleugenol); fractional extraction and separation modelling and kinetic studies; scale-up	CO_2	80–120 bar 35–50 °C	1994 1997	[167] [149]
Ocimum gratissimum (basil), leaves	Basil oil (thymol, carvacrol)	CO_2	276 bar 60 °C	1998	[168]
Origanum majorana (majoram), leaves	Majoram oil (cis-sabinene hydrat, cis-sabinene hydrat acetate); fractional separation; kinetic studies	CO_2	100 bar 50 °C	1992	[169]
Peumus boldus (boldo), leaves	Boldo oil; fractional extraction	CO_2 + hexane CO_2 + acetone CO_2 + methanol	75 °C 80 bar	1997	[170]
Pimenta dioica (pimento), leaves, berries	Pimento oil (cineol, eugenol, myrcene, caryophyllene)	CO_2	276 bar 60 °C	1997 1997	[171] [172]
Piper nigrum (black pepper), seeds	Oleoresins (piperine); modelling and kinetic studies	CO_2	280 bar 24–60 °C	1995	[173]

Table 3 (continued)

Matrix	Targets	Gas	Conditions	Year	Ref.
Rosa damascena (rose), concrete	Rose oil (phenylethanol, citronellol, phenylethyl acetate); fractional separation	CO_2	80–160 bar 40/45 °C	1996 1997	[174] [175]
Rosmarinus officinalis (rosemary), leaves	Rosemary oil; modelling	CO_2	100–160 bar 37/47 °C	1997	[176]
Salvia officinalis (sage), herb, leaves	Composition of oleoresins (triglycerides, sage essential oil, water); modelling and kinetic studies fractional separation	CO_2	90–250 bar 18–50 °C	1996 1995 1996	[113] [177] [178]
Salvia sclarea (clary sage), herb	Clary sage oil (sclareol)	CO_2	100 bar 40 °C	1999	[179]
Salvia triloba (three-lobed sage), leaves	Three-lobed sage oil (cineole, camphor)	CO_2	80 bar 40 °C	1999	[180]
Syzygium aromaticum (clove), bud	Clove bud oil (caryophyllene, eugenol, eugenyl-acetate); modelling and kinetic studies	CO_2	90–241 bar 35/50 °C	1995 1997	[181] [182]
Tagetes minuta	Essential oil (limonene, cis-ocimeme, dihydrotagetone, cis-ocimonene, trans anethol); fractional separation	CO_2	80 bar 40 ° C	1999	[183]
Tanacetum parthenium (feverfew), flowers	Feverfew oil (camphor, chrysanthenyl acetate, parthenolide)	CO_2	100–400 bar 40–60 °C	1999	[184]
Tangor murcote x *Citrus sinensis* (hybrid), peels	Essential oil; fractional extraction	CO_2 + hexane CO_2 + ethyl ether CO_2 + acetone	91 bar 60/75 °C static + dynamic	1998	[185]
Teucrium polium (germander), leaves, flowers	Germander oil (pinene, caryophyllene)	CO_2	100 bar 40 °C	1999	[186]
Thymus vulgaris (thyme), herb and leaves	Thyme oil (thymol, carvacrol); fractional extraction and separation; modelling	CO_2	80–400 bar 40 °C	1996 2000 2000	[158] [187] [188]
Vanilla planifolia (vanilla), beans	Vanilla oleoresin (vanillin, hydroxybenzaldehyde)	CO_2	100–130 bar 33–36 °C	1991	[189]
Zingiber officinalis (ginger), rhizome	Ginger oil (gingerol), oleoresins; selective extraction of pungent compounds or simultaneous extraction of aromatic compounds; fractional separation; kinetic studies	CO_2 CO_2 + ethanol	60–295 bar 0–80 °C	1995 1997 1998 1990 1997	[190] [191] [192] [193] [149]

are available in pharmaceutical formulations. Furthermore *Chamomilla recutita* is used as a spasmolytic and anti-inflammatory drug and eugenol gained from *Syzygium aromaticum* is applied as a preservative and antiseptic drug. A comprehensive overview of literature dealing with SFE of essential oils in analytical and preparative scale is given in Table 2 and Table 3, respectively.

4.3. Other pharmacologically active compounds

In Tables 4 and 5 plants or substances are summarized with a pharmacological principle that is neither due to their vegetable nor to their essential oil content. Thus a lot of different chemical structures with different physico-chemical properties are intended to be isolated. Whereas vegetable and essential oils are often lipophilic enough and therefore extractable with pure carbon dioxide, for those more polar components mainly in analytical scale a lot of different modifiers are added to the supercritical fluid. In preparative scale almost only pure or ethanol-modified carbon dioxide is used because of the non-toxic properties of these mixtures. Beside from active pharmacological extracts there are extracts, which serve as natural colourants, antioxidants or preservatives. Literature about SFE of these constituents is summarized in Table 4 for analytical scale and in Table 5 for preparative scale.

5. Supercritical fluid extraction from pharmaceutical preparations

Supercritical fluid extraction is emerging as a rapid, efficient and selective sample preparation method for routine analysis of active ingredients or excipients in pharmaceutical or cosmetic preparations. The extraction method offers the opportunity for on-line coupling with a chromatographic analysis-system like HPLC or GC/MS; a combination with supercritical fluid chromatography (SFC) is possible as well.

The content of preservatives, especially parabens, in cosmetics is interesting because of their potency to induce allergic contact dermatitis. The extraction from commercial creams, lotions or milks has been carried out using pure [291] or acetonitrile-modified [292] carbon dioxide. Anklam and Müller extracted vanillin as a flavouring compound from lozenges [293]. Furthermore SFE has been applied as a sample preparation method for the determination of active ingredients in solid formulations, e.g. for caffeine in analgesic tablets [293], megestrol acetate in

Table 4: Other pharmacologically active compounds – analytical scale

Plant/matrix	Target	Gas	Condition	Year	Ref.
Ancistrocladus korupensis, leaves	Anti-HIV-alkaloids (michellamine A and B)	CO_2 + methanol	455 bar 32–80 °C	1997	[194]
Apium graveolens (wild celery)	Phototoxic furanocoumarins (psoralen); effect of water content	CO_2	120–250 bar 35–80 °C	1997	[195]
Archangelica officinalis (garden angelica), fruits	Furanocoumarins (xanthotoxin, bergapten, imperatorin); fractional extraction	CO_2	80–500 bar 40–100 °C	1996 1996	[196] [73]
Artemisia annua, herb	Antimalaric sesquiterpenlactones (artemisinin and artemisinic acid)	CO_2 + methanol CO_2 + ethanol CO_2 + water CO_2 + toluol	150 bar 50 °C	1997 1997	[197] [198]
Bixa orellana (annatto), seeds pure bixin	Carotenoid pigments (bixin); solubility of bixin; natural food colourant (trans-bixin)	CO_2 + soybean oil CO_2 + ethanol CO_2 + chloroform CO_2 + acetonitril CO_2 + methanol	207–606 bar 40–80 °C	1991 1997	[199] [200]
Brosimum gaudichaudii, bark roots	Furanocoumarins (bergapten, psoralen); triterpenes (α-, β-amyrin)	CO_2	80 bar 60 °C	1993	[201]
Capsicum annuum (paprika)	Capsaicin and dihydrocapsaicin; natural food colourants (β-carotene); fractional extraction	CO_2 CO_2 + acetone, CO_2 + ethanol	140–600 bar 40/50 °C	1994 1999	[202] [203]
Cedrela toona (cedar), wood	Tetracyclic triterpenoids (cedrelone, a limonoid); modelling and kinetic studies	CO_2 + methanol	300/350 bar 40 °C	1996	[204]
Chlorella vulgaris, alga	Carotenoids, lipids	CO_2	200/350 bar 40/55 °C	1995	[205]
Coffea arabica (coffee), beans, soaked with water/unsoaked, brew of roasted coffee beans	Decaffeination rates; modelling of the extraction process; coffee aroma compounds	CO_2 + water CO_2	47–250 bar 40–80 °C	1992 1998	[206] [207]
Colchicum autumnale (meadow saffron), seeds	Colchicine	CO_2 + ether CO_2 + acetonitril CO_2 + ethanol CO_2 + acetone CO_2 + methanol	200–400 bar 40 °C	1999	[208]
Corn, spiked	Recovery of aflatoxins	CO_2	517 bar 65 °C	1996	[209]
Crotalaria spectabilis, seeds	Fractionation of pyrrolizidine alkaloids (monocrotalines) and lipids; solubility data in the cross-over region	CO_2 + ethanol	100–275 bar 35–55 °C	1988	[210]
Curcuma longa (tumeric), rhizomes	Extractability of curcumin; spectrophotometric measurements	CO_2 + ethanol	180–240 bar 40 °C	2000	[211]
Daucus carota (carrots); carrots and press cake	Antioxidant vitamins (α-, β-carotene); kinetic studies and diffusion models	CO_2 + ethanol CO_2 + methanol CO_2 + chloroform N_2O	303–606 bar 30–70 °C	1995 1997 1998 1996	[212] [213] [214] [215]
Digitalis lanata (foxglove), leaves	Cardiac glycosides (digoxin, acetyldigoxin, digitoxin, gitoxin)	CO_2 + methanol HFKW134a + methanol Fluoroform + methanol	380/404 bar 40–100 °C	1996 1997	[216] [217]
Dilophus ligulatus, algae material	Colour of extracts; antifungal compounds fractional extraction	CO_2	80–280 bar 35–55 °C	1991 1991	[218] [219]
Dioscorea nipponica, tuber	Diosgenin	CO_2	132–217 bar 33–60 °C	1995	[220]
Dorstenia bryoniifolia, rhizomes	Furanocoumarins (bergapten, psoralen); triterpenes (α-, β-amyrin)	CO_2	80 bar 60 °C	1993	[201]
Erythroxylum coca (coca), leaves	Cocaine; kinetic studies	CO_2 + methanol + water	150–250 bar 40–100 °C	2000	[221]
Eucalyptus globulus (eucalyptus), wood	Lipids (sterols, squalene, fatty acids)	CO_2 + methanol	100–250 bar 40–75 °C	2000	[222]

Table 4 (continued)

Plant/matrix	Target	Gas	Condition	Year	Ref.
Ginkgo biloba (maidenhair tree), extracts from leaves and fruits; standardized extract solutions and phytopharmaceuticals	Fractional extraction of fatty acids and ginkgolic acids; ginkgolides and bilobalide	CO_2 CO_2 + methanol CO_2 + ethyl acetate	110–339 bar 45/55 °C	1993 1996	[223] [224]
Glycine maximus (soy) products: miso, tofu, soy flour, soy meal	Anti-carcinogenic isoflavones (daizein, genistein)	CO_2 + methanol, CO_2 + chloroform	405–608 bar 50 °C	1996	[225]
Ilex paraguarensis (mate), leaves	Purine alkaloids (caffeine, theophylline, theobromine); solubility studies	CO_2	255 bar 70 °C	1999	[226]
Ipomoea batatas (sweet potatoes), root	Carotenoids (β-carotene)	CO_2	138–414 bar 38–48 °C	1993	[227]
Maclura pomifera (osage orange), root bark	Flavanones and xanthones (the latter with cytotoxic, antitumor activity)	CO_2 + methanol	405 bar 40–100 °C	1999	[228]
Magnolia officinalis (magnolia), bark	Neolignans (magnolol, honokiol) for the treatment of abdominal distension	CO_2 + methanol	245 bar 40/60 °C	1998	[229]
Magnolia virginiana (magnolia), flowers	Neolignans (methoxyhonokiol, magnolol, biphenyl ether)	CO_2 + methanol, CO_2 + chloroform	405 bar 40 °C	1995	[230]
Nicotiana tabacum (tabacco), leaves	Nicotine; dynamic modelling and kinetic studies	CO_2 + methanol	200/300 bar 50 °C	1996	[231]
Olea europaea (olive), leaves	Natural antioxidants (phenolic compounds)	CO_2 + methanol	155–334 bar 80–120 °C	1998	[232]
Oryza sativum (rice), bran	Extraction of oryzanol	CO_2	689 bar 30–75 °C	2000	[233]
Paeonia suffruticosa, cortex	Deoxyschisandrin, paeonol; kinetic studies	CO_2 + methanol	250 bar 40/60 °C	2000	[242]
Passiflora edulis (passion fruit), leaves	Glycosylated flavonoids	CO_2 + methanol CO_2 + ethanol CO_2 + ethyl acetate CO_2 + chloroform	101 bar 70 °C	1997	[234]
Paulinia cupana (guarana), seeds (45% water)	Caffeine; modelling of caffeine solubility and extraction rate	CO_2	136–272 bar 35–55 °C	1996	[235]
Piper methysticum (kava), root	Kava lactones (kavain, methysticin); fractional extraction	CO_2 + ethanol	250–450 bar 60 °C	1997 1999	[236] [237]
Rosmarinus officinalis (rosemary), extracts and leaves	Deodorizing of antioxidant rosemary extracts (carnosic acid, carnosol)	CO_2 CO_2 + ethanol CO_2 + water CO_2 + acetic acid CO_2 + extraction solvent	100–383 bar 40–120 °C	1998 1997	[238] [239]
Salvia miltiorrhiza bunge (tan-shen), root	Tanshinone IIA	CO_2 + methanol	100–250 bar 60 °C	1998	[240]
Schisandra chinensis, fruits, leaves, stems, seeds	Lignans (schisandrols, schisandrins) that improve liver function; kinetic studies; effect of plant matrix	CO_2 CO_2 + methanol CO_2 + ethanol	136–400 bar 40–80 °C	1998 2000 1999 1997	[241] [242] [243] [244]
Scopolia japonica, herb and roots Scopolamine and hyoscyamine pure substances	Tropane alkaloids (hyoscyamine, scopolamine); extraction of alkaloid-salts using basified modifiers	CO_2 + diethylamine/ methanol or diethylamine/water	102–340 bar 60 °C	1999	[245]
Scutellaria baicalensis, roots, soaked with ethanol	Flavonoids (baicalein, baicalin, wogonin) used in Chinese medicine	CO_2 + ethanol CO_2 + methanol CO_2 + water	197–400 bar 40–70 °C	1996 1999	[246] [247]
Senecio inaequidens *Senecio cordatus* (groundsel)	Pyrrolizidine alkaloids (senecionine, seneciphylline)	CO_2 + methanol	100/150 bar 50–60 °C	1991	[248]
Solanum lycopersicum (tomatoes)	Isolation of natural dyes (lycopene and β-carotene)	CO_2 + hexane CO_2 + chloroform	172–275 bar 40–80 °C	2000	[249]
Tamarindus indica (tamarind), seed coat	Natural antioxidants	CO_2 + ethanol	100–300 bar 40–80 °C	1995	[250]

Table 4 (continued)

Plant/matrix	Target	Gas	Condition	Year	Ref.
Taxus baccata *Taxus brevifolia* (yew), needles, bark	Anti-cancer taxanes (taxol, taxicin); kinetic studies	CO_2 + methanol CO_2 + ethanol	183–400 bar 45/50 °C	1993 1992	[251] [252]
Taxus cuspidata (yew), needles	Paclitaxel and baccatin III with antineoplastic activity	CO_2 + methanol CO_2 + ethyl acetate CO_2 + dichlomethane CO_2 + ethyl ether	100–300 bar 35–70 °C	1996	[253]
Theobroma cacao (cacao), beans	Pyrazines; influence of pod storage period	CO_2 + methanol CO_2 + dichlormethane	60–200 bar 60 °C	1997	[254]
Triticum aestivum (wheat), germ	Oil enriched in tocopherols	CO_2	250 bar 40 °C	1989	[255]
Uncaria tormentosa, root	Oxindole alkaloids; fractional extraction	CO_2 + methanol	250 bar 60 °C	1997	[256]
Vitis vinifera (white grape), seeds	Phenolic compounds (catechins: gallic acid, catechin, epicatechin), lipids, fatty acids, sterols fractional extraction; solubility data	CO_2 + ethanol CO_2 + methanol	140–450 bar 35–60 °C	1999 1999 2000 2000	[257] [258] [259] [260]

cancer-treatment-tablets [294], benzodiazepines in tablets or capsules [295], tocopherol in tablets or powders [296, 297] and vitamin K_1 in powders [298]. Several authors made a comparison to model matrices for pseudoephedrine from tablets and from a spiked-sand matrix [299], for felodipine from tablets and from a spiked-cotton matrix [300] and for carotene and tocopherol from tablets and a spiked-cellulose matrix [301]. An example for the extraction of polar drugs (sulfamethoxazole and trimethoprim) from a commercial infusion, that represents an aqueous and therefore strongly polar matrix, is given by Mulcahey and Taylor [302].

6. Modelling of the extraction processes

Mathematical models are applied to give a quantitative description of the kinetics of an extraction process. They are generally based on equations describing mass transport phenomena and include specified parameters which can be fitted to extraction data, e.g. yield or content within a specified period of time. In general the extracted material is regarded as one pseudocomponent. A few simplified ideas and approaches on extraction models are discussed below.

According to Brunner [4, 303] the extraction process can be divided into three steps:

- Transportation of substances within the solid material onto the surface (solid phase),
- Transition of substances from the solid into the fluid (interface solid-fluid),
- Transportation of substances with the bulk of extraction gas (fluid phase).

Simplified models do not consider all the three steps but combine some of them to one single step. Assuming a steady state process without any flow of the fluid phase the amount of extract transported per unit of time is proportional to the mass transfer area and to a specified concentration gradient, as required for any mass transport, e.g. laws of Fick or law of Noyes-Whitney. The proportionality constant is given by a mass transfer coefficient for either the mass transport within the solid phase to the interface solid-fluid or the transport from there to the bulk fluid. The reciprocal values of these mass transfer coefficients can be summarized to a reciprocal total mass transfer coefficient. Since mass transfer resistance in the solid is dominant the total mass transfer coefficient is approximately represented by the mass transfer coefficient in the solid phase.

Supercritical extraction processes are usually applied in the dynamic mode and therefore more complex models have to be applied which are also based on the above mentioned steps of mass transfer during the extraction process. They are initially derived from equations describing heat transfer phenomena. One model is the one-dimensional dispersion-single particle model [4, 304]. It is based on a mass balance considering solid and fluid phase. For the latter one plug flow is assumed, represented by a convection-term as well as an one-dimensional axial mass transport by diffusion, usually called axial dispersion. This term is derived from Fick's second law with an axial dispersion coefficient as proportionality constant. The solid phase is considered as a single, spherical and isotropic particle. Then a modified Fick's second law can be used to express the concentration profile within a solid particle. The proportionality factor is determined by the diffusion coefficient of extracted material in the solid material. At the solid-fluid interface both the model for the fluid phase and for the solid phase has to be coupled. If the solvent does not change its phase at the interface (homogeneous extraction) the concentration of extracted substances at the interface must be equal. If the solvent changes its phase (heterogeneous extraction) a partition coefficient has to be taken into account. This is usually given by the thermodynamic equilibrium partition coefficient, which is the quotient of concentration in the fluid and in the solid at the interface solid-fluid. The above-mentioned approaches result in more or less complex equations from which boundary conditions can be derived. Similar to the laws of heat transfer they can be simplified by combining specified parameters to dimensionless groups. They are named Fourier-, Biot-, Bodenstein- and Peclet-number [4]. An approach of solving the differential equations is made by Goodarznia and Eikani [304]. To get an optimized modelling system some more effects occurring during the extraction process have to be considered, e.g. radial distribution of flow velocity, radial and axial concentration profiles in the solid bed, distribution of particle size, etc. [4]. A rough survey of literature dealing with special approaches on modelling should be given below.

Table 5: Other pharmacologically active compounds – preparative scale

Matrix	Targets	Gas	Conditions	Year	Ref.
Achillea millefolium (yarrow), herb	Cholagoga substances; kinetic studies	CO_2	100–400 bar 25–60 °C	1999	[261]
Allium cepa (onion), bulbs	Onion flavours	CO_2	207 bar 37 °C	1995	[262]
Aluminium balls, spiked with carotene	Recovery and degradation of carotene; solubility data	CO_2	300 bar 40–60 °C	2000	[263]
Azadirachta indica (neem), seeds	Neem seed oil (azadirachtin, nimbin, salannin)	CO_2 + methanol	69–344 bar 55 °C	1997	[264]
Bixa orellana (annatto), seeds	Natural food colourants (bixin, norbixin)	CO_2	207–345 bar 50/60 °C	1991	[265]
Capsicum annuum (paprika)	Natural food colourants (carotenes, capsanthin) flavour compounds (capsaicin); frational extraction; enrichment of antioxidative vitamines (carotenoid, tocopherol); kinetic studies and phase equilibria data of CO_2-capsaicin-carotene	CO_2 Propane	90–400 bar 20–60 °C 30–50 bar 25 °C	1998 1999 1999	[266] [267] [268]
Chrysanthemum cinerariaefolium (pyrethrum), flowers	Insecticides (pyrethrin I/II);	CO_2	83–284 bar 40 °C	1995	[269]
Citrus reticulata (mandarin), peels	Enrichment of carotenes; kinetic studies	CO_2 Propane	100–400 bar 35–55 °C 30–50 bar 25 °C	1999	[267]
Citrus sinensis (orange), peels	Enrichment of carotenes; kinetic studies	CO_2 Propane	100–400 bar 35–55 °C 30–50 bar 25 °C	1999	[267]
Cod liver oil/carotene-mixture	Enrichment of carotene; solubility data	CO_2	200–300 bar 40–60 °C	2000	[270]
Elaeis guineensis (palm), palm oil pressing residue and palm tree leaves	Antioxidant vitamins (carotene, tocopherol); free fatty acid content; phase equilibria data of CO_2-tocopherol	CO_2	300–500 bar 70 °C	1995	[271]
Ephedra sinica (ephedra), herb	Cuticular waxes, (nonacosan-10-ol, α-amyrin acetate, squalene, stigmasterol); kinetic studies and scale up	CO_2	100–350 bar 35–50 °C	1996 1997	[272] [273]
Glycine maximus (soy), oil deodorizer distillate, seeds	Tocopherols, fatty acids, sterols, squalene; kinetic studies	CO_2	241–700 bar 40–90 °C	2000 1996	[274] [275]
Hordeum vulgare (barley), fruits	Antioxidant vitamines (tocopherols, tocotrienols); fractional extraction	CO_2 Propane	79–127 bar 40 °C	1998	[276]
Ilex paraguariensis (maté), leaves	Methylxanthines (caffeine, theophylline, theobromine); fractional extraction; kinetic studies	CO_2	138/255 bar 40/70 °C	2000	[277]
Magnolia grandiflora (magnolia), leaves	Sesquiterpene lactones (parthenolide, costunolide); sesquiterpene (cyclocolorenone)	CO_2 Propane	75 bar 40/50 °C 45 bar 40/50 °C	1995	[278]
Mauritia flexuosa (buriti), fruit pulp	Enrichment of carotene; modelling and kinetic studies	CO_2	200/300 bar 40/55 °C	1999	[279]
Medicago sativa (alfalfa), leaf protein concentrate	Natural colourants (carotene, lutein, pheophytin a/b); kinetic studies	CO_2	100–700 bar 40 °C	1988	[280]
Morus alba (mulberry), root bark	Higher molecular compounds (nonacosan-10-ol, α-amyrin acetate, squalene, stigmasterol)	CO_2	100–300 bar 35–60 °C	1997	[273]
Nicotiana tabacum (tobacco), leaf bits, powder, aqueous nicotine solutions	Nicotine; modelling and kinetic studies	CO_2	150–300 bar 50–70 °C	1998	[281]
Oil deodorizer distillate	Enrichment of tocopherols; phase equilibria data	CO_2 + ethanol	130–250 bar 50–90 °C	1991	[282]

Table 5 (continued)

Matrix	Targets	Gas	Conditions	Year	Ref.
Olea europaea (olive), olive oil deodorizer distillate, pomace	Enrichment of squalene; phase equilibria data; antioxidative vitamines (tocopherols); fractional separation	CO_2 CO_2 + ethanol	100–350 bar 40–60 °C	2000 2000	[283] [284]
Oryza sativum (rice), bran	Enrichment of tocopherol; kinetic studies	CO_2	250–700 bar 40/80 °C	1996	[275]
Rosmarinus officinalis (rosemary), leaves	Natural antioxidants (rosmanol, carnosolic acid, carnosol); removal of essential oil	CO_2 CO_2 + ethanol	300–500 bar 40–60 °C	1995 2000	[285] [286]
Salvia officinalis (sage), leaves	Natural antioxidants (carnosolic acid, carnosol); removal of essential oil	CO_2	500 bar 60 °C	1995	[285]
shark liver oil	Enrichment of squalene and diacylglycerylesters; fractional extraction; modelling	CO_2 + ethanol CO_2	250 bar 60 °C	2000 2000	[270] [283]
Silybum marianum (St. Mary's thistle), herb	Cholagoga substances; kinetic studies	CO_2	100–400 bar 25–60 °C	1999	[261]
Solanum lycopersicum (tomato), paste	Natural food colorants (carotene, lycopene); fractional separation	CO_2 + ethanol	200–300 bar 35–65 °C	2000	[287]
Spirodela polyrhiza (greater duckweed), entire plant	Higher molecular compounds (nonacosan-10-ol, α-amyrin acetate, squalene, stigmasterol)	CO_2	100–300 bar 35–60 °C	1997	[273]
Stevia rebaudiana (stevia), leaves	Diterpenic glycosides (rebaudiosides, stevioside); frational extraction; modelling and kinetic studies	CO_2 + ethanol CO_2 + water CO_2 + ethanol + water	120/200 bar 16–45 °C	2000	[288]
Taraxacum officinalis (dandelion), roots	Cholagoga substances; kinetic studies	CO_2	100–400 bar 25–60 °C	1999	[261]
Theobroma cacao (cocoa), beans, butter, nibs	Xanthines (theobromine, caffeine); phase equilibria data of CO_2-(ethanol)-theobromine CO_2-(ethanol)-caffeine CO_2-(ethanol)-cocoa butter	CO_2 + ethanol	80–300 bar 40–95 °C	1992 1996	[289] [290]
Triticum aestivum (wheat), germ	Enrichment of tocopherol; kinetic studies	CO_2	250–700 bar 40/80 °C	1996	[275]
Valeriana officinalis (valerian), root	Cholagoga substances; kinetic studies	CO_2	100–400 bar 25–60 °C	1999	[261]

A more simplified approach to extractions from packed beds is made by neglecting any axial dispersion coefficient and either internal diffusion within the solid material or mass transfer resistance at the interface [305]. Sovova describes a model for the extraction of vegetable oil that combines easily accessible solute of broken cells at the beginning of the extraction with enclosed solute in intact cells [306]; solutions of the differential equations are also proposed. Reverchon and Marrone use a similar approach for the extraction of various oil seeds [307]. It takes into account axial dispersion and distinguishes between free and tied solute. The proportion of broken cells in the solid phase is considered as well. Starting from experimental results on the extraction of alkaloids from *Crotalaria spectabilis* by ethanol-modified carbon dioxide [210] a mathematical model is derived [308]. It combines thermodynamic models of ethanol-carbon dioxide mixtures with simplified mass transfer models of the solute, without considering a single particle. The same mass transfer model is applied for the extraction of vegetable oils [309, 310]. Isotropic monosized spheres as well as concentration of extracted material in the fluid approximately to zero is assumed by Bartle et al. [311, 312]. The concentration profile within a solid particle is integrated over the whole particle volume. They also consider solubility effects in this dynamic extraction model [312]. A single-particle model according to Fick's second law for the extraction of essential oils is extended to a whole bed extraction model [313]. It is assumed that each particle has the same volume and consequently the mass transfer of a packed bed can be summarized from all single particles. Some models dealing with more than one component (multicomponent models) during the extraction process are described in [314] with a special regard on the shrinking-core model. It is applied to the extraction of vegetable as well as essential oils and takes into consideration that the diffusion length within a single particle increases during the extraction process. Goto et al. [315] give a similar approach for multicomponent mixtures. Some authors have applied completely different approaches for modelling the extraction process like a desorption model from the surface of an inert matrix described by Pawliszyn [316].

7. Phase equilibria data

7.1. Determination of phase equilibria data

Phase equilibria data are an important tool for design and viability of supercritical fluid extraction with or without application of modifiers. A theoretical and practical survey of phase equilibria measurement is given by Brunner [4].

There are three methods available to determine high-pressure phase equilibria data, which are briefly discussed below.

7.1.1. Static-analytical method

Solid or liquid solutes are placed in a thermostated high-pressure equilibrium cell. The supercritical solvent (pure or modified gas) is pumped into the equilibrium cell until the desired pressure is reached. In most cases the adjustment of equilibrium state is accelerated by stirring or recirculating of either supercritical phase or both liquid and supercritical phase. Dissolving of solute in the supercritical solvent causes a pressure drop, therefore equilibrium state can be assumed if no pressure decrease can be determined anymore (usually varies between one and some hours). When equilibrium is reached agitation is stopped and the phases separate due to their difference in density. Samples are taken from each phase by expanding a small amount of each phase through a thin capillary into a flask. The amount of solvent (gas) is measured volumetrically; the mass of solute can be determined either gravimetrically in case of one component or by a special analytical method (HPLC, GC) in case of multicomponent solutes. The equilibrium cells have a relatively high volume (about 1000 cm^3) and thus high amounts of solute and solvent are required as well. Since samples of the equilibrium phases are analysed separately, phase equilibria measurement of multicomponent systems favours the static-analytical method. Errors occur especially at sample drawing, e.g. pressure drop and residues in sample lines [4].

7.1.2. Synthetic method

A synthetic type apparatus consists of a thermostated high-pressure equilibrium cell with at least two windows for viewing into the interior part of the cell. The volume of the cell can be varied, usually by means of a movable piston inside the equilibrium cell. The content of the cell is stirred magnetically. Since the composition of the phases are not analysed, well-known amounts (determined by weighing the cell) of solid or liquid solute as well as supercritical solvent are brought into the equilibrium cell. When the desired temperature is reached, the pressure inside the equilibrium cell is changed by removing the piston while keeping the temperature constant. Usually the pressure is increased until only one single phase can be observed. Then the pressure is continuously decreased. Depending on the starting composition of the system additional phases will appear at certain pressures. The syn-

Table 6: Phase equilibira data of vegetable oil compounds

Substances	Conditions	Gas/method	Year	Ref.
Behenic acid	80–160 bar 35–45 °C	CO_2 + methanol static-analytical	1999	[318]
Caprylic acid (ester with glycerol)	50–322 bar 40–120 °C	CO_2 static-analytical/synthetic	1997	[319]
Fatty acids	20–120 bar 40–80 °C	CO_2 Ethane static-analytical	1991	[320]
Fatty acids; fatty acid methyl esters	38–288 bar 40/60 °C	CO_2 static-analytical	1990	[321]
Fish oil ethyl esters	90–250 bar 40–80 °C	CO_2 static-analytical	1999	[322]
Glycerides	50–210 bar 25–75 °C	CO_2 synthetic	1993	[323]
Laurylic acid (esters with glycerol)	150–400 bar 35–60 °C	CO_2 dynamic	1993	[324]
Milk fat	100–310 bar 40/60 °C	CO_2 static-analytical	1992	[325]
Oleic acid	96–200 bar 35/45 °C	CO_2 dynamic	1991	[326]
Oleic acid/methyl oleate-mixture	45–262 bar 40/60 °C	CO_2 static-analytical	1993	[327]
Olive oil	138–302 bar 40–80 °C	CO_2 static-analytical	1996	[328]
Palm oil	50–106 bar 50–80 °C	CO_2 synthetic	1987	[329]
Squalene; free fatty acids and -esters	230 bar 57–97 °C	CO_2 static-analytical	1999	[330]
Stearic acid	90–165 bar 45 °C	CO_2 + acetic acid CO_2 + methyl acetate static-analytical	1997	[331]
Triglycerides	150–350 bar 40 °C	CO_2 dynamic	1992	[332]
Triglycerides; fatty acids	80–300 bar 40 °C	CO_2 dynamic	1998	[333]

Table 7: Phase equilibria data of essential oil compounds

Substances	Conditions	Gas/Method	Year	Ref.
Camphor; fenchone; limonene; pinene	60–126 bar 40–60 °C	CO_2 static-analytical	1999	[334]
Citral; limonene	70–100 bar 37–50 °C	CO_2 synthetic	1995	[335]
Citral; limonene	30–110 bar 35–50 °C	CO_2 dynamic	1989	[336]
Lemon oil; limonene/ citral-mixture	50–106 bar 50–80 °C	CO_2 synthetic	1987	[329]
Limonene; linalool	69–111 bar 45/55 °C	CO_2 dynamic	2000	[337]
Orange oil	71–137 bar 50–70 °C	CO_2 synthetic	2000	[338]

thetic method uses small equilibrium cells and thus small sample amounts. It is suitable for measuring phase equilibria of binary systems or the determination of boundary lines in multicomponent systems. Sources of error are the determination of total composition at the beginning and the measurement of the equilibrium pressure [4].

7.1.3. Dynamic method

The solute, usually mixed up with an inert matrix, is placed in a thermostated equilibrium cell. Unloaded pure or modified solvent is set to the desired temperature and pressure and passes continuously through the equilibrium cell. During the short residence time of the supercritical solvent in the cell, equilibrium concentration is assumed to be adjusted. Saturated gas leaves the equilibrium cell and is depressurised to atmospheric pressure. Measuring of gas volume and analysing of solute can be done analogically to the static analytical method. Because of accumulation of the solute, the dynamic method can be used even if solubility in the supercritical phase is very low. The greatest problem is to establish equilibrium during the short residence time of the supercritical solvent. The method is used particularly for one-component solutes. For multicomponent solutes changes in the solute due to different equilibrium solubilities as well as in the supercritical solvent have to be taken into account [4].

7.2. Phase equilibria data in the literature

For a lot of substances and multicomponent mixtures that are relevant in SFE, phase equilibria data are published over a wide pressure range. For more polar substances phase equilibria data including modifiers are also available. Depending on the system and target of the measurement either the static-analytical, synthetic or dynamic method has been applied. Literature dealing with phase equilibria data of vegetable oil, essential oil and other pharmacologically active compounds are compiled in Table 6, Table 7 and Table 8, respectively.

8. Other applications of supercritical fluids

8.1. Supercritical fluid chromatography

Supercritical fluid chromatography (SFC) is a separation method, which has been derived from HPLC. It can be utilized in analytical and in preparative scale as well. The mobile phase consists of a supercritical or a near-critical fluid. Pure gases can be used, but mostly they are combined with more or less polar modifiers. Stationary phases are mainly adopted from well-established sorbents used in HPLC. Detailed reviews about both, carbon dioxide based mobile phase, including also phase equilibria data [19], and stationary phases [359] are available. Brunner gives a detailed overview of SFC as separation method [4]. Starting from general aspects of SFC he describes technical requirements and theoretical approaches for scale-up. An actual review about applications of SFC has been published by Chester and Pinkston [360].

8.2. Particle design

In the last few years particle design by means of supercritical fluids has gained more and more importance. Depending on the method and conditions micron or submicron particles can be obtained, as well as particles with alternative morphological or solid state forms. The supercritical fluid, which is required either as solvent or as antisolvent, consists of a pure or modified gas, which is mostly carbon dioxide. General reviews on particle formation by supercritical fluids are available [361–363]. Particle formation processes can be divided into three main groups [362], which should be briefly described.

8.2.1. Precipitation from supercritical solutions

Solid material is dissolved in a supercritical fluid under high pressure. This solution is rapidly expanded, thus the density decreases suddenly. With declining density the solvent capacity is reduced by orders of magnitude so that micron or submicron particles are formed by homogenous nucleation. If the solution is rapidly expanded to atmospheric pressure, the supercritical solvent suddenly evaporates and strong aerosolation effects occur in addition. This procedure is called rapid expansion from supercritical solution (RESS). The yields are rather small, due to the very low solubility of organic or biological material in supercritical fluids, particularly carbon dioxide. Special surveys of the RESS process are available [364, 365]. Alessi et al. give a review about particle production of steroid drugs by the RESS process [366].

8.2.2. Precipitation with supercritical fluids as antisolvent

The low solubility of organics in supercritical solution is overcome by dissolving the organic material in conventional liquid solvents. Starting from these solutions particles can be formed in two different ways. The first one is called gas antisolvent (GAS); solution and supercritical fluid are brought together in a vessel and mixed up. Due to a relatively high solubility of supercritical fluids in organic solvents, the volume of the solution is expanded, thus the density decreases. The solvent capacity is reduced by orders of magnitude and fine solid particles precipitate out of the solution because of the antisolvent effect. For the second way at least three terms are common. Precipitation from compressed antisolvents (PCA) is the general expression but supercritical antisolvent (SAS) process and aerosol spray extraction system (ASES) are also used. The solution is sprayed by a fine nozzle into the supercritical fluid as antisolvent and fine particles are formed by precipitation. A special application of this type is the solution enhanced dispersion by supercritical fluids (SEDS). The

Table 8: Phase equilibria data of pharmacologically active compounds

Substances	Conditions	Gas/Method	Jahr	Ref.
Antibiotics (polycyclic ethers)	140–400 bar 60–80 °C	CO_2 + methanol CO_2 + water static-analytical	1992	[339]
Antioxidants (ascorbic acid, gallic acid, butylhydroxyanisole, tocopherol)	130–250 bar 35–60 °C	CO_2 dynamic	1999	[340]
Caffeine (partition coefficient)	80–300 bar 40–60 °C	CO_2 + water static-analytical	1996	[341]
Capsaicin	70–400 bar 25–60 °C	CO_2 static-analytical	1992	[342]
Carbonic acids	23–240 bar 35–120 °C	CO_2 synthetic	2000	[343]
Chlorophyll	120–200 bar 35 °C	CO_2 Ethane synthetic	1990	[344]
Colourants (carotene, lutein, capsanthin, bixin, curcumin, pheophytin)	50–800 bar 15–55 °C	CO_2 + ethanol synthetic	1991	[345]
Coumarins	74–405 bar 40–100 °C	CO_2 dynamic	1990	[346]
Coumarins	85–250 bar 35–50 °C	CO_2 dynamic	1998	[347]
Estradiol; fluorouracil	100–220 bar 35–55 °C	CO_2 + ethanol dynamic	2000	[348]
Gingerol	127–196 bar 50/65 °C	CO_2 static-analytical	1990	[349]
Lutein diesters	115–316 bar 40 °C	CO_2 + several modifiers dynamic	2000	[350]
Monosaccharides (glucose, fructose)	40–214 bar 35–75 °C	CO_2 static-analytical	1994	[351]
Penicilline	100–350 bar 40–60 °C	CO_2 dynamic	1999	[352]
Phenoxymethylpenicilline	80–280 bar 32–52 °C	CO_2 dynamic	1991	[353]
Phenylacetic acid	84–195 bar 35/45 °C	CO_2 dynamic	1990	[354]
Resveratrol	80–140 bar 40 °C	CO_2 + ethanol dynamic	2001	[355]
Salicylic acid	87–157 bar 35/45 °C	CO_2 + ethanol static-analytical	1996	[356]
Steroids (progesterone, testosterone, cholesterol)	80–250 bar 35–60 °C	CO_2 + N_2O dynamic	1992	[357]
Vanillan	84–195 bar 35/45 °C	CO_2 dynamic	1990	[354]
Vanillin (partition coefficient)	80–300 bar 40–60 °C	CO_2 + water static-analytical	1996	[341]
Wool wax	250–650 bar 31–150 °C	CO_2 static-analytical	1997	[358]

organic solution and the supercritical antisolvent are brought together in the mixing chamber of the nozzle, before being finely dispersed in a precipitation vessel [362]. A detailed review about the SAS process is given by Reverchon [367].

8.2.3. Precipitation from gas saturated solutions

Compared to the above-mentioned methods, precipitations from gas-saturated solutions (PGSS) are scarcely applied. A supercritical fluid is dissolved in the molten solute to a high-pressure supercritical fluid saturated solution. This solution is expanded by a nozzle to atmospheric conditions where the volatile gas evaporates and fine particles are formed [362].

References

1 Cagniard de la Tour, C.: Ann. chim. phys. **2**, 127 (1822)
2 Hannay, J. B.; Hogarth, J.: Proc. Roy. Soc. **29**, 324 (1879)
3 Stahl, E.; Quirin, K.-W.; Gerard, D.: Verdichtete Gase zur Extraktion und Raffination, Springer-Verlag, Berlin/Heidelberg 1987
4 Brunner, G.: Gas Extraction, Steinkopff-Verlag, Darmstadt/Springer-Verlag, New Vork 1994
5 Smith, R. M.: Pure & Appl. Chem. **65**, 2397 (1993)
6 Drake, B. D.; Smith, R. L. Jr.: J. Supercrit. Fluids **3**, 162 (1990)

7 Yoon, J.-H.; Lee, H.-S.; Lee, H.: J. Chem. Eng. Data **38**, 53 (1993)
8 Page, S. H.; Goates, S. R.; Lee, M. L.: J. Supercrit. Fluids **4**, 109 (1991)
9 Suzuki, K.; Sue, H.; Itou, M.; Smith, R. L.; Inomata, H.; Arai, K.; Saito, S.: J. Chem. Eng. Data **35**, 63 (1990)
10 Semenova, A. I.; Emel'yanova, E. A.; Tsimmerman, S. S.; Tsiklis, D. S.: Russ. J. Phys. Chem. **53**, 1428 (1979)
11 Jennings, D. W.; Lee, R.-J.; Teja, A. S.: J. Chem. Eng. Data **36**, 303 (1991)
12 Gilbert, M. L.; Paulaitis, M. E.: J. Chem. Eng. Data **31**, 296 (1986)
13 Lim, J. S.; Lee, Y. Y.; Chung, H. S.: J. Supercrit. Fluids **7**, 219 (1994)
14 Chen, H.-I.; Chang, H.-Y.; Huang, E. T. S.; Huang, T.-C.: Ind. Eng. Chem. Res. **39**, 4849 (2000)
15 King, M. B.; Mubarak, A.; Kim, J. D.; Bott, T. R.: J. Supercrit. Fluids **5**, 296 (1992)
16 Wendland, M.; Hasse, H.; Maurer, G.: J. Supercrit. Fluids **7**, 245 (1994)
17 Adrian, T.; Hasse, H.; Maurer, G.: J. Supercrit. Fluids **9**, 19 (1996)
18 Wendland, M.; Hasse, H.; Maurer, G.: J. Supercrit. Fluids **6**, 211 (1993)
19 Page, S. H.; Sumpter, S. R.; Lee, M. L.: J. Microcol. Sep. **4**, 91 (1992)
20 Machado, N. T.; Brunner, G.: Ciênc. Tecnol. Aliment. **17**, 361 (1997)
21 Brunner, G: J. Supercrit. Fluids **13**, 283 (1998)
22 Lack, E.; Marr, R.: Sep. Sci. Technol. **23**, 63 (1988)
23 Smith, R. L.; Inomata, H.; Kanno, M.; Arai, K.: J. Supercrit. Fluids **15**, 145 (1999)
24 Reverchon, E.; Osséo, L. S.: JAOCS **71**, 1007 (1994)
25 Buskov, S.; Sörensen, H.; Sörensen, J. C.; Sörensen, S.: Pol. J. Food Nutr. Sci. **6/47**, 117 (1997)
26 Brühl, L.; Matthäus, B.: Fresenius J. Anal. Chem. **364**, 631 (1999)
27 Kuk, M. S.; Hron, R. J. Sr.: J. Am. Oil Chem. Soc. **71**, 1353 (1994)
28 Esquivel, M. M.; Bernardo-Gil, G.: J. Supercrit. Fluids **6**, 91 (1993)
29 Kim, H.-J.; Lee, S.-B.; Park, K.-A.; Hong, I.-K.: Sep. Purif. Technol. **15**, 1 (1999)
30 Illes, V.; Szalai, O.; Then, M.; Daood, H.; Perneczky, S.: J. Supercrit. Fluids **10**, 209 (1997)
31 Szentmihalyi, K.; Then, M.; Illes, V.; Perneczky, S.; Sandor, Z.; Lakatos, B.; Vinkler, P.: Z. Naturforsch. **53**, 779 (1998)
32 Roy, B. C.; Goto, M.; Hirose, T.: Int. J. Food Sci. Technol. **31**, 137 (1996)
33 Cheung, P. C. K.: Food Chem. **65**, 399 (1999)
34 Cheung, P. C. K.; Leung, A. Y. H.; Ang, P. O. Jr.: J. Agric. Food Chem. **46**, 4228 (1998)
35 Walker, T. H.; Cochran, H. D.; Hulbert, G. J.: J. Am. Oil Chem. Soc. **76**, 595 (1999)
36 Cygnarowicz-Provost, M.; O'Brien, D. J.; Boswell, R. T.; Kurantz, M. J.: J. Supercrit. Fluids **8**, 51 (1995)
37 Esquivel, M. M.; Bandarra, N. M.; Fontan, I.; Bernardo Gil, M. G.; Batista, I.; Nunes, M. L.; Empis, J. A.: Lebensm.-Wiss. Technol. **30**, 715 (1997)
38 Taylor, S. L.; King, J. W.: J. Chromatogr. Sci. **38**, 91 (2000)
39 Boselli, E.; Caboni, M. F.: J. Supercrit. Fluids **19**, 45 (2000)
40 Higashidate, S.; Yamauchi, Y.; Saito, M.: J. Chromatogr. **515**, 295 (1990)
41 Türkay, S.; Burford, M. D.; Sangün, M. K.; Ekinci, E.; Bartle, K. D.; Clifford, A. A.: J. Am. Oil Chem. Soc. **73**, 1265 (1996)
42 Dunford, N. T.; Temelli, F.; LeBlanc, E.: J. Food Sci. **62**, 289 (1997)
43 Stastova, J.; Jez, J.; Bartlova, M.; Sovova, H.: Chem. Eng. Sci. **51**, 4347 (1996)
44 Reverchon, E.; Kazinuas, A.; Marrone, C.: Chem. Eng. Sci. **55**, 2195 (2000)
45 Sovova, H.; Kucera, J.; Jez, J.: Chem. Eng. Sci. **49**, 415 (1994)
46 Shobha, S. V.; Ravindranath, B.: J. Agric. Food Chem. **39**, 2214 (1991)
47 De Franca, L. F.; Christensen, T.; Brunner, G.: J. Supercrit. Fluids **14**, 67 (1998)
48 Dunford, N. T.; Temelli, F.: J. Am. Oil Chem. Soc. **72**, 1009 (1995)
49 Przybylski, R.; Lee, Y.-C.; Kim, I.-H.: Lebensm.-Wiss. Technol. **31**, 687 (1998)
50 Temelli, F.: J. Food Sci. **57**, 440 (1992)
51 Alexander, W. S.; Brusewitz, G. H.; Maness, N. O.: J. Food Sci. **62**, 762 (1997)
52 Ronyai, E.; Simandi, B.; Tömösközi, S.; Deak, A.; Vigh, L.; Weinbrenner, Z.: J. Supercrit. Fluids **14**, 75 (1998)
53 Bulley, N. R.; Labay, L.; Arntfield, S. D.: J. Supercrit. Fluids **5**, 13 (1992)
54 Wu, W.; Hou, Y.: J. Supercrit. Fluids **19**, 149 (2001)
55 Franca, L. F.; Meireles, M. A. A.: Cienc. Tecnol. Aliment. **17**, 384 (1997)
56 Fleck, U.; Tiegs, C.; Brunner, G.: J. Supercrit. Fluids **14**, 67 (1998)
57 King, J. W.; Sahle-Demessie, E.; Temelli, F.; Teel, J. A.: J. Supercrit. Fluids **10**, 127 (1997)
58 Montanari, L.; Fantozzi, P.; Snyder, J. M.; King, J. W.: J. Supercrit. Fluids **14**, 87 (1999)
59 Araujo, M. E.; Machado, N. T.; Franca, L. F.; Meireles, M. A. A.: Braz. J. Chem. Eng. **17**, 297 (2000)
60 Perrut, M.; Clavier, J. Y.; Poletto, M.; Reverchon, E.: Ind. Eng. Chem. Res. **36**, 430 (1997)
61 Bittencourt Spricigo, C.; Teixeira Pinto, L.; Bolzan, A.; Ferreira Novais, A.: J. Supercrit. Fluids **15**, 253 (1999)
62 Favati, F.; King, J. W.; Mazzanti, M.: Am. Oil Chem. Soc. **68**, 422 (1991)
63 Zizovic, I.; Skala, D.; Ristic, M.: J. Serb. Chem. Soc. **63**, 883 (1998)
64 Simoes, P. C.; Carmelo, P. J.; Pereira, P. J.; Lopes, J. A.; Nunes da Ponte, M.; Brunner, G.: J. Supercrit. Fluids **13**, 337 (1998)
65 Devittori, C.; Gumy, D.; Kusy, A.; Colarow, L.; Bertoli, C.; Lambelet, P.: JAOCS **77**, 573 (2000)
66 Passey, C. A.; Gros-Louis, M.: J. Supercrit. Fluids **6**, 255 (1993)
67 Marrone, C.; Poletto, M.; Reverchon, E.; Stassi, A.: Chem. Eng. Sci. **53**, 3711 (1998)
68 del Valle, J. M.; Bello, S.; Thiel, J.; Allen, A.; Chordia, L.: Braz. J. Chem. Eng. **17**, 335 (2000)
69 Eggers, R.; Ambrogi, A.; Schnitzler, J. von: Braz. J. Chem. Eng. **17**, 329 (2000)
70 Manninen, P.; Pakarinen, J.; Kallio, H.: J. Agric. Food Chem. **45**, 2533 (1997)
71 Molero Gomez, A.; Martinez de la Ossa, E.: JAOCS **77**, 969 (2000)
72 Sass-Kiss, A.; Czukor, B.; Gao, Y.; Stefanovits, P.; Boross, F.: J. Sci. Food Agric. **76**, 189 (1998)
73 Gawdzik, J.; Mardarowicz, M.; Suprynowicz, Z.; Kawka, S.; Wolski, T.: J. High Resolut. Chromatogr. **19**, 237 (1996)
74 Cassel, E.; Frizzo, C. D.; Vanderlinde, R.; Atti-Serafini, L.; Lorenzo, D.; Dellacassa, E.: Ind. Eng. Chem. Res. **39**, 4803 (2000)
75 Ma, X.; Yu, X.; Zheng, Z.; Mao, J.: Chromatographia **32**, 40 (1991)
76 Kallio, H.; Kerrola, K.; Alhonmäki, P.: J. Agric. Food Chem. **42**, 2478 (1994)
77 Smith, R. M.; Burford, M. D.: J. Chromatogr. **600**, 175 (1992)
78 Smith, R. M.; Burford, M. D.: J. Chromatogr. Sci. **32**, 265 (1994)
79 Miller, K. G.; Poole, C. F.; Chichila, T. M. P.: J. High Resolut. Chromatogr. **18**, 461 (1995)
80 Lozano, P.; Delgado, D.; Gomez, D.; Rubio, M.; Iborra, J. L.: J. Biochem. Biophys. Methods **43**, 367 (2000)
81 Gopalan, B.; Goto, M.; Kodama, A.; Hirose, T.: J. Agric. Food Chem. **48**, 2189 (2000)
82 Ma, X.; Yu, X.; Han, J.: Phytochem. Anal. **6**, 292 (1995)
83 Hawthorne, S. B.; Riekkola, M.-L.; Serenius, K.; Holm, Y.; Hiltunen, R.; Hartonen, K.: J. Chromatogr. **634**, 297 (1993)
84 Ghassempour, A.; Arshadi, M. R.; Mamaghani, M.: Sepu **16**, 463 (1998)
85 Ruberto, G.; Biondi, D.; Renda, A.: Phytochem. Anal. **10**, 241 (1999)
86 Verschuere, M.; Sandra, P.; David, F.: J. Chromatogr. Sci. **30**, 388 (1992)
87 Liu, L. K.: Anal. Commun. **33**, 175 (1996)
88 Ozek, T.; Bozan, B.; Baser, K. H. C.: Chem. Nat. Comp. **34**, 668 (1998)
89 Walker, D. F. G.; Bartle, K. D.; Breen, D. G. P. A.; Clifford, A. A.; Costiou, S.: Analyst **119**, 2789 (1994)
90 Akgün, M.; Akgün, N. A.; Dincer, S.: Ind. Eng. Chem. Res. **39**, 473 (2000)
91 Padukka, I.; Bhandari, B.; D'Arcy, B.: J. Food Comp. Anal. **13**, 59 (2000)
92 Dauksas, E.; Venskutonis, P. R.; Sivik, B.: J. Agric. Food Chem. **46**, 4347 (1998)
93 Dauksas, E.; Venskutonis, P. R.; Sivik, B.: J. Supercrit. Fluids **15**, 51 (1999)
94 Roy, B. C.; Goto, M.; Kodama, A.; Hirose, T.: J. Chem. Tech. Biotechnol. **67**, 21 (1996)
95 Ammann, A.; Hinz, D. C.; Addleman, R. S.; Wai, C. M.; Wenclawiak, B. W.: Fresenius J. Anal. Chem. **364**, 650 (1999)
96 Moldao-Martins, M.; Trigo, R.; Nolasco, M. A.; Bernardo Gil, M. G.; Beirao da Costa, M. L.: Dev. Food Sci. **40**, 133 (1998)
97 Kim, K. H.; Hong, J.: Sep. Sci. Technol. **35**, 315 (2000)
98 Ondarza, M.; Sanches, A.: Chromatographia **30**, 16 (1990)
99 Orav, A.; Kailas, T.; Koel, M.: J. Essent. Oil Res. **10**, 387 (1998)
100 [100] Ferreira, S. R. S.; Nikolov, Z. L.; Doraiswamy, L. K.; Meireles, M. A. A.; Petenate, A. J.: J. Supercrit. Fluids **14**, 235 (1999)
101 Coelho, J. A. P.; Mendes, R. L.; Provost, M. C.; Cabral, J. M. S.; Novais, J. M.; Palavra, A. M. F.: ACS Symp. Ser. **670**, 101 (1997)
102 Heikes, D. L.: J. Chromatogr. Sci. **32**, 253 (1994)
103 Esquivel, M. M.; Ribeiro, M. A.; Bernardo Gil, M. G.: J. Supercrit. Fluids **14**, 129 (1999)
104 Clifford, A. A.; Basile, A.; Al-Saidi, S. H. R.: Fresenius J. Anal. Chem. **364**, 635 (1999)
105 Sagrero-Nieves, L.; Bartley, J. P.; Provis-Schwede, A.: J. Essent. Oil Res. **6**, 547 (1994)
106 Subra, P.; Vega-Bancel, A.; Reverchon, E.: J. Supercrit. Fluids **12**, 43 (1998)

107 Bartley, J. P.; Foley, P.: J. Sci. Food Agric. **66**, 365 (1994)
108 Roy, B. C.; Goto, M.; Hirose, T.: Ind. Eng. Chem. Res. **35**, 607 (1996)
109 Sass-Kiss, A.; Simandi, B.; Gao, Y.; Boross, F.; Vamos-Falusi, Z.: J. Sci. Food Agric. **76**, 320 (1998)
110 Nykänen, I.; Nykänen, L.; Alkio, M.: J. Essent. Oil Res. **3**, 229 (1991)
111 Doneanu, C.; Anitescu, G.: J. Supercrit. Fluids **12**, 59 (1998)
112 Simandi, B.; Oszagyan, M.; Lemberkovics, E.; Petri, G.; Kery, A.; Fejes, S.: J. Essent. Oil Res. **8**, 305 (1996)
113 Catchpole, O. J.; Grey, J. B.; Smallfield, B. M.: J. Supercrit. Fluids **9**, 273 (1996)
114 Mishra, R. R.; Tiwari, K. K.: Pafai J. **16**, 13 (1994)
115 Sovova, H.; Komers, R.; Kucera, J.; Jez, J.: Chem. Eng. Sci. **49**, 2499 (1994)
116 Baysal, T.; Starmans, D. A. J.: J. Supercrit. Fluids **14**, 225 (1999)
117 Vuorela, H.; Holm, Y.; Hiltunen, R.; Harvala, T.; Laitinen, A.: Flavour Fragrance J. **5**, 81 (1990)
118 Zekovic, Z. P.: J. Essent. Oil Res. **12**, 85 (2000)
119 Pekic, B.; Zekovic, Z.; Tolic, A.: J. Serb. Chem. Soc. **59**, 249 (1994)
120 Reverchon, E.; Senatore, F.: J. Agric. Food Chem. **42**, 154 (1994)
121 Pekic, B.; Zekovic, Z.; Petrovic, L.; Tolic, A.: Sep. Sci. Technol. **30**, 3567 (1995)
122 Pekic, B.; Zekovic, Z.; Tolic, A.: J. Serb. Chem. Soc. **60**, 439 (1995)
123 Pekic, B.; Zekovic, Z.; Tolic, A.: J. Serb. Chem. Soc. **62**, 531 (1997)
124 Scalia, S.; Giuffreda, L.; Pallado, P.: J. Pharm. Biomed. Anal. **21**, 549 (1999)
125 Della Porta, G.; Reverchon, E.; Chouchi, D.; Barth, D.: J. Essent. Oil Res. **9**, 515 (1997)
126 Reverchon, E.; Della Porta, G.; Lamberti, G.: J. Supercrit. Fluids **14**, 115 (1999)
127 Poiana, M.; Fresa, R.; Mincione, B.: Flavour Fragrance J. **14**, 358 (1999)
128 Reverchon, E.; Iacuzio, G.: Chem. Eng. Sci. **52**, 3553 (1997)
129 Kondo, M.; Goto, M.; Kodama, A.; Hirose, T.: Ind. Eng. Chem. Res. **39**, 4745 (2000)
130 Sato, M.; Goto, M.; Kodama, A.; Hirose, T.: Chem. Eng. Sci. **53**, 4095 (1998)
131 Sato, M.; Goto, M.; Hirose, T.: Ind. Eng. Chem. Res. **35**, 1906 (1996)
132 Poiana, M.; Sicari, V.; Mincione, B.: J. Essent. Oil Res. **10**, 145 (1998)
133 Poiana, M.; Sicari, V.; Mincione, B.: Flavour Fragrance J. **13**, 125 (1998)
134 Sato, M.; Goto, M.; Kodama, A.; Hirose, T.: ACS Symp. Ser. **670**, 119 (1997)
135 Sato, M.; Kondo, M.; Goto, M.; Kodama, A.; Hirose, T.: J. Supercrit. Fluids **13**, 311 (1998)
136 Mira, B.; Blasco, M.; Subirats, S.; Berna, A.: J. Supercrit. Fluids **9**, 238 (1996)
137 Sargenti, S. R.; Lancas, F. M.: J. Chromatogr. Sci. **36**, 169 (1998)
138 Mira, B.; Blasco, M.; Berna, A.; Subirats, S.: J. Supercrit. Fluids **14**, 95 (1999)
139 Lee, Y.-W.; Lee, C. H.; Kim, J.-D.; Lee, Y. Y.; Row, K. H.: Sep. Sci. Technol. **35**, 1069 (2000)
140 Budich, M.; Heilig, S.; Wesse, T.; Leibküchler, V.; Brunner, G.: J. Supercrit. Fluids **14**, 105 (1999)
141 Pino, J. A.; Garcia, J.; Martinez, M. A.: J. Essent. Oil Res. **8**, 373 (1996)
142 Anitescu, G.; Doneanu, C.; Radulescu, V.: Flavour Fragrance J. **12**, 173 (1997)
143 Eikani, M. H.; Goodarznia, I.; Mirza, M.: Flavour Fragrance J. **14**, 29 (1999)
144 Chassagnez-Mendez, A. L.; Machado, N. T.; Araujo, M. E.; Maia, J. G.; Meireles, M. A. A.: Ind. Eng. Chem. Res. **39**, 4729 (2000)
145 Chassagnez-Mendez, A. L.; Correa, N. C. F.; Franca, L. F.; Machado, N. T.; Araujo, M. E.: Braz. J. Chem. Eng. **17**, 315 (2000)
146 Sargenti, S. R.; Lancas, F. M.: Chromatographia **46**, 285 (1997)
147 Gopalakrishnan, N.; Narayanan, C. S.: J. Agric. Food Chem. **39**, 1976 (1991)
148 Della Porta, G.; Porcedda, S.; Marongiu, B.; Reverchon, E.: Flavour Fragrance J. **14**, 214 (1999)
149 D'Alpaos, M.; Traldi, P.; Tassinato, G.; Pallado, P.: Riv. Ital. EPPOS Spec. Num., 236 (1997)
150 Della Porta, G.; Taddeo, R.; D'Urso, E.; Reverchon, E.: Lebensm.-Wiss. Technol. **31**, 454 (1998)
151 Barth, D.; Chouchi, D.; Della Porta, G.; Reverchon, E.; Perrut, M.: J. Supercrit. Fluids **7**, 177 (1994)
152 Reverchon, E.; Daghero, J.; Marrone, C.; Mattea, M.; Poletto, M.: Ind. Eng. Chem. Res. **38**, 3069 (1999)
153 Simandi, B.; Deak, A.; Ronyai, E.; Yanxiang, G.; Veress, T.; Lemberkovics, E.; Then, M.; Sass-Kiss, A.; Vamos-Falusi, Z.: J. Agric. Food Chem. **47**, 1635 (1999)
154 Langezaal, C. R.; Chandra, A.; Katsiotis, S. T.; Scheffer, J. J. C.; de Haan, A. B.: J. Sci. Food Agric. **53**, 455 (1990)
155 Reverchon, E.; Della Porta, G.; Gorgoglione, D.: J. Supercrit. Fluids **8**, 60 (1995)
156 Rao, G. V. R.; Srinivas, P.; Sastry, S. V. G. K.; Mukhopadhyay, M.: J. Supercrit. Fluids **5**, 19 (1992)
157 Reverchon, E.; Della Porta, G.: J. Agric. Food Chem. **43**, 1654 (1995)
158 Oszagyan, M.; Simandi, B.; Sawinsky, J.; Kery, A.; Lemberkovics, E.; Fekete, J.: Flavour Fragrance J. **11**, 157 (1996)
159 Adasoglu, N.; Dincer, S.; Bolat, E.: J. Supercrit. Fluids **7**, 93 (1994)
160 Pino, J. A.; Garcia, J.; Martinez, M. A.: J. Essent. Oil Res. **9**, 341 (1997)
161 Reis-Vasco, E. M. C.; Coelho, J. A. P.; Palavra, A. M. F.: Chem. Eng. Sci. **55**, 2917 (2000)
162 Reis-Vasco, E. M. C.; Coelho, J. A. P.; Palavra, A. M. F.: Flavour Fragrance J. **14**, 156 (1999)
163 Pino, J. A.; Garcia, J.; Martinez, M. A.: J. Essent. Oil Res. **11**, 191 (1999)
164 Barton, P.; Hughes, R. E. Jr.; Hussein, M. M.: J. Supercrit. Fluids **5**, 157 (1992)
165 Reverchon, E.; Ambruosi, A.; Senatore, F.: Flavour Fragrance J. **9**, 19 (1994)
166 de Haan, A. B.; de Graauw, J.; Schaap, J. E.; Badings, H. T.: J. Supercrit. Fluids **3**, 15 (1990)
167 Reverchon, E.; Osseo, L. S.; Gorgoglione, D.: J. Supercrit. Fluids **7**, 185 (1994)
168 Pino, J. A.; Garcia, J.; Martinez, M. A.: J. Essent. Oil Res. **10**, 575 (1998)
169 Reverchon, E.: J. Supercrit. Fluids **5**, 256 (1992)
170 Sargenti, S. R.; Lancas, F. M.: J. High Resolut. Chromatogr. **20**, 511 (1997)
171 Pino, J. A.; Garcia, J.; Martinez, M. A.: J. Essent. Oil Res. **9**, 689 (1997)
172 Garcia-Fajardo, J.; Martinez-Sosa, M.; Estarron-Espinosa, M.; Vilarem, G.; Gaset, A.; De Santos, J. M.: J. Essent. Oil Res. **9**, 181 (1997)
173 Sovova, H.; Jez, J.; Bartlova, M.; Stastova, J.: J. Supercrit. Fluids **8**, 295 (1995)
174 Reverchon, E.; Della Porta, G.: J. Supercrit. Fluids **9**, 199 (1996)
175 Reverchon, E.; Della Porta, G.; Gorgoglione, D.: Flavour Fragrance J. **12**, 37 (1997)
176 Coelho, L. A. F.; Oliveira, J. V.; d'Avila, S. G.; Vilegas, J. H. Y.; Lancas, F. M.: J. High Resolut. Chromatogr. **20**, 431 (1997)
177 Reverchon, E.; Taddeo, R.: J. Supercrit. Fluids **8**, 302 (1995)
178 Reverchon, E.: AIChE J. **42**, 1765 (1996)
179 Ronyai, E.; Simandi, B.; Lemberkovics, E.; Veress, T.; Patiaka, D.: J. Essent. Oil Res. **11**, 69 (1999)
180 Ronyai, E.; Simandi, B.; Veress, T.; Lemberkovics, E.; Patiaka, D.: J. Essent. Oil Res. **11**, 499 (1999)
181 Fuh, M.-R. S.; Pan, W. H.; Hsieh, I. J.; Chuo, C.-M.: Am. Lab. **27**, 36 (1995)
182 Reverchon, E.; Marrone, C.: Chem. Eng. Sci. **52**, 3421 (1997)
183 Daghero, J.; Mattea, M.; Reverchon, E.; Della Porta, G.; Senatore, F.: Acta Hort. **503**, 21 (1999)
184 Kery, A.; Ronyai, E.; Simandi, B.; Lemberkovics, E.; Keve, T.; Deak, A.; Kemeny, S.: Chromatographia **49**, 503 (1999)
185 Sargenti, S. R.; Lancas, F. M.: J. Microcolumn Sep. **10**, 213 (1998)
186 Eikani, M. H.; Goodarznia, I.; Mirza, M.: J. Essent. Oil Res. **11**, 470 (1999)
187 Zekovic, Z.; Lepojevic, Z.; Vujic, D.: Chromatographia **51**, 175 (2000)
188 Vieira de Melo, S. A. B.; Costa, G. M. N.; Garau, R.; Casula, A.; Pittau, B.: Braz. J. Chem. Eng. **17**, 367 (2000)
189 Nguyen, K.; Barton, P.; Spencer, J. S.: J. Supercrit. Fluids **4**, 40 (1991)
190 Yonei, Y.; Ohinata, H.; Yoshida, R.; Shimizu, Y.; Yokoyama, C.: J. Supercrit. Fluids **8**, 156 (1995)
191 D'Alpaos, M.; Traldi, P.; Pallado, P.; Gorgoglione, D.: Riv. Ital. EPPOS **Spec. Num.**, 200 (1997)
192 Badalyan, A. G.; Wilkinson, G. T.; Chun, B.-S.: J. Supercrit. Fluids **13**, 319 (1998)
193 Kandiah, M.; Spiro, M.: Int. J. Food Sci. Technol. **25**, 328 (1990)
194 Ashraf-Khorassani, M.; Taylor, L. T.: Anal. Chim. Acta **347**, 305 (1997)
195 Järvenpää, E. P.; Jestoi, M. N.; Huopalahti, R.: Phytochem. Anal. **8**, 250 (1997)
196 Gawdzik, J.; Kawka, S.; Mardarowicz, M.; Suprynowicz, Z.; Wolski, T.: Herba Pol. **42**, 26 (1996)
197 Kohler, M.; Haerdi, W.; Christen, P.; Veuthey, J.-L.: J. Chromatogr., A **785**, 353 (1997)
198 Kohler, M.; Haerdi, W.; Christen, P.; Veuthey, J.-L.: Phytochem. Anal. **8**, 223 (1997)
199 Degnan, A. J.; Elbe, J. H. von; Hartel, R. W.: J. Food Sci. **56**, 1655 (1991)

200 Anderson, S. G.; Nair, M. G.; Chandra, A.; Morrison, E.: Phytochem. Anal. **8**, 247 (1997)
201 Vilegas, J. H. Y.; Lancas, F. M.; Vilegas, W.; Pozetti, G. L.: Phytochem. Anal. **4**, 230 (1993)
202 Yao, J.; Nair, M. G.; Chandra, A.: J. Agric. Food Chem. **42**, 1303 (1994)
203 Jaren-Galan, M.; Nienaber, U.; Schwartz, S. J.: J. Agric. Food Chem. **47**, 3558 (1999)
204 Modey, W. K.; Mulholland, D. A.; Raynor, M. W.: Phytochem. Anal. **7**, 1 (1996)
205 Mendes, R. L.; Fernandes, H. L.; Coelho, J. P.; Reis, E. C.; Cabral, J. M. S.; Novais, J. M.; Palavra, A. F.: Food Chem. **53**, 99 (1995)
206 Peker, H.; Srinivasan, M. P.; Smith, J. M.; McCoy, B. J.: AIChE J. **38**, 761 (1992)
207 Ramos, E.; Valero, E.; Ibanez, E.; Reglero, G.; Tabera, J.: J. Agric. Food Chem. **46**, 4011 (1998)
208 Ruibin, F.; Shihong, Z.: Sepu **17**, 249 (1999)
209 Holcomb, M.; Thompson, H. C. Jr.; Cooper, W. M.; Hopper, M. L.: J. Supercrit. Fluids **9**, 118 (1996)
210 Schaeffer, S. T.; Zalkow, L. H.; Teja, A. S.: AIChE J. **34**, 1740 (1988)
211 Baumann, W.; Rodrigues, S. V.; Viana, L. M.: Braz. J. Chem. Eng. **17**, 323 (2000)
212 Barth, M. M.; Zhou, C.; Kute, K. M.; Rosenthal, G. A.: J. Agric. Food Chem. **43**, 2876 (1995)
213 Chandra, A.; Nair, M. G.: Phytochem. Anal. **8**, 244 (1997)
214 Subra, P.; Castellani, S.; Jestin, P.; Aoufi, A.: J. Supercrit. Fluids **12**, 261 (1998)
215 Vega, P. J.; Balaban, M. O.; Sims, C. A.; O'Keefe, S. F.; Cornell, J. A.: J. Food Sci. **61**, 757 (1996)
216 Moore, W. N.; Taylor, L. T.: J. Nat. Prod. **59**, 690 (1996)
217 Moore, W. N.; Taylor, L. T.: Phytochem. Anal. **8**, 238 (1997)
218 Subra, P.; Boissinot, P.: J. Chromatogr. **543**, 413 (1991)
219 Subra, P.; Tufeu, R.; Garrabos, Y.; Boissinot, P.: J. Supercrit. Fluids **4**, 244 (1991)
220 Liu, B.; Lockwood, G. B.; Gifford, L. A.: J. Chromatogr., A **690**, 250 (1995)
221 Brachet, A.; Christen, P.; Gauvrit, J.-Y.; Longeray, R.; Lanteri, P.; Veuthey, J.-L.: J. Biochem. Biophys. Methods **43**, 353 (2000)
222 Gonzalez-Vila, F. J.; Bautista, J. M.; Gutierrez, A.; Del Rio, J. C.; Gonzalez, A. G.: J. Biochem. Biophys. Methods **43**, 345 (2000)
223 Verotta, L.; Peterlongo, F.: Phytochem. Anal. **4**, 178 (1993)
224 Beek, van, K. R.; Moore, L. G.: Text. Chem. Color. **29**, 66 (1997)
225 Chandra, A.; Nair, M. G.: Phytochem. Anal. **7**, 259 (1996)
226 Saldana, M. D. A.; Mohamed, R. S.; Baer, M. G.; Mazzafera, P.: J. Agric. Food Chem. **47**, 3804 (1999)
227 Spanos, G. A.; Chen, H.; Schwartz, S. J.: J. Food Sci. **58**, 817 (1993)
228 Da Costa, C. T.; Margolis, S. A.; Benner, B. A. Jr.; Horton, D.: J. Chromatogr., A **831**, 167 (1999)
229 Dean, J. R.; Liu, B.; Price, R.: Phytochem. Anal. **9**, 248 (1998)
230 Chandra, A.; Nair, M. G.: Planta Med. **61**, 192 (1995)
231 Fischer, M.; Jefferies, T. M.: J. Agric. Food Chem. **44**, 1258 (1996)
232 Le Floch, F.; Tena, M. T.; Rios, A.; Valcarcel, M.: Talanta **46**, 1123 (1998)
233 Xu, Z.; Godber, J. S.: JAOCS **77**, 547 (2000)
234 Moraes, M. L.; Vilegas, J. H. Y.; Lancas, F. M.: Phytochem. Anal. **8**, 257 (1997)
235 Mehr, C. B.; Biswal, R. N.; Collins, J. L.; Cochran, H. D.: J. Supercrit. Fluids **9**, 185 (1996)
236 Lopez-Avila, V.; Benedicto, J.: J. High Resolut. Chromatogr. **20**, 555 (1997)
237 Ashraf-Khorassani, M.; Taylor, L. T.; Martin, M.: Chromatographia **50**, 287 (1999)
238 Lopez-Sebastian, S.; Ramos, E.; Ibanez, E.; Bueno, J. M.; Ballester, L.; Tabera, J.; Reglero, G.: J. Agric. Food Chem. **46**, 13 (1998)
239 Tena, M. T.; Valcarcel, M.; Hidalgo, P. J.; Ubera, J. L.: Anal. Chem. **69**, 521 (1997)
240 Dean, J. R.; Liu, B.; Price, R.: J. Chromatogr. A, **799**, 343 (1998)
241 Choi, Y. H.; Kim, J.; Jeon, S. H.; Yoo, K.-P.; Lee, H.-K.: Chromatographia **48**, 695 (1998)
242 Dean, J. R.; Liu, B.: Phytochem. Anal. **11**, 1 (2000)
243 Kim, Y.; Choi, Y. H.; Chin, Y.-W.; Jang, Y. P.; Kim, Y. C.; Kim, J.; Kim, J. Y.; Joung, S. N.; Noh, M. J.; Yoo, K.-P.: J. Chromatogr. Sci. **37**, 457 (1999)
244 Lojkova, L.; Slanina, J.; Mikesova, M.; Taborska, E.; Vejrosta, J.: Phytochem. Anal. **8**, 261 (1997)
245 Choi, Y. H.; Chin, Y.-W.; Kim, J.; Jeon, S. H.; Yoo, K.-P.: J. Chromatogr., A **863**, 47 (1999)
246 Mishima, K.; Wada, N.; Uchiyama, H.; Nagatani, M.; Choi, W. S.; Kitazaki, H.; Takai, T.: Solvent Extr. Res. Dev. **8**, 231 (1996)
247 Lin, M.-C.; Tsai, M.-J.; Wen, K.-C.: J. Chromatogr. A **830**, 387 (1999)
248 Bicchi, C.; Rubiolo, P.; Frattini, C.; Sandra, P.; David, F.: J. Nat. Prod. **54**, 941 (1991)
249 Cadoni, E.; De Giorgi, M. R.; Medda, E.; Poma, G.: Dyes Pigm. **44**, 27 (2000)
250 Tsuda, T.; Mizuno, K.; Ohshima, K.; Kawakishi, S.; Osawa, T.: J. Agric. Food Chem. **43**, 2803 (1995)
251 Heaton, D. M.; Bartle, K. D.; Rayner, C. M.; Clifford, A. A.: J. High Resolut. Chromatogr. **16**, 666 (1993)
252 Jennings, D. W.; Deutsch, H. M.; Zalkow, L. H.; Teja, A. S.: J. Supercrit. Fluids **5**, 1 (1992)
253 Chun, M.-K.; Shin, H.-W.; Lee, H.: J. Supercrit. Fluids **9**, 192 (1996)
254 Sanagi; M. M.; Hung, W. P.; Yasir, S. M.: J. Chromatogr. A **785**, 361 (1997)
255 Saito, M.; Yamauchi, Y.; Inomata, K.; Kottkamp, W.: J. Chromatogr. Sci. **27**, 79 (1989)
256 Lopez-Avila, V.; Benedicto, J.; Robaugh, D.: J. High Resolut. Chromatogr. **20**, 231 (1997)
257 Palma, M.; Taylor, L. T.: J. Chromatogr. A **849**, 117 (1999)
258 Palma, M.; Taylor, L. T.; Varela, R. M.; Cutler, S. J.; Cutler, H. G.: J. Agric. Food Chem. **47**, 5044 (1999)
259 Murga, R.; Ruiz, R.; Beltran, S.; Cabezas, J. L.: J. Agric. Food Chem. **48**, 3408 (2000)
260 Palma, M.; Taylor, L. T.; Zoecklein, B. W.; Douglas, L. S.: J. Agric. Food Chem. **48**, 775 (2000)
261 Gamse, T.; Marr, R.: Wiss. Ber. – Forschungszent. Karlsruhe, 201 (1999)
262 Guyer, D. E.; Sinha, N. K.: J. Sci. Food Agric. **69**, 457 (1995)
263 Cocero, M. J.; Gonzalez, S.; Perez, S.; Alonso, E.: J. Supercrit. Fluids **19**, 39 (2000)
264 Johnson, S.; Morgan, E. D.: Phytochem. Anal. **8**, 228 (1997)
265 Chao, R. R.; Mulvaney, S. J.; Sanson, D. R.; Hsieh, F.-H.; Tempesta, M. S.: J. Food Sci. **56**, 80 (1991)
266 Skerget, M.; Knez, Z.; Novak, Z.; Bauman, D.: Acta Aliment. **27**, 149 (1998)
267 Daood, H. G.; Biacs, P. A.; Illes, V.; Gnayfeed, M. H.: Wiss. Ber. – Forschungszent. Karlsruhe **FZKA 6271**, 185 (1999)
268 Illes, V.; Daood, H. G.; Biacs, P. A.; Gnayfeed, M. H.; Meszaros, B.: J. Chromatogr. Sci. **37**, 345 (1999)
269 Pan, W. H. T.; Chang, C.-C.; Su, T.-T.; Lee, F.; Fuh, M.-R. S.: Talanta **42**, 1745 (1995)
270 Catchpole, O. J.; Grey, J. B.; Noermark, K. A.: J. Supercrit. Fluids **19**, 25 (2000)
271 Birtigh, A.; Johannsen, M.; Brunner, G.: J. Supercrit. Fluids **8**, 46 (1995)
272 Choi, Y. H.; Kim, J.; Noh, M. J.; Park, E. M.; Yoo, K.-P.: Korean J. Chem. Eng. **13**, 216 (1996)
273 Choi, Y. H.; Kim, J.; Noh, M. J.; Choi, E. S.; Yoo, K.-P.: Phytochem. Anal. **8**, 233 (1997)
274 Chang, C. J.; Chang, Y.-F.; Lee, H.; Lin, J.; Yang, P.-W.: Ind. Eng. Chem. Res. **39**, 4521 (2000)
275 King, J. W.; Favati, F.; Taylor, S. L.: Sep. Sci. Technol. **31**, 1843 (1996)
276 Colombo, M. L.; Corsinin, A.; Mossa, A.; Sala, L.; Stanca, M.: Phytochem. Anal. **9**, 192 (1998)
277 Saldana, M. D. A.; Mohamed, R. S.; Mazzafera, P.: Braz. J. Chem. Eng. **17**, 251 (2000)
278 Castaneda-Acosta, J.; Cain, A. W.; Fischer, N. H.; Knopf, F. C.: J. Agric. Food Chem. **43**, 63 (1995)
279 Ferreira de Franca, L.; Reber, G.; Meireles, M. A. A.; Machado, N. T.; Brunner, G.: J. Supercrit. Fluids **14**, 247 (1999)
280 Favati, F.; King, J. W.; Friedrich, J. P.; Eskins, K.: J. Food Sci. **53**, 1532 (1988)
281 Rincon, J.; De Lucas, A.; Garcia, M. A.; Garcia A.; Alvarez, A.; Carnicer, A.: Sep. Sci. Technol. **33**, 411 (1998)
282 Brunner, G.; Malchow, T.; Stürken, K.; Gottschau, T.: J. Supercrit. Fluids **4**, 72 (1991)
283 Catchpole, O. J.; Simoes, P.; Grey, J. B.; Nogueiro, E. M. M.; Carmelo, P. J.; Nunes da Ponte, M.: Ind. Eng. Chem. Res. **39**, 4820 (2000)
284 Ibanez, E.; Palacios, J.; Senorans, F. J.; Santa-Maria, G.; Tabera, J.; Reglero, G.: J. Am. Oil Chem. Soc. **77**, 187 (2000)
285 Gerard, D.; Quirin, K.-W.; Schwarz, E.: Food Marketing & Technology (1995)
286 Ibanez, El; Cifuentes, A.; Crego, A. L.; Senorans, F. J.; Cavero, S.; Reglero, G.: J. Agric. Food Chem. **48**, 4060 (2000)
287 Baysal, T.; Ersus, S.; Starmans, D. A. J.: J. Agric. Food Chem. **48**, 5507 (2000)
288 Pasquel, A.; Meireles, M. A. A.; Marques, M. O. M.; Petenate, A. J.: Braz. J. Chem. Eng. **17**, 271 (2000)
289 Li, S.; Hartland, S.: J. Supercrit. Fluids **5**, 7 (1992)
290 Li, S.; Hartland, S.: J. Am. Oil Chem. Soc. **73**, 423 (1996)
291 Scalia, S.; Games, D. E.: Analyst **117**, 839 (1992)
292 Wang, S.-P.; Chang, C.-L.: Anal. Chim. Acta **377**, 85 (1998)
293 Anklam, E.; Müller, A.: Pharmazie **50**, 364 (1995)
294 Dean, J. R.; Lowdon, J.: Analyst **118**, 747 (1993)
295 Lawrence, J. K.; Larsen, A. K.; Tebbett, I. R.: Anal. Chim. Acta **288**, 123 (1994)

296 Becerra, G.; Menolasina, S.; Salvador, A.: J. High Resolut. Chromatogr. **22**, 300 (1999)
297 Salvador, A.; Jaime, M. A.; Guardia, M. de la; Becerra, G.: Anal. Commun. **35**, 53 (1998)
298 Schneiderman, M. A.; Sharma, A. K.; Mahanama, K. R. R.; Locke, D. C.: J. Assoc. Off. Anal. Chem. **71**, 815 (1988)
299 Eckard, P. R.; Taylor, L. T.: J. High Resolut. Chromatogr. **22**, 469 (1999)
300 Howard, A. L.; Shah, M. C.; Ip, D. P.; Brooks, M. A.; Strode, J. T. III; Taylor, L. T.: J. Pharm. Sci. **83**, 1537 (1994)
301 Scalia, S.; Ruberto, G.; Bonina, F.: J. Pharm. Sci. **84**, 433 (1995)
302 Mulcahey, L. J.; Taylor, L. T.: Anal. Chem. **64**, 981 (1992)
303 Brunner, G.: Ber. Bunsenges. Phys. Chem. **88**, 887 (1984)
304 Goodarznia, I.; Eikani, M. H.: Chem. Eng. Sci. **53**, 1387 (1998)
305 Del Valle, J. M.; Napolitano, P.; Fuentes, N.: Ind. Eng. Chem. Res. **39**, 4720 (2000)
306 Sovova, H.: Chem. Eng. Sci. **49**, 409 (1994)
307 Reverchon, E.; Marrone, C.: J. Supercrit. Fluids **19**, 161 (2001)
308 Schaeffer, S. T.; Zalkow, L. H.; Teja, A. S.: J. Supercrit. Fluids **2**, 15 (1989)
309 Bulley, N. R.; Fattori, M.; Meisen, A.; Moyls, L.: JAOCS **61**, 1362 (1984)
310 Lee, A. K. K.; Bulley, N. R.; Meisen, A.: JAOCS **63**, 921 (1986)
311 Bartle, K. D.; Clifford, A. A.; Hawthorne, S. B.; Langenfeld, J. J.; Miller, D. J.; Robinson, R.: J. Spercrit. Fluids **3**, 143 (1990)
312 Bartle, K. D.; Boddington, T.; Clifford, A. A.; Hawthorne, S. B.: J. Supercrit. Fluids **5**, 207 (1992)
313 Reverchon, E.; Donsi, G.; Osseo, L. S.: Ind. Eng. Chem. Res. **32**, 2721 (1993)
314 Tezel, A.; Hortacsu, A.; Hortacsu, Ö.: J. Supercrit. Fluids **19**, 3 (2000)
315 Goto, M.; Hirose, T.; McCoy, B. J.; J. Supercrit. Fluids **7**, 61 (1994)
316 Pawliszyn, J.: J. Chromatogr. Sci. **31**, 31 (1993)
317 Bartle, K. D.; Clifford, A. A.; Hawthorne, S. B.; Langenfeld, J. J.; Miller, D. J.; Robinson, R.: J. Supercrit. Fluids **3**, 143 (1990)
318 Guan, B.; Liu, Z.; Han, B.; Yan, H.: J. Supercrit. Fluids **14**, 213 (1999)
319 Borch-Jensen, C.; Mollerup, J.: J. Supercrit. Fluids **10**, 87 (1997)
320 Peter, S.; Jakob, H.: J. Supercrit. Fluids **4**, 166 (1991)
321 Zou, M.; Yu, Z. R.; Kashulines, P.; Rizvi, S. S. H.; Zollweg, J. A.: J. Supercrit. Fluids **3**, 23 (1990)
322 Riha, V.; Brunner, G.: J. Supercrit. Fluids **15**, 33 (1999)
323 Hammam, H.; Sivik, B.: J. Supercrit. Fluids **6**, 223 (1993)
324 Ashour, I.; Hammam, H.: J. Supercrit. Fluids **6**, 3 (1993)
325 Yu, Z.-R.; Rizvi, S. S. H.; Zollweg, J. A.: J. Supercrit. Fluids **5**, 123 (1992)
326 Foster, N. R.; Yun, S. L. J.; Ting, S. S. T.: J. Supercrit. Fluids **4**, 127 (1991)
327 Yu, Z.-R.; Zou, M.; Bhaskar, A. R.; Rizvi, S. S. H.; Zollweg, J. A.: J. Supercrit. Fluids **6**, 63 (1993)
328 Simoes, P. C.; Brunner, G.: J. Supercrit. Fluids **9**, 75 (1996)
329 Kalra, H.; Chung, S. Y.-K.; Chen, C.-J.: Fluid Phase Equilib. **36**, 263 (1987)
330 Buß, D.; Brunner, G.: Wiss. Ber. – Forschungszent. Karlsruhe **FZKA 6271**, 197 (1999)
331 Zhong, M.; Han, B.; Yan, H.: J. Supercrit. Fluids **10**, 113 (1997)
332 Hammam, H.: J. Supercrit. Fluids **5**, 101 (1992)
333 Bamberger, T.; Erickson, J. C.; Cooney, C. L.: J. Chem. Eng. Data **33**, 327 (1988)
334 Akgün, M.; Akgün, N. A.; Dincer, S.: J. Supercrit. Fluids **15**, 117 (1999)
335 Marteau, P.; Obriot, J.; Tufeu, R.: J. Supercrit. Fluids **8**, 20 (1995)
336 Di Giacomo, G.; Brandani, V.; Del Re, G.; Mucciante, V.: Fluid Phase Equilib. **52**, 405 (1989)
337 Berna, A.; Chafer, A.; Monton, J. B.: J. Chem. Eng. Data **45**, 724 (2000)
338 Stuart, G. R.; Dariva, C.; Oliveira, J. V.: Braz. J. Chem. Eng. **17**, 181 (2000)
339 Maxwell, R. J.; Hampson, J. W.; Cygnarowicz-Provost, M.: J. Supercrit. Fluids **5**, 31 (1992)
340 Cortesi, A.; Kikic, I.; Alessi, P.; Turtoi, G.; Garnier, S.: J. Supercrit. Fluids **14**, 139 (1999)
341 Brudi, K.; Dahmen, N.; Schmieder, H.: J. Supercrit. Fluids **9**, 146 (1996)
342 Knez, Z.; Steiner, R.: J. Supercrit. Fluids **5**, 251 (1992)
343 Byun, H.-S.; Kim, K.; McHugh, M. A.: Ind. Eng. Chem. Res. **39**, 4580 (2000)
344 Subra, P.; Tufeu, R.: J. Supercrit. Fluids **3**, 20 (1990)
345 Jay, A. J.; Steytler, D. C.; Knights, M.: J. Supercrit. Fluids **4**, 131 (1991)
346 Calvey, E. M.; Page, S. W.: J. Supercrit. Fluids **3**, 115 (1990)
347 Choi, Y. H.; Kim, J.; Noh, M. J.; Choi, E. S.; Yoo, K.-P.: Chromatographia **47**, 93 (1998)
348 Guney, O.; Akgerman, A.: J. Chem. Eng. Data **45**, 1049 (2000)
349 Spiro, M.; Kandiah, M.: Int. J. Food Sci. Technol. **25**, 566 (1990)
350 Naranjo-Modad, S.; Lpez-Munguia, A.; Vilarem, G.; Gaset, A.; Barzana, E.: J. Agric. Food Chem. **48**, 5640 (2000)
351 Yau, J.-S.; Tsai, F.-N.: J. Supercrit. Fluids **7**, 129 (1994)
352 Gordillo, M. D.; Blanco, M. A.; Molero, A.; Martinez de la Ossa, E.: J. Supercrit. Fluids **15**, 183 (1999)
353 Ko, M.; Shah, V.; Bienkowski, P. R.; Cochran, H. D.: J. Supercrit. Fluids **4**, 32 (1991)
354 Wells, P. A.; Chaplin, R. P.; Foster, N. R.: J. Supercrit. Fluids **3**, 8 (1990)
355 Berna, A.; Chafer, A.; Monton, J. B.: J. Supercrit. Fluids **19**, 133 (2001)
356 Ke, J.; Mao, C.; Zhong, M.; Han, B.; Yan, H.: J. Supercrit. Fluids **9**, 82 (1996)
357 Kosal, E.; Lee, C. H.; Holder, G. D.: J. Supercrit. Fluids **5**, 169 (1992)
358 Jones, F. W.; Bateup, B. O.; Dixon, D. R.; Gray, S. R.: J. Supercrit. Fluids **10**, 105 (1997)
359 Poole, C. F.: J. Biochem. Biophys. Methods **43**, 3 (2000)
360 Chester, T. L.; Pinkston, J. D.: Anal. Chem. **72**, 129R (2000)
361 Subramaniam, B.; Rajewski, R. A.; Snavely, K.: J. Pharm. Sci. **86**, 885 (1997)
362 Palakodaty, S.; York, P.: Pharm. Res. **16**, 976 (1999)
363 Subra, P.; Jestin, P.: Powder Technol. **103**, 2 (1999)
364 Donsi, G.; Reverchon, E.: Pharm. Acta Helv. **66**, 170 (1991)
365 Phillips, E. M.; Stella, V. J.: Int. J. Pharm. **94**, 1 (1993)
366 Alessi, P.; Cortesi, A.; Kikic, I.; Foster, N. R.; Macnaughton, S. J.; Colombo, I.: Ind. Eng. Chem. Res. **35**, 4718 (1996)
367 Reverchon, E.: J. Supercrit. Fluids **15**, 1 (1999)

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