

POLYMERNÍ MONOLITY

Pokročilá
kapalinová
chromatografie



Jiří Urban, Ústav chemie, Přírodovědecká fakulta, Masarykova univerzita, Brno, urban@chemi.muni.cz

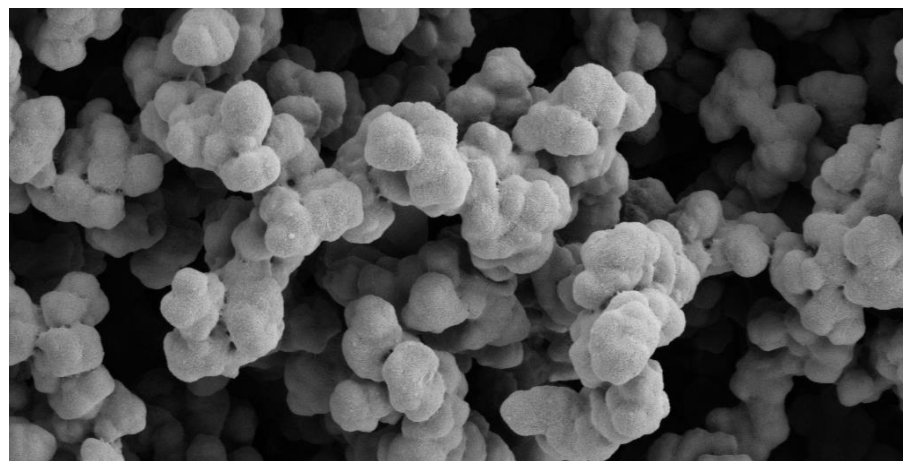
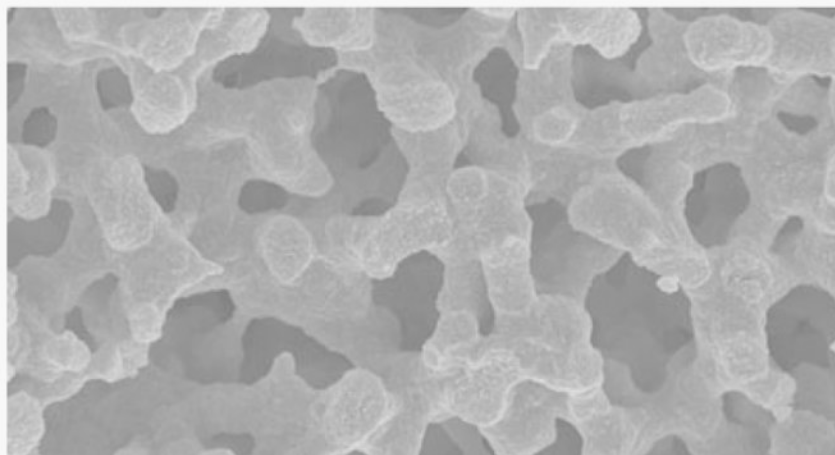
SVĚTOVÉ MONOLITY



www.crystalinks.com



Peter Polichronis



RADIKÁLOVÁ POLYMERACE



HISTORIE

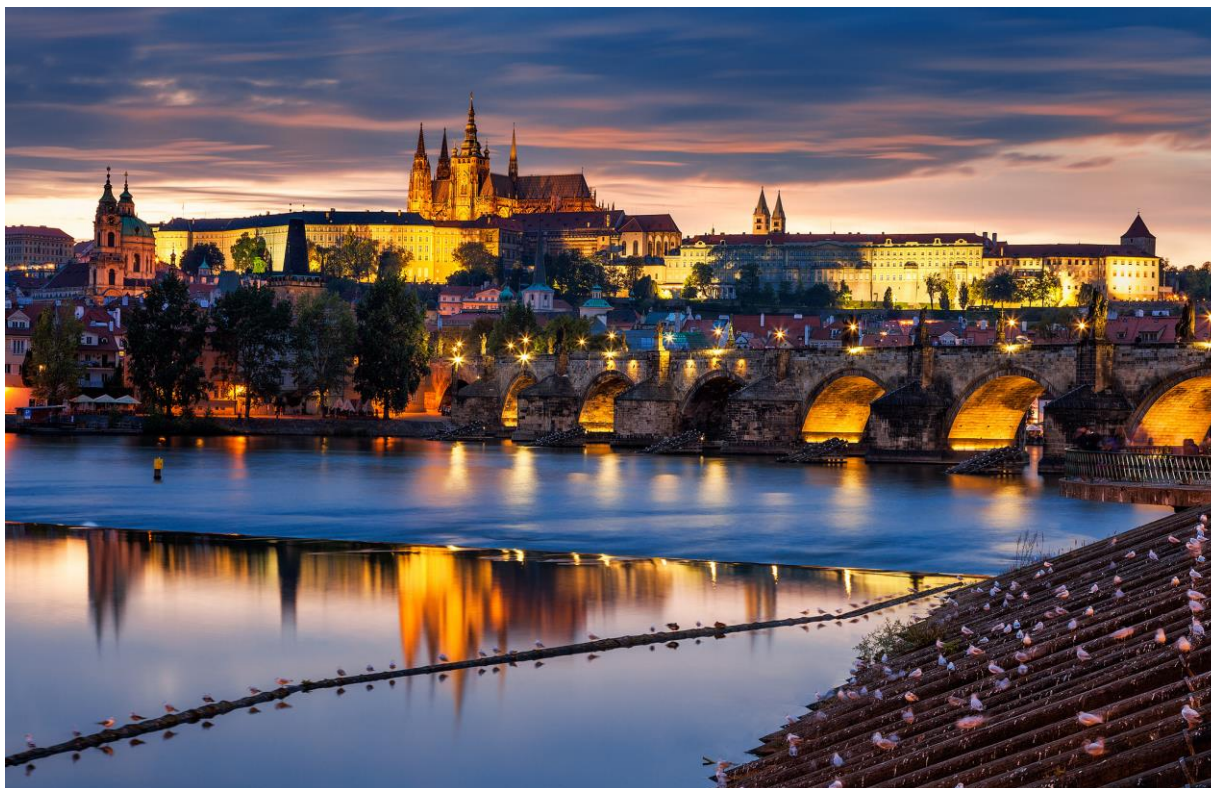
2-hydroxyethyl-methakrylátové gely

- 22% vodný roztok
- Nízká permeabilita (4 ml/h)
- Nízká účinnost

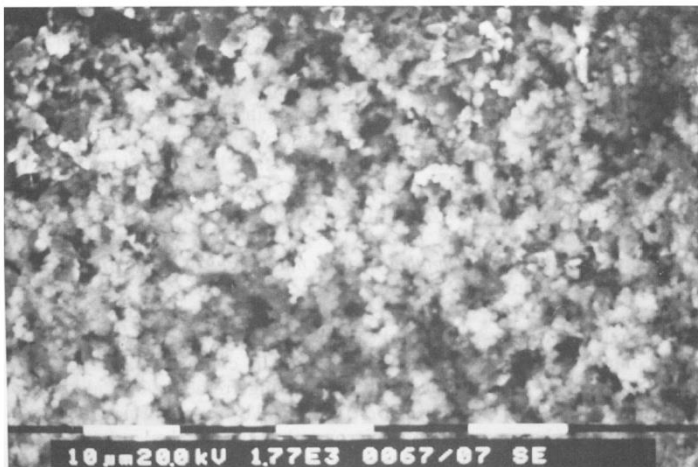
Kubín M., Špaček P., Chromeček R.: Collect. Czech. Chem. Commun. 32, 3881 (1967).

Polyuretany

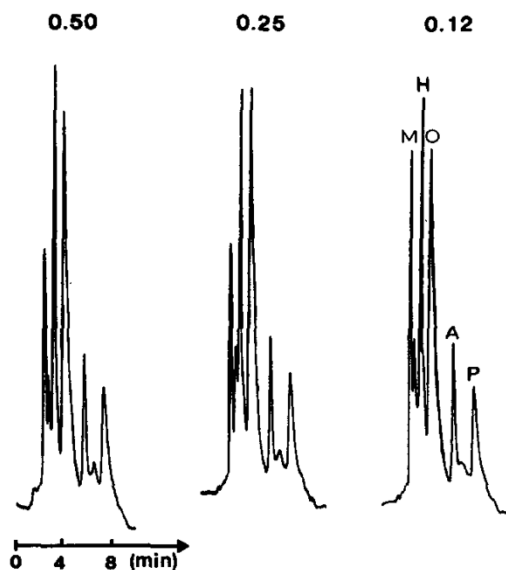
- Polyurethanové pěny s otevřenými póry připravené napěněním *in situ*
- Silikagel
- Ross W. D., Jefferson R. T.: J. Chrom. Sci. 8, 386 (1970).
- Hansen L. C., Sievers R. E.: J. Chromatogr. 99, 123 (1974).



STLAČENÉ GELY

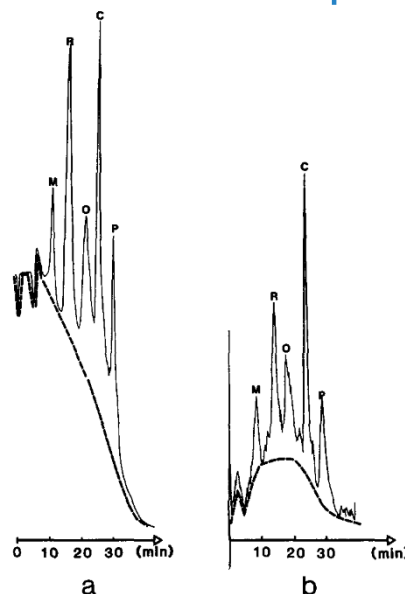


Vliv průtoku mobilní fáze



- Polymerace vodného roztoku N,N'-methylenebisakrylamidu a kyseliny akrylové v přítomnosti anorganické soli, nejčastěji síranu amonného
- Stlačení na méně než 10 % původního objemu
- Separace bílkovin v iontově-výměnném módu

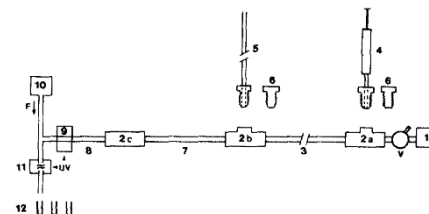
(neočekávané) zvýšení permeability
po stlačení gelu



Lineární gradient

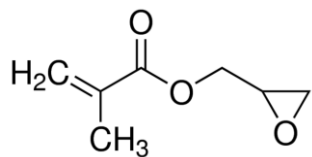
2.25 – 0.25 M síran amonný

- myoglobin (M)
- ribonuclease (R)
- ovalbumin (O)
- α -chymotrypsinogen A (C)
- phycoerythrin (P)

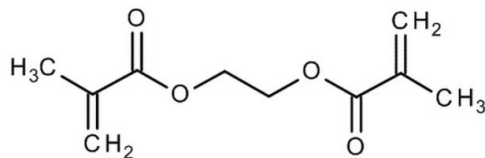


MEMBRÁNOVÁ CHROMATOGRRAFIE

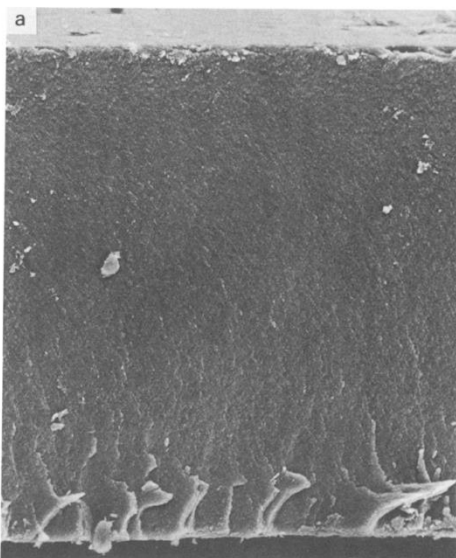
Pro separace vysokomolekulárních látek je potřeba velmi krátká kolona (B.G. Belenkii)



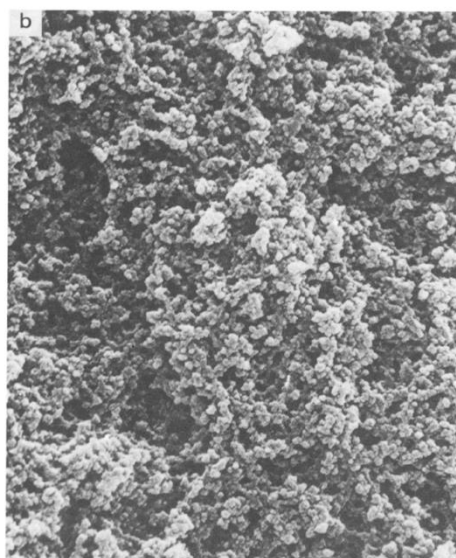
Glycidyl metakrylát



Ethylen dimetakrylát

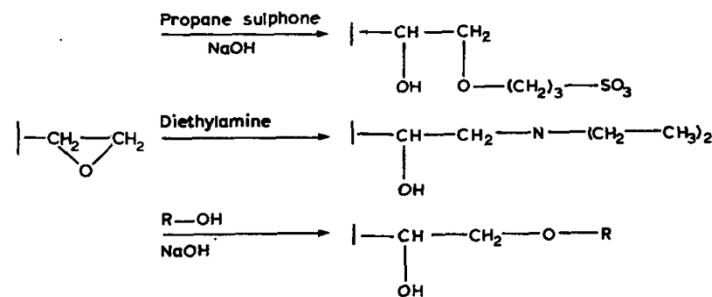


800x



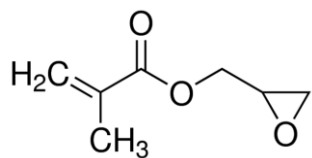
5400x

Reaktivní povrch

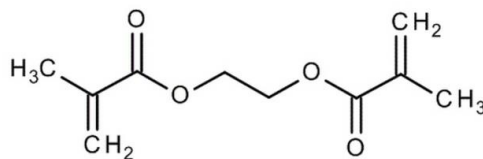


MEMBRÁNOVÁ CHROMATOGRAFIE

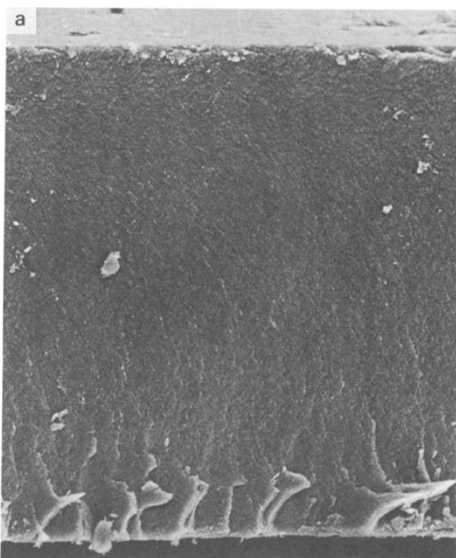
~~Pro separace vysokomolekulárních látek je potřeba velmi krátká kolona (B.G. Belenkii)~~



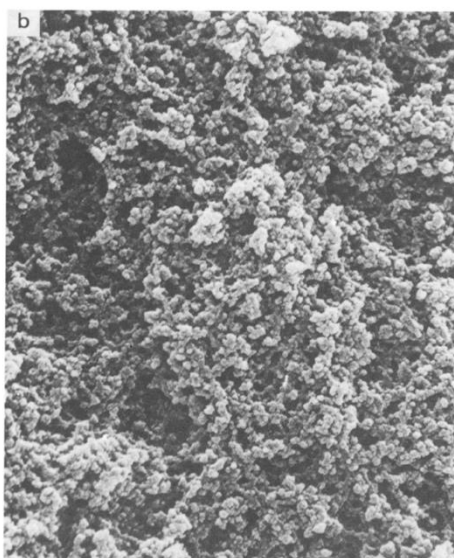
Glycidyl metakrylát



Ethylen dimetakrylát

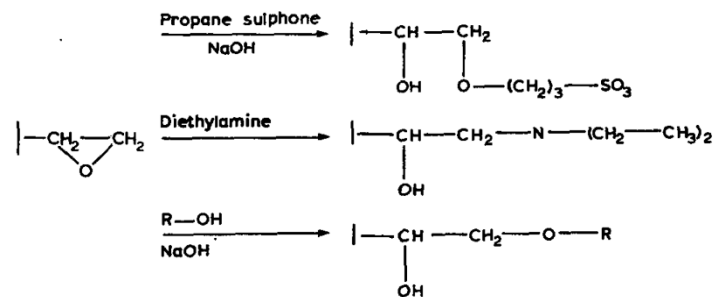


800x



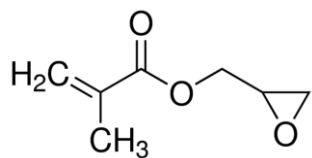
5400x

Reaktivní povrch

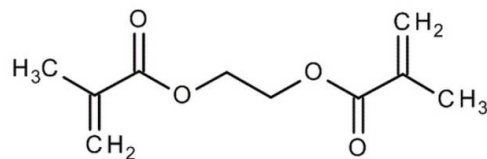


MEMBRÁNOVÁ CHROMATOGRAFIE

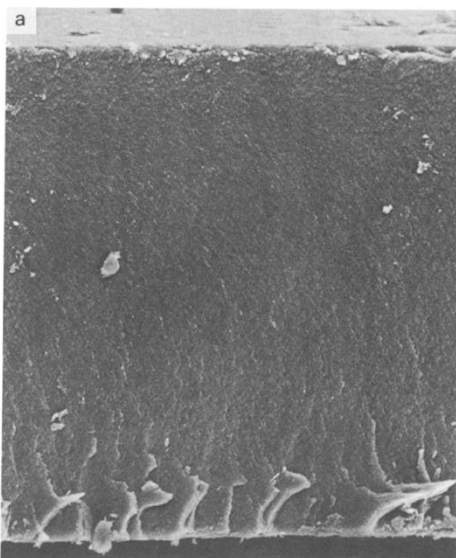
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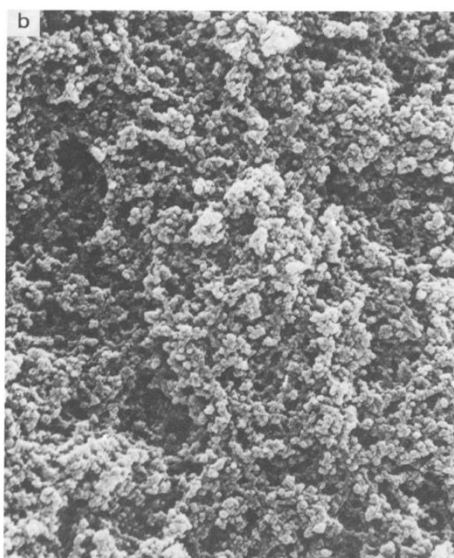
Glycidyl metakrylát



Ethylen dimetakrylát

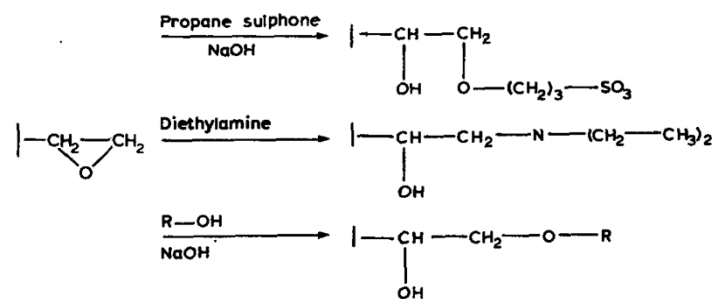


800x



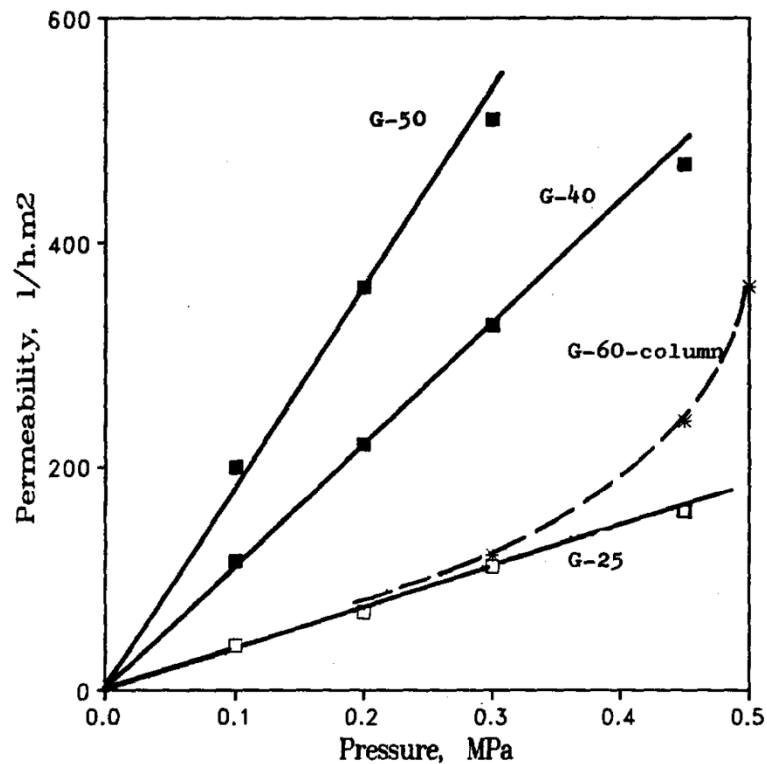
5400x

Reaktivní povrch

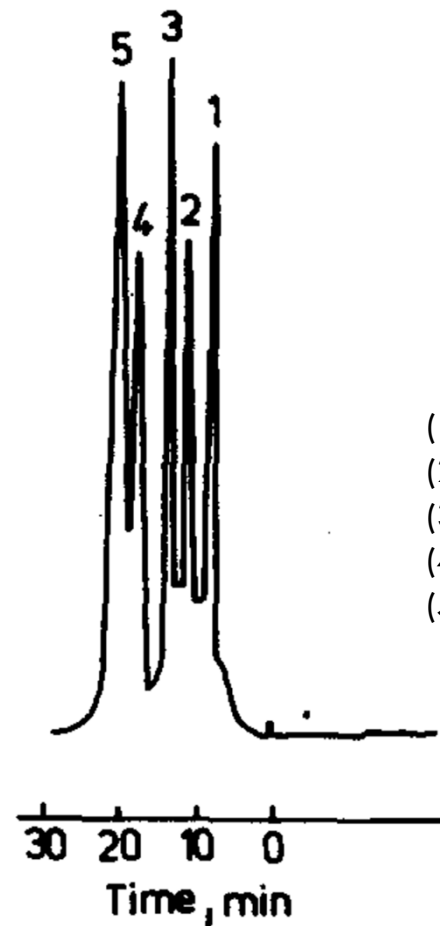


MEMBRÁNOVÁ CHROMATOGRAFIE

Vliv složení polymerační směsi
(rostoucí koncentrace GMA)



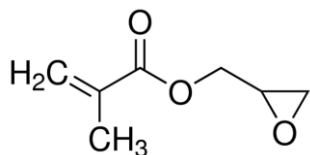
Separace bílkovin



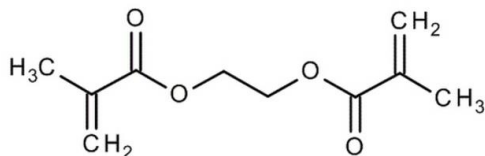
Membrána
1 x 20 mm

- (1) Myoglobin
- (2) Ovalbumin
- (3) Ribonucleáza A
- (4) Lysozym
- (5) Chymotrypsinogen

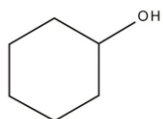
POLYMERNÍ MONOLITY



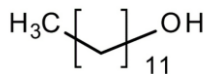
Glycidyl metakrylát



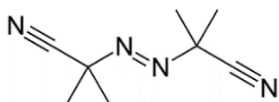
Ethylen dimetakrylát



Cyklohexanol

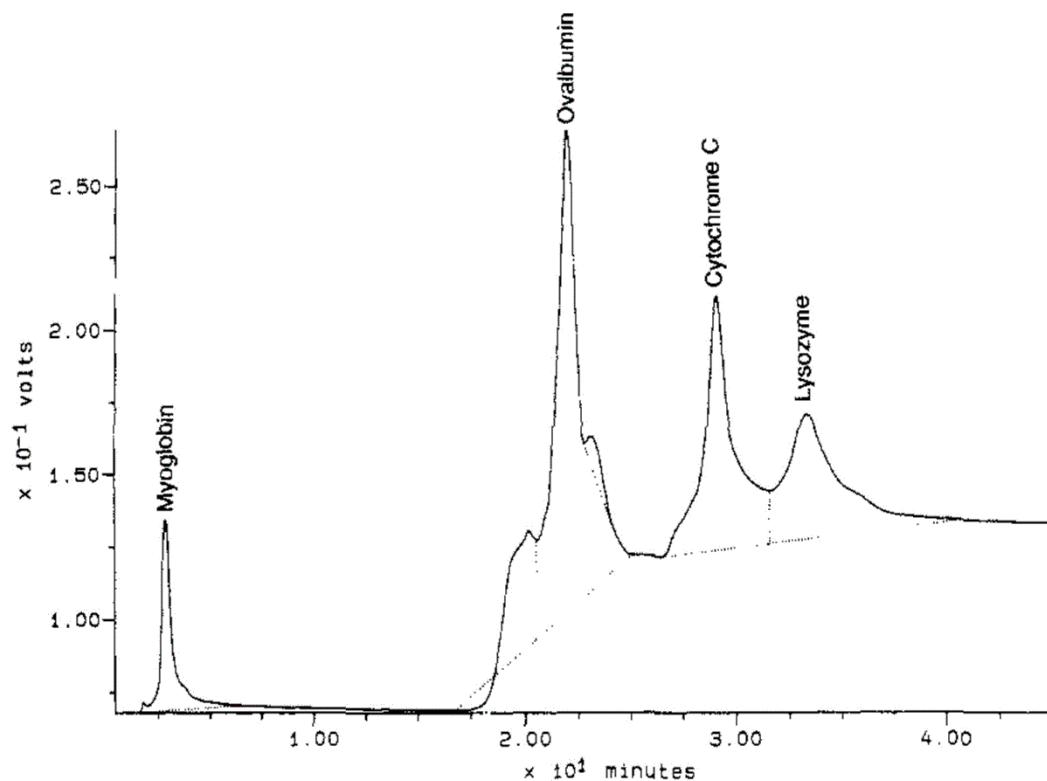


Dodekanol



Azobisisobutyronitril

Chromatografie iontové výměny
modifikováno (diethylamino)hydroxypropylem



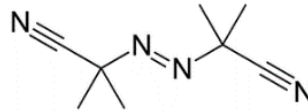
6 h při 70 °C
30 x 8 mm

RADIKÁLOVÁ POLYMERACE

Fáze polymerace

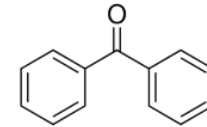
- 1) Iniclace
- 2) Propagace
- 3) Terminace

Azobisisobutyronitril

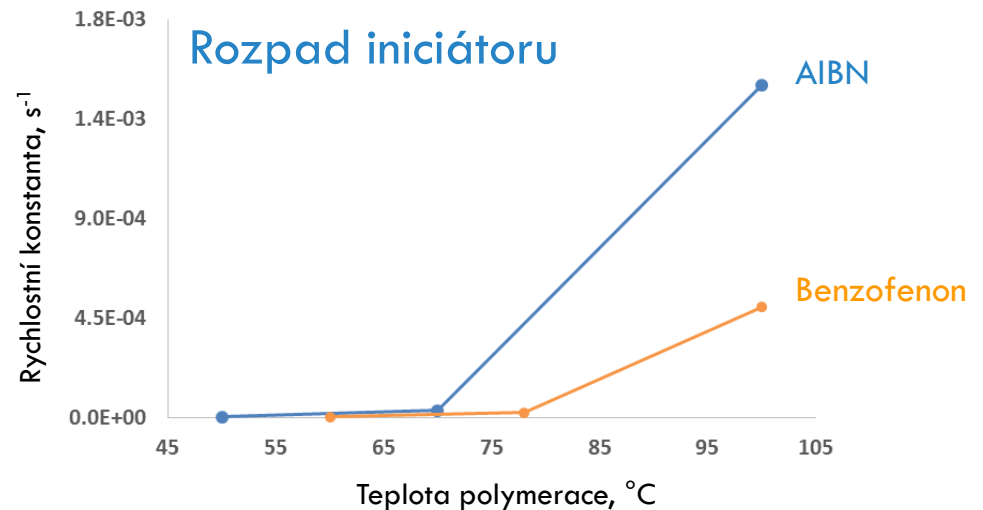
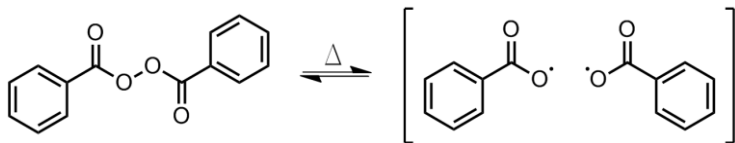
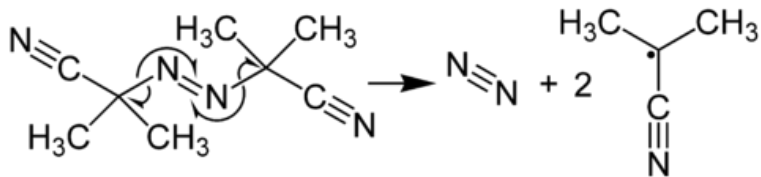


Teplotou iniciovaná polymerace

Benzofenon



UV zářením iniciovaná polymerace



POLYMERNÍ MONOLITY

Modifikace vnitřní stěny



Kapilára



Iniciace

UV, T, ...

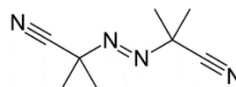


Monolitická kapilární kolona

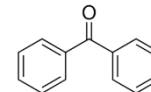
Polymerační směs

- Funkční monomery (ST, CMS, BMA, GMA, ...)
- Síťující monomery (DVB, EDMA, ...)
- Porogenní rozpouštědla (alkoholy, ...)
- Iniciátor (AIBN, benzofenon)

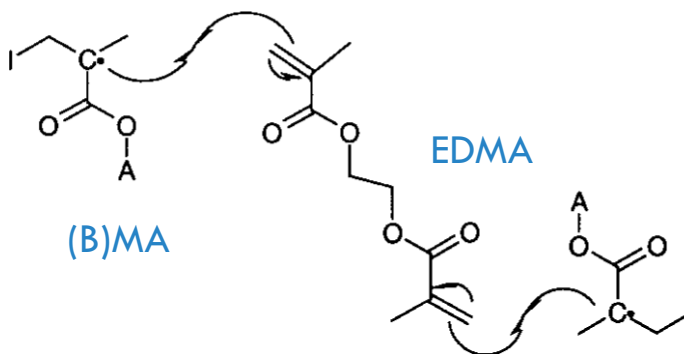
azobisisobutyronitril



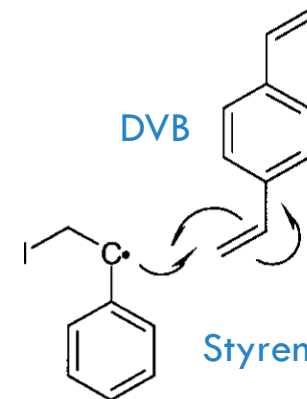
benzofenon



Polymetakryláty

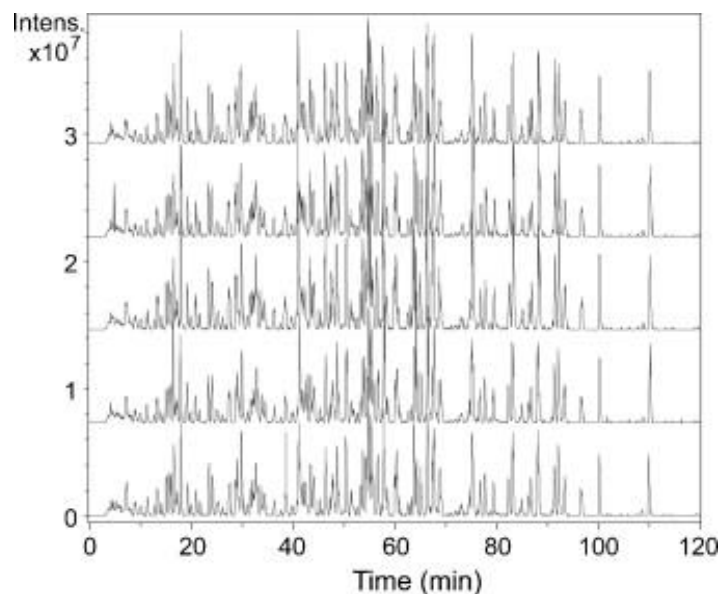


Styren-divinylbenzen



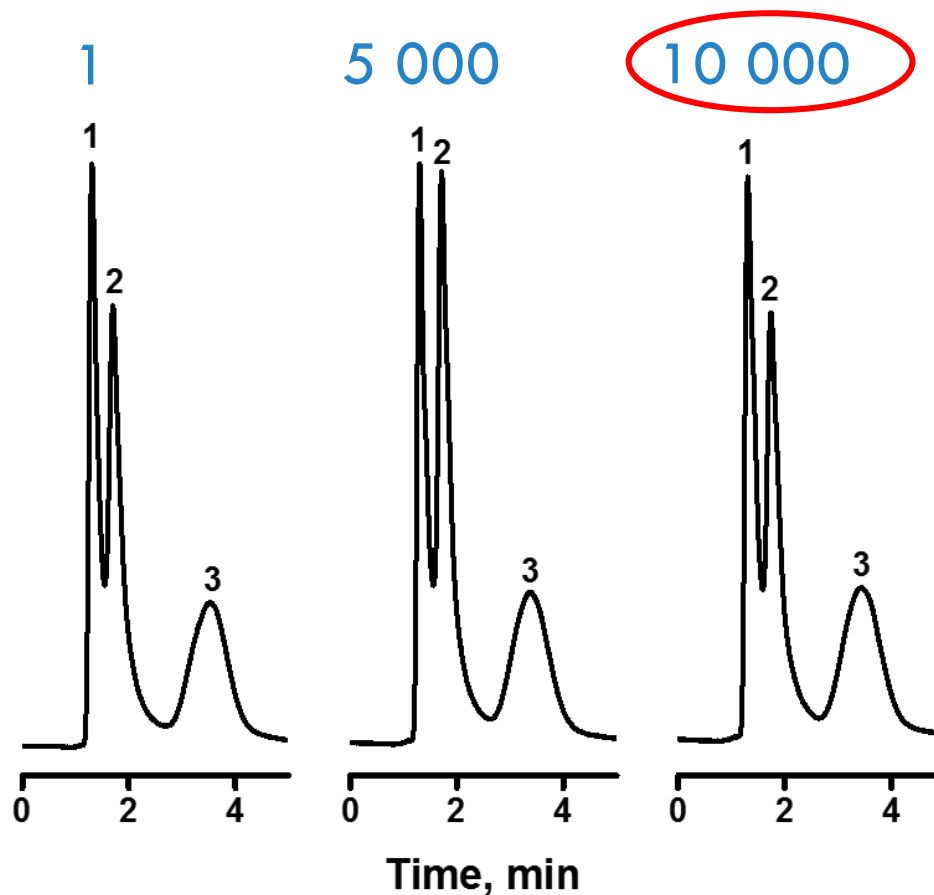
ROBUSTNOST MONOLITŮ

Digest šesti proteinů, monolit 250 mm



J. Chromatogr. A, 1217 (2010) 6610 – 6615.

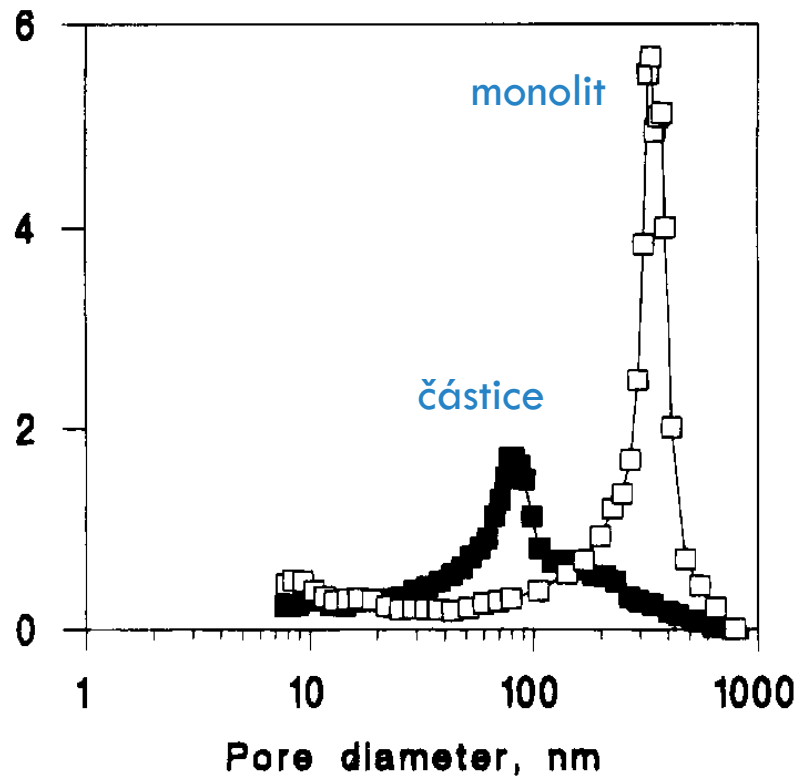
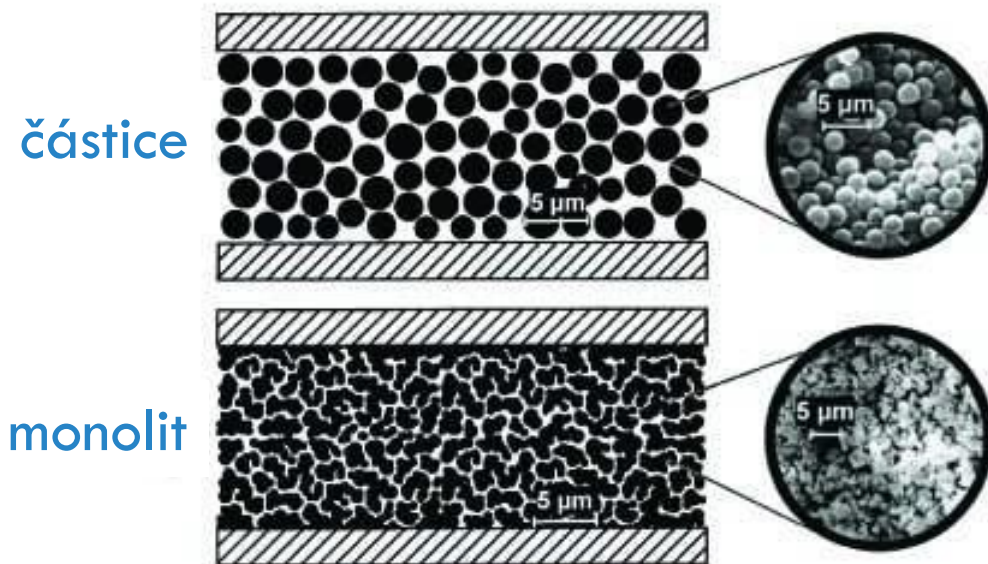
Izokratická separace malých molekul



Stabilní a reprodukované výsledky
(stále ale pouze výzkumné laboratoře)

Fenol (1), toluen (2), and thiomočovina(3) v 98% ACN,
průtok 10 μ l/min. J. Sep. Sci. 2013, 36, 2806–2812

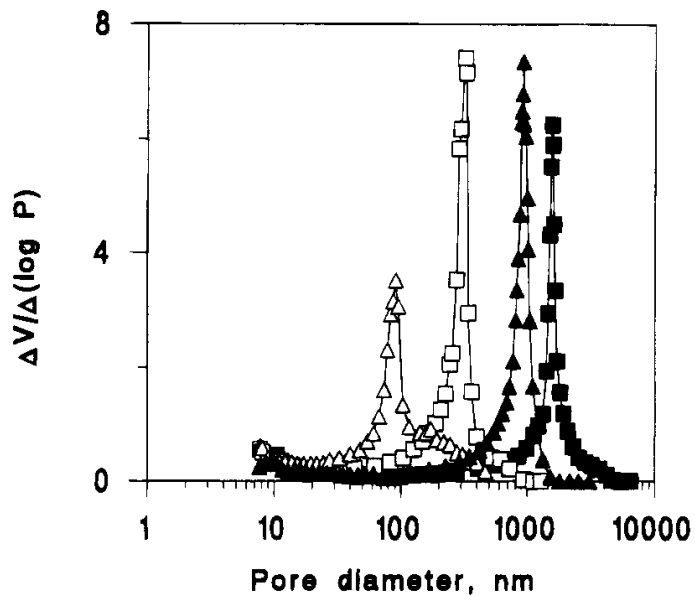
DISTRIBUCE PÓRŮ



Dramaticky jiná porézní struktura a distribuce pórů

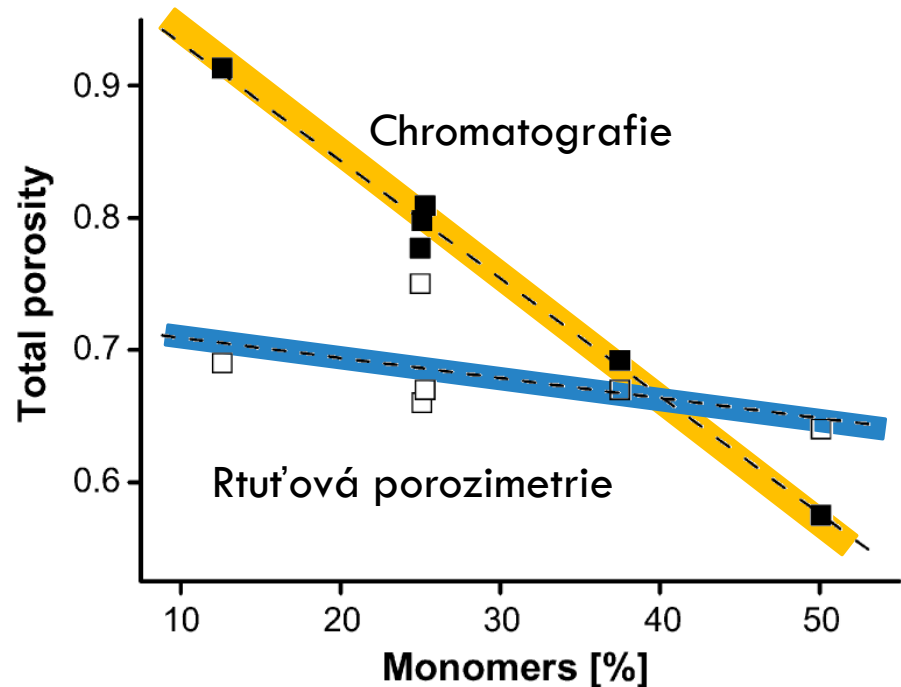
PORÉZNÍ VLASTNOSTI

Rtuťová porozimetrie



Chem. Mater. 7 (1995) 707.

Chromatografická porozimetrie

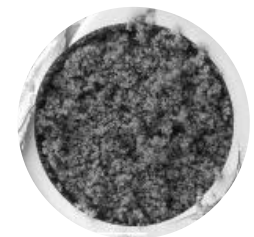


Suchý vs. nabobtnaný stav

Porozita, aktivní povrch, distribuce pórů



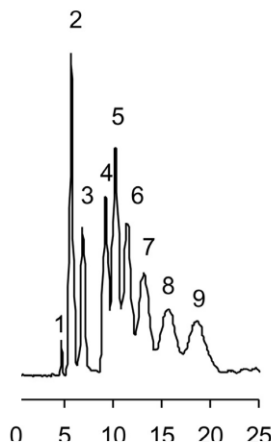
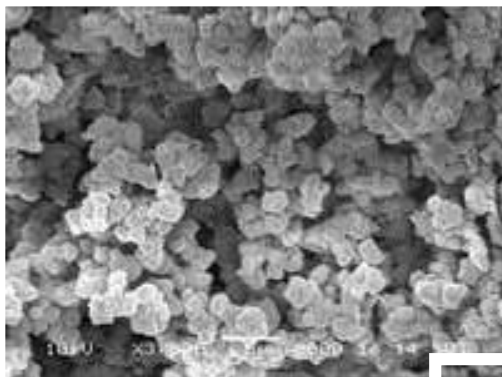
vs.



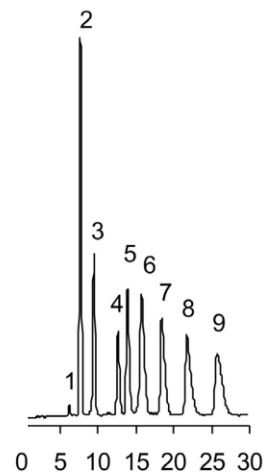
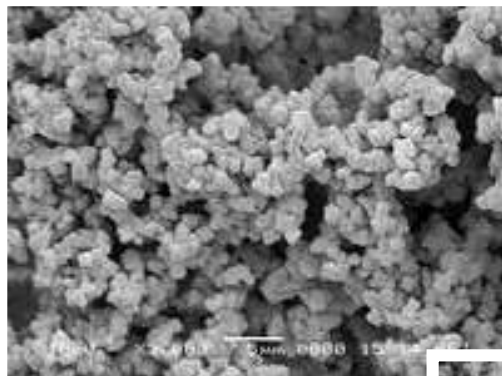
KONTROLA MORFOLOGIE

Vliv koncentrace 1,4-butandiolu
v polymerační směsi

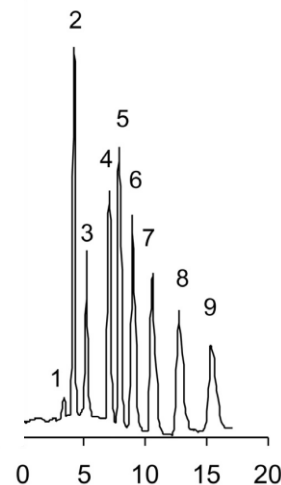
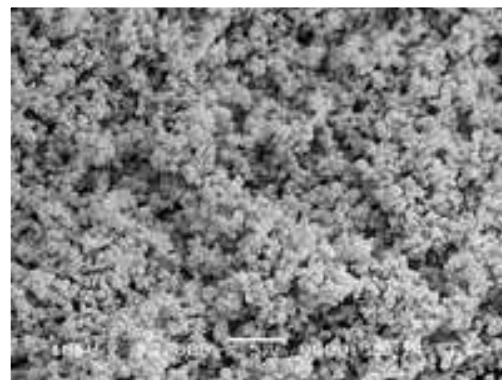
30%



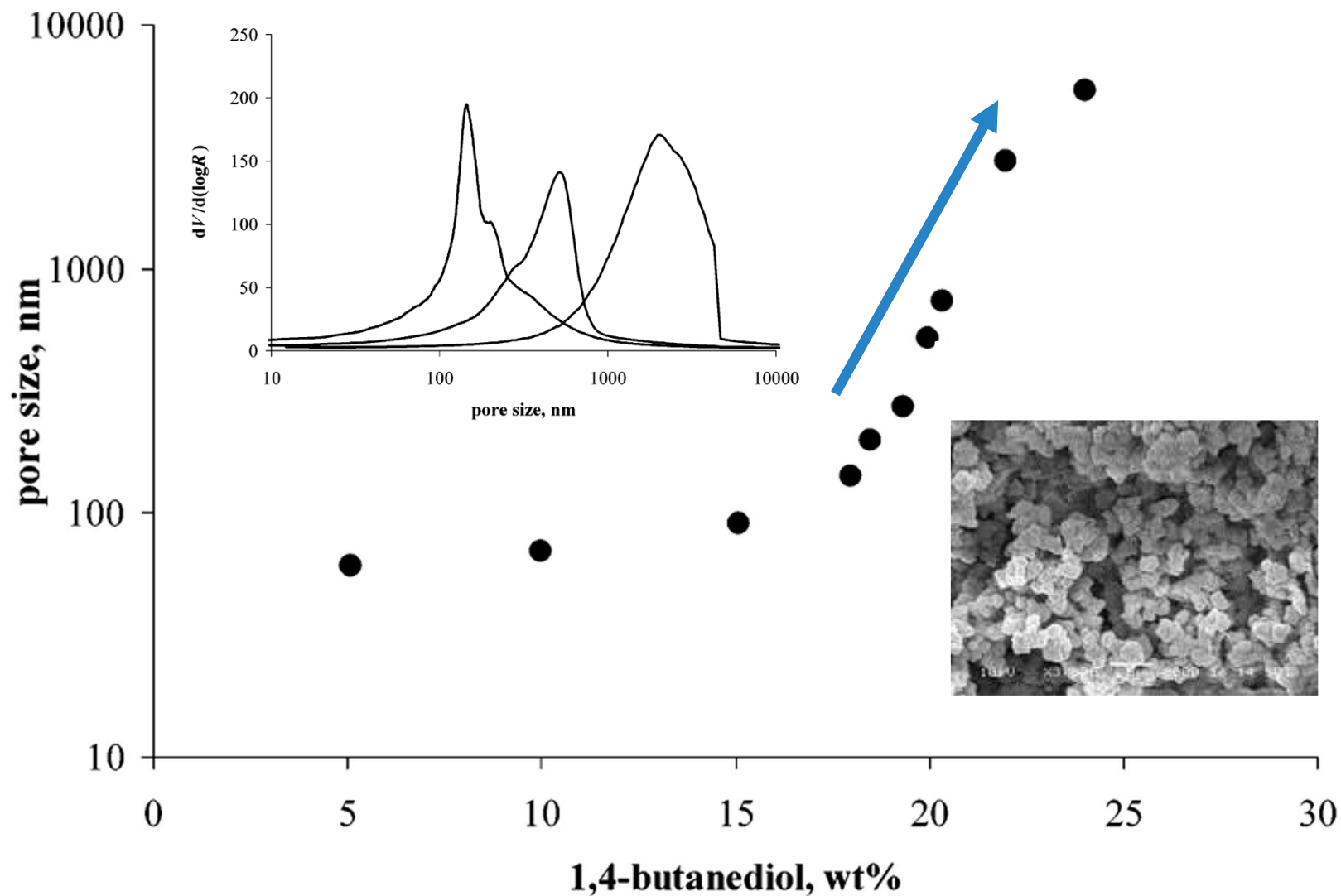
29%



28%



DOMINANTNÍ PRŮTOČNÉ PÓRY



MAKROPORÉZNÍ MONOLITY

Monolit

50 x 4.6 mm

Polymer

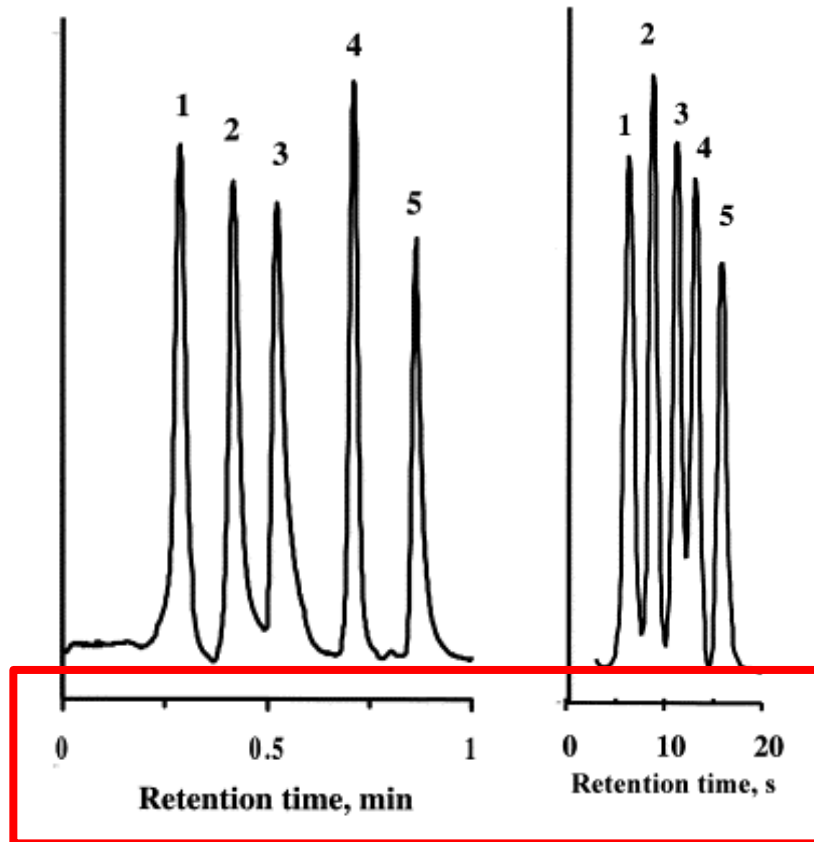
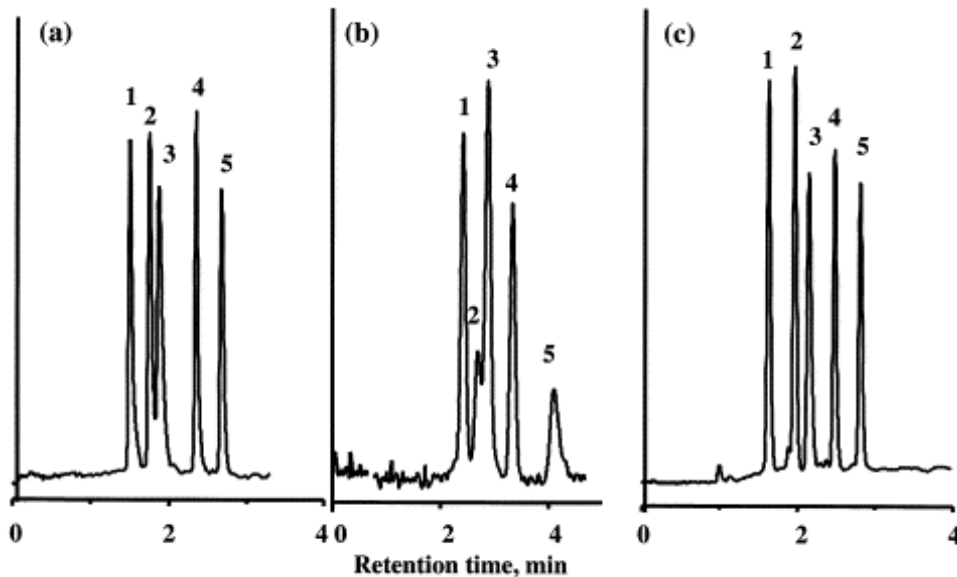
150 x 4.6 mm, 10 μm

C8 Silikagel

150 x 4.6 mm, 5 μm

Peptidy

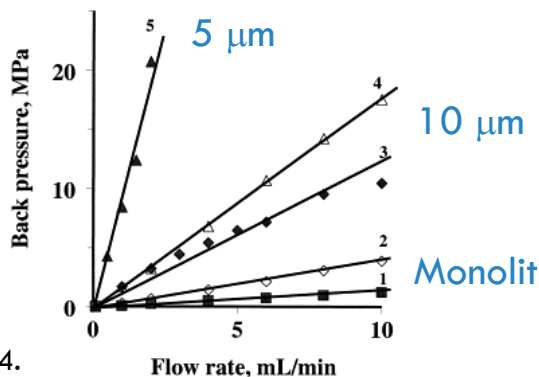
Proteiny



Nízký
pracovní tlak



Rychlý průtok
mobilní fáze

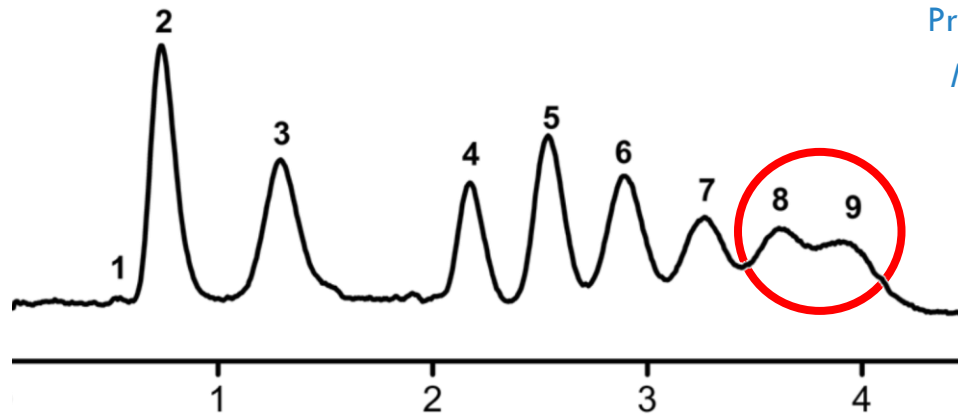
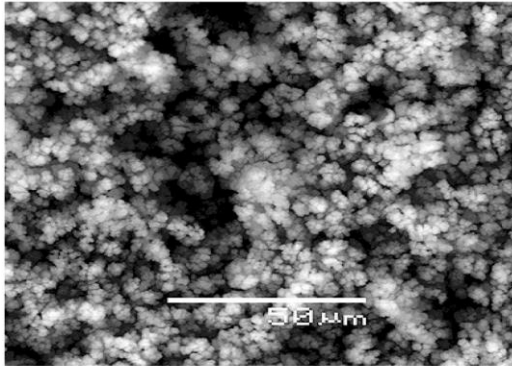


Rychlé gradientové separace

VLIV MALÝCH PÓRŮ

Gradientová eluce malých molekul

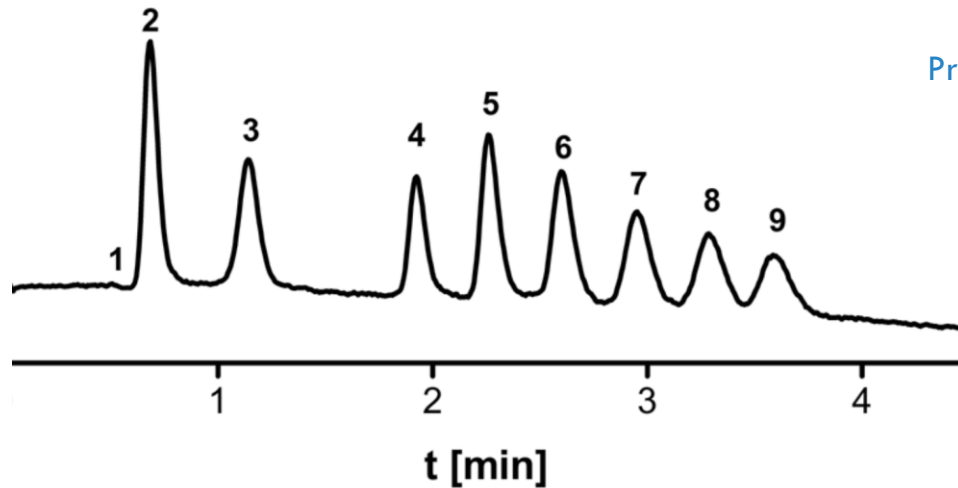
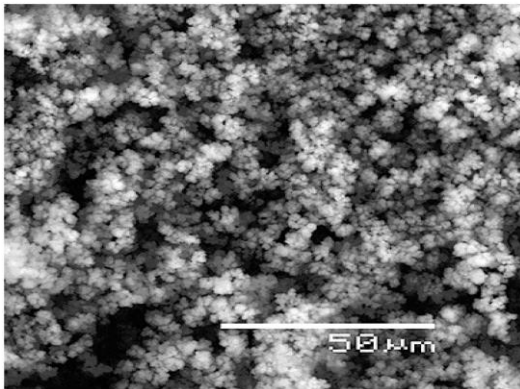
A



Průtočné póry 54.0%

Mezopóry 12.0%

B

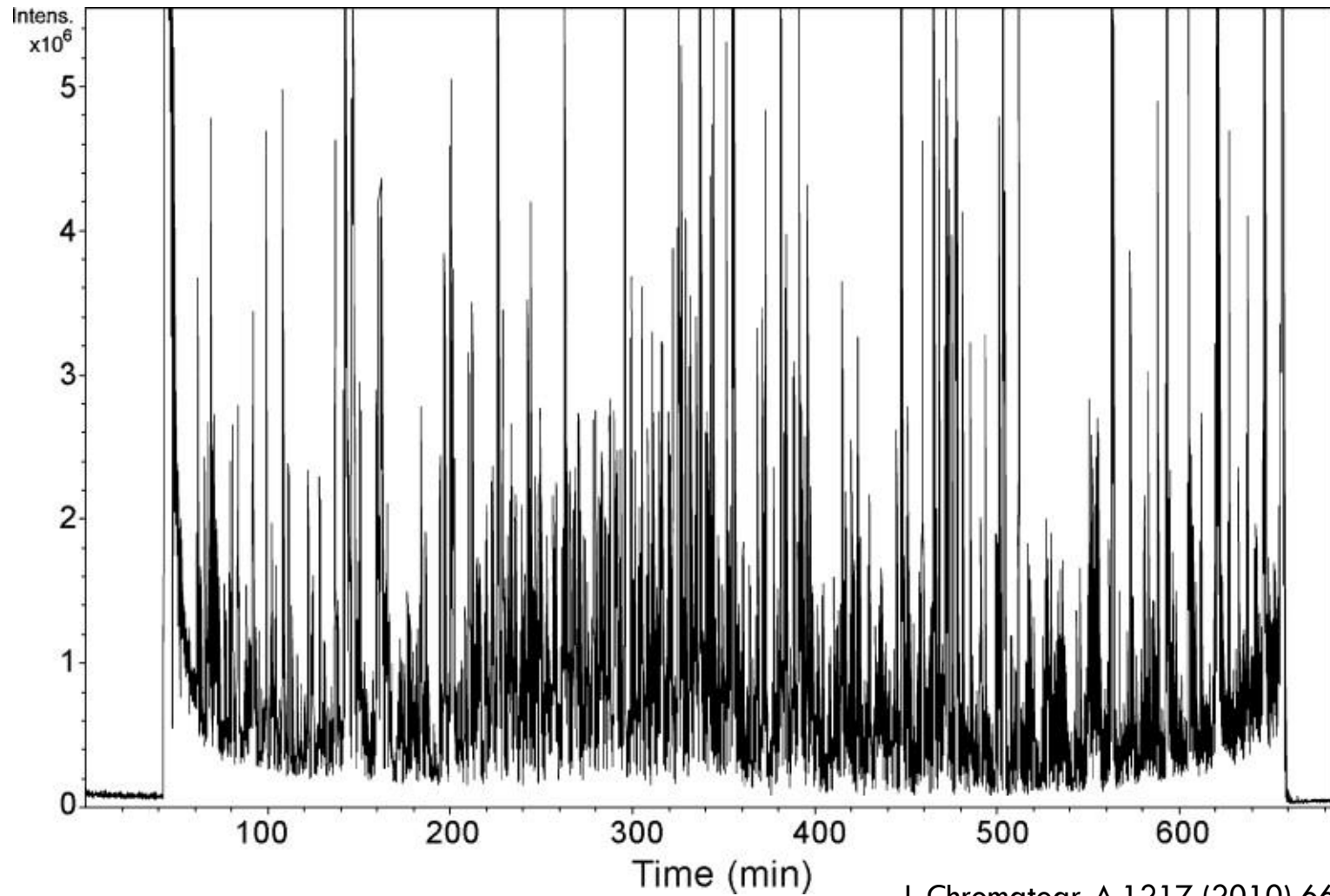


Průtočné póry 61.9%

Mezopóry 7.6%

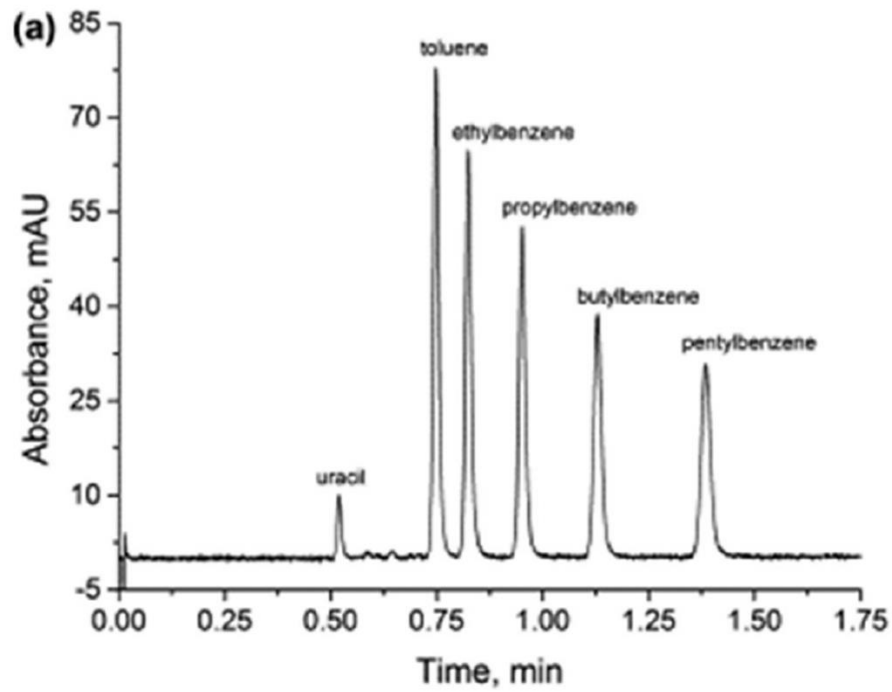
E. COLI DIGEST

1 m monolit, píková kapacita 1038

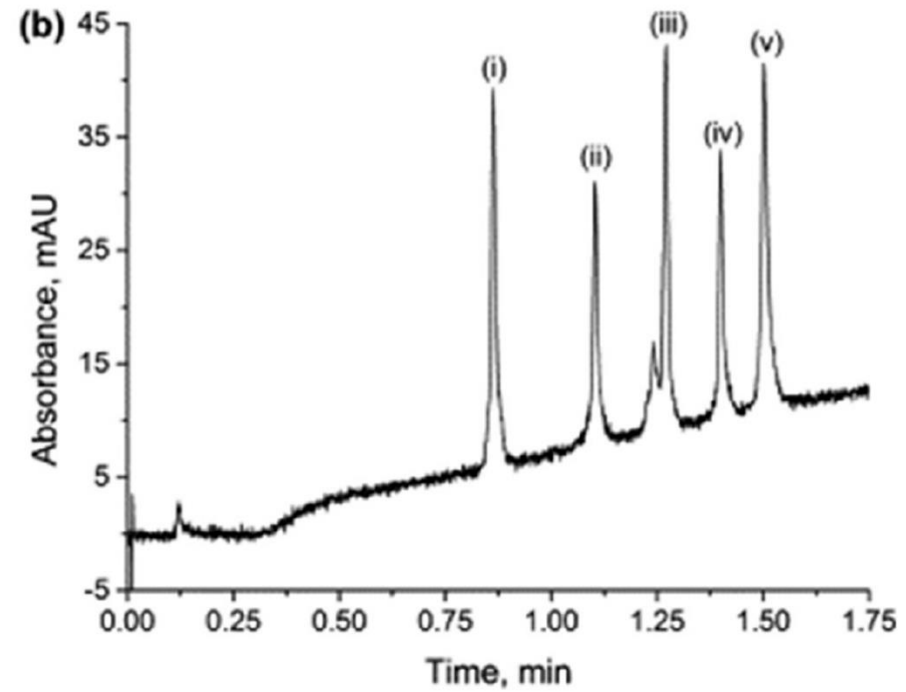


MONOLITICKÉ STACIONÁRNÍ FÁZE

Anorganické monolity
malé molekuly



Organické monolity
velké molekuly



KONTROLA VLASTNOSTÍ

- Polymerační teplota
- Čas polymerace
- Složení polymerační směsi

Funkční monomer

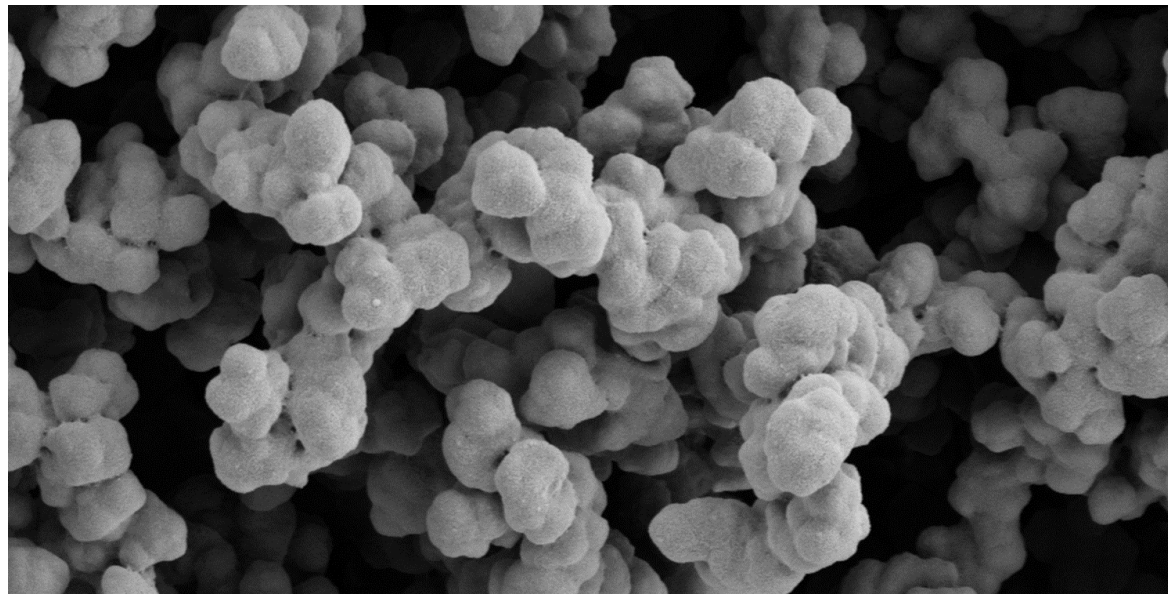
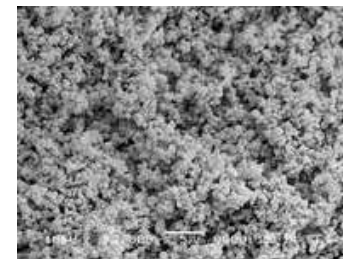
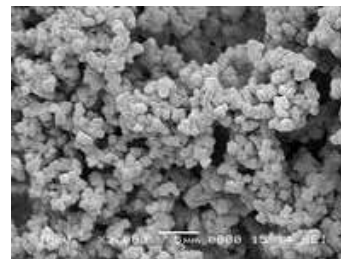
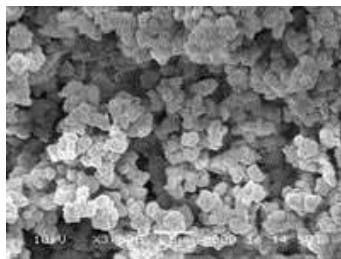
Síťující monomer

- Porogenní rozpouštědla
- Modifikace povrchu

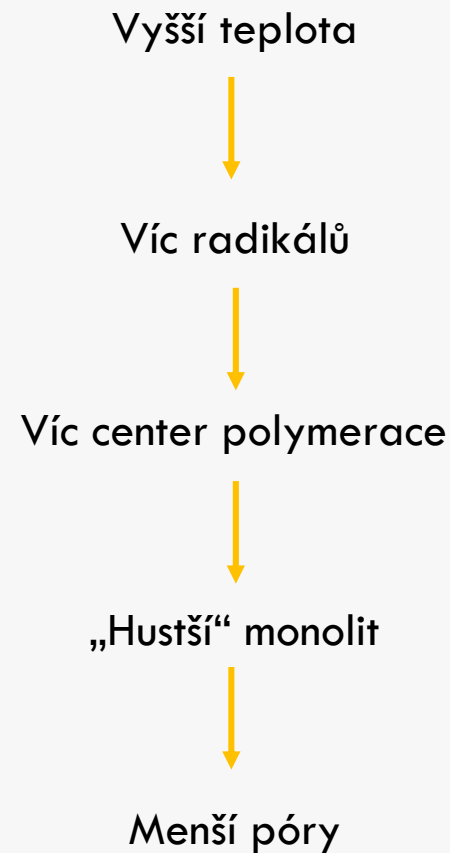
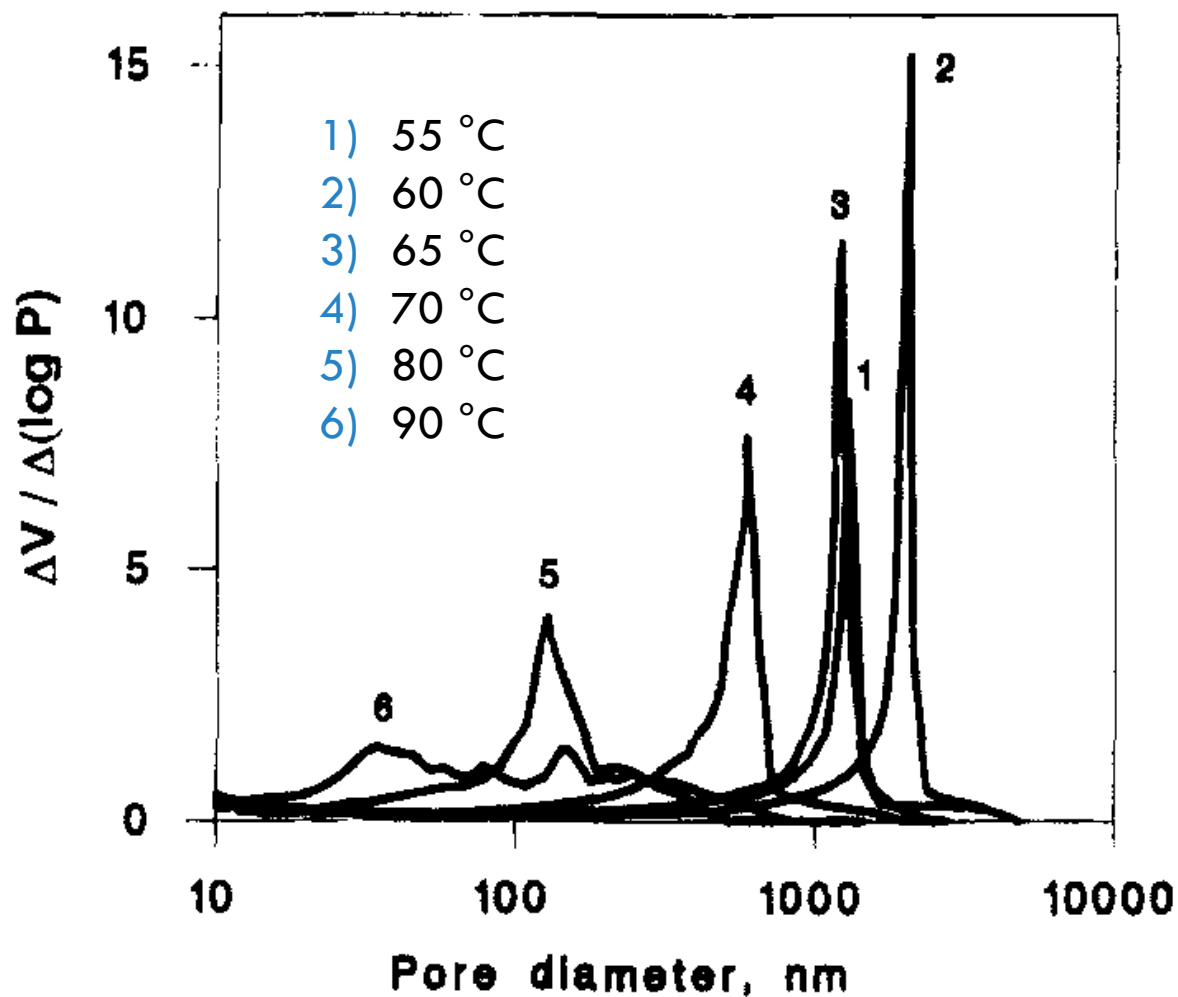
Chemická

Grafting

- Kompozitní materiály
- Planární uspořádání

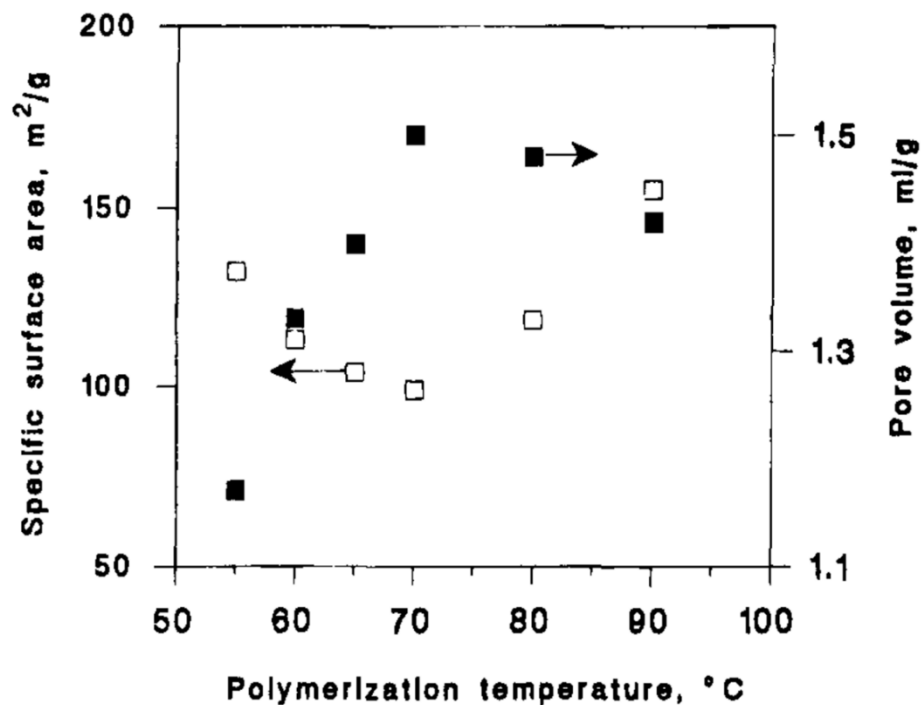


TEPLOTA POLYMERACE

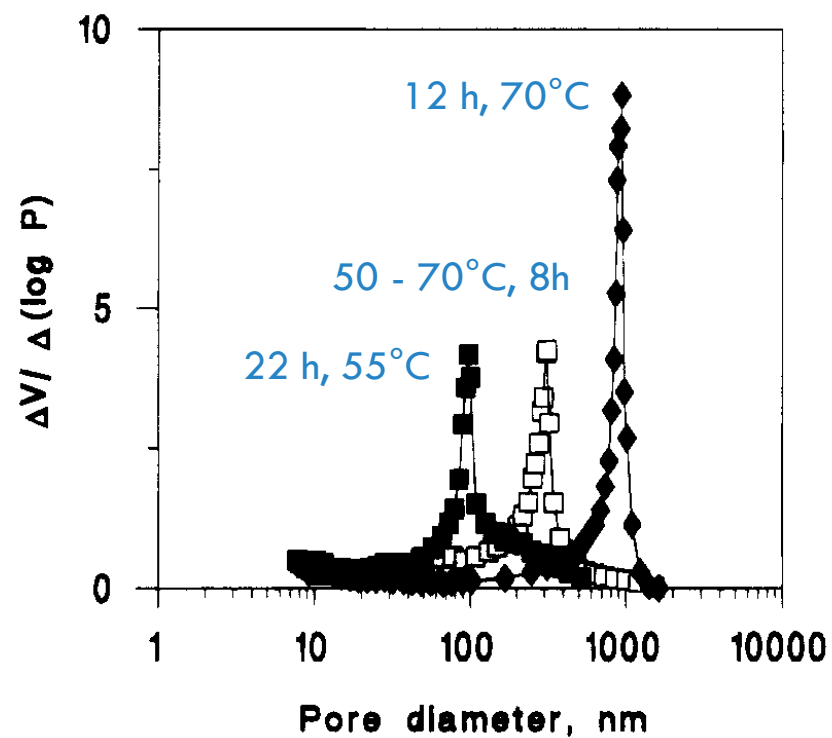


TEPLOTA POLYMERACE

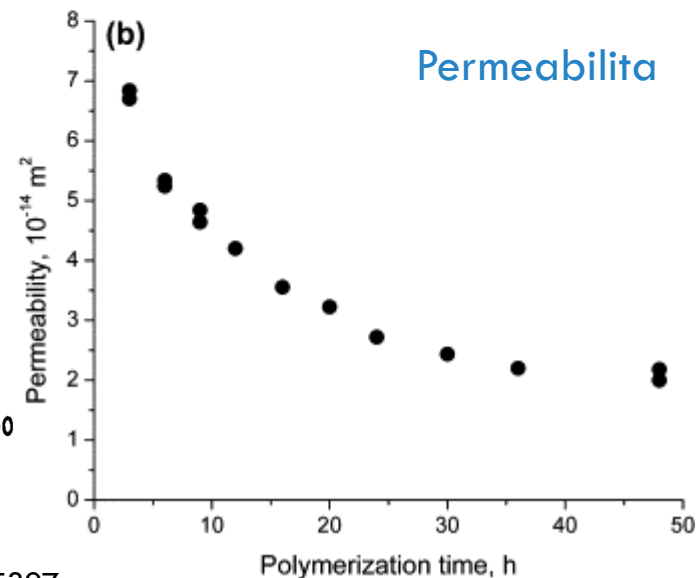
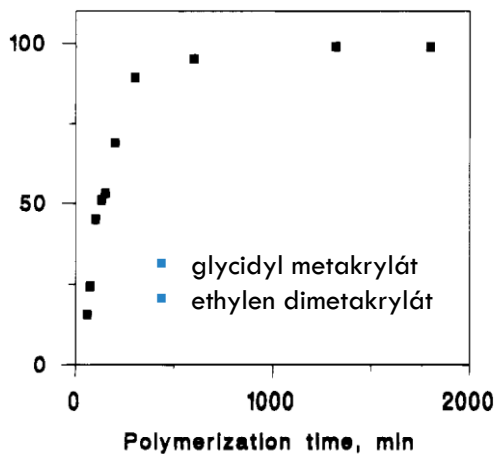
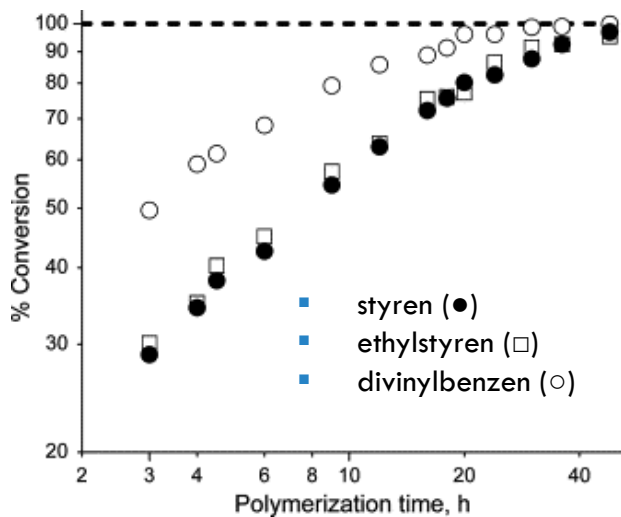
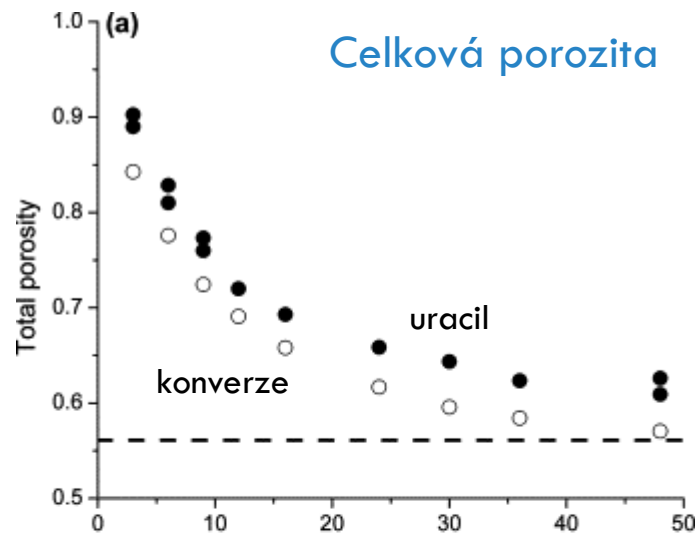
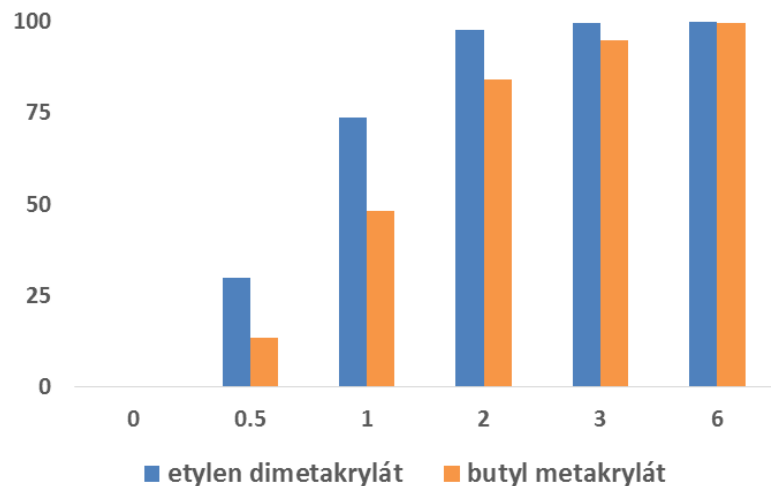
Povrch & Objem pórů



Distribuce pórů



KONVERZE POLYMERACNÍ REAKCE

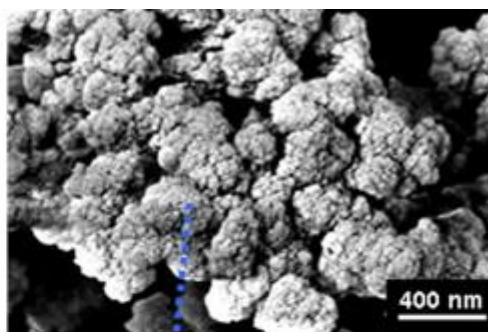
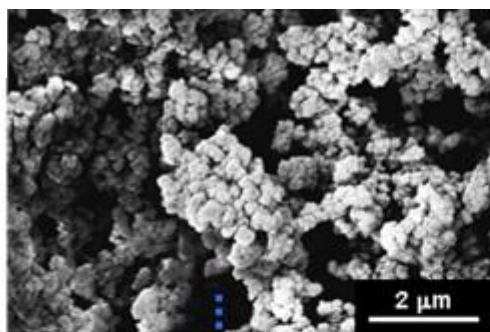


KONVERZE POLYMERÁČNÍ REAKCE

Morfologie a kvalita separace

poly(styren-co-divinylbenzen)

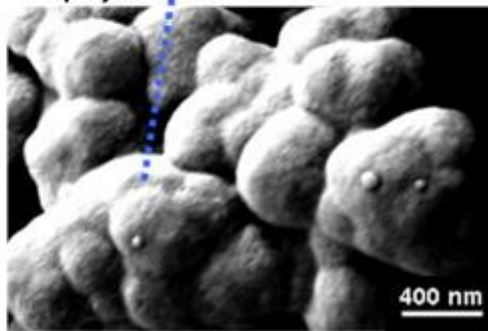
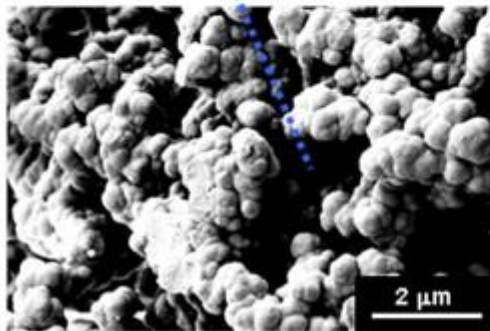
3 h



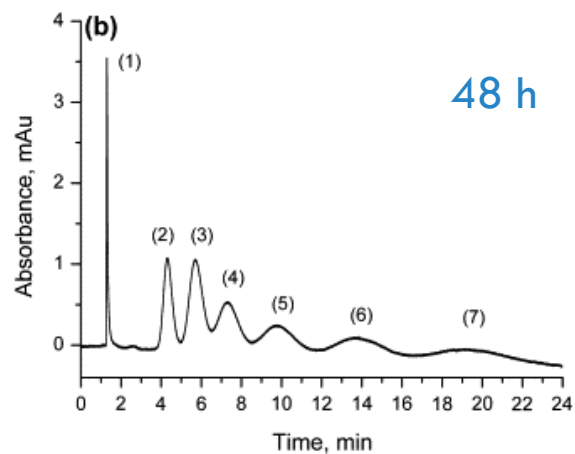
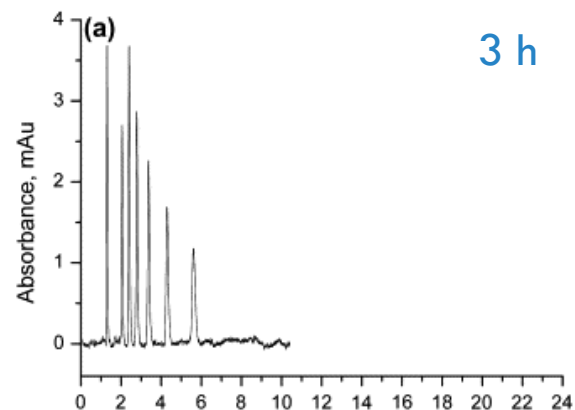
Macropores

Mesopores

48 h



Less cross-linked polymer

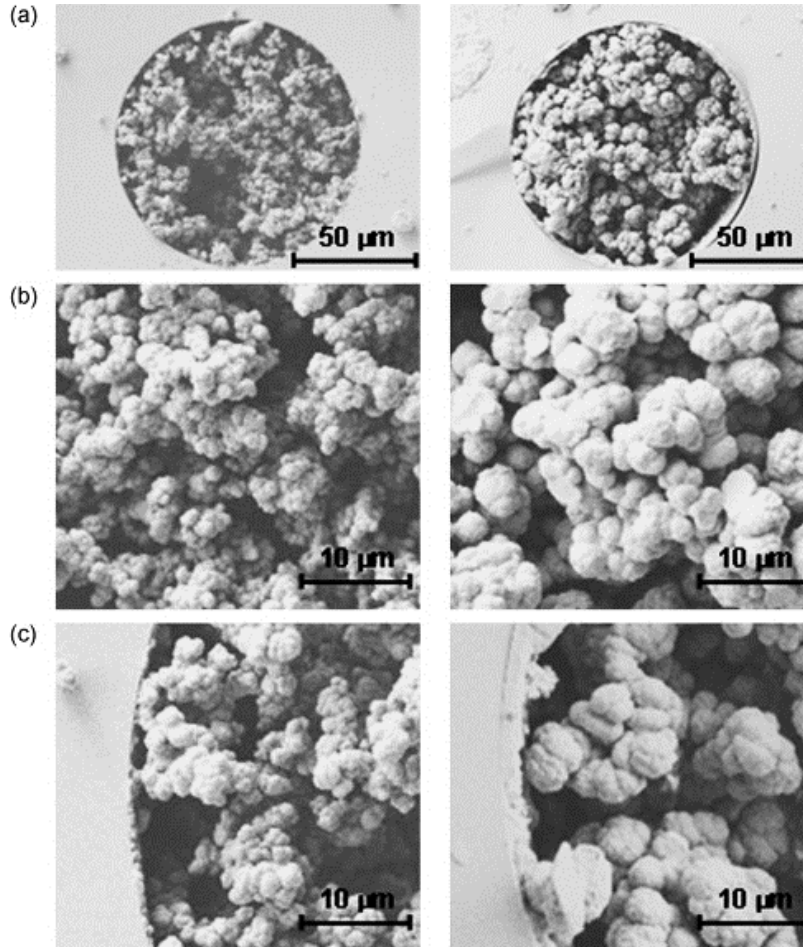


KONVERZE POLYMERÁČNÍ REAKCE

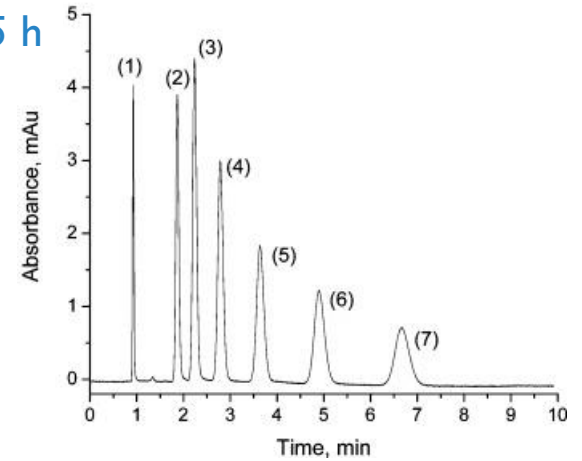
0.5 h

48 h

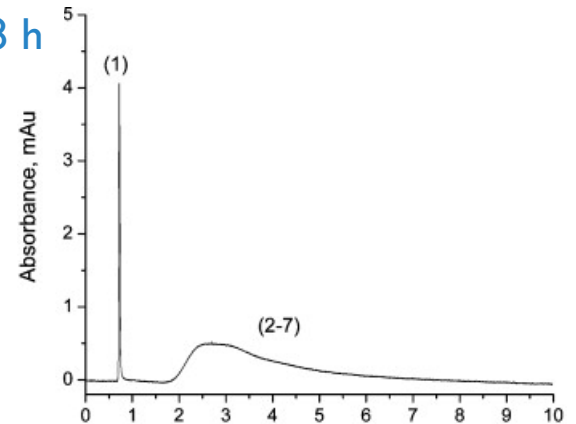
poly(butyl metakrylát-co-ethylen dimetakrylát)



0.5 h

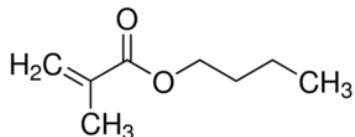


48 h

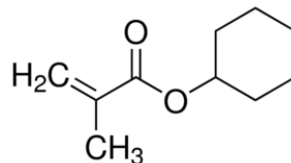


FUNKČNÍ MONOMER

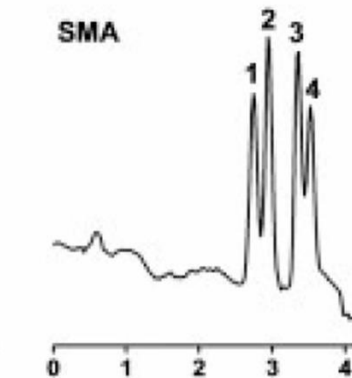
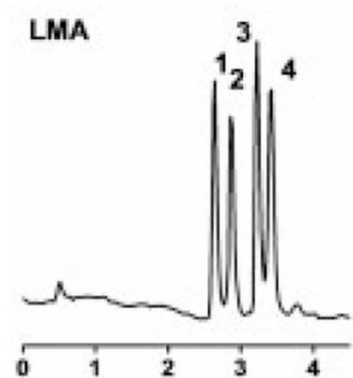
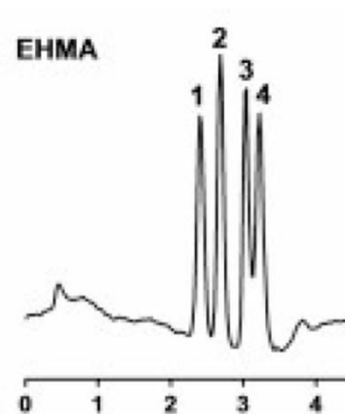
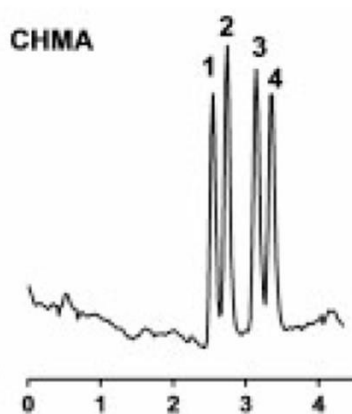
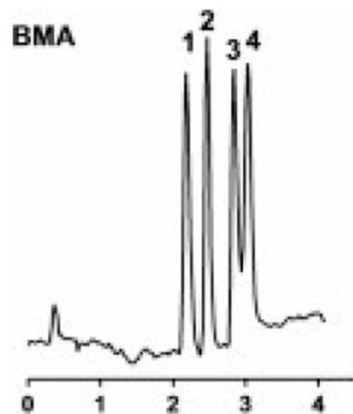
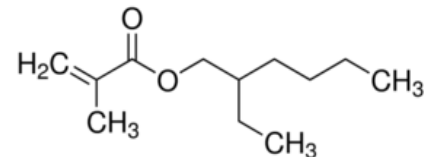
Butyl metakrylát (BMA)



Cyklohexyl metakrylát (CHMA)

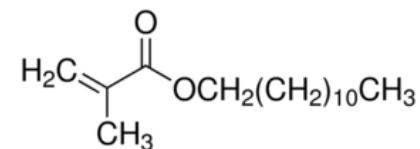


Ethylhexyl metakrylát (EHMA)

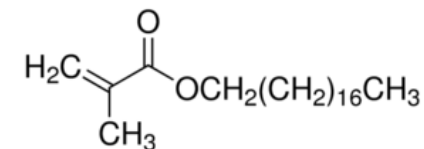


Time, min

Lauryl metakrylát (LMA)



Stearyl metakrylát (SMA)

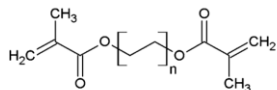


- (1) Insulin
- (2) Cytochrom c
- (3) BSA
- (4) β -laktoglobulin.

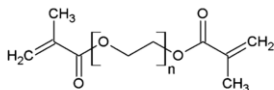
**Nutnost (neustálé)
optimalizace
složení směsi**

SÍŤUJÍCÍ MONOMER

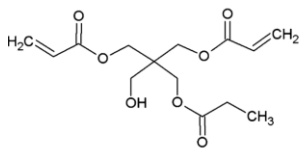
1 - 3



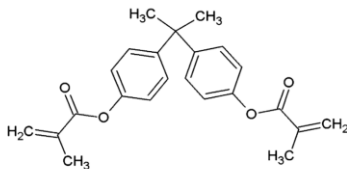
4 - 6



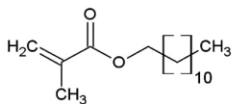
7



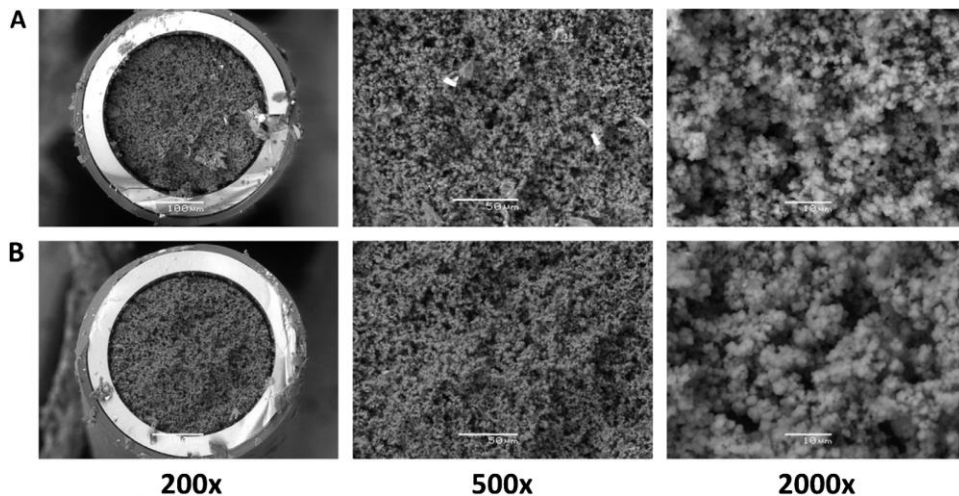
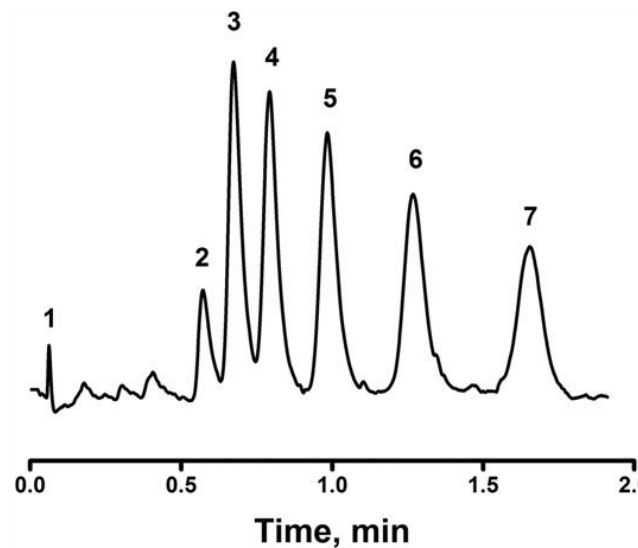
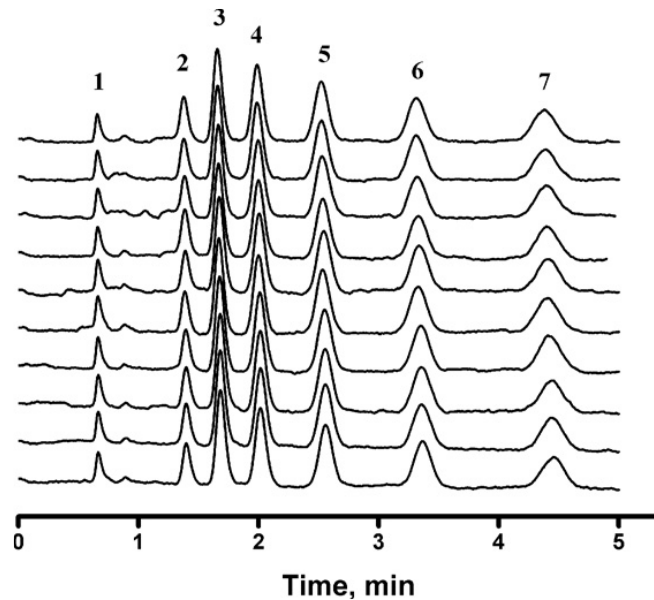
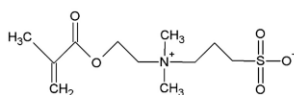
8



9

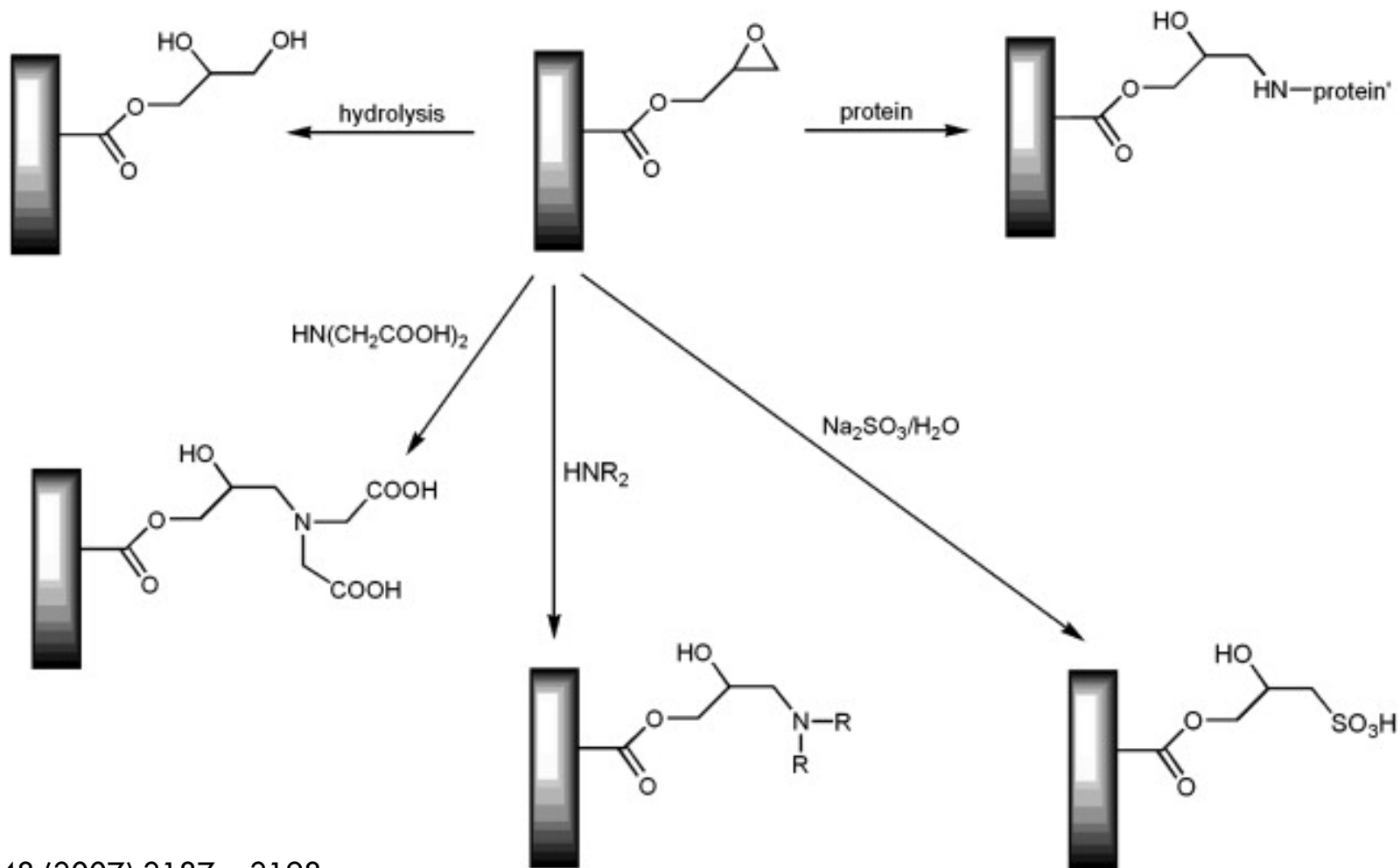


10



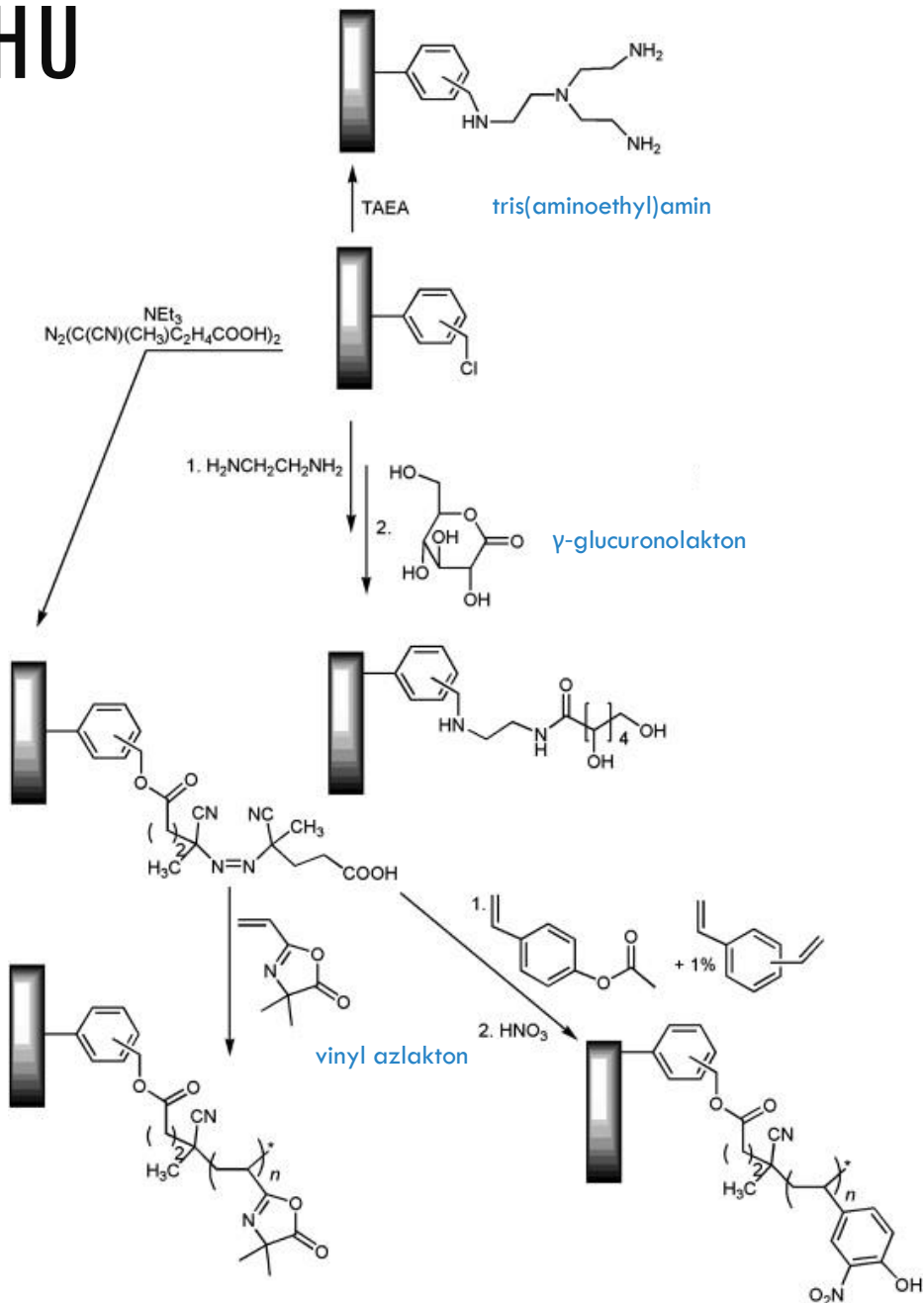
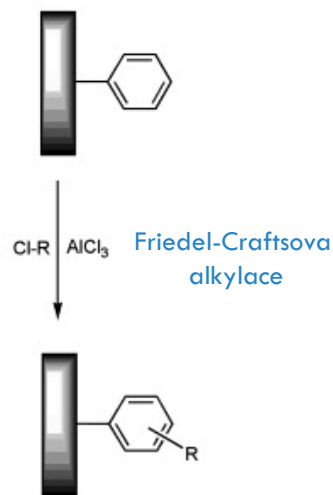
MODIFIKACE POVRCHU

Glycidyl metakrylát

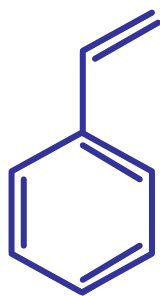


MODIFIKACE POVRCHU

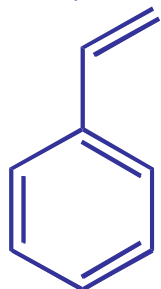
Styren & Chlormethylstyren



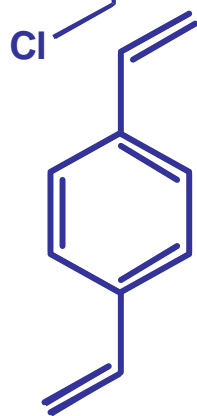
OBECNÝ MONOLIT



Styrene (ST)



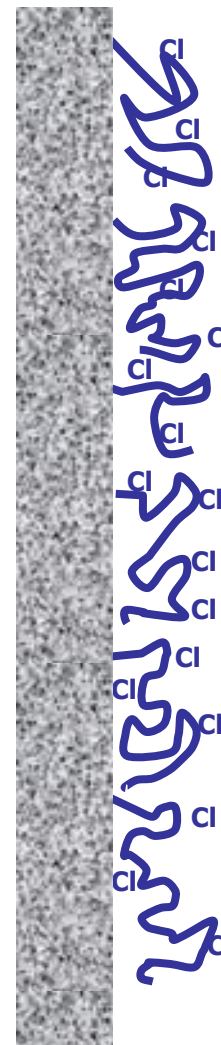
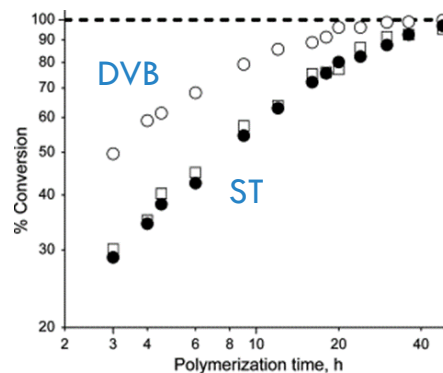
Chloromethylstyrene (CMS)



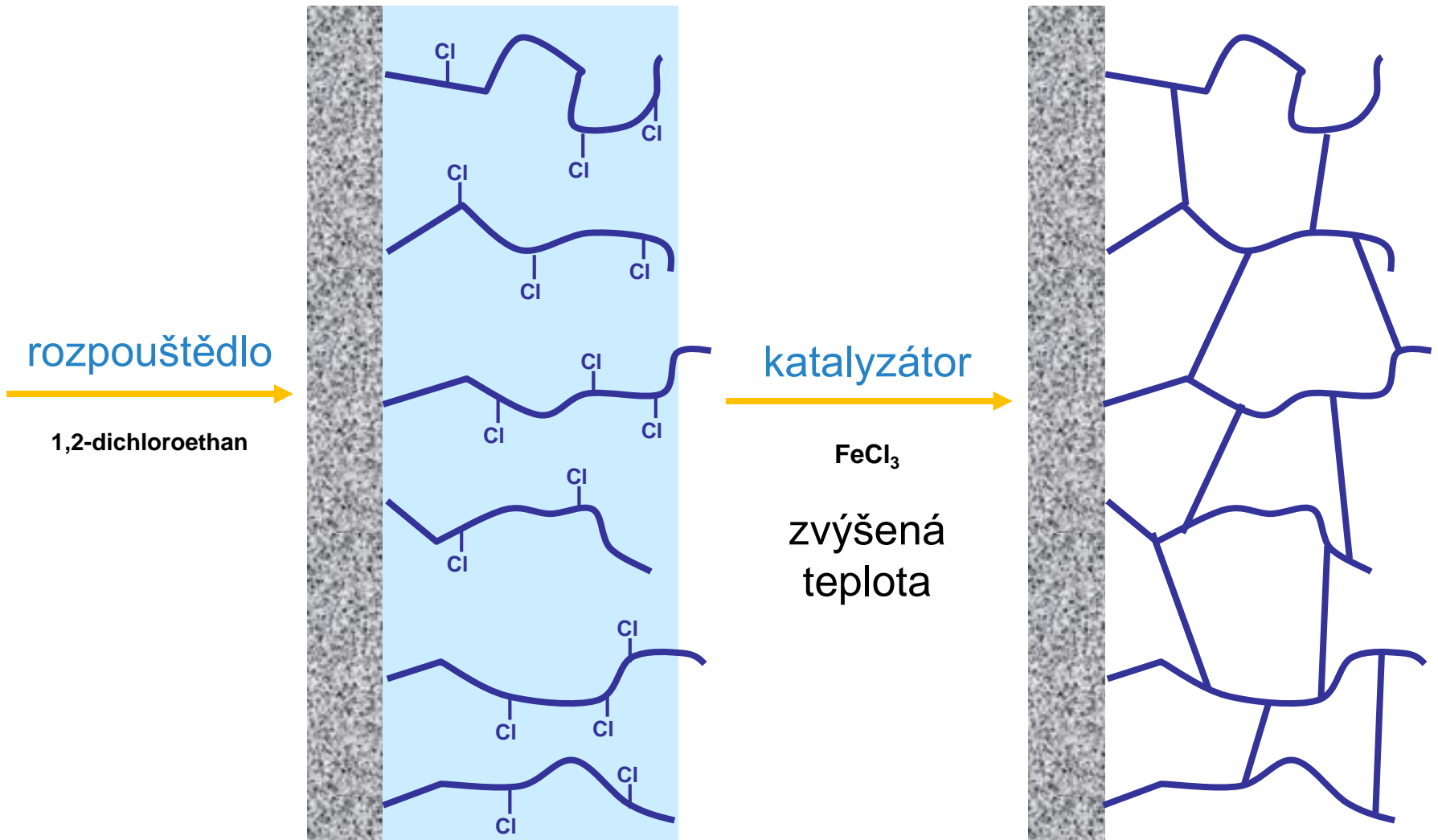
Divinylbenzene (DVB)

Toluene (TOL)
Dodecanol (DOD)
AIBN

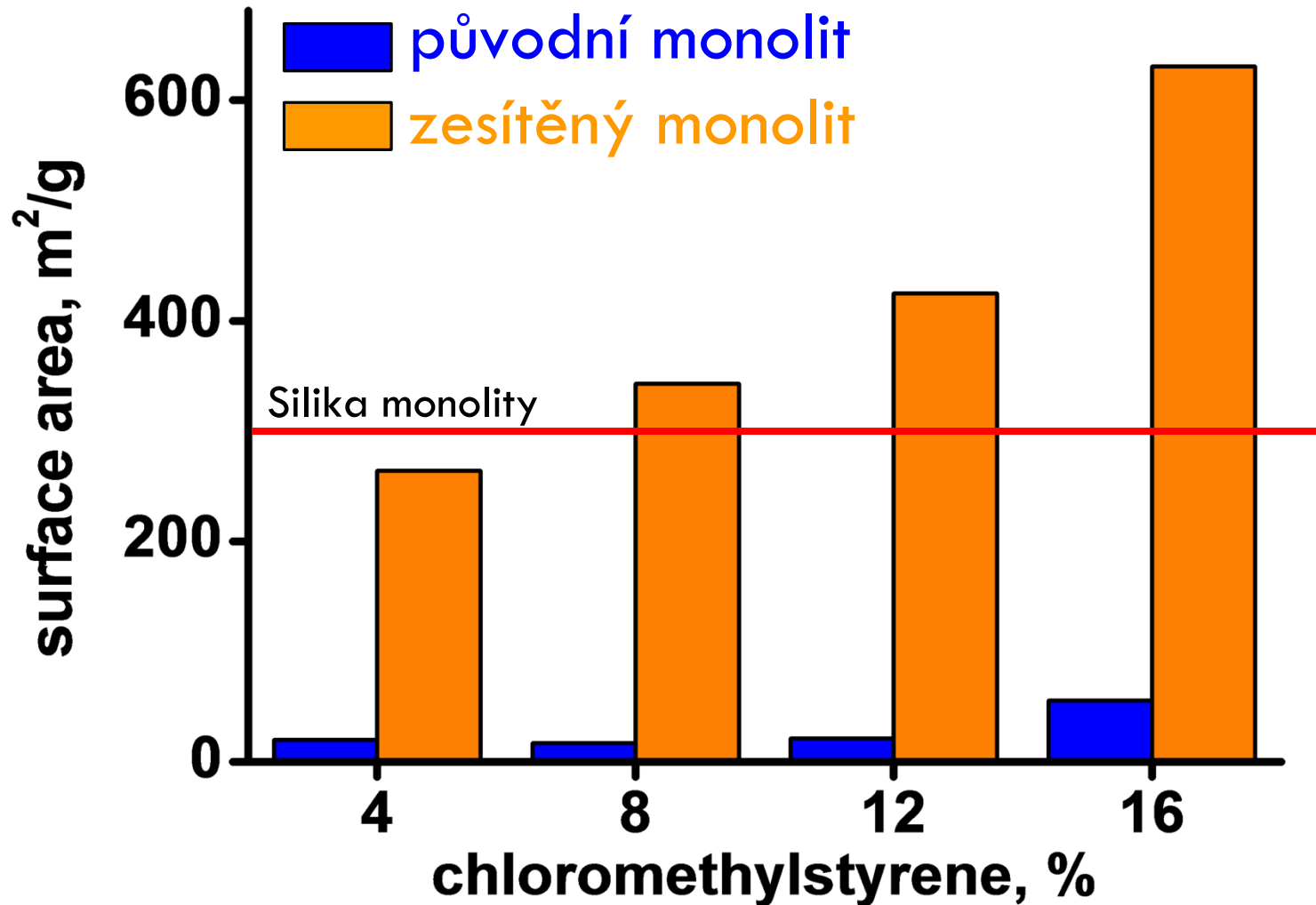
70 °C, 20 h



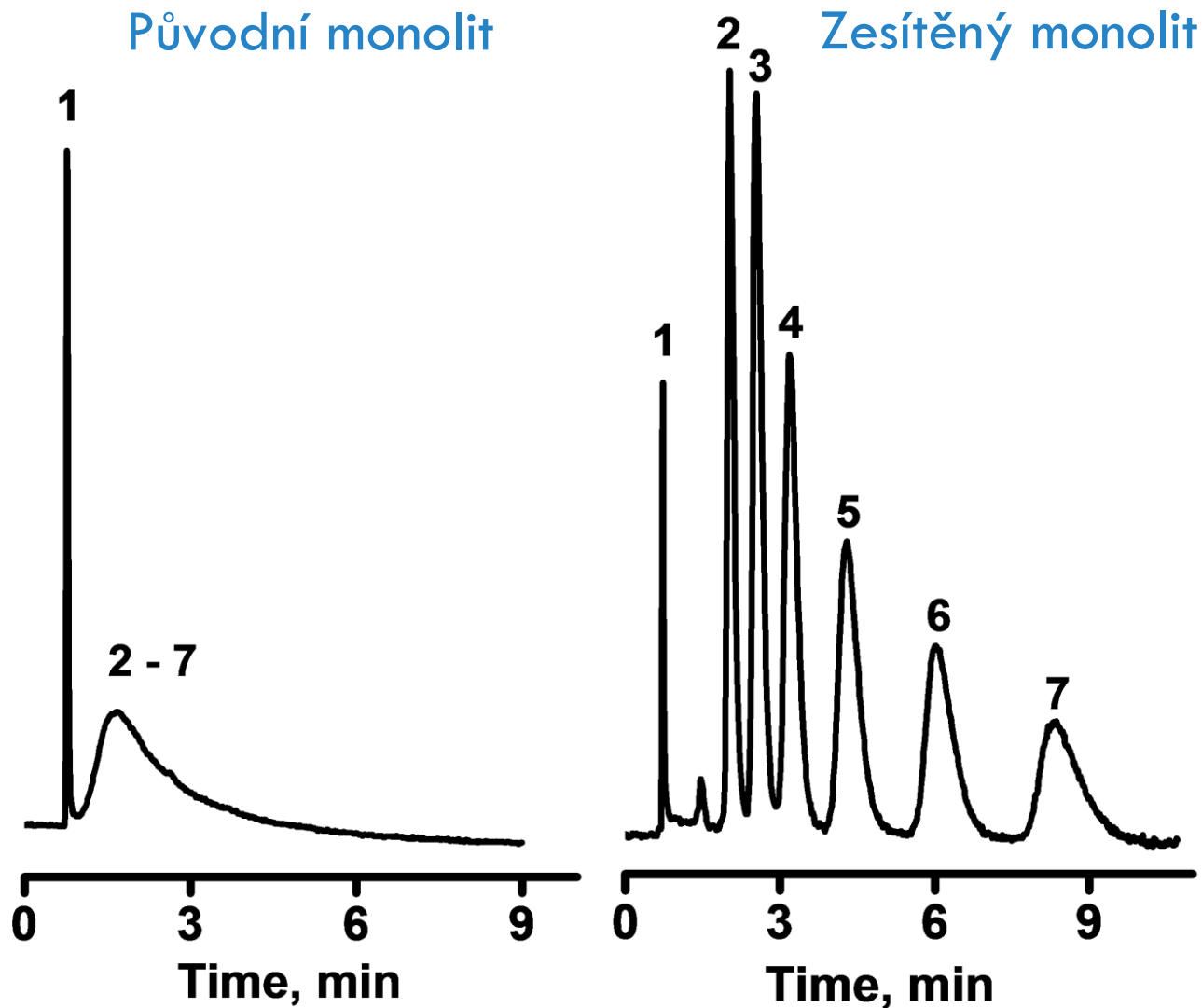
ZESÍTĚNÍ (HYPERCROSSLINKING)



ZVÝŠENÍ AKTIVNÍHO POVRCHU



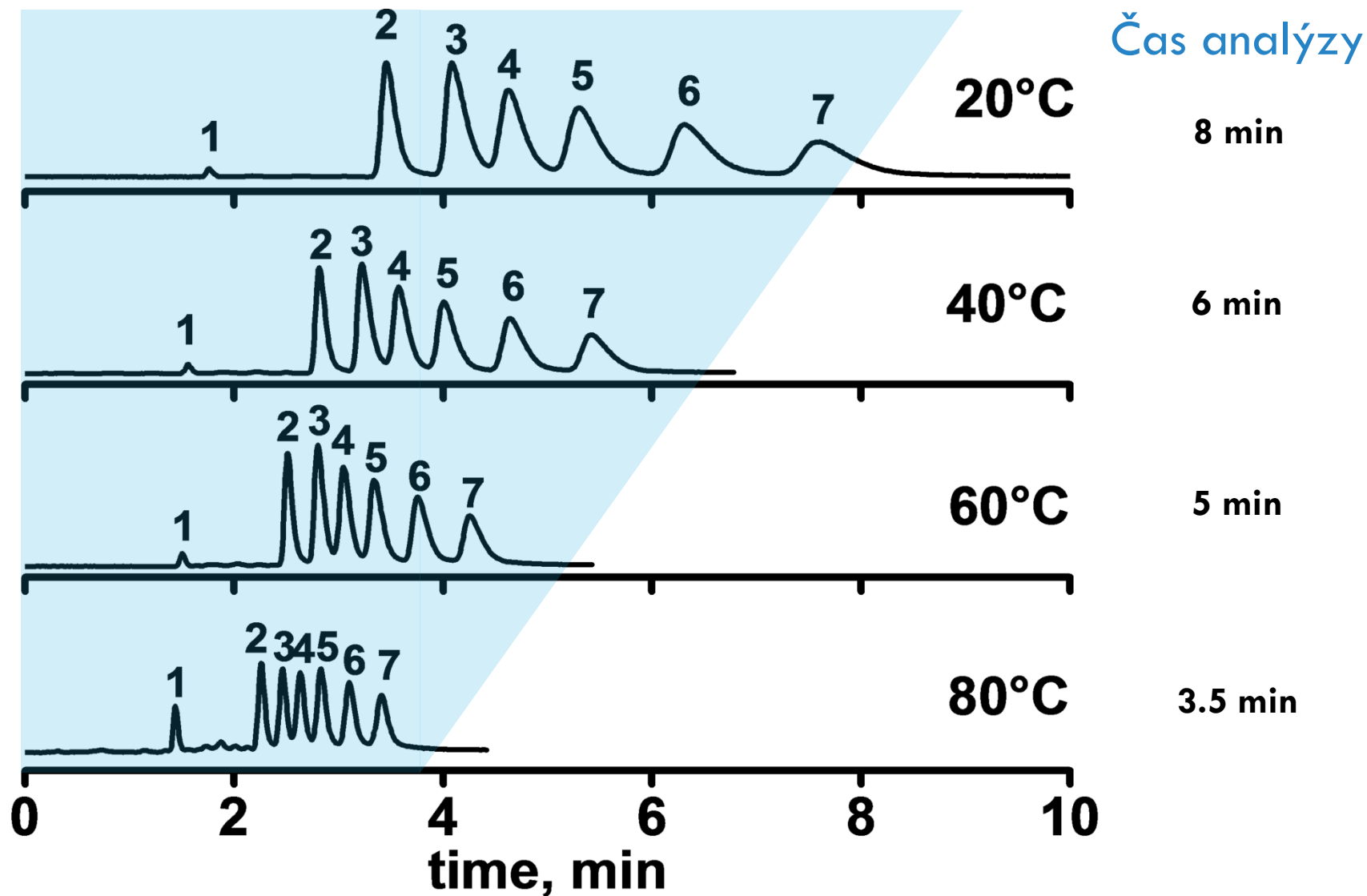
ZLEPŠENÍ SELEKTIVITY



Kolona 170 × 100 mm,
80% ACN, 1.5 $\mu\text{L}/\text{min}$,
16.4 MPa, 254 nm

- 1 – uracil
- 2 – benzen
- 3 – toluen
- 4 – ethylbenzen
- 5 – propylbenzen
- 6 – butylbenzen
- 7 – amylbenzen

TEPLOTA ANALÝZY

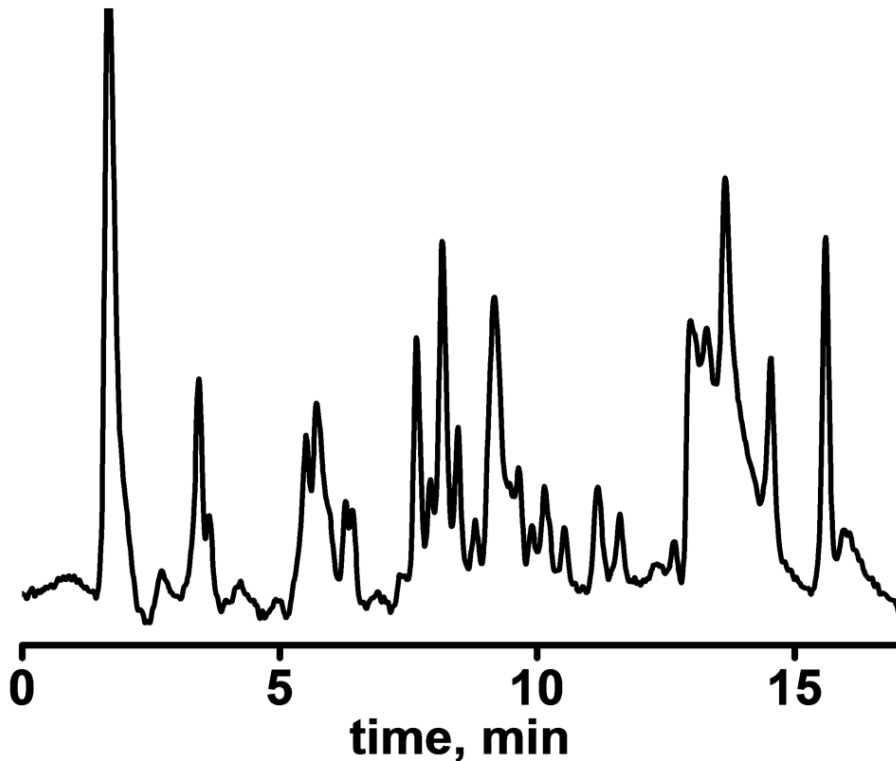


Mobilní fáze 20% vody, 20% THF a 60% ACN, průtok 0.5 $\mu\text{L}/\text{min}$. UV detekce při 254 nm.

Látky: 1 – uracil, 2 – benzen, 3 – toluen, 4 – ethylbenzen, 5 – propylbenzen, 6 – butylbenzen, 7 – amylbenzen.

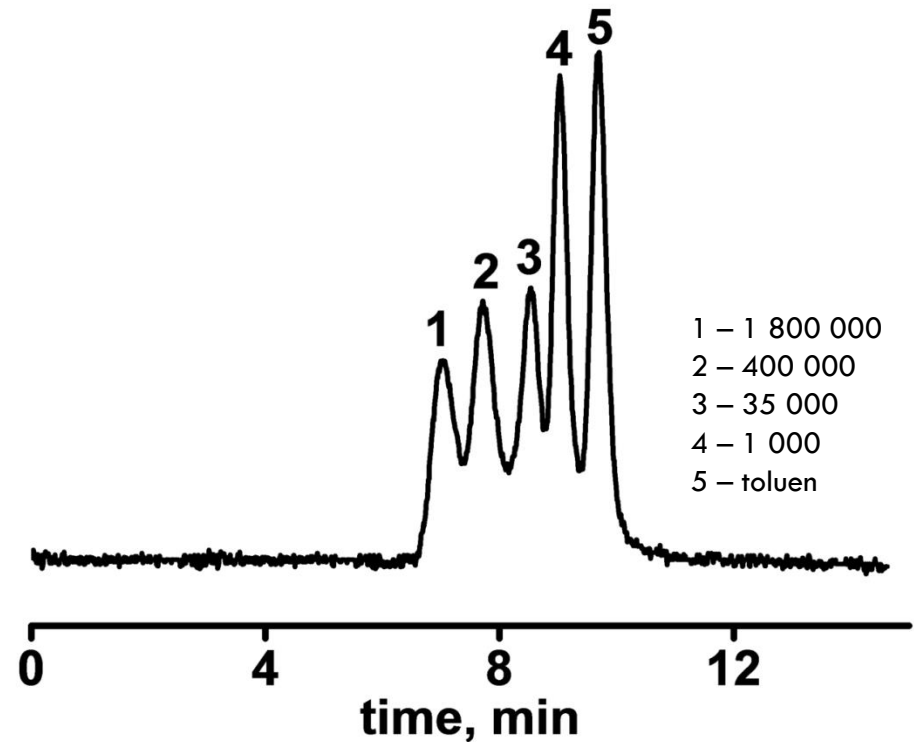
UNIVERZÁLNÍ KOLONA(?)

Gradientová eluce tryptic digestu
(Cytochrome C)



Column modified for 2 h at 90°C, length 130 mm, flow rate 0.5 μ L/min, gradient 5 - 40% ACN in 0.1% aqueous formic acid in 10 min, starting pressure 240 bar, gradient delay 5 min, UV detection

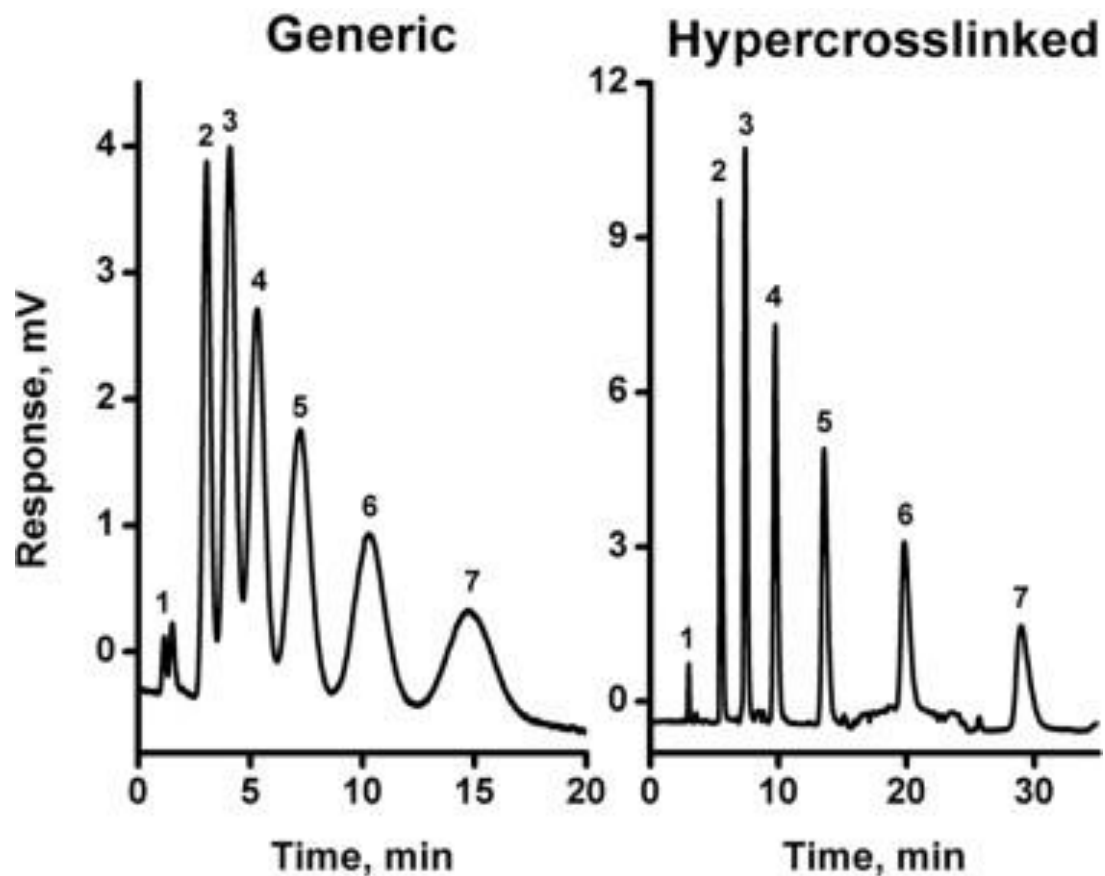
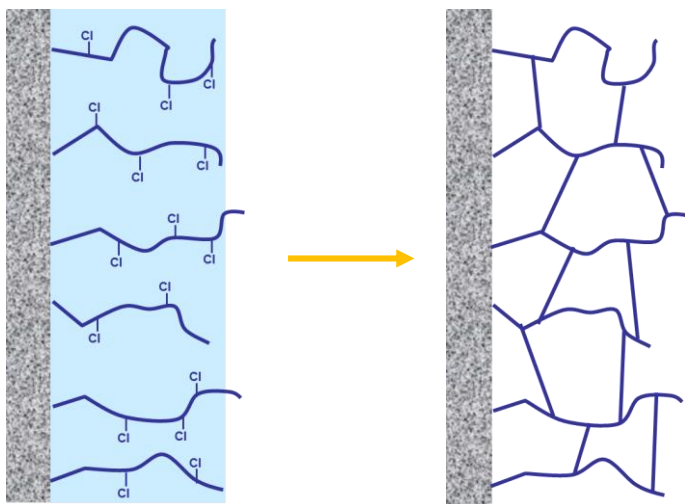
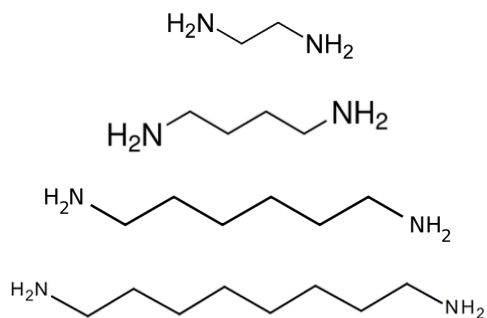
Size-exclusion separace polymerů



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

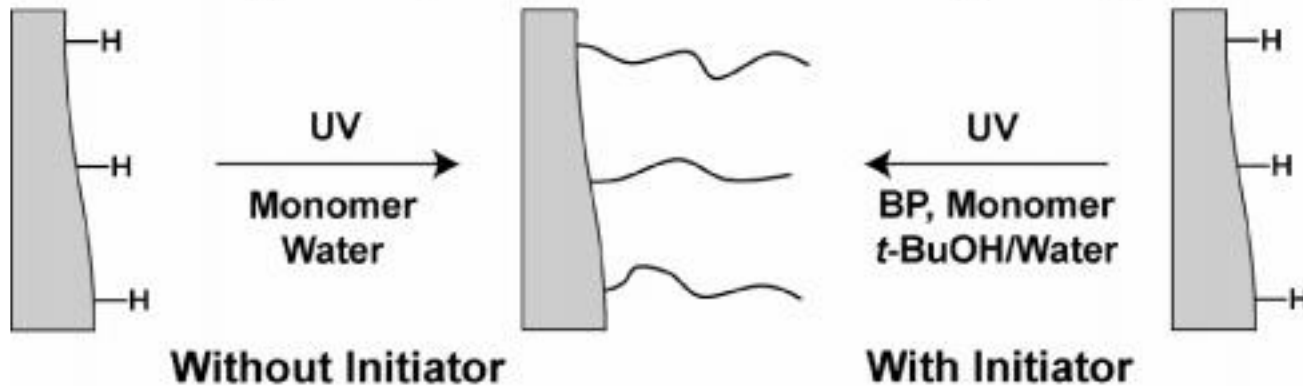
ZESÍTĚNÍ NUKLEOFILNÍ SUBSTITUCÍ

Diaminoalkany



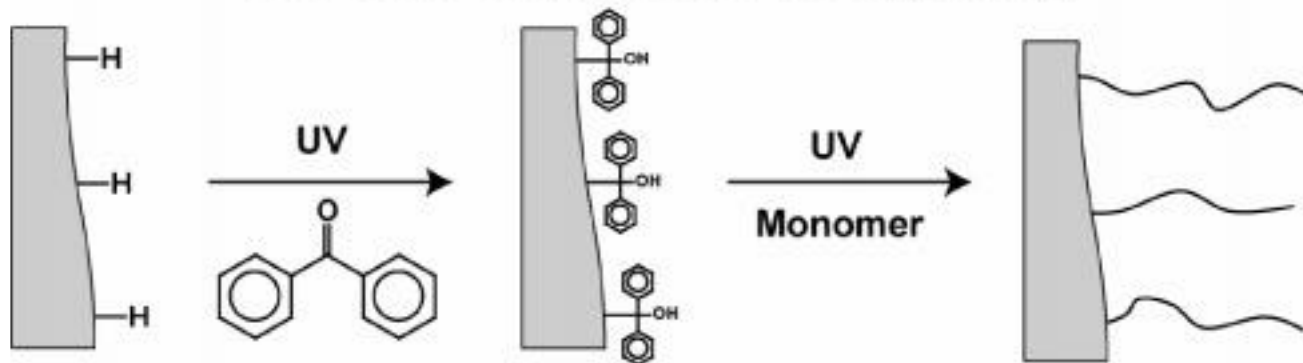
ROUBOVÁNÍ (GRAFTING)

Jednokrokový grafting



- Horší kontrola modifikace
- Možnost ucpání pórů

Dvoukrokový grafting



- Cílená modifikace
- Maximální pokrytí povrchu

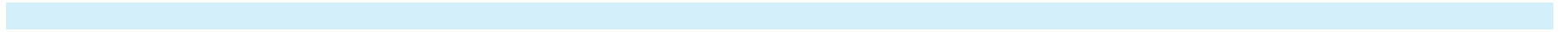
DVOUKROKOVÝ GRAFTING

Původní monolit

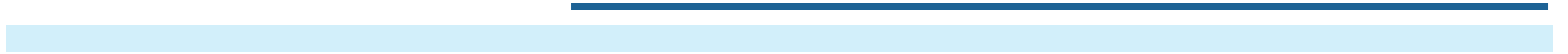


Benzofenon

Původní monolit



UV záření



Aktivovaný povrch



DVOUKROKOVÝ GRAFTING

Aktivovaný povrch



Vybraný funkční monomer



UV záření

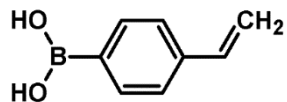


Modifikovaný povrch

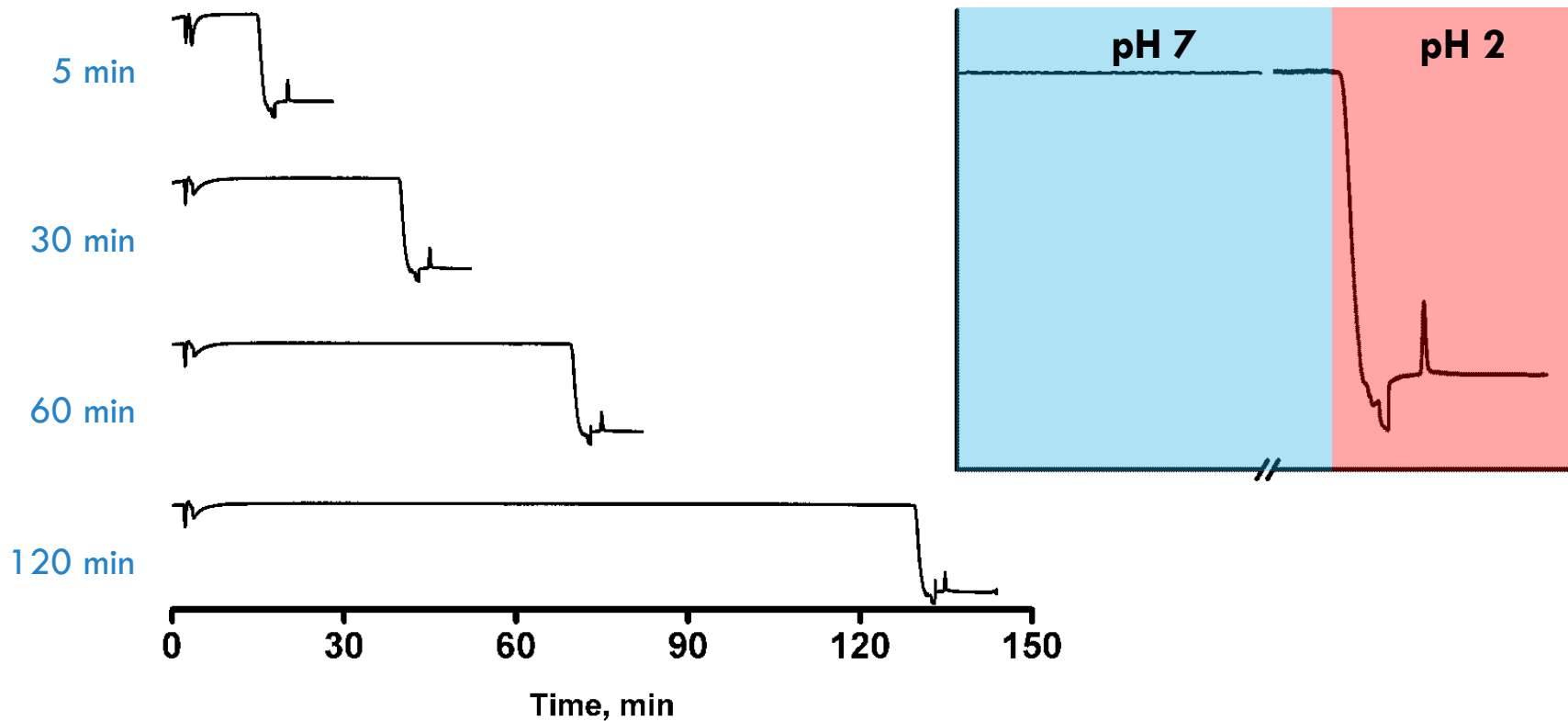
Původní povrch



KONTROLOVANÝ ZÁCHYT ANALYTŮ

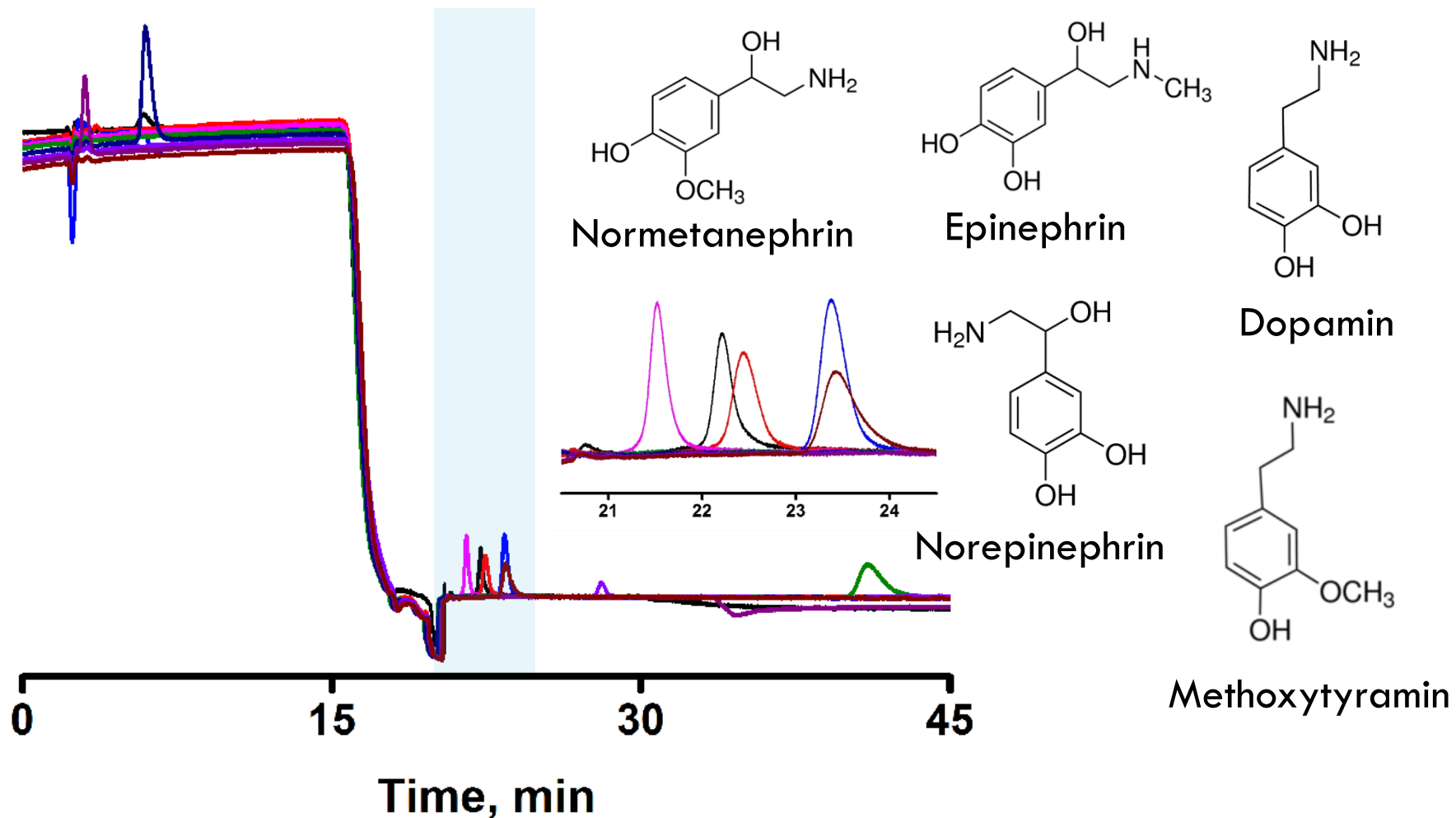


4-vinylphenyl kyselina boritá



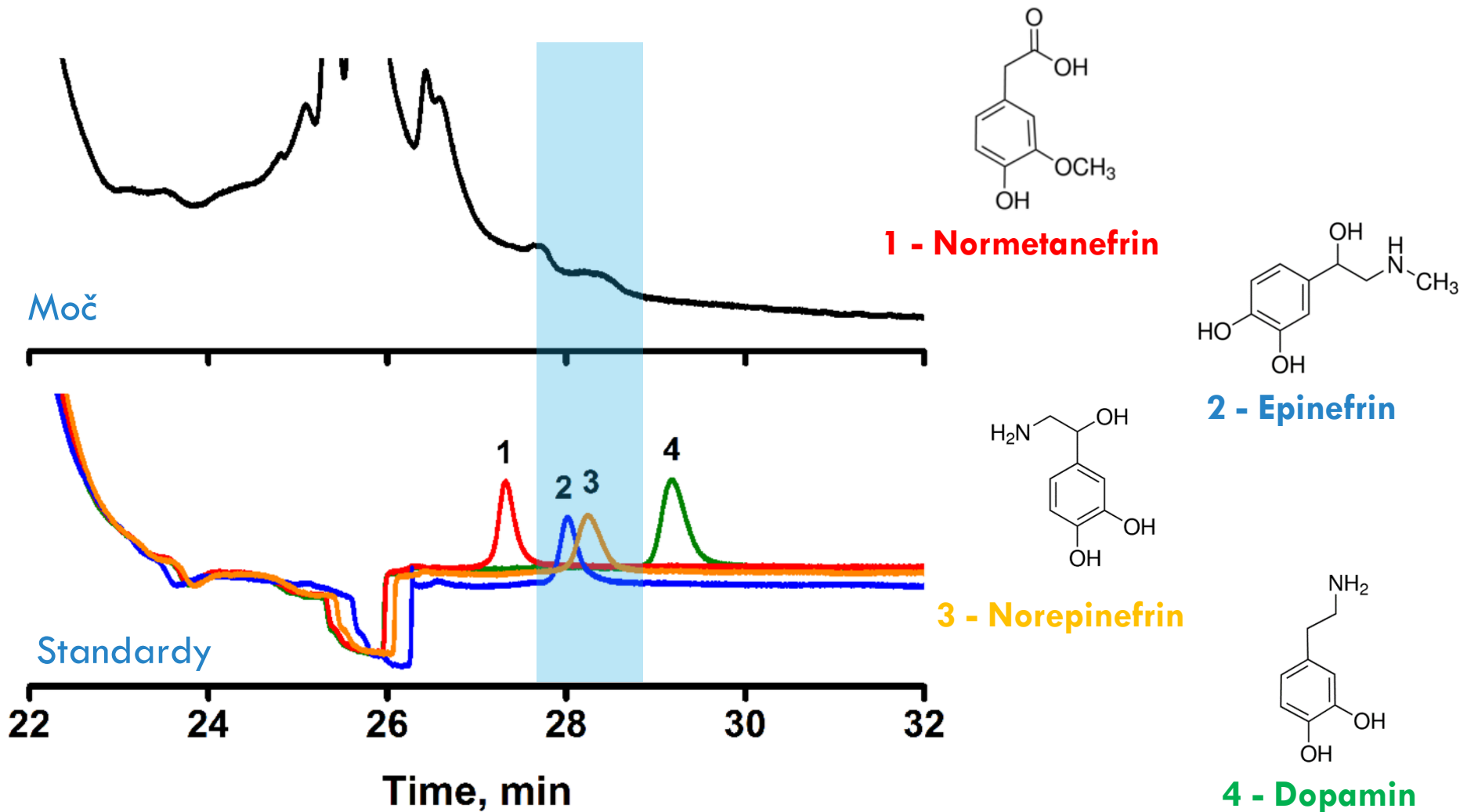
pH 7 – 5 mM HEPES | **pH 2** – 2% acetonitril + 0.1% TFA, dopamin, 0.35 $\mu\text{l}/\text{min}$

EXTRAKCE A ANALÝZA NEUROTRANSMITERŮ

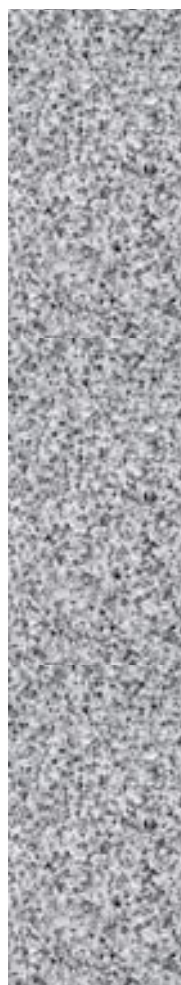


pH 7 – 5 mM HEPES | pH 2 – 2% acetonitril + 0.1% TFA, dopamin, 0.35 μ l/min

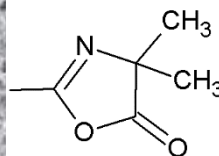
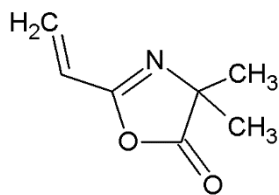
STANOVENÍ NEUROTRANSMITERŮ V MOČI



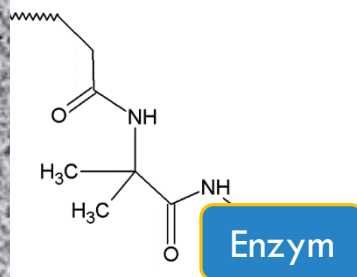
ENZYMAZTICKÉ REAKTORY (IMER_S)



Dvoukrokový
grafting
(vinyl azlacton)



Immobilizace
enzymu

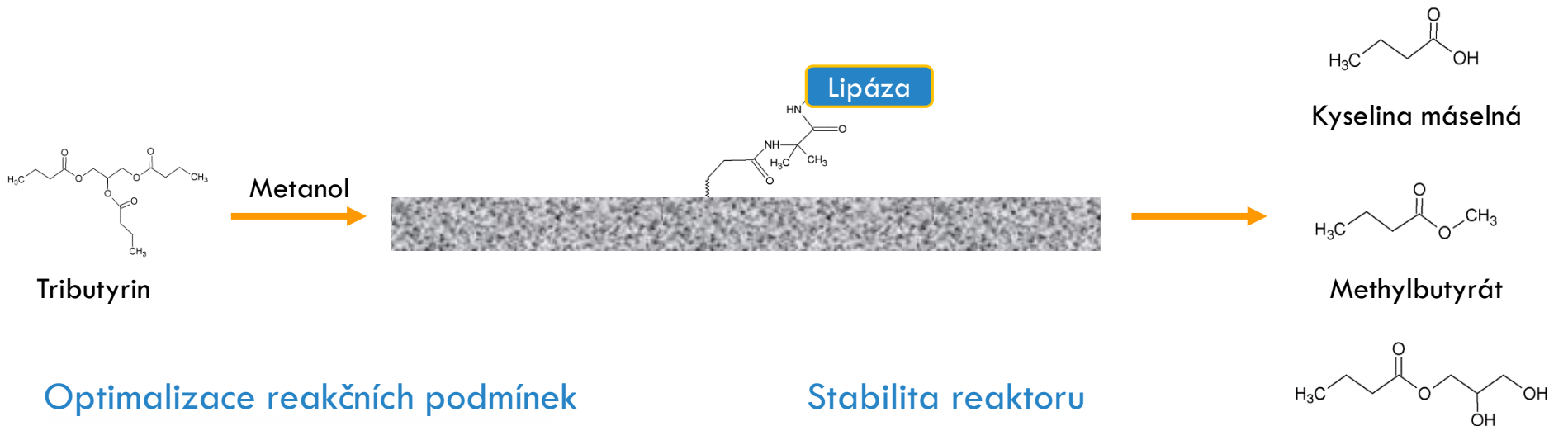


Monolit

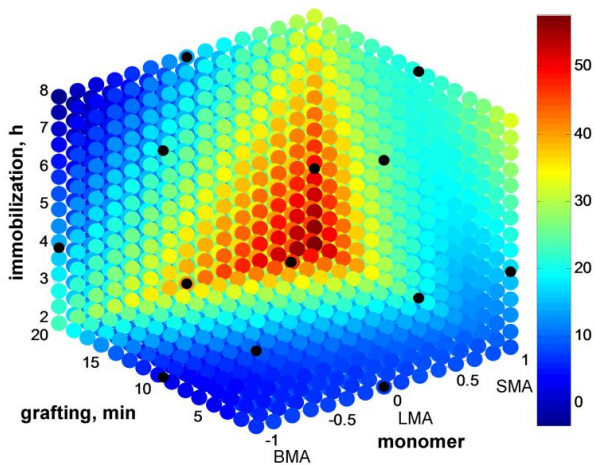
Aktivovaný
monolit

Immobilizovaný Monolitický
Enzymatický Reaktor

PŘÍPRAVA A CHARAKTERIZACE

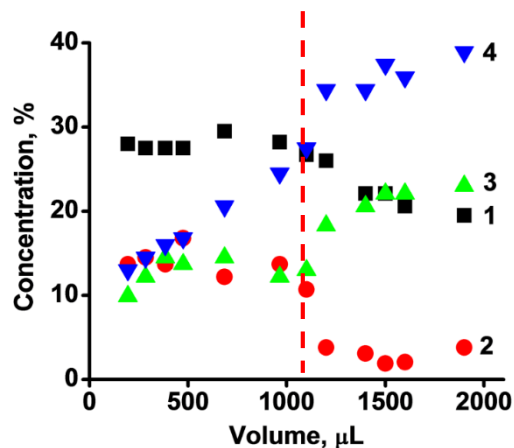


Optimalizace reakčních podmínek



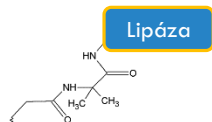
- Stearyl metakrylát
- 20 min VAZ
- 2 h lipase

Stabilita reaktoru

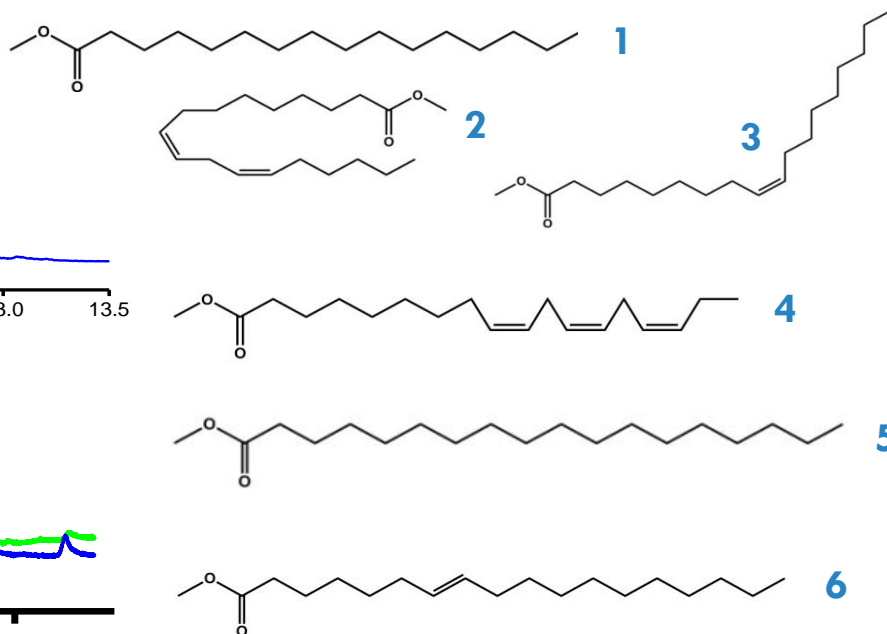
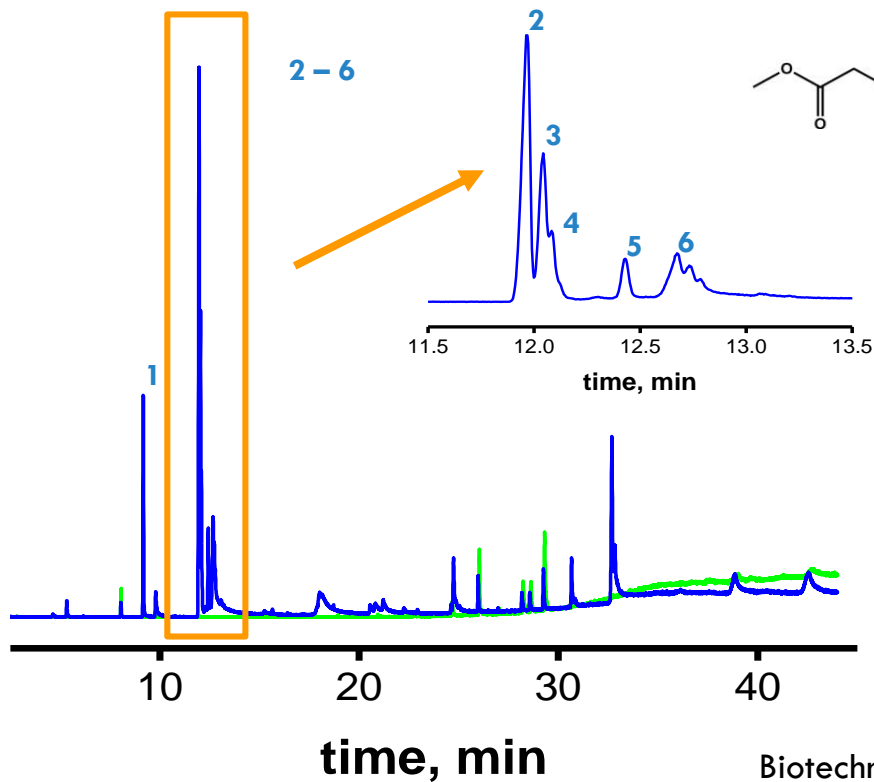


Enzymatický reaktor
pracující v čistě
organickém prostředí

„SYNTÉZA“ BIODIESLU

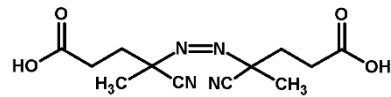
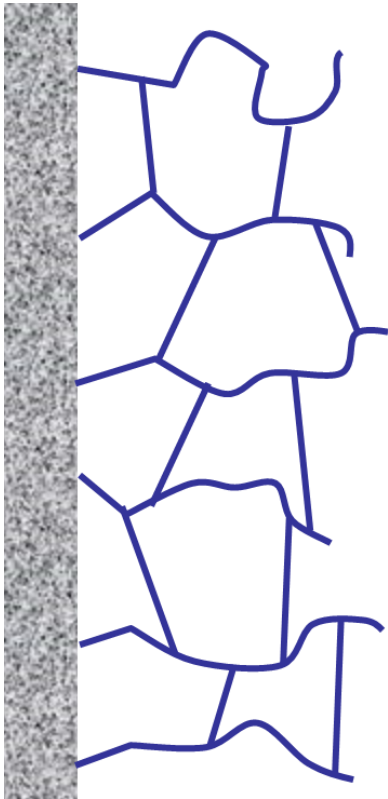


~~Metylestry
mastných kyseliny
biodiesel~~



TEPLOTNÍ GRAFTING

Zesíťný monolit

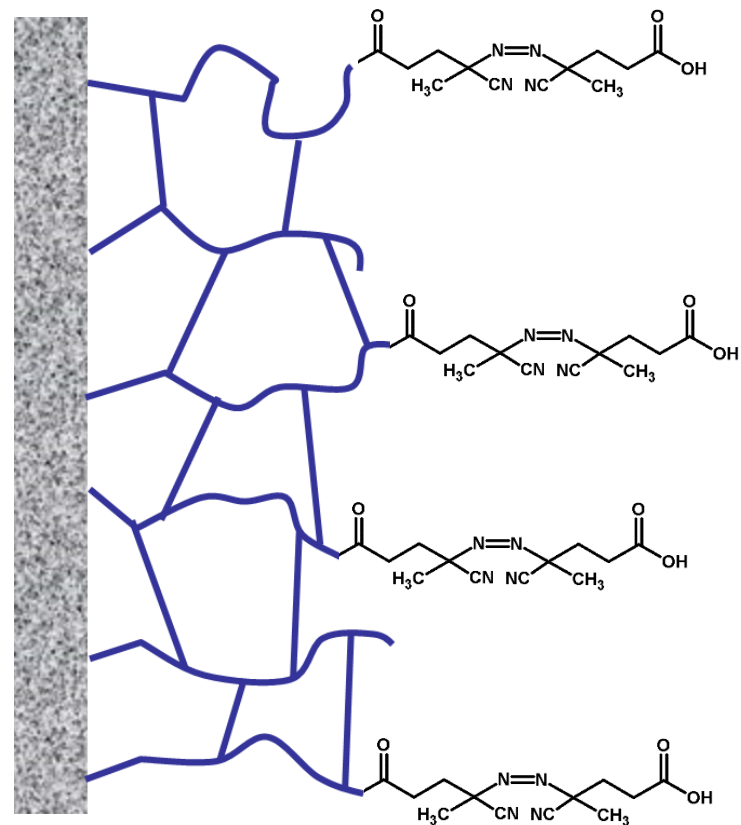


ACVA



TEA
DMF
laboratorní teplota
1 – 20 h

Aktivovaný povrch

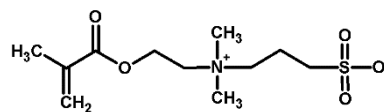
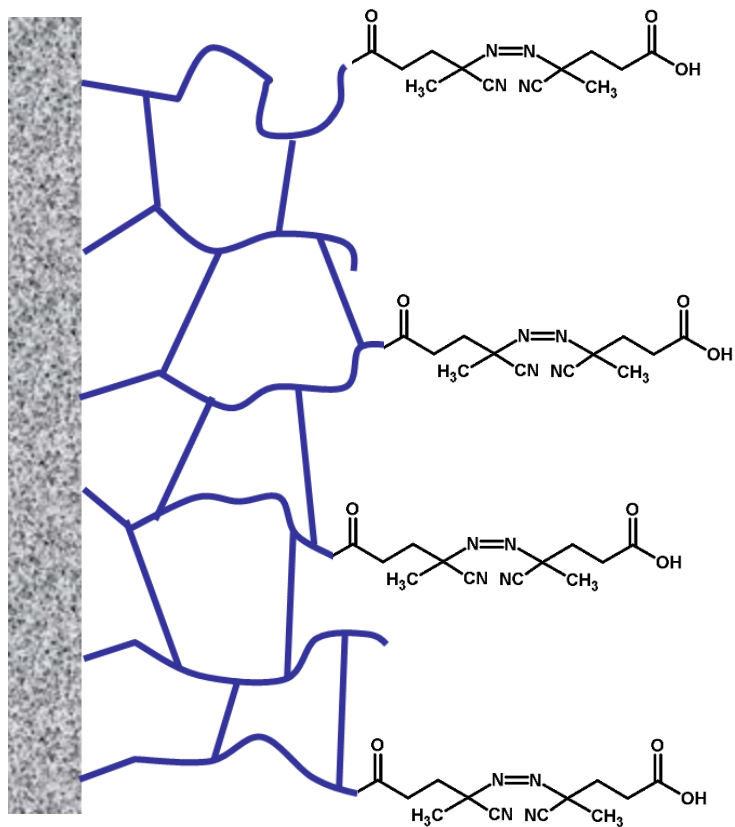


TEA – triethylamin, DMF - dimethylformamid

ACVA – 4,4'-azobis(4-kyanovalerová kyselina)

TEPLOTNÍ GRAFTING

Aktivovaný povrch

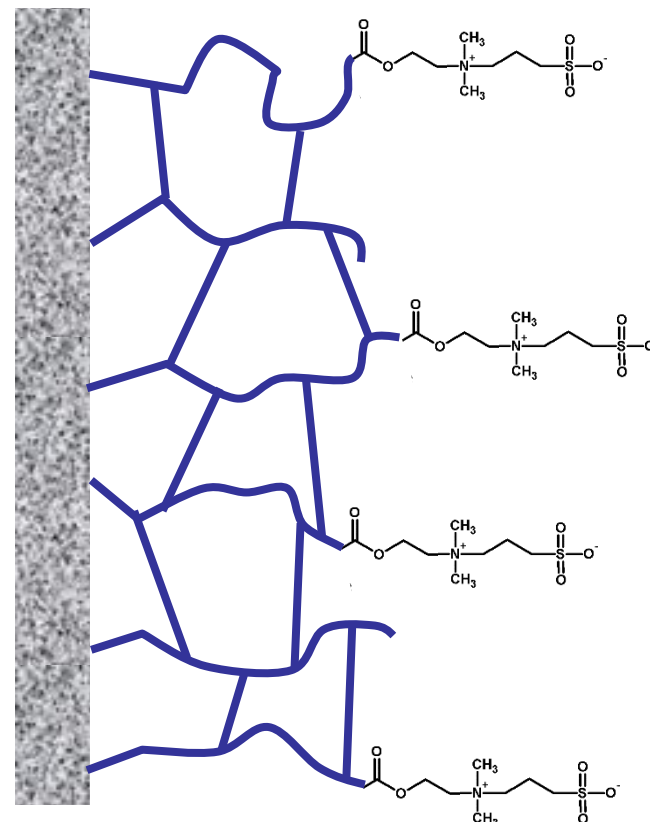


MEDSA



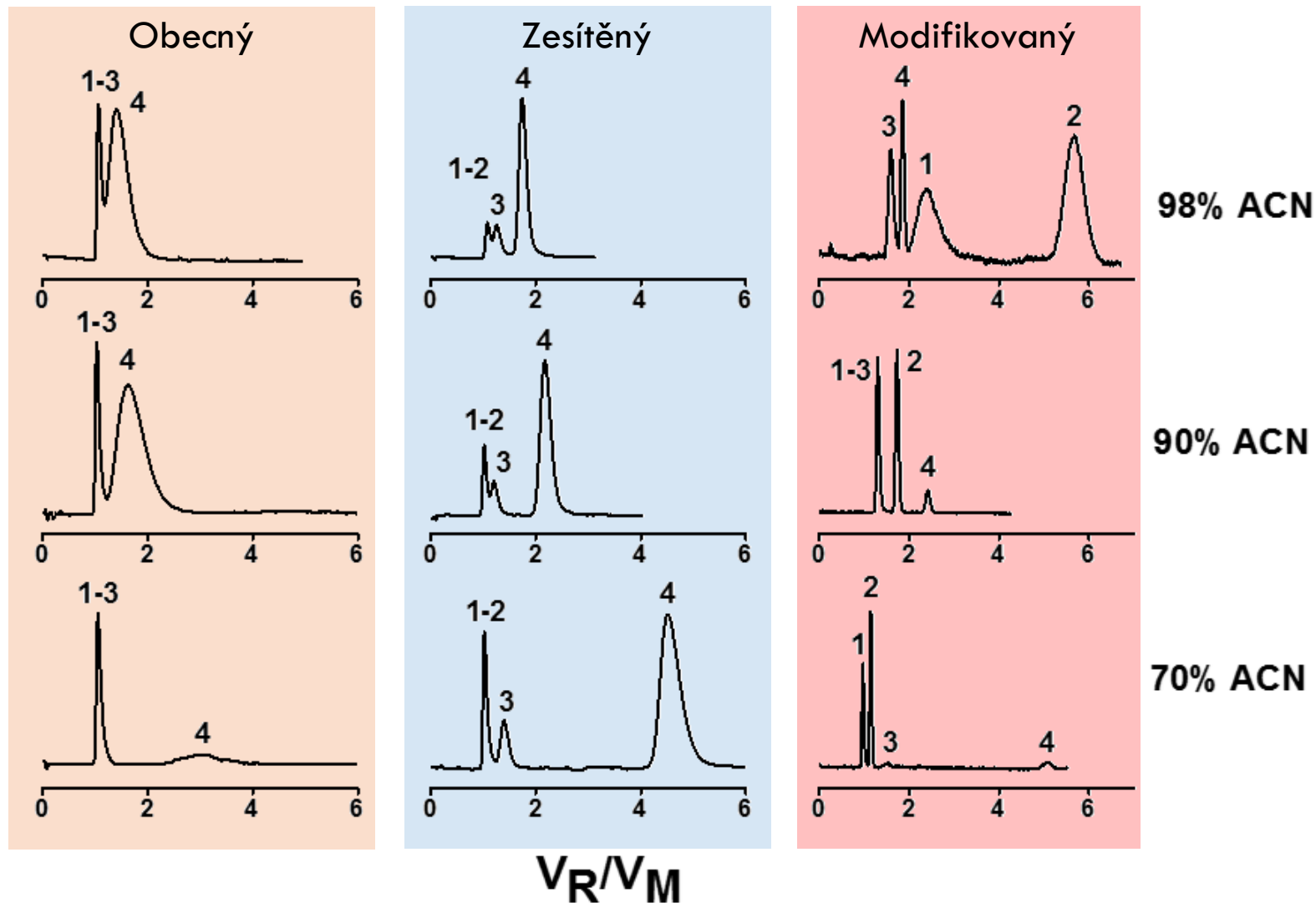
Metanol
50 – 90°C
1 – 24 h

Modifikovaný povrch



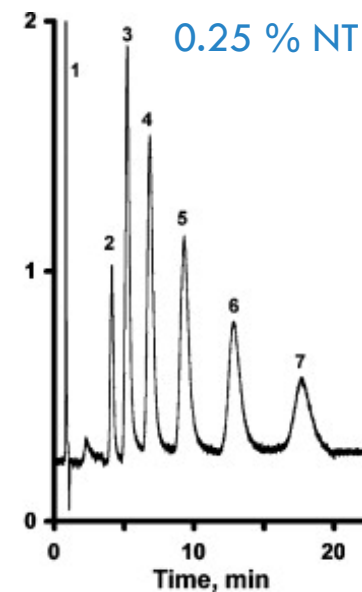
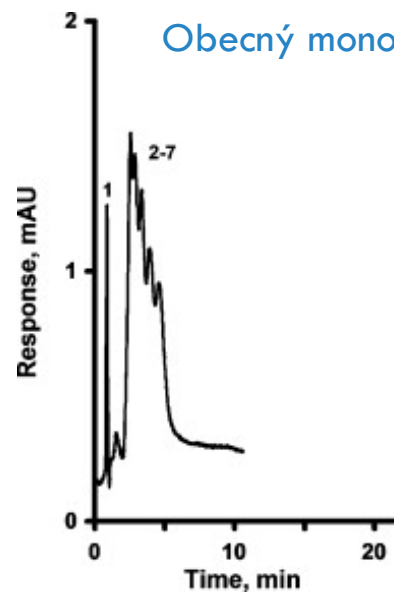
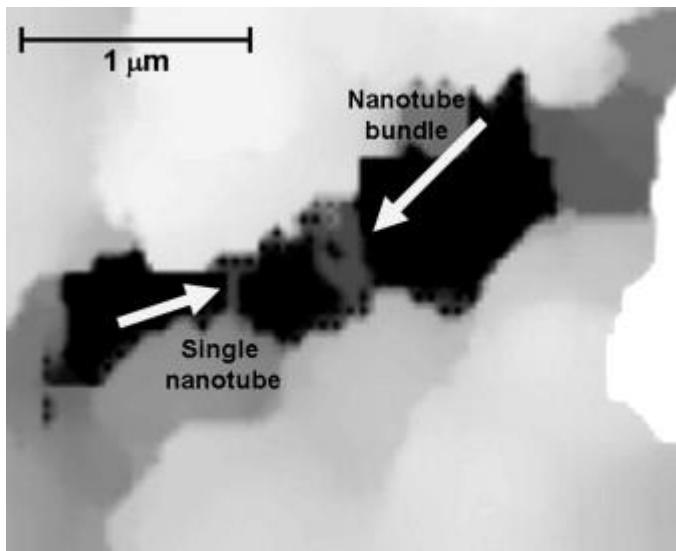
MEDSA - [2-(methacryloyloxy)-ethyl]-dimethyl-(3-sulfopropyl)-ammonium hydroxide

DUÁLNÍ RETENČNÍ MECHANISMUS

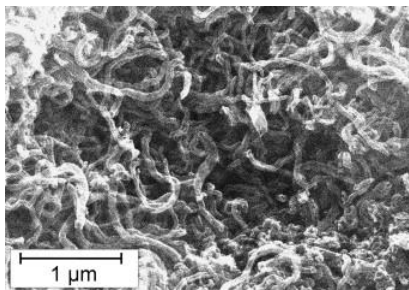


Uracil (1), thiomocovina (2), fenol (3) a toluen (4)

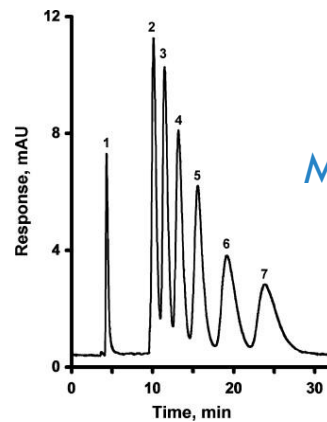
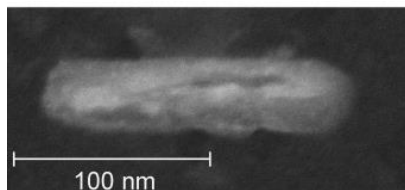
MODIFIKACE NANOČÁSTICEMI



„Směs“ nanotrubiček

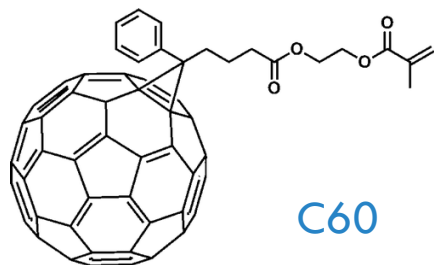


„Nařezané“ NT



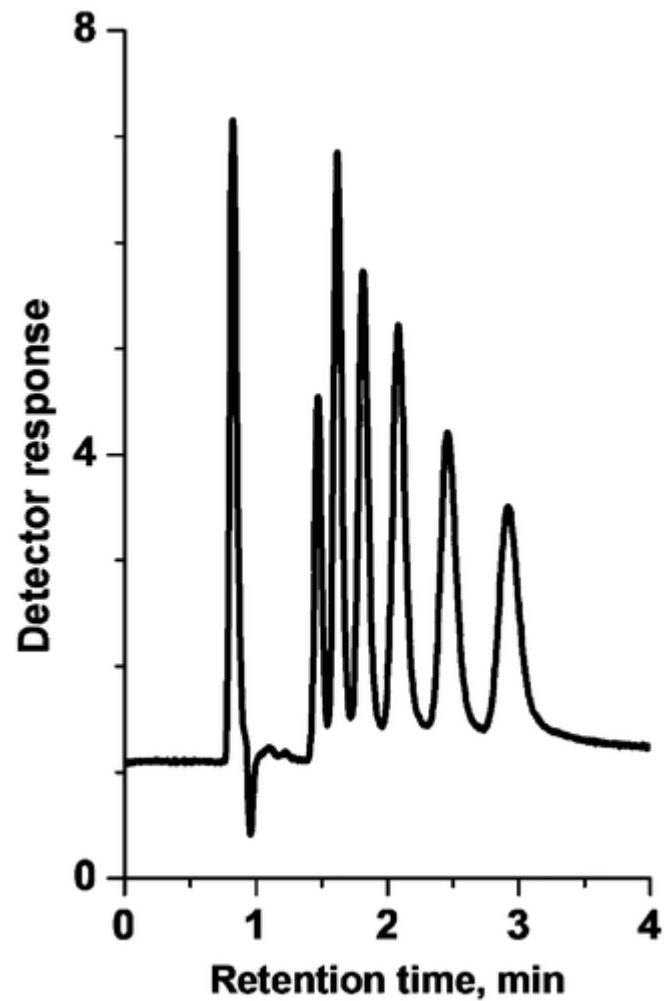
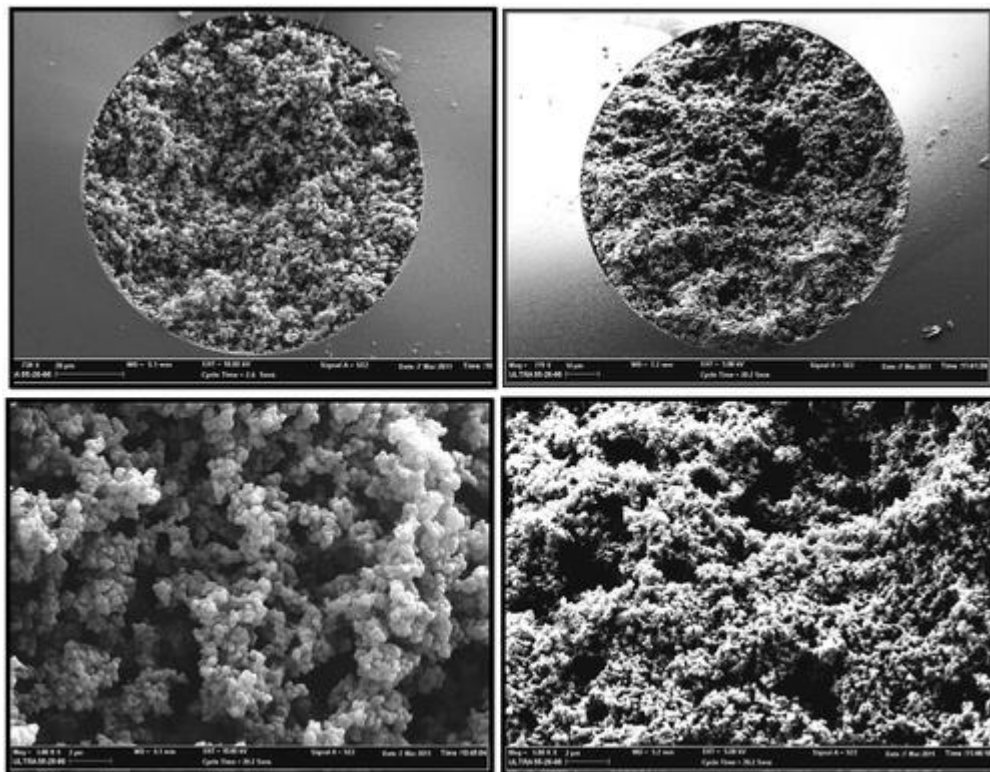
Modifikovaný povrch

MODIFIKACE NANOČÁSTICEMI

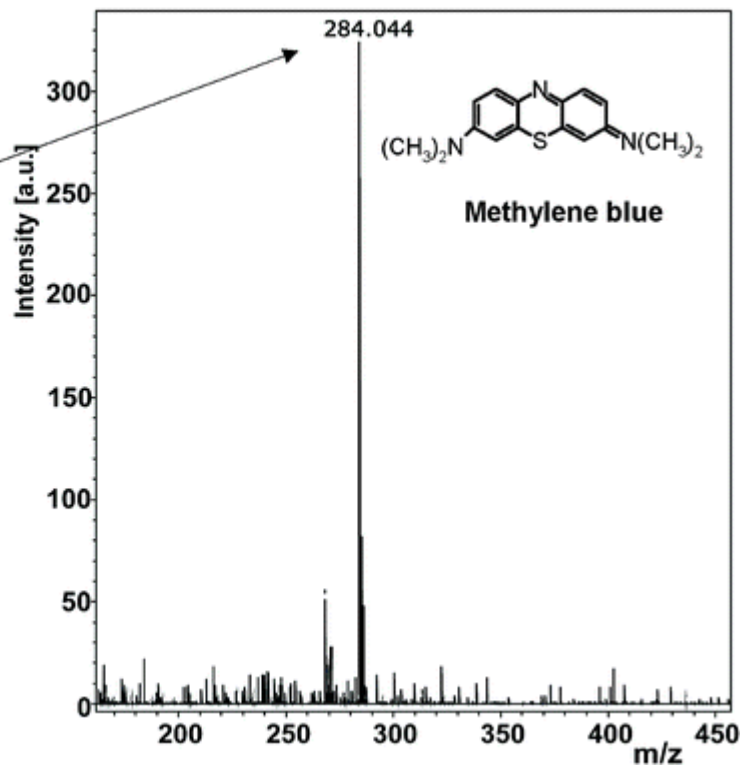
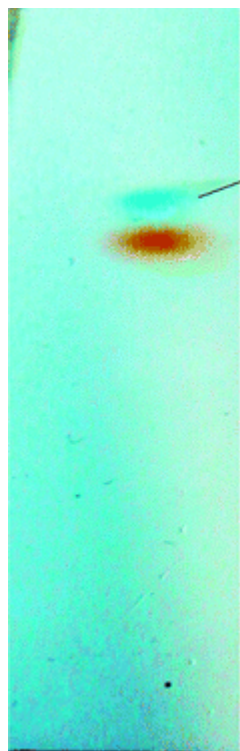
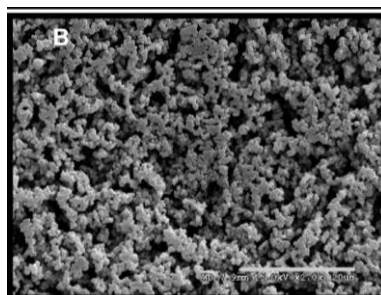
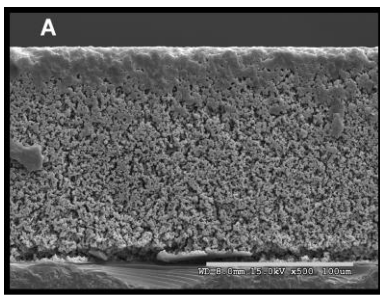


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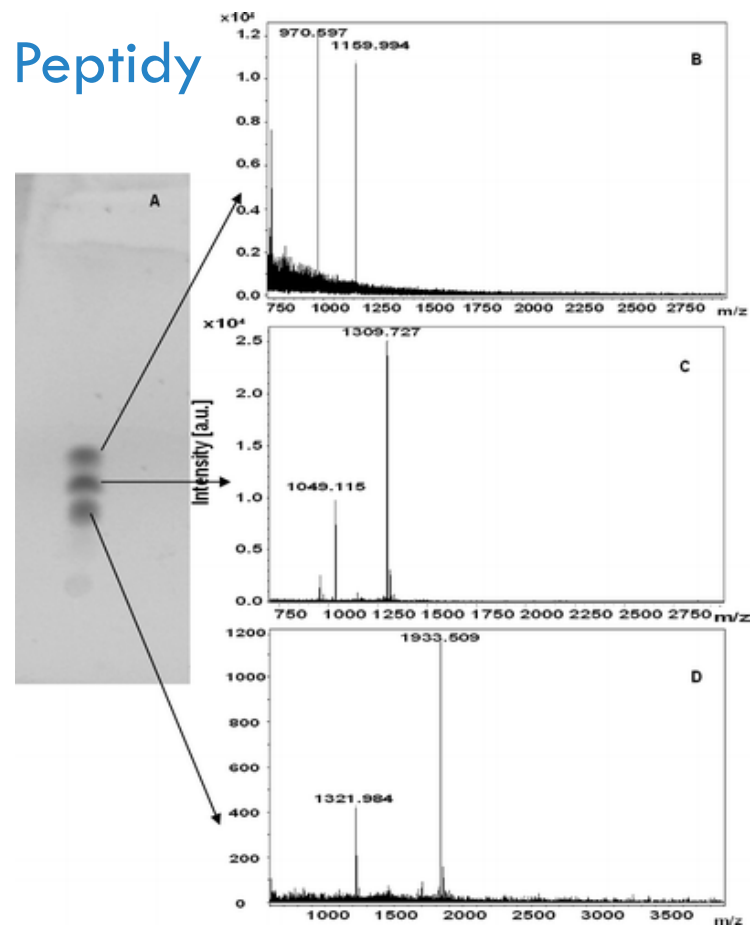
1 %



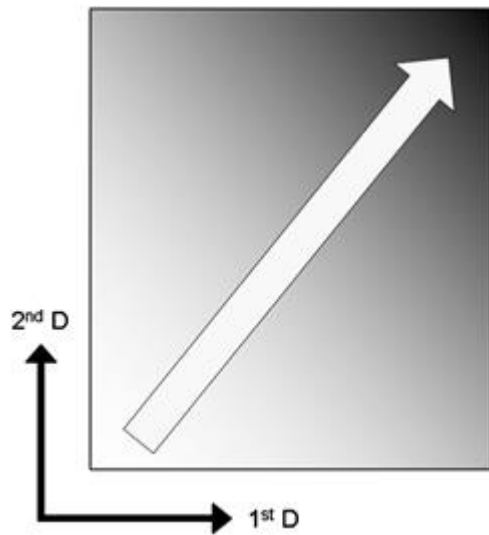
TENKOVRESTEVNÁ CHROMATOGRAFIE



Peptidy



TENKOVRESTEVNÁ 2D CHROMATOGRAFIE

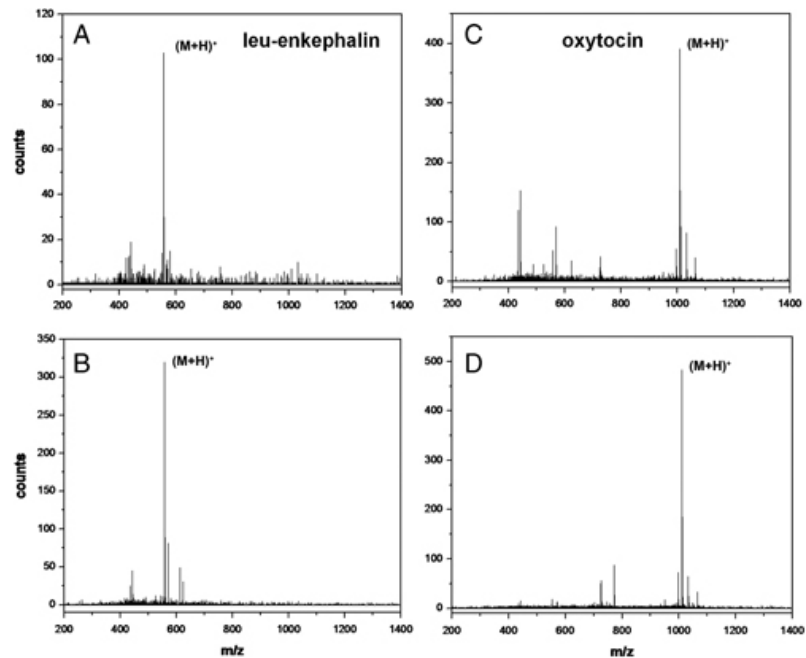
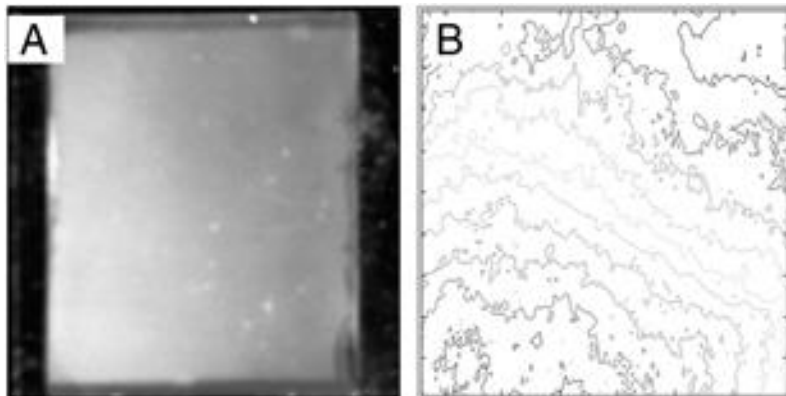
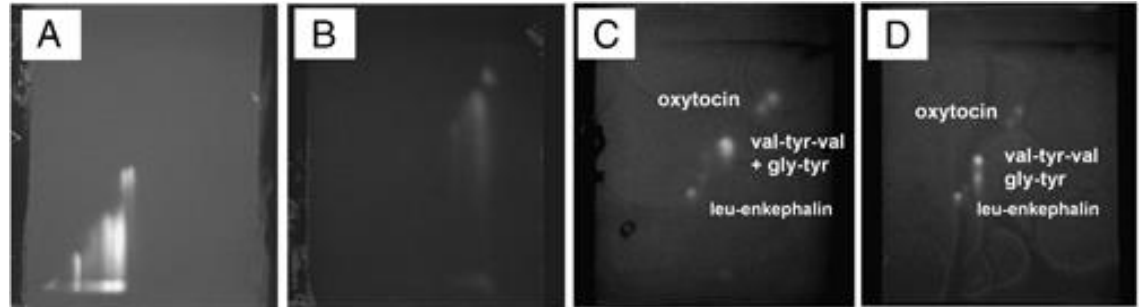


Monolit

Hydrolyza

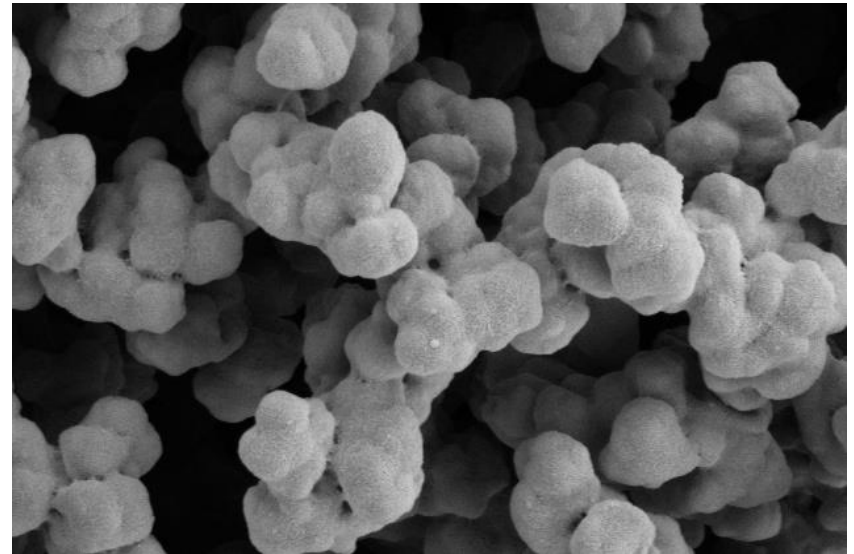
LMA

LMA gradient



POLYMERNÍ MONOLITY

- Jednoduchá příprava
- Snadná modifikace
- Úprava vlastností
- Kontrola selektivity
- Integrované systémy





*„Science is simply the word we use
to describe a method of organizing
our curiosity“*

Tim Minchin