Phytochemistry

Extraction, Isolation And Separation of Physiologically Active Natural Compounds

Extraction Techniques

- Spontaneous isolation without extraction.
- Usually necessary at least one extraction and one purification step for compound isolation.
- Not only separation of high molecular compounds, but also removal of low molecular impurities disturbing late stages of separation and purification.
- *Matrix effect.*

Extraction – **Fick´s Diffusion Law**

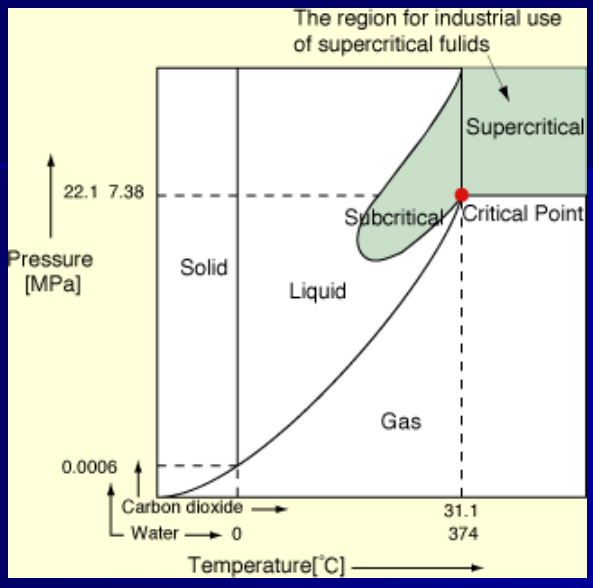
$$dc/dt = - (DA/h) . (c_0 - c)$$

- **dc/dt** - diffusion speed
- **D** - coefficient of diffusion (depending on temperature and particle size)
- **A** - interface
- **h** – diffusion layer
- **(c_0 - c)** – concentration gradient

- **Extraction process depends on**:
- Type of drug and its form (*Matrix effect*)
  - grade of disintegrations according to the diameters holes of sieve
  - grinding and mill apparatus
  - cutting apparatus
- Weight ratio of drug and extraction medium
- Humidity of drug
- Extraction method
- Extraction medium and its composition

Supercritical Fluid Extraction (SFE)

- Extraction process is running with help of supercritical fluids.
- **Supercritical fluid**
  - Pressure and temperature crosses critical levels.
  - Its physical properties are on the cross between gases and liquids.
  - Density close to liquids → good solvation ability.
  - Diffusion constant close to gases → rapid transfer of mass.
  - Viscosity lower then liquids → advantage especially for supercritical fluid chromatography.
  - Low surface tension → easy material penetration.



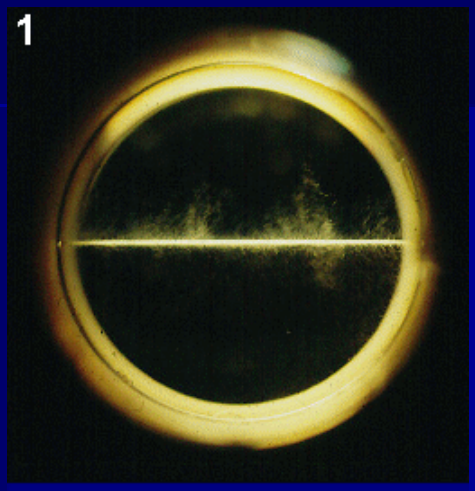
Phase diagram for water and carbon dioxide

Characteristics	Density (kg/m^3)	Viscosity (cP)	Diffusivity (mm^2/s)
Gas	1	0.01	1-10
SCF	100-800	0.05-0.1	0.01-0.1
Liquid	1000	0.5-1.0	0.001

Component	P_c [bar]	T_c [^oC]
CO_2	73.8	31.1
N_2O	72.4	36.4
NH_3	112.7	132.5
SO_2	78.8	157.6
SF_6	37.6	45.5
Xe	58.3	16.6

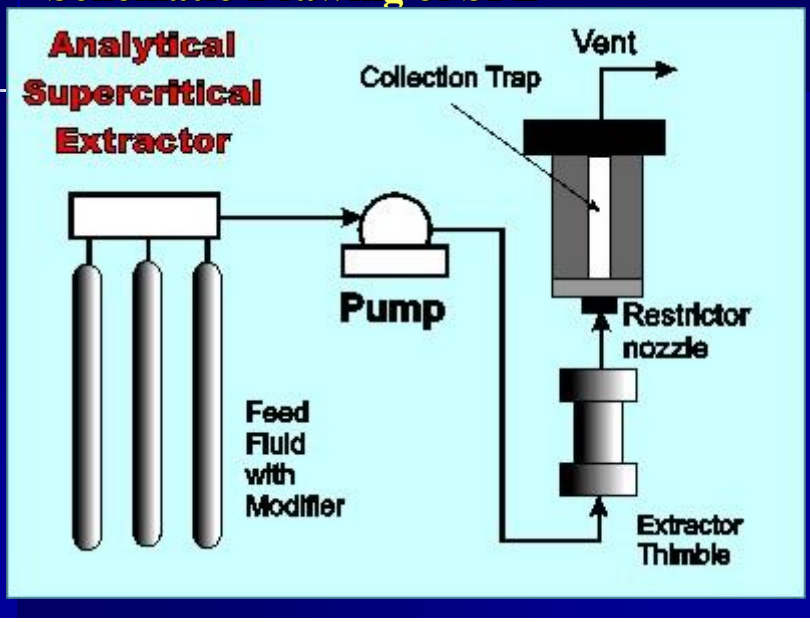
Liquid	Critical temperature (Kelvin)	Critical pressure (bar)
CO_2	304.1	73.8
Ethan	305.4	48.8
Ethylen	282.4	50.4
Propan	369.8	42.5
Propylen	364.9	46.0
Trifluoromethan	299.3	48.6
Chlrortrifluoromethan	302.0	38.7
Trichlorofluoromethan	471.2	44.1
NH_3	405.5	113.5
H_20	647.3	221.2
Cyclohexan	553.5	40.7
n-Pentan	469.7	33.7
Toluen	591.8	41.0

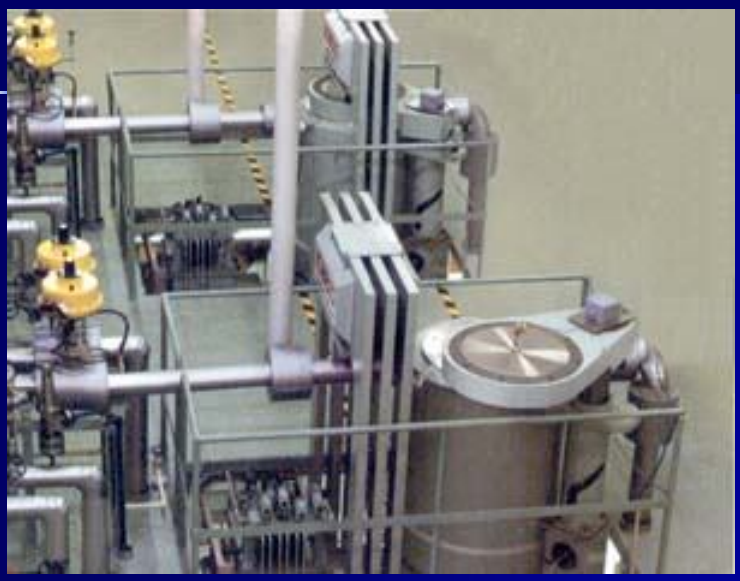
Generation of Supercritical Fluid



Below the critical parameters, two distinct phases exist

Schematic Drawing of SFE





- SFE Advantages:
  - Fine technique.
  - In ideal case no need of organic solvents.
  - Ecological harmlessness.
  - Relatively cheap.
  - Rapid.
  - Possibility of automatization.
  - Solvation power affected by changes of pressure.

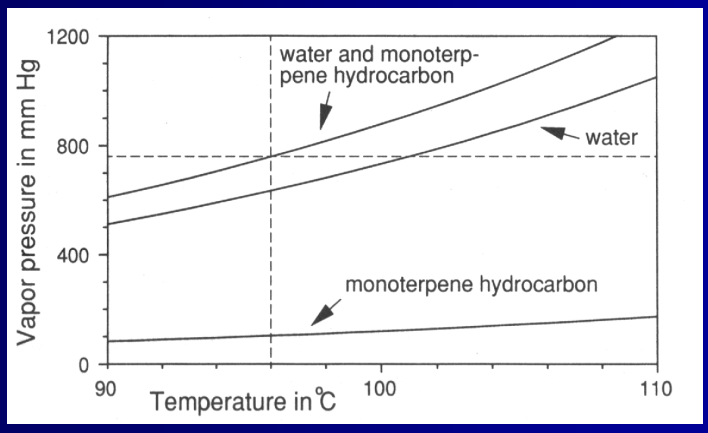
- SFE Disadvantages:
  - Less suitable for more polar compounds.
  - More demanding on equipment.
  - Need to use high pressures.
  - Less suitable for extraction of plant leaves.
  - Problems with extraction tuning.
  - Difficult to extract fresh material (water content).

- CO_2 – inflammable, non-explosive, easy available, cheap, ecological harmlessness, useful supercritical area, (T=31.1 ºC; P=7.28 MPa), suitable for extraction of less polar (hydrophobic) compounds (volatile oils, oils, waxes, carotenoids etc.)
  - **Used for:**
    - Hop extraction.
    - De-caffeinization of coffee (production of caffeine).
    - Extraction of taxol (Paclitaxel TM) from *Taxus brevifolia.*
    - Extraction of essential oils and spices.
    - Non-pharmaceutical purposes.

Distillation with Water Steam (Hydrodistillation)

- Method suitable for isolation of water insoluble volatile compounds.
- Common content of volatile compounds in plant material – > 1 % of weight – difficult SFE or solvent extraction.
- Selective.
- Simple.
- Pure.

Principles of Steam Water Distillation



- Advantages:
  - Selective for volatile compounds.
  - Simple apparatus.
  - Very cheap method.
  - Only water used as a „solvent“.
  - Useable in preparative scale.
  - Extract obtained very pure.

- Disadvantages:
  - Utilizable for non-polar (hydrophobic) volatile compounds.
  - Possibility of material decomposition (presence of water, high temperatures).
  - Improper for very low quantities of starting material.
  - Quantitative extraction time consummating.
  - Possibility of loss of polar compounds from total bulk of compounds.

Principles of Water Steam Distillation

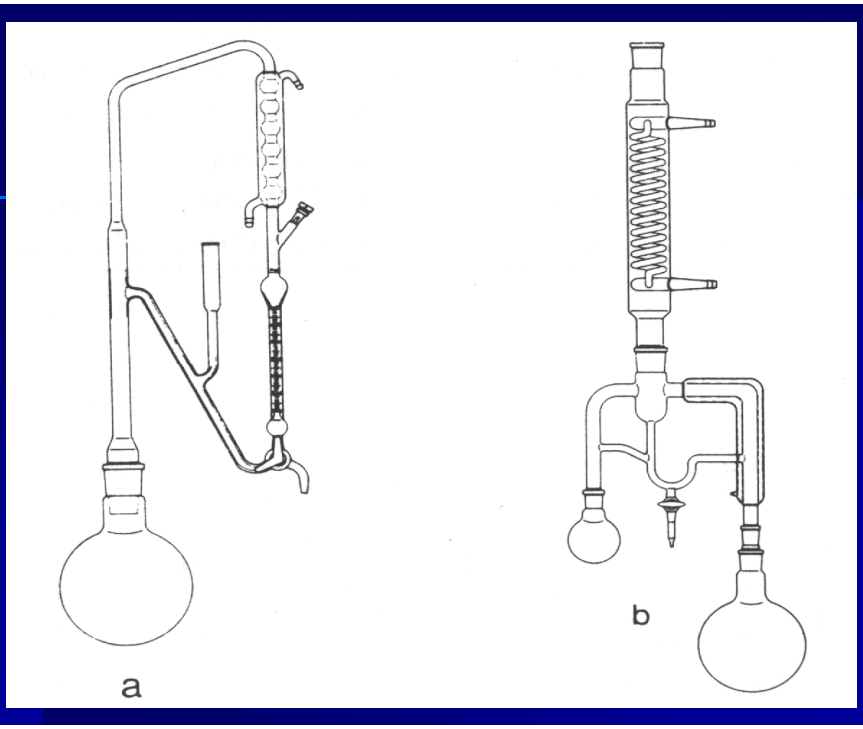
- Possible forms:
  - 1. „True“ water steam distillation.
  - 2. Hydrodistillation.

– True water steam distillation:

- Steam is generated separately in „steam generator“.
- Steam is forced down into material.
- Used in industry.

– Hydrodistillation:

- Plant material is suspended in boiling water.
- Water steam is generated *in situ*.
- Clevenger apparatus. Suitable for laboratory purposes.



- Affection by matrix effect:
  - If are volatile compounds (like essential oils) located on surface of plant material (trichomes, glandules…) – *matrix* effect is weak or not present, distillation is very rapid.
  - If are volatile compounds present deeper inside the material, distillation is slower and it is affected by matrix effect and diffusion.
- Special case – steam distillation extraction (SDE):
  - Used not only water steam.
  - Mixtures diethyl ether/water 1:1, pentane/water 1:1.
  - Volatile non-polar compounds are dissolved in organic solvent, consequently two phases arise.

Separation and Purification Techniques

- Shake out (liquid-liquid extraction).
- Precipitation.
- Crystallization.
- Chromatography.

Shake out (liquid-liquid extraction).

- Following after the extraction.
- Based on different solubility in immiscible or limited miscible solvents.
- Coarse separation of compounds to fractions with similar polarity, or creation of complexes and salts.
- Coarse extract are usually not suitable directly for chromatographic separation:
  - Wide spectrum of compounds.
  - Very variant polarity.
  - Bad solubility in common chromatographic solvents.
- On analytical scale often replaced by SPE.

- Advantages:
  - Without necessity of special equipment.
  - Good capacity.
  - Possibility of fine tuning.
  - No irreversible adsorption.
  - Solvents could be chosen directly according to the compounds characteristics.

- Disadvantages:
  - Limited ability of separation.
  - Formation of problematic inter-phases and emulsions.
  - No possibility of automatization.

- Examples of common mixtures and their use:
  - Water: diethyl ether (petroleum ether)
  - Water: chloroform
  - Water: n-butanol
  - Methanol: hexane

  - Isolation of alkaloids.
  - De-fatting of extracts.
  - Separation of steroids.
  - Separation of glycosides from aglycones.

Precipitation

- Selective removal of group of target analytes or impurities.
- Followed by decantation, filtration and/or centrifugation.
- Employing the formation and dissolution of well defined precipitates.
- In present time:
  - Isolation of alkaloids (Mayer reagent, Valser reagent).
  - Separation of polyphenolic compounds (lead acetate, PVPP).

Crystallization

- Final purification step.
- Possible in range from milligrams to tons.
- Suitable solvent – usually chosen by method trial and error.
- Target compounds usually less soluble than impurities.
- In present time of lower importance
- Stays necessary for some identification methods (X-ray).