Compressed fluids in analytical separation methods

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employing the results and presentations by Pavel Karásek, Josef Planeta, Elena Varaďová Ostrá, Jaroslav Pól, Barbora Hohnová, Lenka Šťavíková, Marie Horká, Dana Moravcová and Karel Šlais

Structure

topic outline – why compressed fluids in separations ?

1) supercritical fluid chromatography (SFC)
2) supercritical fluid extraction (SFE)
3) extraction with organic solvents at elevated *T* and *P*

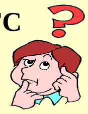
PFE - Pressurized Fluid Extraction
PLE – Pressurized Liquid Extraction
PSE - Pressurized Solvent Extraction
ASE - Accelerated Solvent Extraction

4) extraction with pressurized hot (subcritical) water

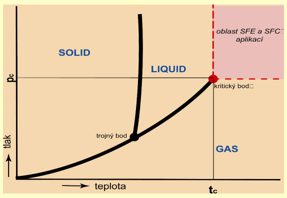
PHWE - Pressurized Hot Water Extraction
SubWE - Subcritical Water Extraction

5) supercritical water *vs.* siliceous surfaces – application in analytical separations

SFE, SFC



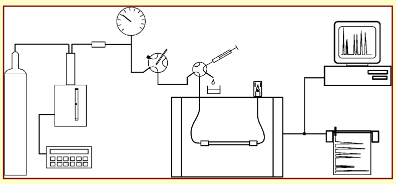
SF = supercritical fluid



– *utilization of fluid properties above their respective T_c and P_c*
– *properties (density, solvent power,…) controlled by P and T*
– *CO_2 T_c ~ 31°C P_c ~ 7.8 MPa*
– *density, solvent power ~ liquids*
– *viscosity, diffusion rate ~ gases*
– *faster than liquid chromatography/extraction*
– *environment-saving – less organic solvents (or none at all)*

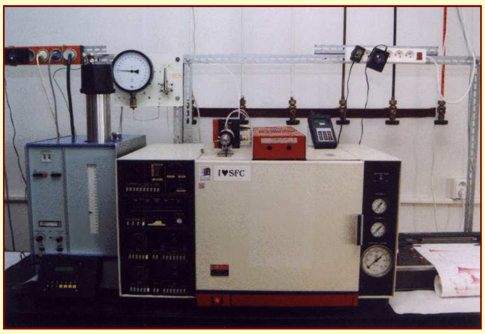
1) Supercritical fluid chromatography (SFC)

* **SFC apparatus**
* **Preparation of columns for SFC (micro HPLC)**
* **Examples of SFC separations**
* **Non-analytical applications – systems with ionic liquids**

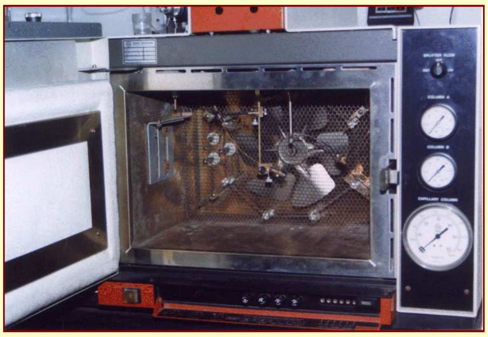


- **Mobile phase (CO_2) in supercritical state: p > 8 MPa, t > 31 °C**
- **(HPLC) High-pressure pump, injection valve**
- **(GC) Flame ionization detector (FID)**
- **(HPLC, GC) columns - packed (ϕ 320 µm – 4,6 mm)**
  capillary (ϕ 50 µm - 100 µm), length 10m
- **(-) Restrictor to control the mobile phase flow rate**

SFC apparatus (modified GC Varian 3700)



Detail vnitřku SFC zařízení

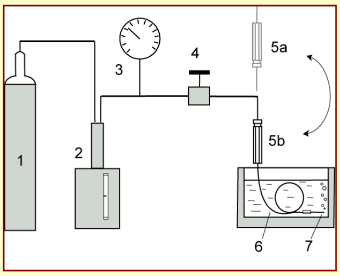


Příprava kapilárních náplňových kolon pro SFC (HPLC)

Požadavky na kolony:

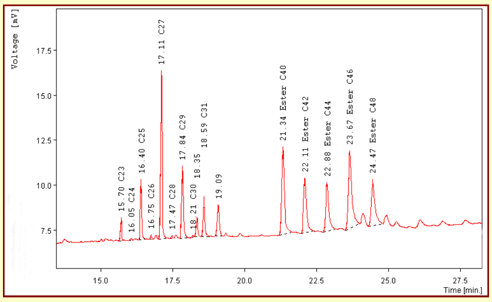
- Náplň sorbent o zrnitosti 3 nebo 5 μm, délka kolony do 1m
- Průměr kolony do 320 μm => F = 4μl/min(liq.), F = 10ml/min(g)
- Pracovní tlak do 40 MPa => nároky na uzavření konců kolon
- Vysoká účinnost vyrobených kolon

Apparatus for filling of packed capillary columns



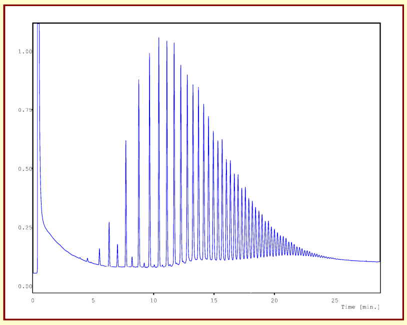
1 – CO_2 cylinder
2 - HPLC pump
3 – manometer
4 - on/off valve,
5 - stainless steel filling reservoir,
6 - fused silica capillary,
7 – restrictor

Examples of SFC separations



SFC separation of Rudolf II seal (beeswax). Column 320 μm x 150 mm, 5 μm Biospher C18, t=80°C, FID 150°C, program 8-35 MPa

Examples of SFC separations



SFC separation of poly(dimethylsiloxane). Column 320 μm x 150 mm, 5 μm Biospher C18, t=80°C, FID 150°C, pressure program 8-35 MPa

SFC today ?

- chiral separations (enantiomers)
- separation and purification in pharmaceutical research/industry

↑

higher throughput than HPLC
(more separations/analyses per unit time

~~pure CO_2~~ → CO_2 + MeOH

SFC as 2nd dimension in 2D chromatographic separations

Ionic Liquids (ILs)

?

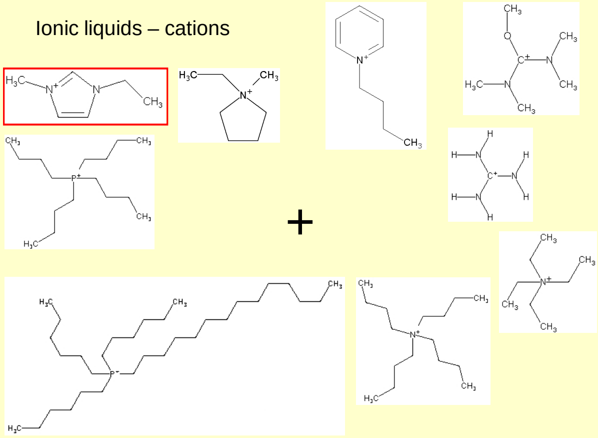
= liquid organic salts (melting point below 100°C)

= liquids composed exclusively of ions (no electroneutral particles)

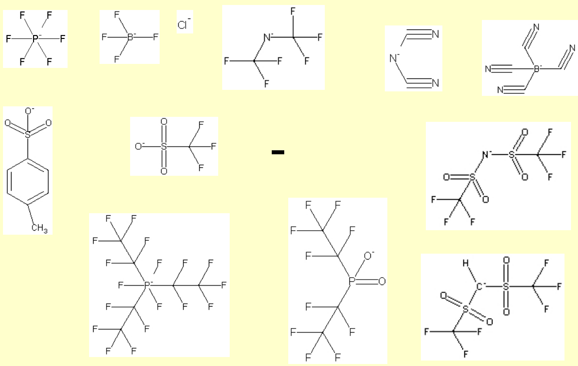
properties of ILs differ markedly from those of common molecular solvents (water, organic solvents)

number of „possible“ ILs = ~10^{18}

Ionic liquids – cations

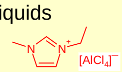


Ionic liquids – anions



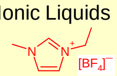
History of Ionic Liquids …1914 …Walden… $[(C_2H_5)_4N][NO_3]$

- 1980s: Chloroaluminate Ionic Liquids
  1st generation



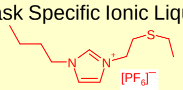
J.S. Wilkes, J.A. Levisky, R.A. Wilson and C.L. Hussey, *Inorg. Chem. 21* (**1982**) 1263-1264.

- 1990s: Air- and moisture-stable Ionic Liquids
  2nd generation



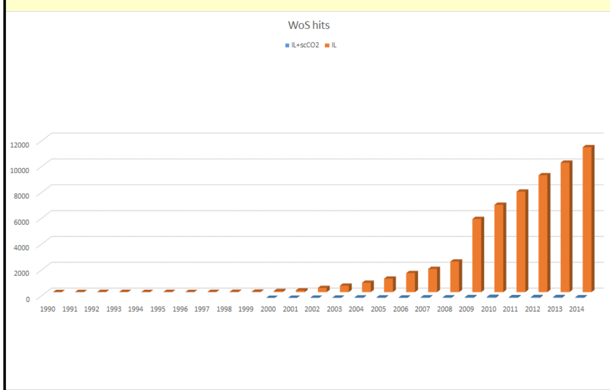
J.S. Wilkes and M.J. Zaworotko, *J. Chem. Soc. Chem. Commun* (**1992**) 965-966.

- 2000s: First examples of „Task Specific Ionic Liquids“
  3rd generation



A.E. Visser, R.P. Swatloski, W.M. Reichert, R. Mayton, S. Sheff, A. Wierzbicki, J.H. Davis, Jr. and R.D. Rogers, *Chem. Commun.* (**2001**) 135-136.

- 2010s: Biodegradable Ionic Liquids
  4th generation

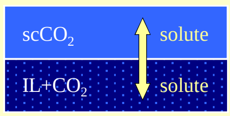


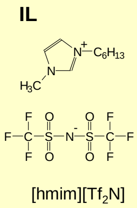
Supercritical fluid chromatography

data on solute partitioning between supercritical CO_2 and ionic liquid

- scientific importance

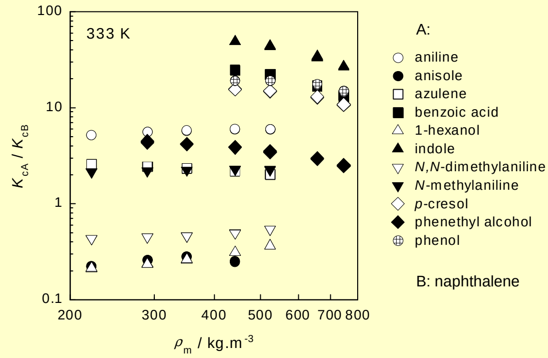
- applications





siloxane polymer (PDMS, M_w ~ 4.8×10^5)   IL

Relative partition coefficients in $[bmim][BF_4]$–$scCO_2$ system



Review papers on SFC applications

a) pharmaceutical analysis

E. Lemasson, S. Bertin, C. West: Use and practice of achiral and chiral supercritical fluid chromatography in pharmaceutical analysis and purification, *J. Separ. Sci.* **2016**, *39*, 212-233; http://dx.doi.org/10.1002/jssc.201501062 .

V. Desfontaine, D. Guillarme, E. Francotte, L. Nováková: Supercritical fluid chromatography in pharmaceutical analysis, *J. Pharm. Biomed. Anal.* **2015**, *113*, 56-71; http://dx.doi.org/10.1016/j.jpba.2015.03.007 .

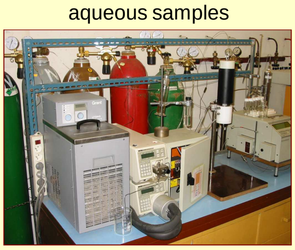
J. M. Plotka, M. Biziuk, C. Morrison, J. Namiesnik: Pharmaceutical and forensic drug applications of chiral supercritical fluid chromatography, *TrAC – Trends Anal. Chem.* **2014**, *56*, 74-89; http://dx.doi.org/10.1016/j.trac.2013.12.012 .

b) food analysis

J. L. Bernal, M. T. Martin, L. Toribio: Supercritical fluid chromatography in food analysis, *J. Chromatogr. A* **2013**, *1313*, 24-36; http://dx.doi.org/10.1016/j.chroma.2013.07.022 .

2) SFE - Supercritical fluid extraction (CO_2)

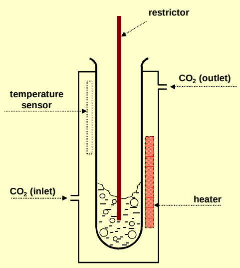
solid samples   aqueous samples

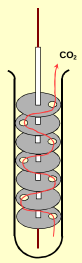


solvent power of CO_2 depends strongly on pressure (density)

? chrom. analysis – decompression – analyte losses – trapping ?

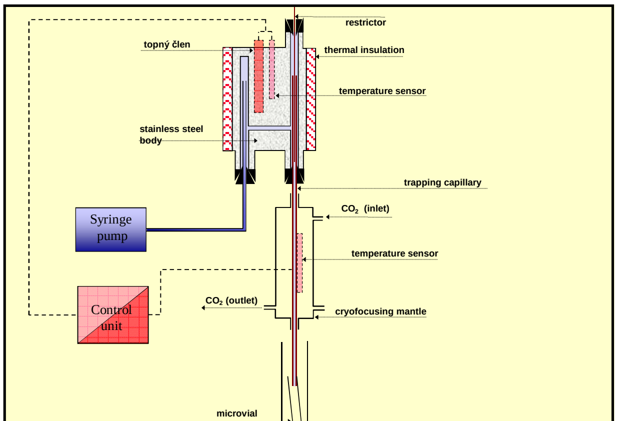
Basic technique of analyte trapping into organic solvent



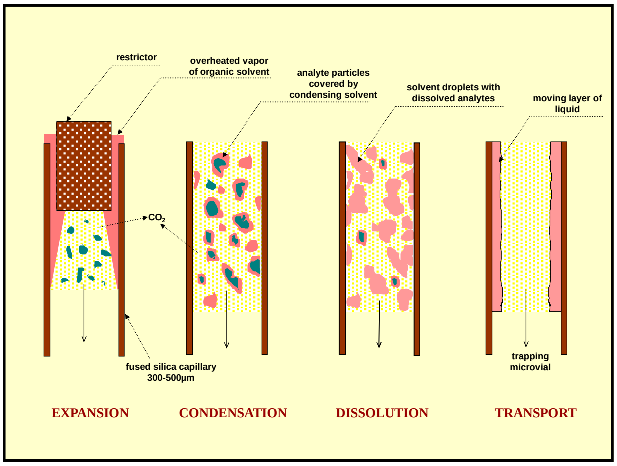


+ simple construction

- high consumption of solvent
- loss of volatile analytes
- restrictor clogging



Apparatus for analyte trapping into overheated vapor of organic solvent



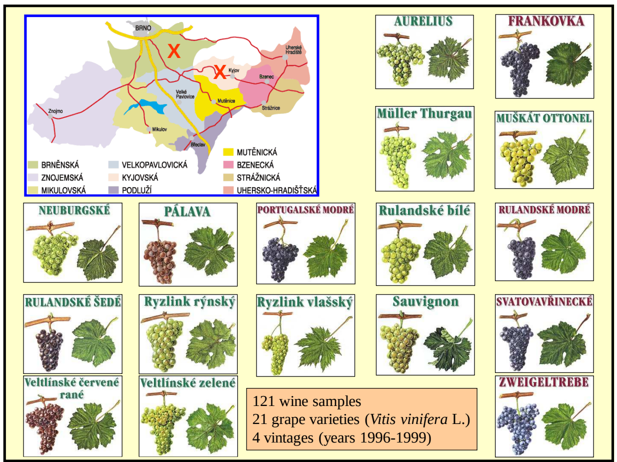
SFE of (pleasing) liquid samples ?

Wine analysis

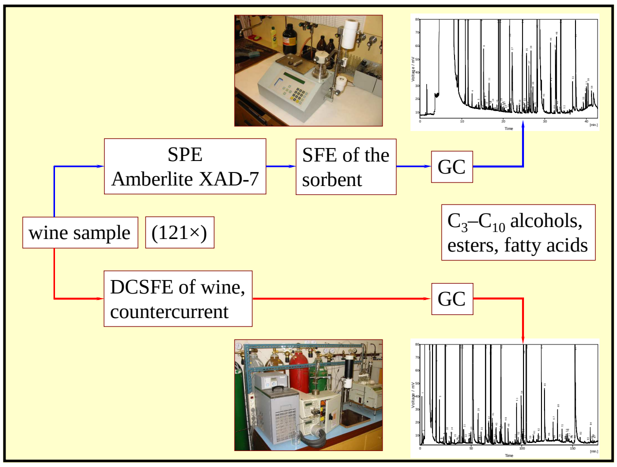
Direct Continuous Supercritical Fluid Extraction as a Novel Method of Wine Analysis: Comparison with Conventional Indirect Extraction and Implications for Wine Variety Identification

Pavel Karásek, Josef Planeta, Elena Varaďová Ostrá, Milena Mikešová, Jan Goliáš, Michal Roth, and Jiří Vejrosta

Journal of Chromatography A **2003**, *1002*, 13-23.



121 wine samples
21 grape varieties (*Vitis vinifera* L.)
4 vintages (years 1996-1999)



Multivariate Statistics

of the wine varieties represented by >=4 wine samples

[cluster analysis] - used to select the 4 samples/variety if more

discriminant analysis - elimination of redundant (= linearly dependent) component peak areas from the input data matrix

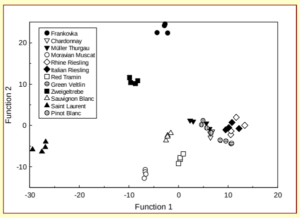
canonical correlation analysis - computation of discriminant functions, i.e., the latent factors differentiating among the wine samples

Info: http://www.statsoft.com/textbook/stathome.html

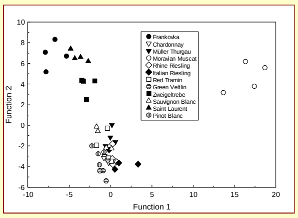
Computation tool: KyPlot spreadsheet SW, Koichi Yoshioka, http://www.qualest.co.jp/Download/KyPlot/kyplot_e.htm , http://www.kyenslab.com/en

Statistical processing (discriminant analysis) of chromatograms

Compared with the procedure involving solid phase extraction (SPE-SFE-GC), direct SFE of wines (DCSFE-GC) provides much clearer discrimination among wine varieties



DCSFE-GC



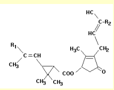
SPE-SFE-GC

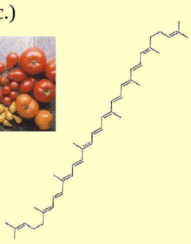
Other analytical applications of direct continuous SFE (DCSFE) of aqueous samples:

•**beer** - aldehydes, ketones, fatty acids, esters; PAH, PCB (DCSFE-GC)

•natural insecticides - **pyrethrines** (*Chrysanthemum cinerariaefolium*) (DCSFE-HPLC)

•**lycopene** (tetraterpene, red dye of tomatoes, etc.) (DCSFE-HPLC)

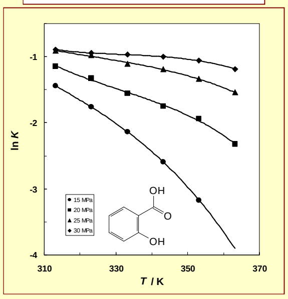




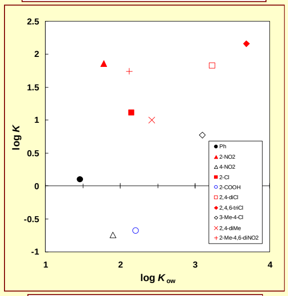
X

Interphase distribution of analytes in H_2O - $scCO_2$ system

Partition Coefficient ($K = y/x$) of Salicylic Acid as a Function of T and P



CO_2–Water Partition Coefficients (333 K, 20 MPa) *vs.* Octanol–Water Partition Coefficients at Ambient Conditions



Red symbols = *ortho*-substituted phenols
Blue symbol = 2 dissociable protons

Review articles on SFE applications

J. A. Mendiola, M. Herrero, A. Cifuentes, E. Ibanez: Use of compressed fluids for sample preparation: Food applications, *J. Chromatogr. A* **2007**, *1152*, 234-246; http://dx.doi.org/10.1016/j.chroma.2007.02.046 .

M. Herrero, J. A. Mendiola, A. Cifuentes, E. Ibanez: Supercritical fluid extraction: Recent advances and applications, *J. Chromatogr. A* **2010**, *1217*, 2495-2511; http://dx.doi.org/10.1016/j.chroma.2009.12.019 .

C. G. Pereira, M. A. A. Meireles: Supercritical Fluid Extraction of Bioactive Compounds: Fundamentals, Applications and Economic Perspectives, *Food Bioprocess. Technol.* **2010**, *3*, 340-372; http://dx.doi.org/10.1007/s11947-009-0263-2 .

J. Azmir, I. S. M. Zaidul, M. M. Rahman, K. M. Sharif, A. Mohamed, F. Sahena, M. H. A. Jakurul, K. Ghafoor, N. A. N. Norulaini, A. K. M. Omar: Techniques for extraction of bioactive compounds from plant materials: A review, *J. Food Eng.* **2013**, *117*, 426-436; http://dx.doi.org/10.1016/j.jfoodeng.2013.01.014 .

M. M. R. de Melo, A. J. D. Silvestre, C. M. Silva: Supercritical fluid extraction of vegetable matrices: Applications, trends and future perspectives of a convincing green technology, *J. Supercrit. Fluids* **2014**, *92*, 115-176; http://dx.doi.org/10.1016/j.supflu.2014.04.007 .

A. R. C. Morais, A. M. D. Lopes, R. Bogel-Lukasik: Carbon Dioxide in Biomass Processing: Contributions to the Green Biorefinery Concept, *Chem. Rev.* **2015**, *115*, 3-27; http://dx.doi.org/10.1021/cr500330z .

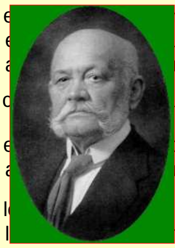
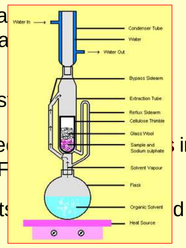
3) Extraction with liquids at elevated temperatures [$T > T_{boil}^{solvent}$] and pressures [$P > P_{sat}^{solvent}(T)$]

organic solvents / blends: PFE, PLE, PSE, ASE

PFE instruments - automated extractors:

Advantages of PFE compared with low-pressure Soxhlet extra

a) higher solubility of analytes because of their higher volatility at elevated temperature

b) e  nalytes from the sa 

e ure (faster mass tra
a ractions)

c) c nd b) results in fas

d) e power may be tune in T
a uch less than in SF

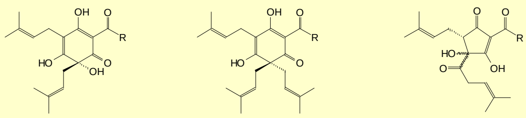
e) l of organic solvents d
l nvironment

f) tighter control of composition when using mixed solvents - unlike the Soxhlet extraction, PFE does not involve any solvent phase transition (vapor-liquid equilibrium)

PFE application – nutrition-relevant substances from plants

1) hops (cones, pellets)

alpha acids (humulones) beta acids (lupulones) isohumulones



R = -CH(CH_3)$_2$, -CH_2CH(CH_3)$_2$, -CH(CH_3)CH_2CH_3

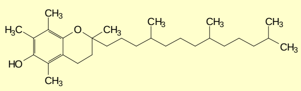
2) "Tea" plants

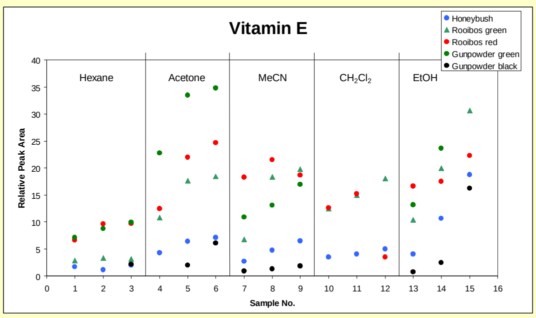
honeybush (*Cyclopia intermedia*) - South Africa

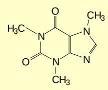
rooibos (*Aspalathus linearis*) - South Africa

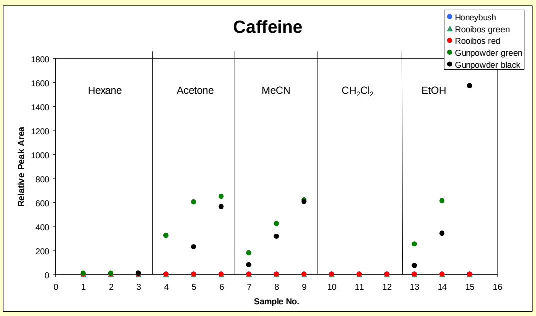
čaj (*Camelia sinensis*) - China

yerba maté (*Ilex paraguayensis*) - South America





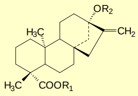




3) Stevioside

Stevia rebaudiana

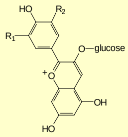
cca 300× sweeter than sucrose



stevioside:
R_1 = *β*-Glc
R_2 = *β*-Glc-*β*-Glc

steviol:
$R_1 = R_2$ = H

4) Antioxidants from grape skins: off-line PFE–EPR



De3glc: $R^1 = R^2$ = OH
Cy3glc: R^1 = OMe, R^2 = OH
Pt3glc: R^1 = OMe, R^2 = H
Pn3glc: $R^1 = R^2$ = OMe
Mv3glc: R^1 = OH, R^2 = H

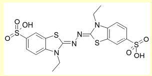
St. Laurent (Svatovavřinecké)
Alibernet

(ground lyophilized skins)

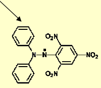
PFE:
MeOH, EtOH, 40–120°C, 15 MPa

antioxidanty from the extracts quench the radicals added to the system

time evolution of EPR signal
~ antioxidant aktivity of extract



2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) or ABTS



2,2-diphenyl-1-picrylhydrazyl (DPPH)

Review papers on PFE applications

a) Food analysis:

A. Mustafa, C. Turner: Pressurized liquid extraction as a green approach in food and herbal plants extraction: A review, *Anal. Chim. Acta* **2011**, *703*, 8-18; http://dx.doi.org/10.1016/j.aca.2011.07.018 .

A. Baiano: Recovery of Biomolecules from Food Wastes - A Review, *Molecules* **2014**, *19*, 14821-14842; http://dx.doi.org/10.3390/molecules190914821 .

C. C. Teo: Pressurized hot water extraction (PHWE), *J. Chromatogr. A* **2010**, *1217*, 2484-2494; http://dx.doi.org/10.1016/j.chroma.2009.12.050 .

S. M. Zakaria, S. M. M. Kamal: Subcritical Water Extraction of Bioactive Compounds from Plants and Algae: Applications in Pharmaceutical and Food Ingredients, *Food Eng. Rev.* **2016**, *8*, 23-34; http://dx.doi.org/10.1007/s12393-015-9119-x .

4) Pressurized hot (subcritical) water extraction

Motivation:
Water is not only the greenest but also the most tuneable solvent (through changes in operating T and P).

Standard conditions (25 °C, 0.1MPa):
NaCl well soluble, benzene nearly insoluble

“Supercritical” conditions (>374 °C, >22.1 MPa):
NaCl ~ insoluble, benzene ~ fully miscible

Applications of high temperature, high pressure water:

a) Supercritical water (t > 374 °C, P > 22 MPa)

supercritical water oxidation, SCWO
supercritical water dissolves SiO_2 – geochemistry, surfaces

b) Subkritická voda (100 °C < t < 374 °C, $P > P^{sat}(t)$)

„environmental remediation“
extraction of plant materials
analytical chemistry – sample preparation
biopolymers – cellulose dissolution, protein hydrolysis
biomass gasification – energy ($CO+H_2$)

Motivation

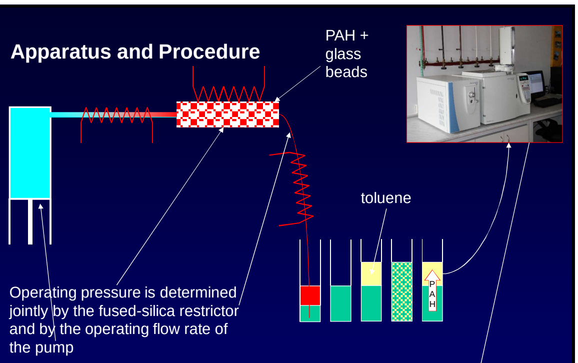
water = the "greenest" and the most "tuneable" solvent

Property	"ambient"	"supercritical"
	25 °C, 0.1 MPa	500 °C, 30 MPa
Density ρ / kg·m^{3}	997.0	115
Cohesive energy density **c** / J·cm^{-3}	2299	35.5
Solubility parameter δ / (J·cm^{-3})$^{1/2}$	47.9	5.96
Internal pressure P_{int} / MPa	169	32
Ion product K_w / (mol·dm^{-3})^{2}	1×10^{-14}	1.57×10^{-23}
Relative permittivity ε_r	78.4	1.68

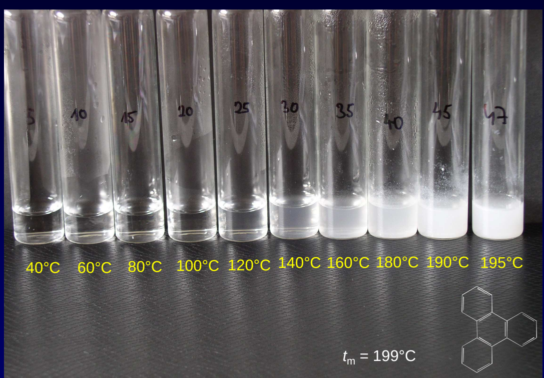
PHWE : 100°C < t < 374°C, $P > P_{sat}$ (t)

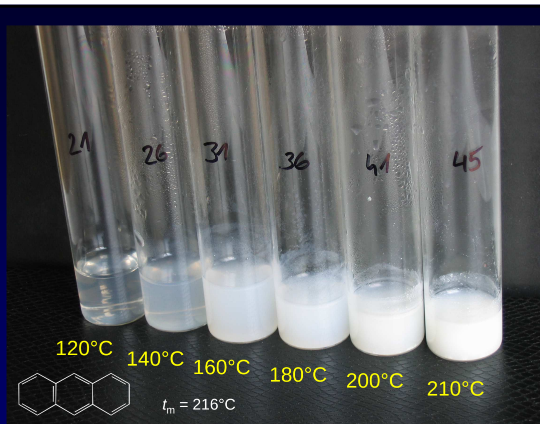
relative wealth of analytical applications of PHWE
×
relative lack of solubility data

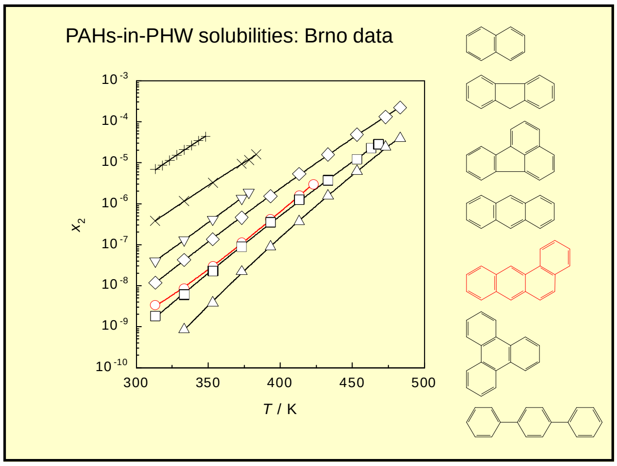
Apparatus and Procedure

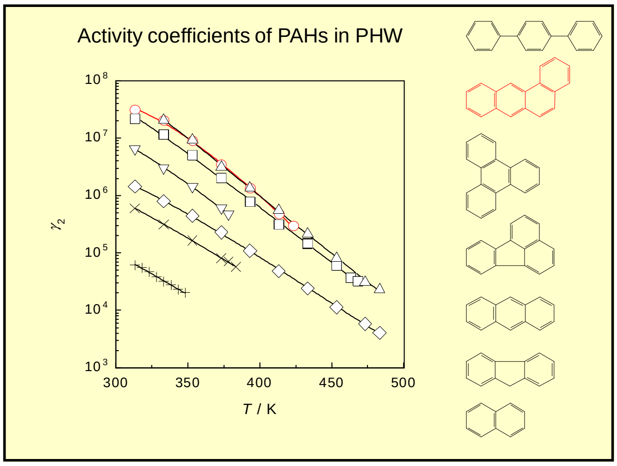


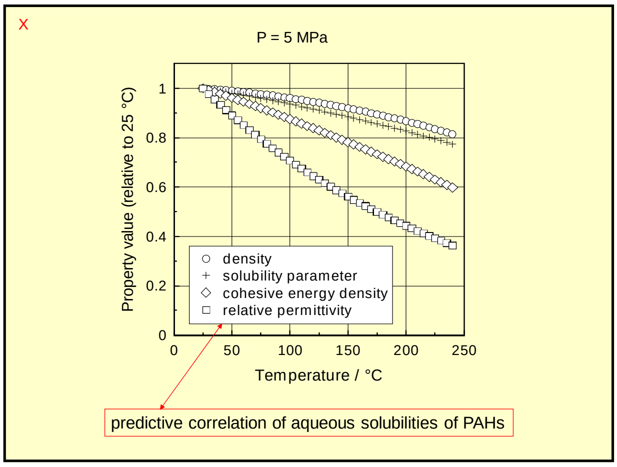
Peak areas of the PAH and the internal standard together with volumetric dilution factors make it possible to obtain the solubility (equilibrium mole fraction) of the PAH in pressurized hot water, x_2

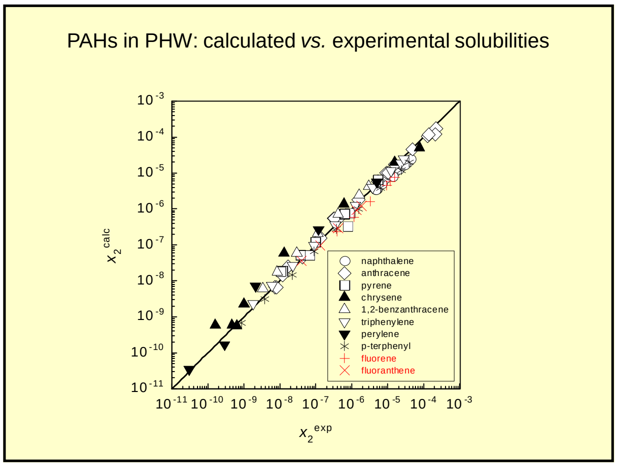


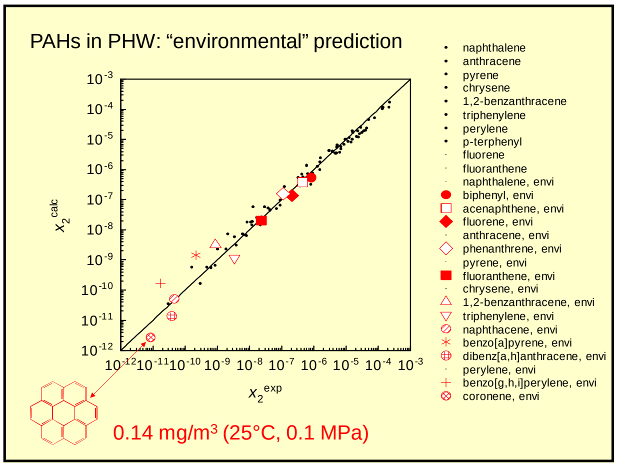


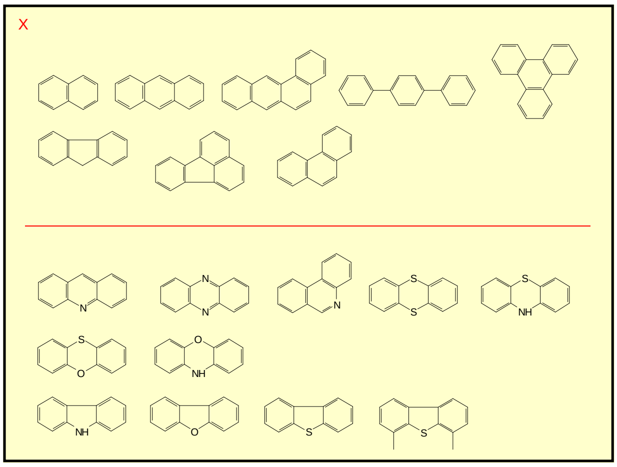

PAHs-in-PHW solubilities: Brno data
x2
T / K
300
350
400
450
500


Activity coefficients of PAHs in PHW
γ2
T / K
300
350
400
450
500

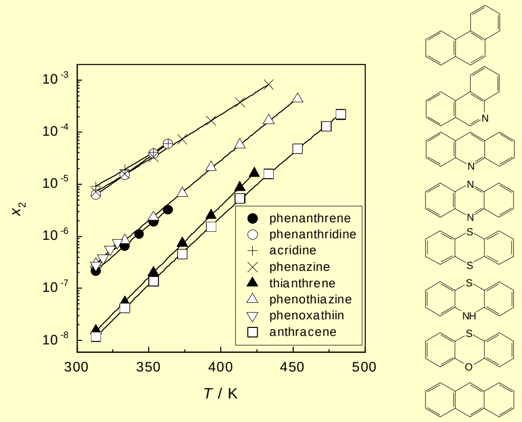

X
P = 5 MPa
Property value (relative to 25 °C)
Temperature / °C
density
solubility parameter
cohesive energy density
relative permittivity
predictive correlation of aqueous solubilities of PAHs


PAHs in PHW: calculated vs. experimental solubilities
naphthalene
anthracene
pyrene
chrysene
1,2-benzanthracene
triphenylene
perylene
p-terphenyl
fluorene
fluoranthene

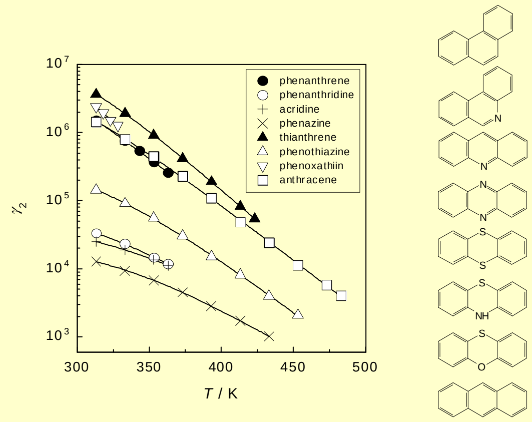

PAHs in PHW: “environmental” prediction
naphthalene
anthracene
pyrene
chrysene
1,2-benzanthracene
triphenylene
perylene
p-terphenyl
fluorene
fluoranthene
naphthalene, envi
biphenyl, envi
acenaphthene, envi
fluorene, envi
anthracene, envi
phenanthrene, envi
pyrene, envi
fluoranthene, envi
chrysene, envi
1,2-benzanthracene, envi
triphenylene, envi
naphthacene, envi
benzo[a]pyrene, envi
dibenz[a,h]anthracene, envi
perylene, envi
benzo[g,h,i]perylene, envi
coronene, envi
0.14 mg/m³ (25°C, 0.1 MPa)


X

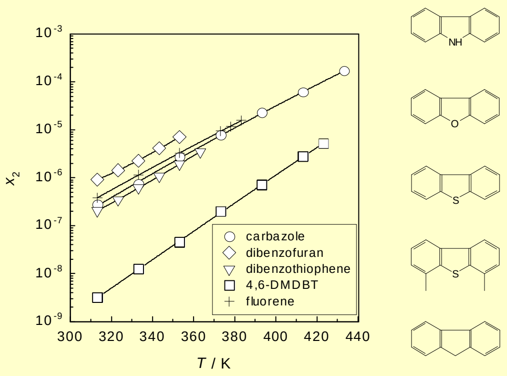
X



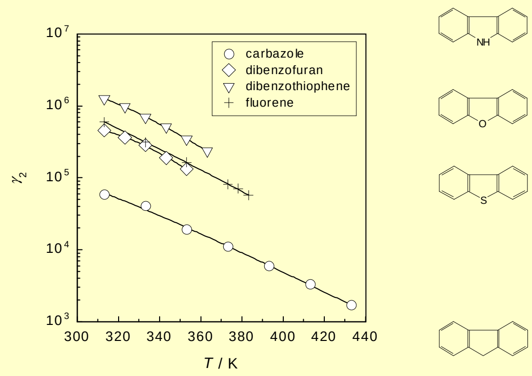
X

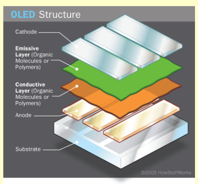


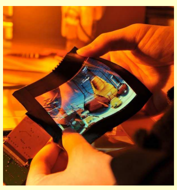
X



X



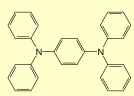




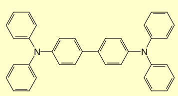
Organic Light Emitting Diode displays

Smartphones, tablets, etc. … OLED displays

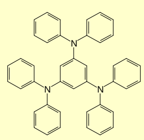
Increasing production ⇒ increasing rate of disposal
Environment ?? … Aqueous solubilities



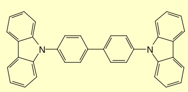
1,4-bis(diphenylamino)benzene



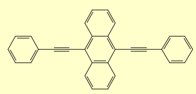
tetra-*N*-phenylbenzidine



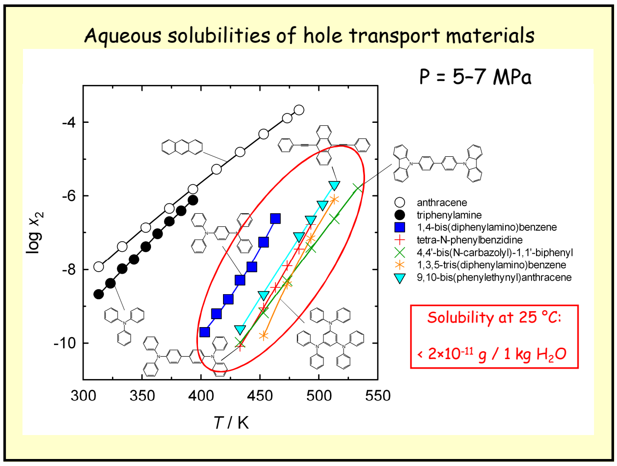
1,3,5-tris(diphenylamino)benzene

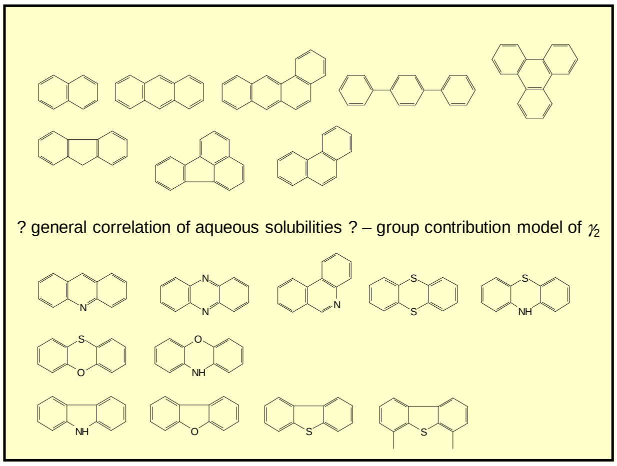


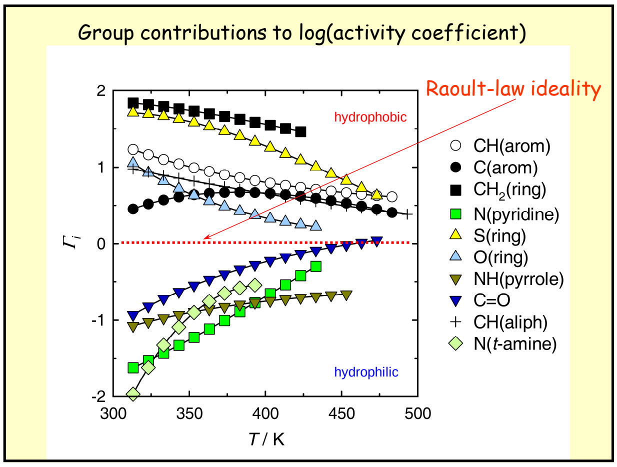
4,4'-bis(*N*-carbazolyl)-1,1'-biphenyl

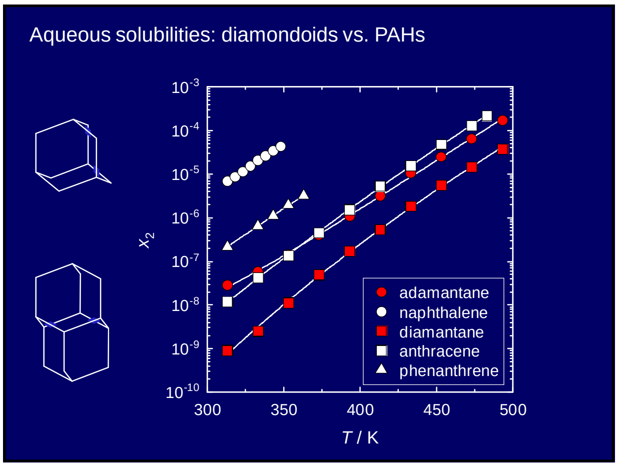


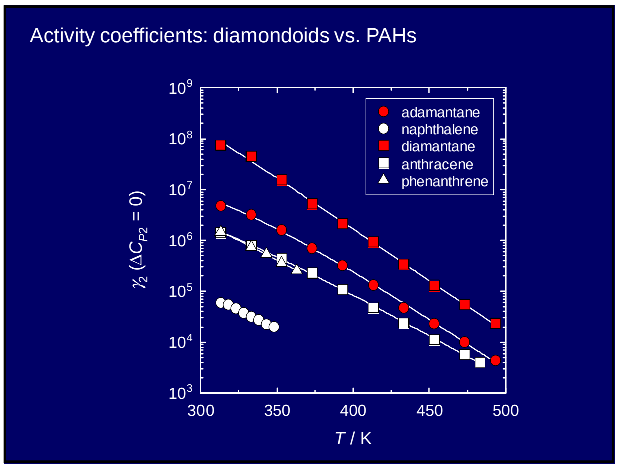
9,10-bis(phenylethynyl)anthracene


Aqueous solubilities of hole transport materials
P = 5-7 MPa
log x_2
T / K
anthracene
triphenylamine
1,4-bis(diphenylamino)benzene
tetra-N-phenylbenzidine
4,4'-bis(N-carbazolyl)-1,1'-biphenyl
1,3,5-tris(diphenylamino)benzene
9,10-bis(phenylethynyl)anthracene
Solubility at 25 °C:
< 2×10^{-11} g / 1 kg H_2O


? general correlation of aqueous solubilities ? – group contribution model of γ_2

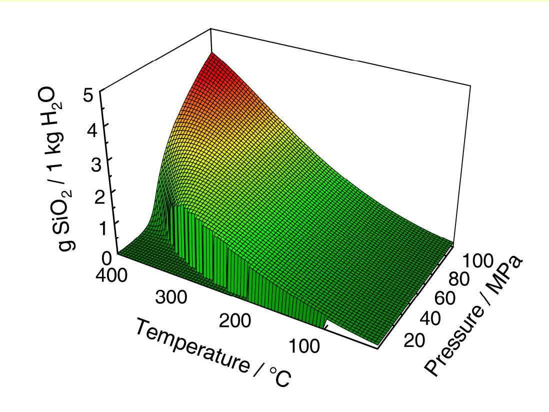

Group contributions to log(activity coefficient)
Raoult-law ideality
hydrophobic
hydrophilic
Γ_i
T / K
CH(arom)
C(arom)
CH_2(ring)
N(pyridine)
S(ring)
O(ring)
NH(pyrrole)
C=O
CH(aliph)
N(t-amine)


Aqueous solubilities: diamondoids vs. PAHs
x_2
T / K
adamantane
naphthalene
diamantane
anthracene
phenanthrene

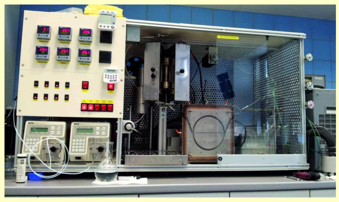

Activity coefficients: diamondoids vs. PAHs
γ_2 (ΔC_{P2} = 0)
T / K
adamantane
naphthalene
diamantane
anthracene
phenanthrene


5) Supercritical water vs. silica surfaces
SiO_2
Quartz
Fused silica
Both quartz and fused silica soluble in very hot water
⇓
Water as silica surface treatment agent for separation devices

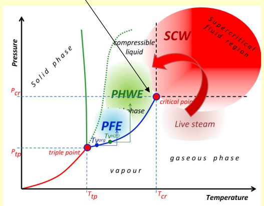
Aqueous solubility of fused silica vs. *T* and *P*

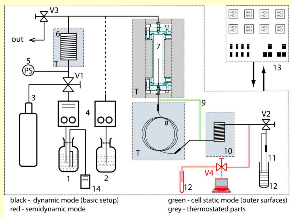


Multi-purpose extractor/reactor for applications of supercritical water (up to 500 °C and 75 MPa)

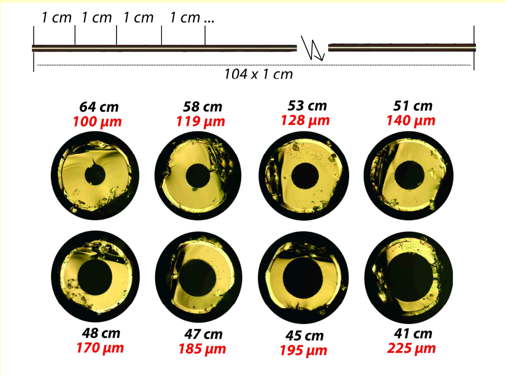


T_c = 374 °C
P_c = 22.1 MPa

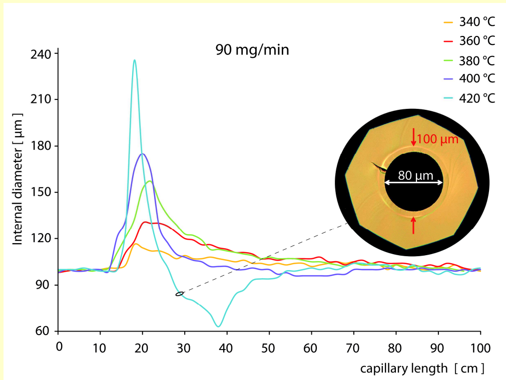




Tapered capillaries

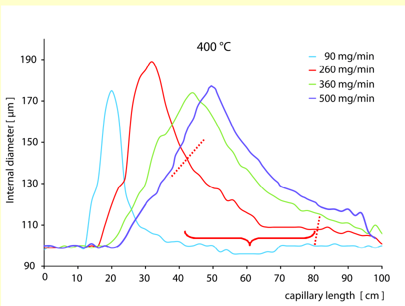


Tapered capillaries – internal diameter profiles

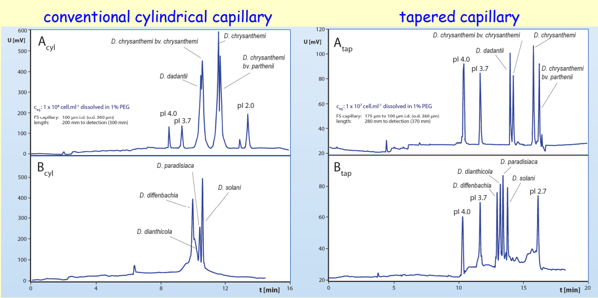


Tapered capillaries – what are they good for ?

Karel Šlais (1995) – theory – tapered capillaries can improve resolution of ampholytes in capillary isoelectric focusing (CIEF)



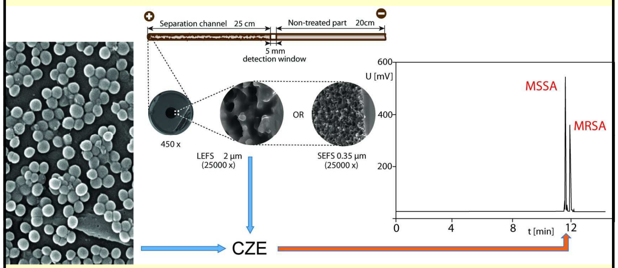
Tapered capillaries – how do they work in CIEF ?



Separation of several Dickeya bacterium species with mutually close isoelectric points by CIEF with 2.0–4.0 pH gradient. Left – conventional cylindrical capillary; Right – tapered capillary.

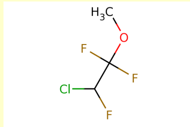
Inner surface-etched capillaries of uniform diameter

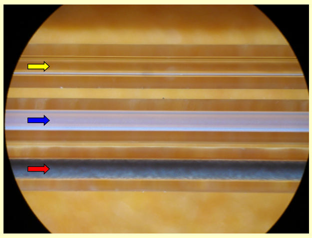
Etching with SCW can also be arranged so that the capillary internal diameter remains constant but the inner surface acquires a "porous" structure" with cavities of defined size



MRSA = methicillin-resistant *Staphylococcus aureus*
MSSA = methicillin-sensitive *Staphylococcus aureus*

Columns - troubles in surface treatment – an example





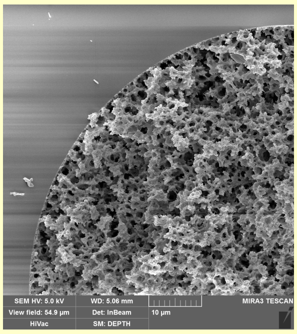
⇨ untreated fused silica capillary (100 µm i.d.)

➡ fused silica capillary after etching with 2-chloro-1,1,2-trifluoroethyl methyl ether (**33 %** of capillary volume filled with the liquid ether, capillary sealed, 320 °C, 12 hours) followed by etching with saturated methanolic solution of ammonium hydrogen difluoride (25 °C, 24 hours)

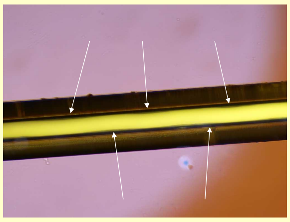
➡ fused silica capillary after etching with 2-chloro-1,1,2-trifluoroethyl methyl ether (**50 %** of capillary volume filled with the liquid ether, capillary sealed, 350 °C, 12 hours); black coloration comes from the carbon soot produced by decomposition of the ether

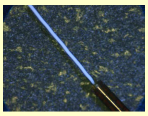
Monolithic silica (capillary) columns

Skill AND good luck

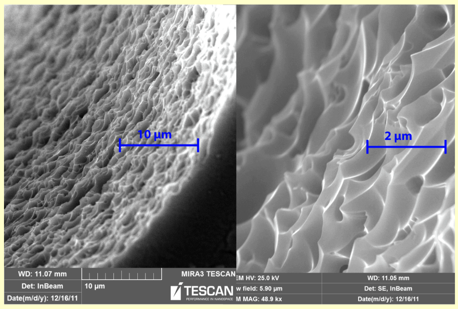


Skill and BAD luck



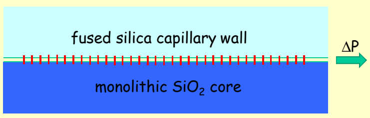


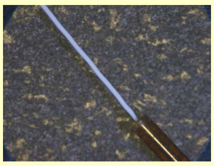
REALLY BAD luck



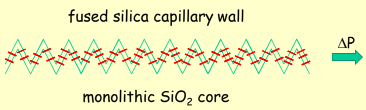
400 °C, 32 MPa, semi-dynamic mode, 20 replacements of SCW inside the capillary

Surface roughening *versus* mechanical stability of the column

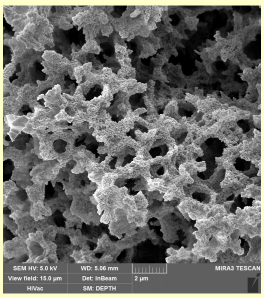


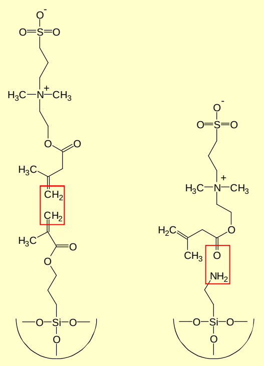


I = Si–O–Si wall–core bonds

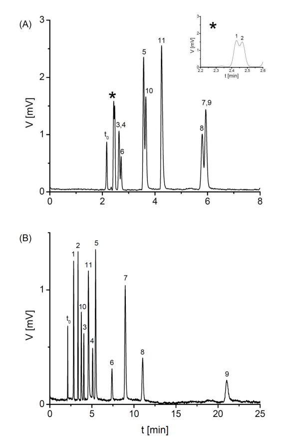


SiO_2 Monolith Modification (for HILIC)





Separation of Nucleic Acid Bases and their Derivatives



Comparison of isocratic elution on bare silica monolithic (A) and sulfoalkylbetaine monolithic (B) capillary columns. Mobile phase: 95% (v/v) ACN/50 mM ammonium formate, pH = 4.5, flow rate 0.5 µl/min; detection: UV 210 nm; sample: toluene (t_0 marker), thymine (1), uracil (2), 2-deoxyuridine (3), 5-methyluridine (4), adenosine (5), uridine (6), cytosine (7), 2-deoxycytidine (8), cytidine (9), 2-deoxyadenosine (10), adenine (11), and adenosine (12).

Thank you for your attention