

CE vs. HPLC analysis methods used for analyzing sugars and sugar derivatives in IL media obtained from lignocellulosic biomass

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Content of the presentation

- Samples
- Experimental procedures
- Challenges caused by ionic liquids (ILs)
- Analysis method comparison
- Disadvantages and advantages of IL process and the analysis methods used

SAMPLES

POKE Workshop, June 11-12, 2013
Stockholm

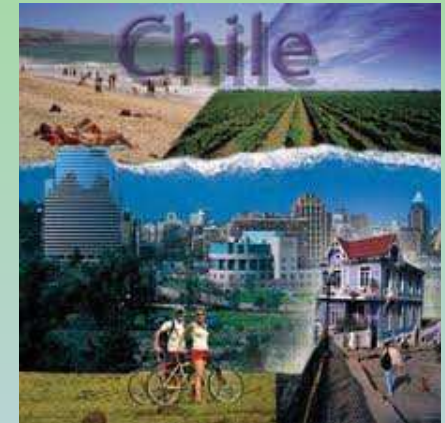
LIGNOCELLULOSICS studied so far ...

Softwood
from
Central Finland:

- **Scots pine**
(*Pinus sylvestris*)
- **Norway spruce**
(*Picea abies*)
- **Silver Birch**
(*Betula pendula*)

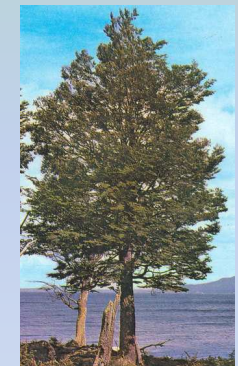


...and in Chile



Forest residues:

- **Eucalyptus**
- **Lenga**
(*Nothofagus pumilio*)



Crops residues:

- **Corn**
- **Wheat straw**

Sample collecting and sampling

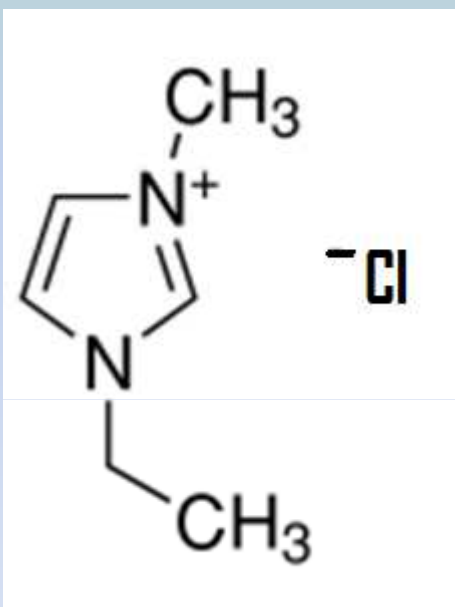
To be considered before collecting sample

- Things that might affect the results

- heart wood vs. sapwood
- growing place (e.g. heavy metals in soil?)
- age of wood
- condition/healthy of wood (fungi etc.)
- height where sample is taken from (1.2 - 1.50 m)
- Usually samples should be free from reaction wood & compression wood, branches and knots

1-Ethyl-3-methylimidazolium chloride

EmimCl or **[emim]⁺ [Cl]⁻**



Molar mass:	146,62 g/mol
Density	1,1120 g/cm ³ (at 80 °C)
Melting point	77-80 °C ← impurities effect
Flash point:	186 °C
Viscosity	47.4 mPas (at 80 °C)
Flame point	515 °C
Solubility in water	∞

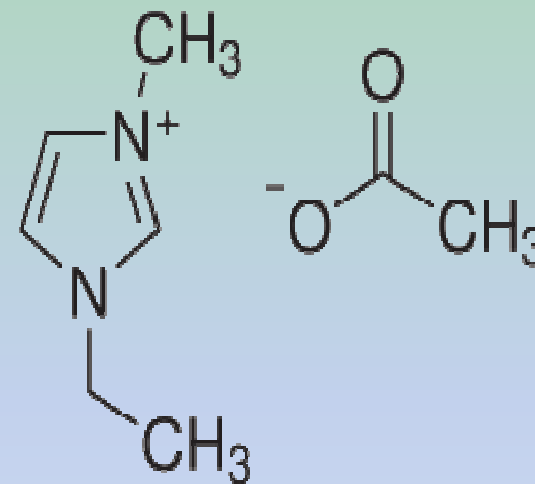
Very hygroscopic

Information concerning
melting point varies in
literature

1-Ethyl-3-methylimidazolium acetate

EmimOAc or **[emim]⁺ [CH₃COO]⁻**

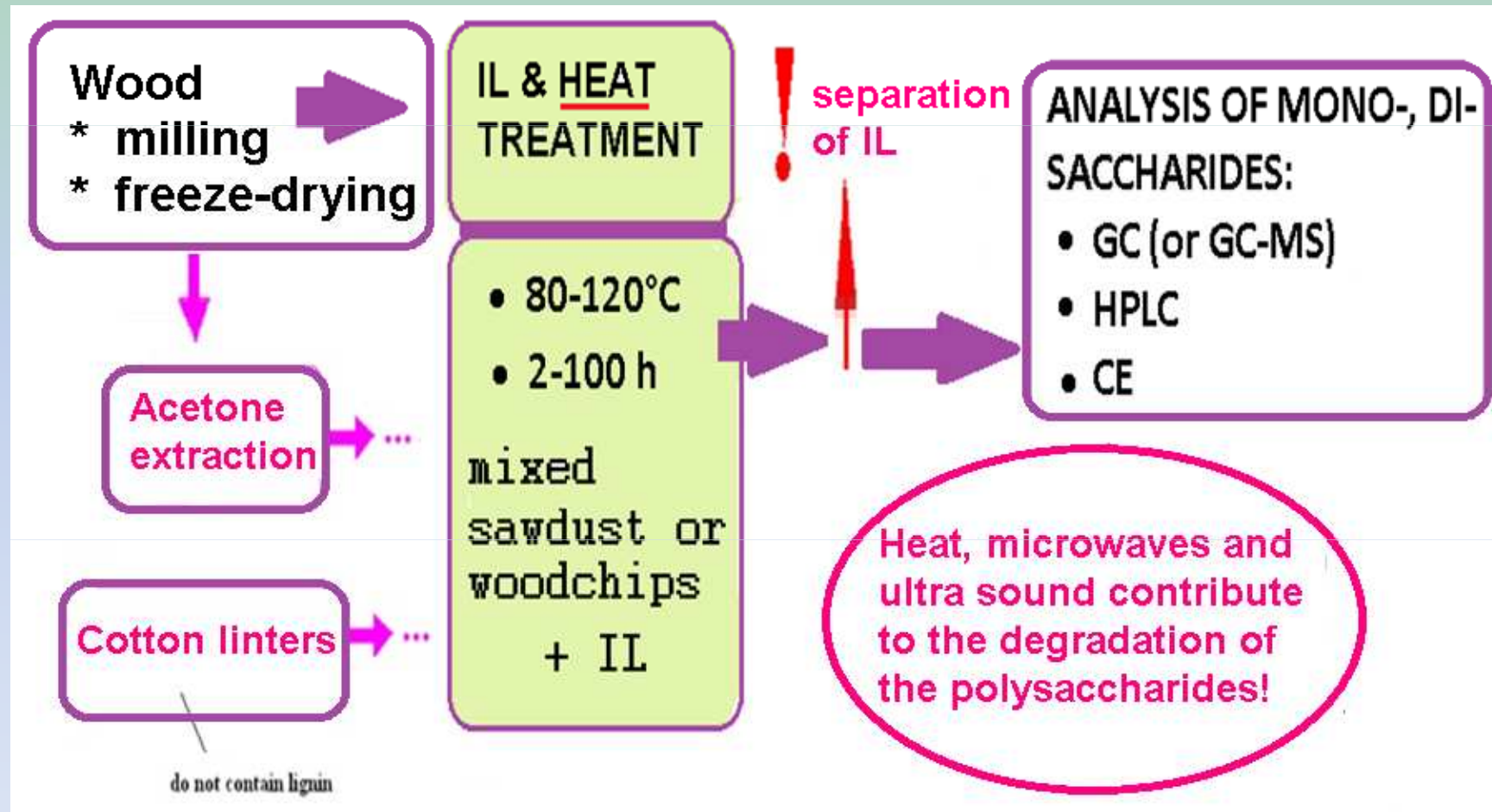
- Molar mass: 170,21 g/mol
- Density 1.027 g/cm³ at 25 °C
- Melting point > 30 °C
- Flash point: 164 °C
- Viscosity **10** mPas (at 80 °C)
- Solubility in water **∞**



EXPERIMENTAL PROCEDURES

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Experimental conditions & procedures:

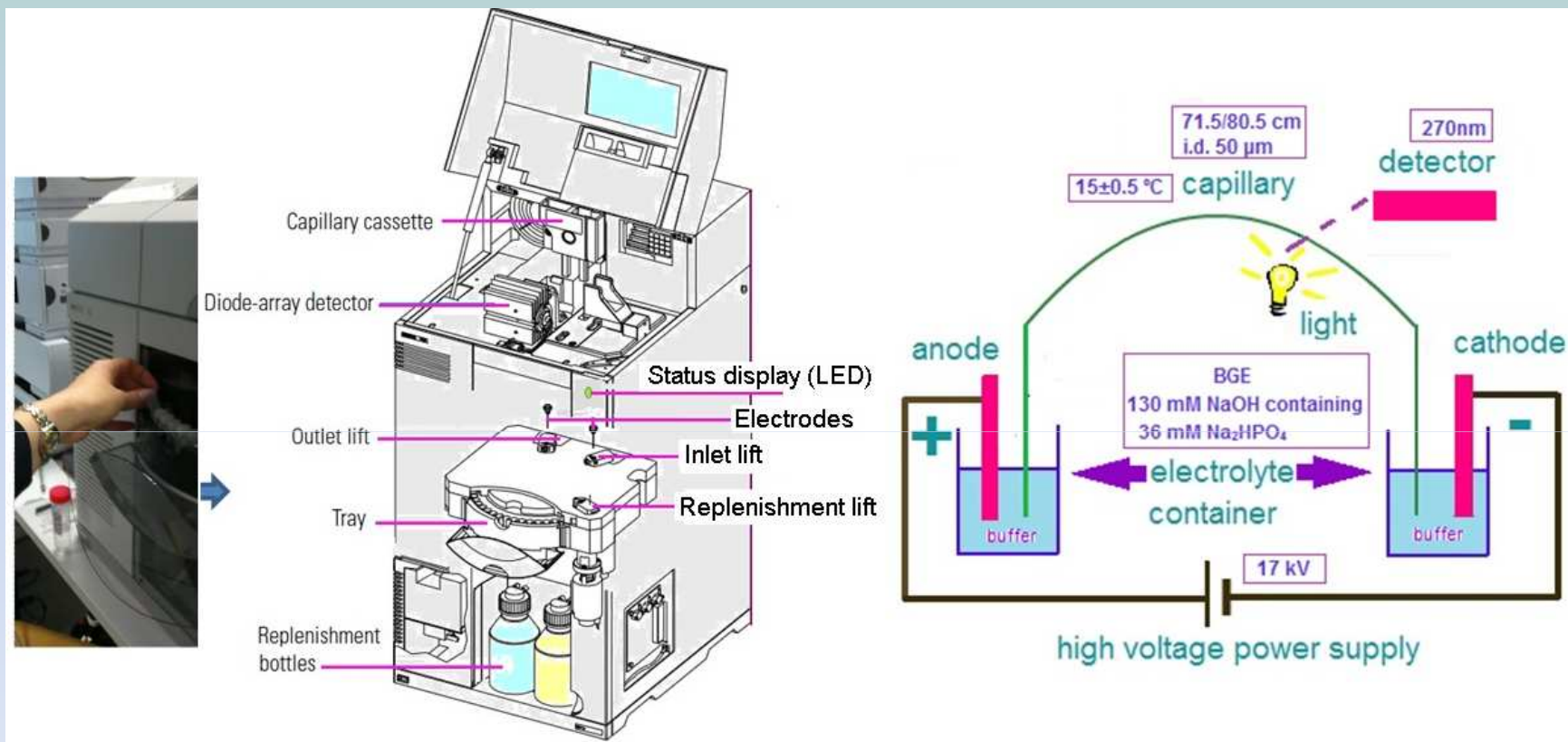


THE ANALYSIS METHODS (CE, GC & HPLC) AND COMPARISON OF THEM IN THIS PARTICULAR CASE

(FOR ANALYSIS OF CARBOHYDRATES & THEIR
DEGRADATION PRODUCTS IN LIGNOCELLULOSIC
SAMPLES IN THE PRESENCE OF IONIC LIQUIDS)

CE (Capillary electrophoresis)

- Separates ions based on their electrophoretic mobility
- Electrophoretic mobility depends on the charge of the molecule, the viscosity, and the atom's radius.



CE (Capillary electrophoresis)

- Detector: diode array UV/Vis
- Background electrolyte/buffer solution:

130 mM NaOH containing 36 mM Na_2HPO_4

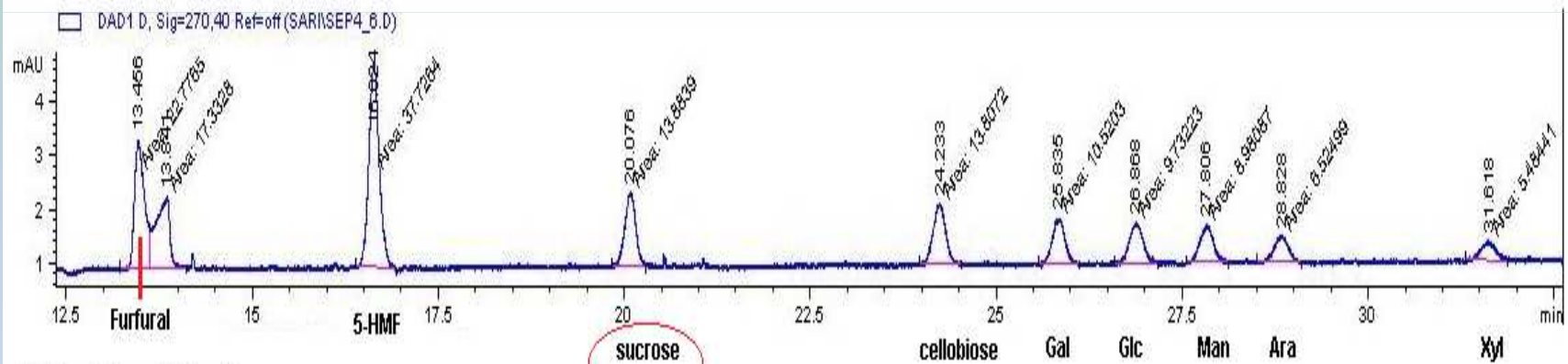
❖ must always be fresh

❖ **Sample derivatization is not needed**

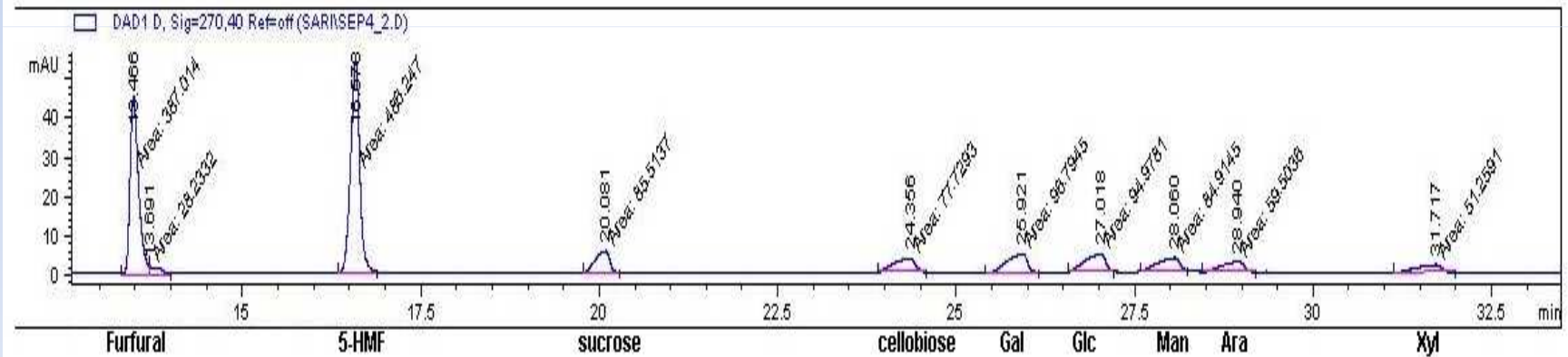
→ instead: dilution, centrifugation & filtration

CE calibration standards

STD mixture 0.10 mM



STD mixture 2.00 mM



GC analysis

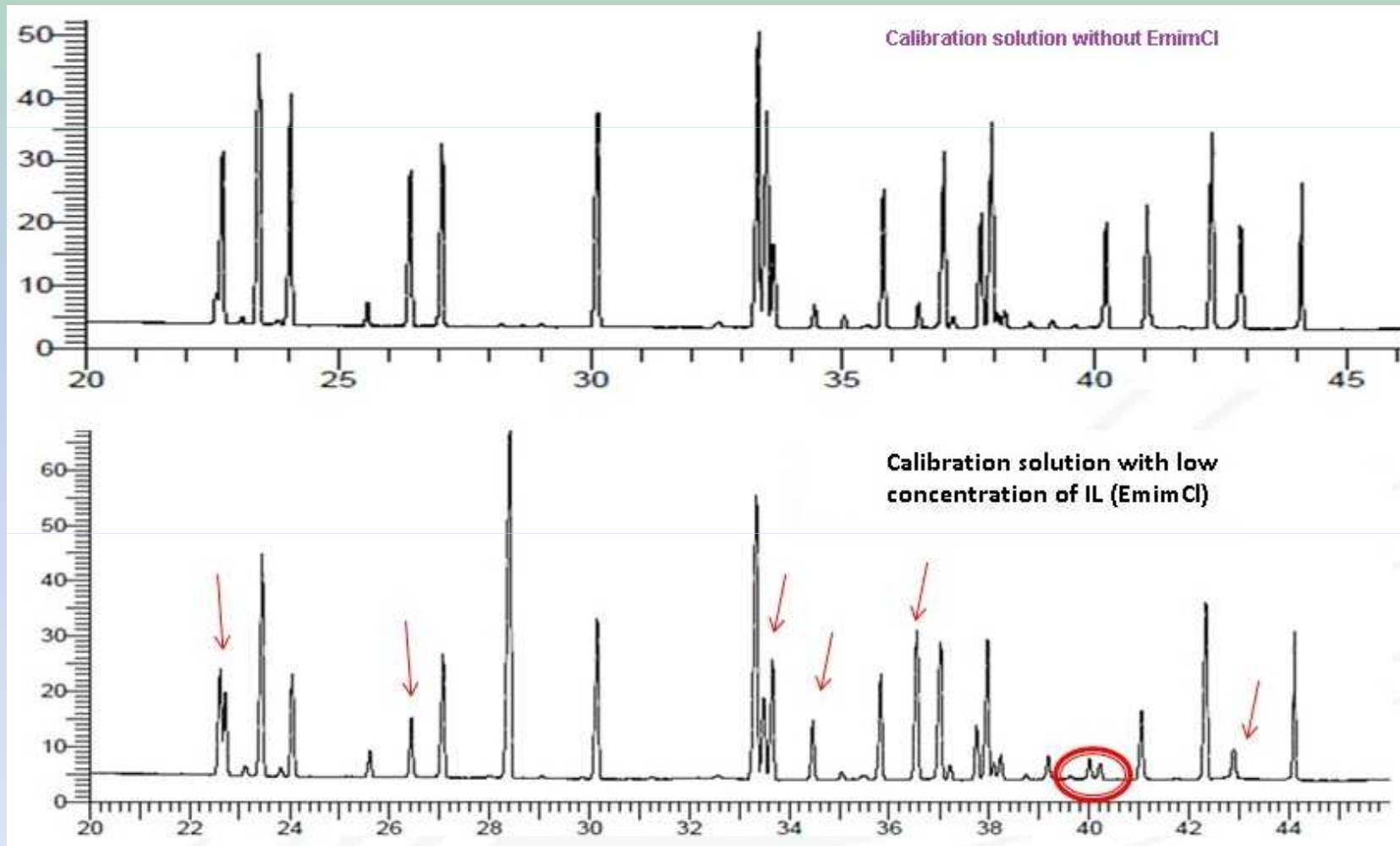
Analysis of carbohydrates on extract Without acid methanolysis

- calibration samples: STD sugars with xylitol in MeOH
- ISTD: xylitol instead of sorbitol
- Evaporation (N_2) + vacuum oven →
- silylation:
pyridine, HMDS and TMCS
- shaking (and ultrasonic bath)



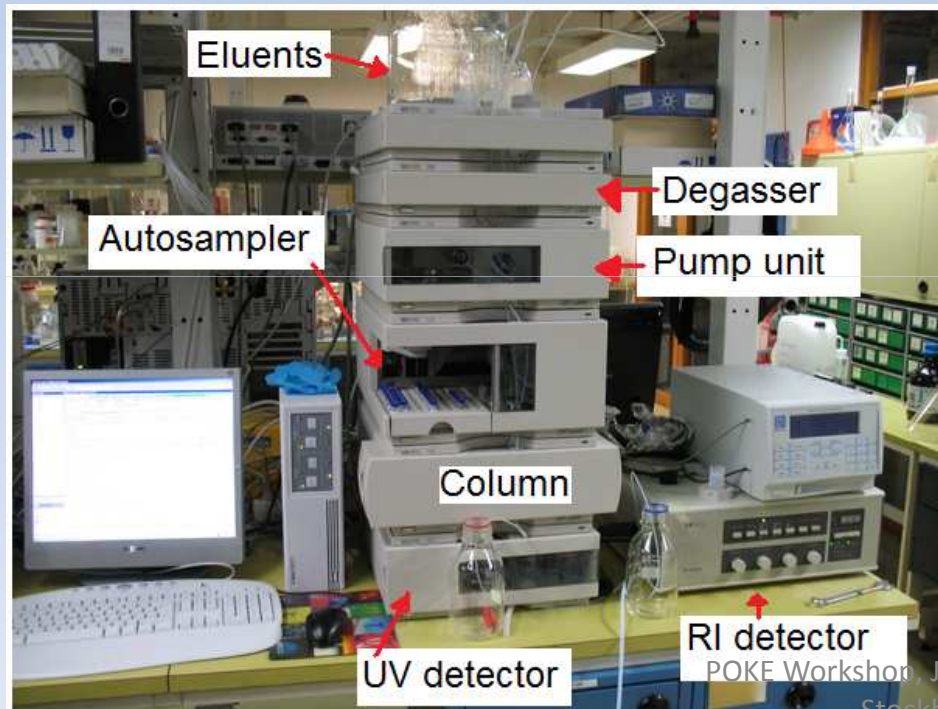
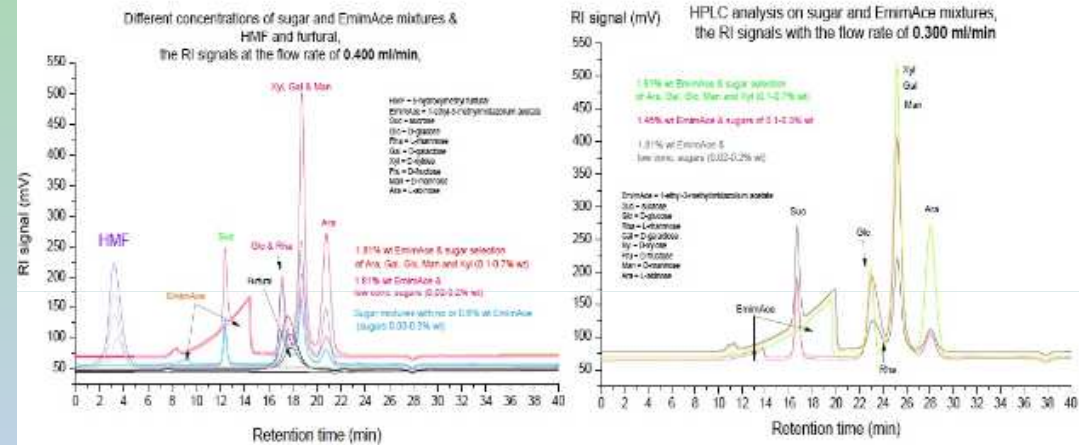
For acid methanolysis:
water-free 2 M HCl in
methanol
ISTD: sorbitol

Reliability problem with GC monosaccharide calibration



HPLC analysis

...Sample results: HPLC analysis



Chromatography involves a mass transfer process involving adsorption

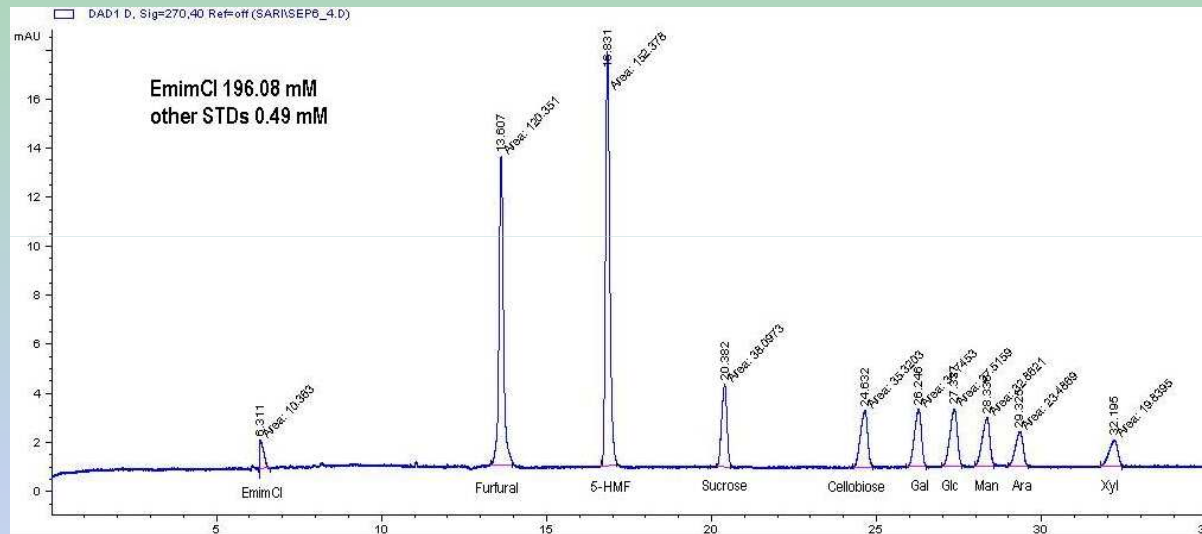
Challenges in monosaccharide analysis

(HPLC)

- anomer mutarotation → wide & split peaks
- loss of reducing sugars (T)
- formation of Schiff bases ($R^1R^2C=NR^3$)
- shortened column lifetime
- long analysis time
- salt interferences

<http://www.sepscience.com/Sectors/Enviro/Articles/521-/Overcoming-Challenges-in-Carbohydrate-Separations>

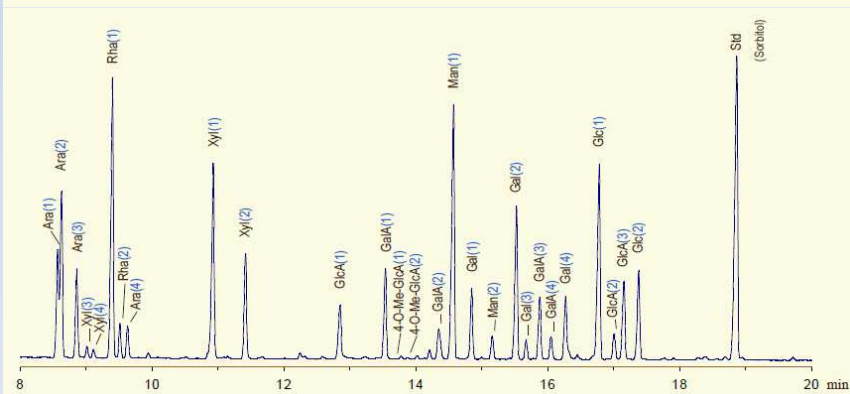
HPLC & GC chromatograms vs. CE electropherogram



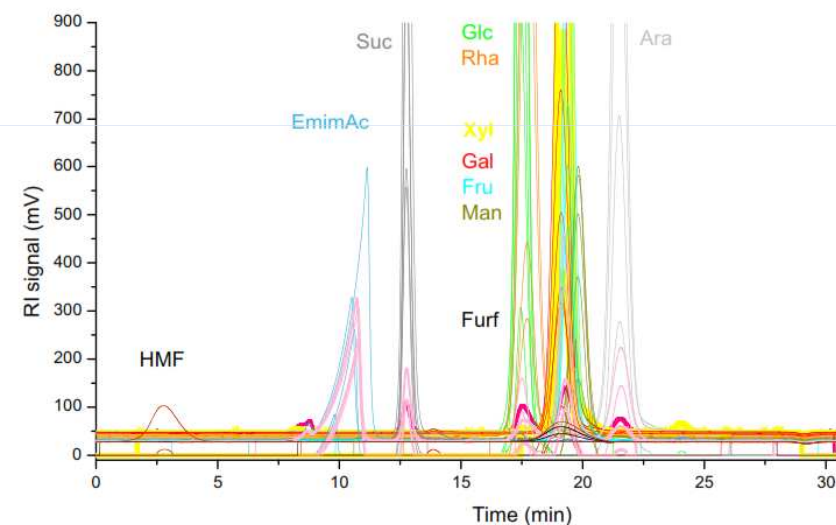
TMS-derivat av metanoliserade sockrar
HP-1, 25 m x 0,20 mm; 100 °C, 4 °C/min, 175 °C, 12 °C/min, 290 °C, 5 min

Kalibrering

Monomera sockrar



HPLC chromatogram for sugar and some other standard solutions



Comparison summary (CE, HPLC, GC)

- CE analysis is the method of choice
- GC (+ STD sugar column) is unreliable when ILs are present: concentration of IL is critical
- HPLC suffers from peak overlapping

Conclusions

IL pretreatment leads to significant formation of HMF/furfurals (like all depolymerization/degradation processes) – good indicator to follow up (e.g. fermentation inhibitors, degree of depolymerization, colorization ...)

THANK YOU!



COST ACTION CM0903



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