One column fits all: Hypercrosslinked monoliths for reversed-phase and size-exclusion capillary liquid chromatography

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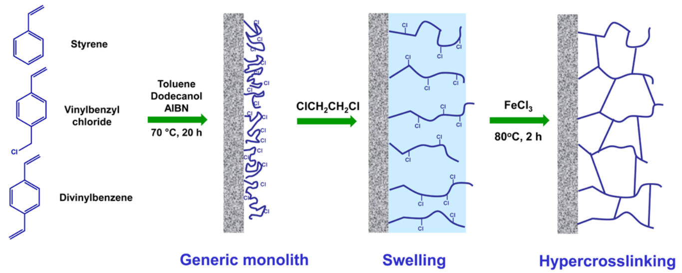
Hypercrosslinking

2nd generation of monolithic phases

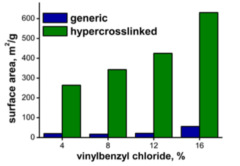
The **unfavorable reactivity** ratios for monomers lead to porous polymer structures that are amenable to hypercrosslinking. The **divinylbenzene polymerizes faster** then monovinyl styrene and vinylbenzyl chloride. Thus, remaining monomer **mixture becomes significantly richer in the monovinyl monomers** as the polymerization reaction approaches completion and affords only slightly crosslinked chains attached to the surface of highly crosslinked microglobular scaffolds. After **solvation with a thermodynamically good solvent**, this **layer can be crosslinked via the Friedel-Crafts reaction**. The polymer chains become fixed in their solvated state during the reaction thus forming pores that persist even after the solvent is removed.



J. Urban, F. Svec, J.M.J. Fréchet, *Anal. Chem.* 82 (2010) 1621.

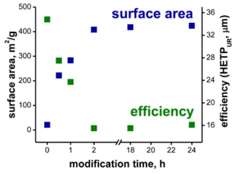
Hypercrosslinking conditions control:

Surface area



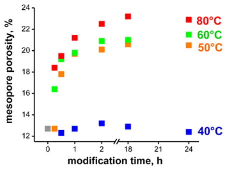
Polymer modified for 24 h at 80°C, styrene 0 - 14%, divinylbenzene 24%, toluene 18%, 1-dodecanol 42%, nitrogen absorption, BET calculation

Column efficiency



Styrene 12%, vinylbenzyl chloride 12%, divinylbenzene 16%, toluene 18%, 1-dodecanol 42%, Surface area from BET calculation, HETP for non-retained uracil

Mesopore porosity



Mesopore porosity calculated from ISEC data. Styrene 21%, vinylbenzyl chloride 7%, divinylbenzene 12%, toluene 19%, 1-dodecanol 41%

Separation of small molecules

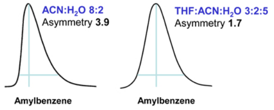
Suppression of peak tailing

Sources of peak tailing

- discontinuous degree of crosslinking
- π interactions on the stationary phase

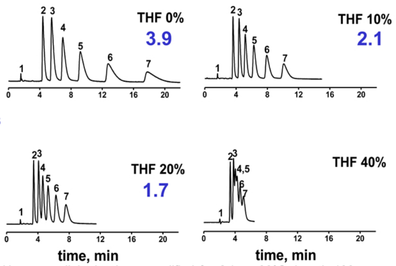
Solution?

THF in mobile phase



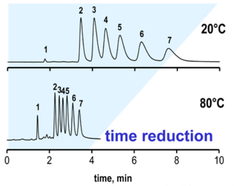
THF in mobile phase significantly reduces peak tailing as well as retention

Assymetry factors for amylbenzene (peak 7)

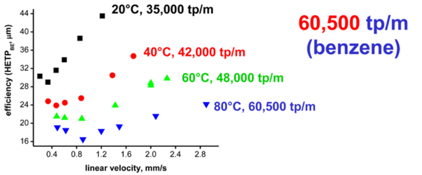


Hypercrosslinked column modified for 2 h at 90°C, length 130 mm, flow rate 0.5 µL/min, ternary mobile phase with 20% water. Uracil, benzene, toluene, ethylbenzene, propylbenzene, butylbenzene, amylbenzene

Effect of increased temperature



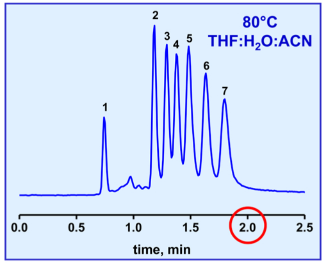
Hypercrosslinked column modified for 2 h at 90°C, length 130 mm, flow rate 0.5 µL/min, mobile phase 20% water, 20% THF, 60% ACN. Uracil, benzene, toluene, ethylbenzene, propylbenzene, butylbenzene, amylbenzene



60,500 tp/m (benzene)

Hypercrosslinked column modified for 2 h at 90°C, length 130 mm, mobile phase 20% water, 20% THF and 60% ACN

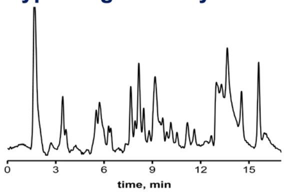
Fast and efficient isocratic separation using high temperature and ternary mobile phase



Hypercrosslinked column modified for 2 h at 90°C, length 130 mm, flow rate1.0 µL/min, pressure 260 bar, mobile phase 20% water, 20% THF and 60% ACN, Uracil, benzene, toluene, ethylbenzene, propylbenzene, butylbenzene, amylbenzene

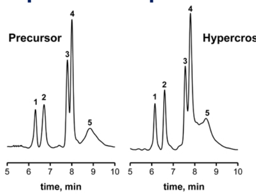
Gradient elution of peptides & proteins

Tryptic digest of cytochrome C



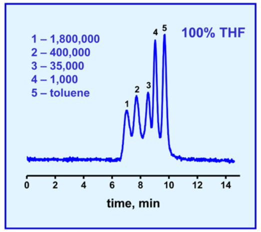
Column modified for 2 h at 90°C, length 130 mm, flow rate 0.5 µL/min, gradient 5 - 40% acetonitrile in 0.1% aqueous formic acid in 10 min, starting presure 240 b, gradient delay 5 min, UV detection

Separation of proteins



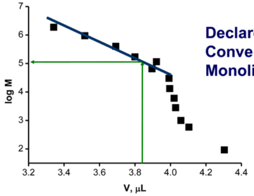
Column length 161 mm (A), 145 mm (B); Mobile phase gradient 5–80% acetonitrile in 0.1% aqueous formic acid in 10 min; flow rate 0.5 µL/min; UV detection at 214 nm. Ribonuclease (1), cytochrome C (2), myoglobin (3), a-chymotrypsin A (4), albumin (5).

Size-exclusion chromatography



Hypercrosslinked column modified for 2 h at 80°C, length 670 mm (320 + 350 mm), flow rate 0.5 µL/min, pressure 205 bar, 100% THF as mobile phase, polystyrene standards and toluene

Molar mass determination



Declared: 99 209
Conventional SEC: 99 459
Monolithic SEC: 107 438

Fast size-exclusion separation of synthetic polymers on organic polymer monoliths

2.5x faster than conventional SEC

Conclusions

- Composition of **polymerization mixture controls the porous properties** of hypercrosslinked monoliths.
- **Addition of THF** to the mobile phase significantly **decreases peak tailing and retention**.
- **Increased temperature allows fast** and **highly efficient separation** of small molecules.
- Hypercrosslinked monoliths are suitable for **fast gradient separations** of peptides
- **Good size-exclusion separation** of synthetic polymers was achieved.

Acknowledgements

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