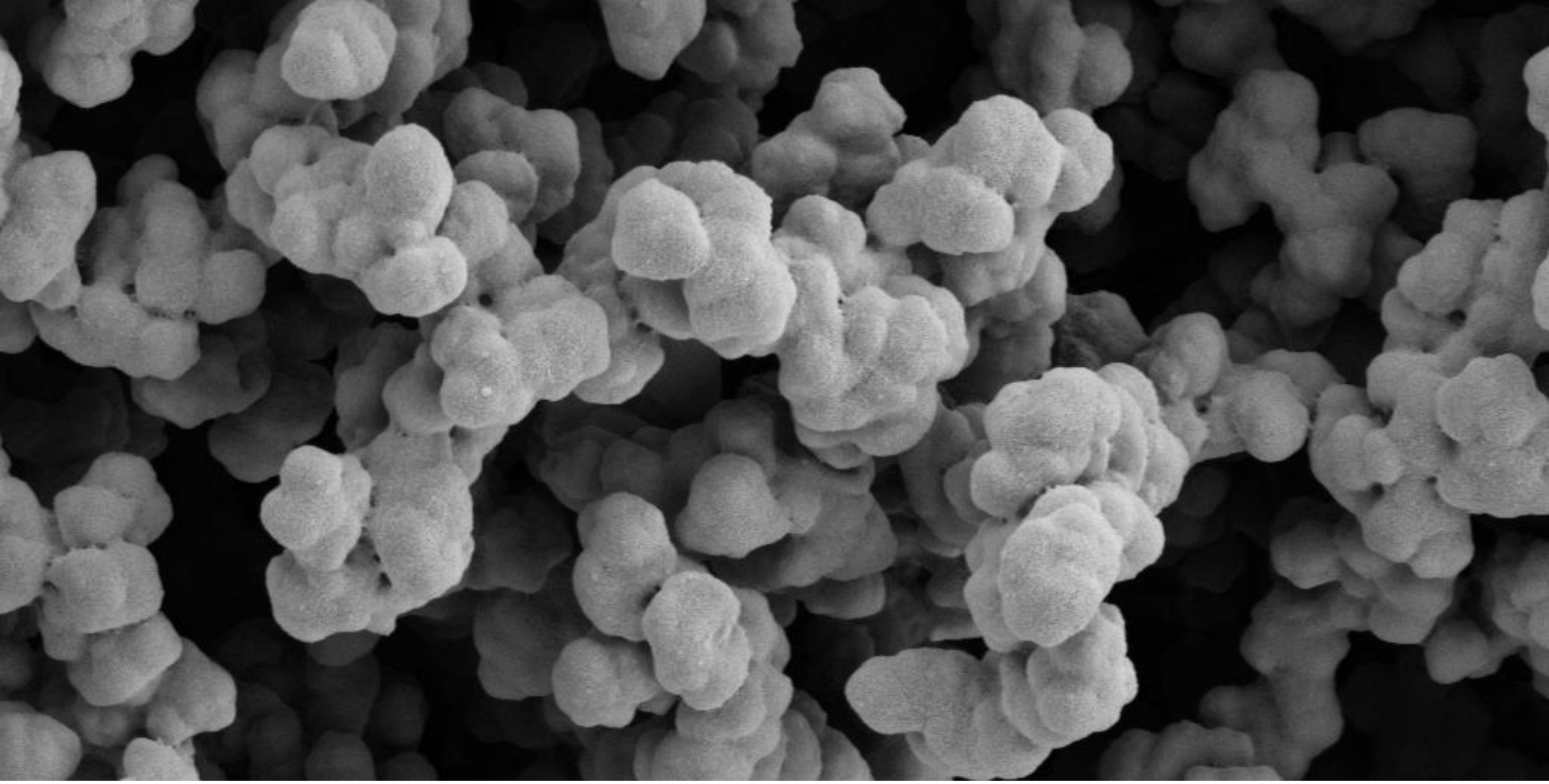


POLYMERNÍ MONOLITY

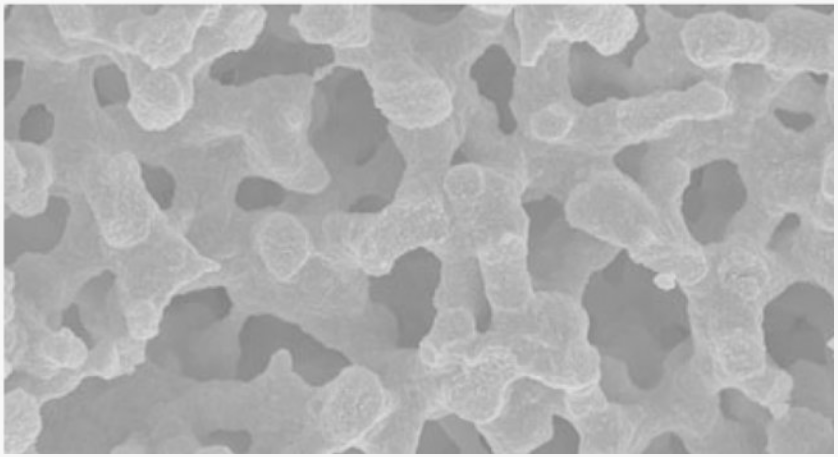
Pokročilá kapalinová chromatografie

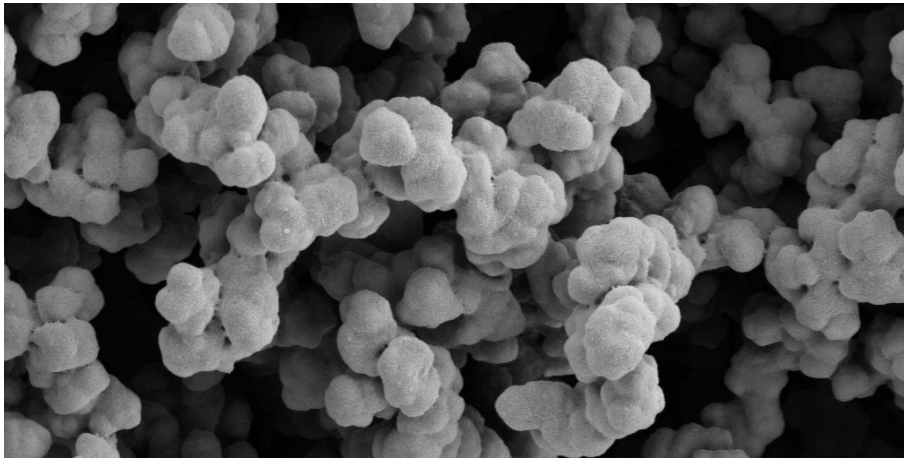
Jiří Urban, Ústav chemie, Přírodovědecká fakulta, Masarykova univerzita, Brno, urban@chemi.muni.cz

SVĚTOVÉ MONOLITY

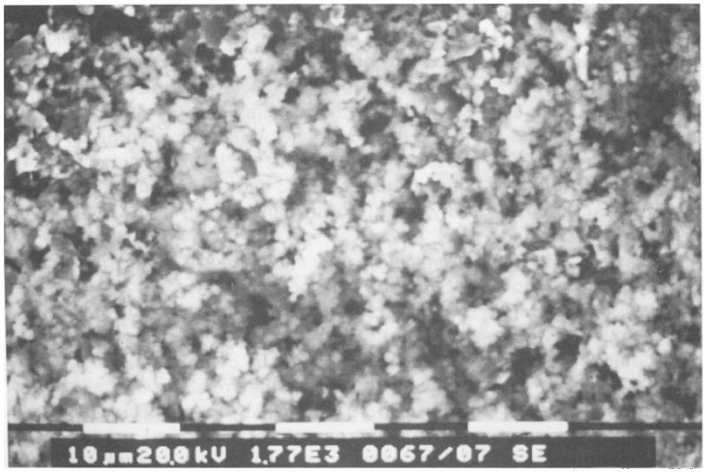
www.crystalinks.com

Peter Polichronis





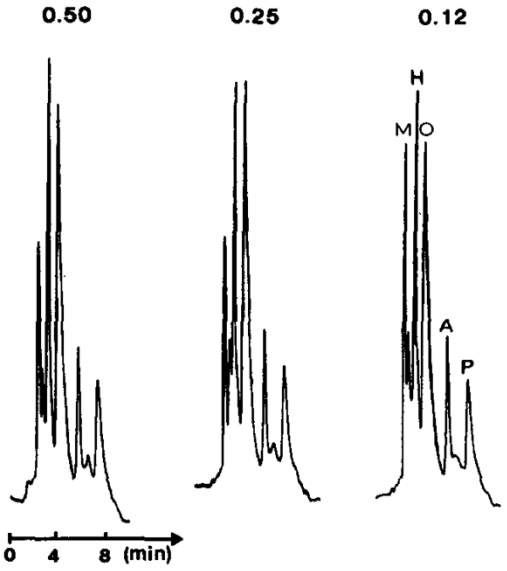
STLAČENÉ GELY

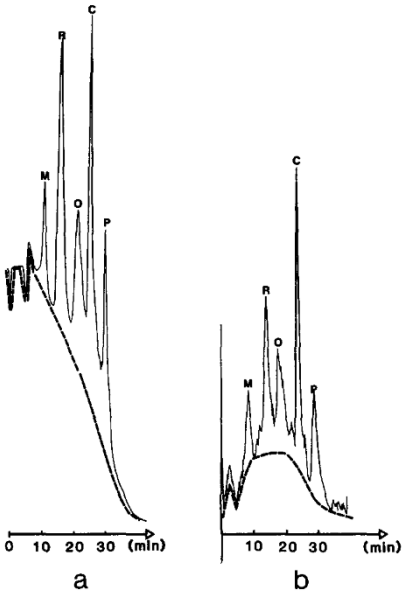


- Polymerace vodného roztoku N,N´-methylenebis-akrylamidu a kyseliny akrylové v přítomnosti anorganické soli, nejčastěji síranu amonného
- Stlačení na méně než 10 % původního objemu
- Separace bílkovin v iontově-výměnném módu

(neočekávané) zvýšení permeability po stlačení gelu

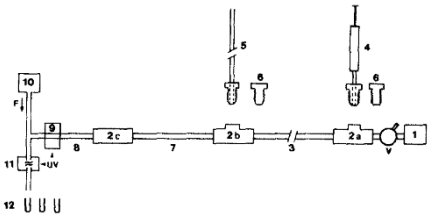
Vliv průtoku mobilní fáze





Lineární gradient
2.25 – 0.25 M síran amonný

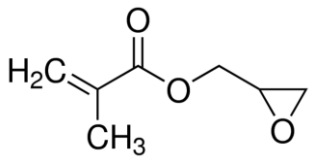
- myoglobin (M)
- ribonuclease (R)
- ovalbumin (O)
- α-chymotrypsinogen A (C)
- phycoerythrin (P)



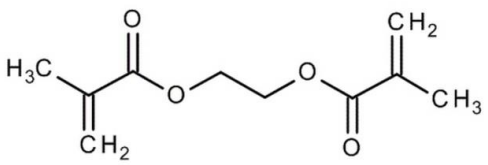
J. Chromatogr., 473 (1989) 273., J. Chromatogr. 586 (1991) 21-26.

MEMBRÁNOVÁ CHROMATOGRAFIE

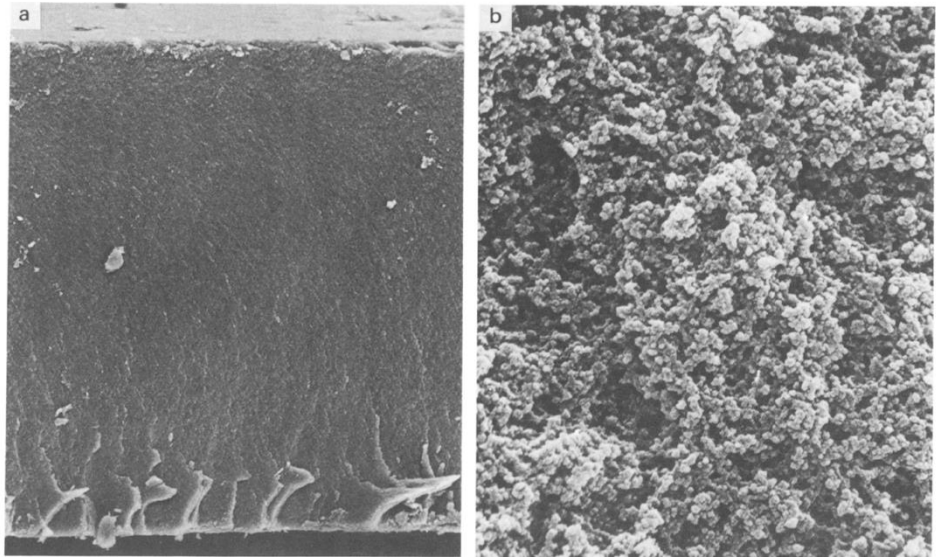
Pro separace vysokomolekulárních látek je potřeba velmi krátká kolona (B.G. Belenkii)



Glycidyl metakrylát

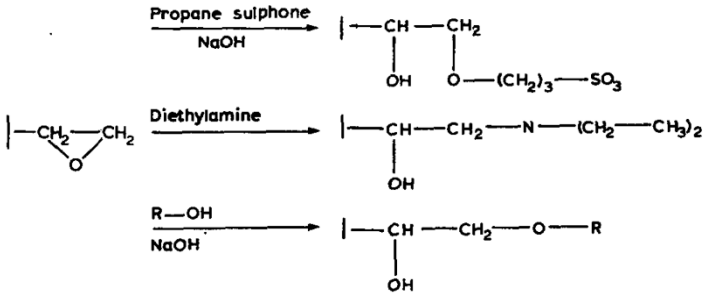


Ethylen dimetakrylát



800x 5400x

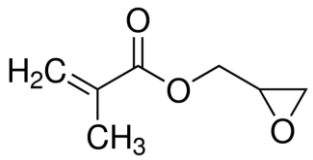
Reaktivní povrch



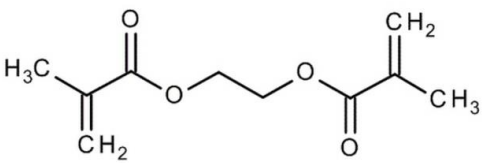
J. Chromatogr. 555 (1991) 97-107.

MEMBRÁNOVÁ CHROMATOGRAFIE

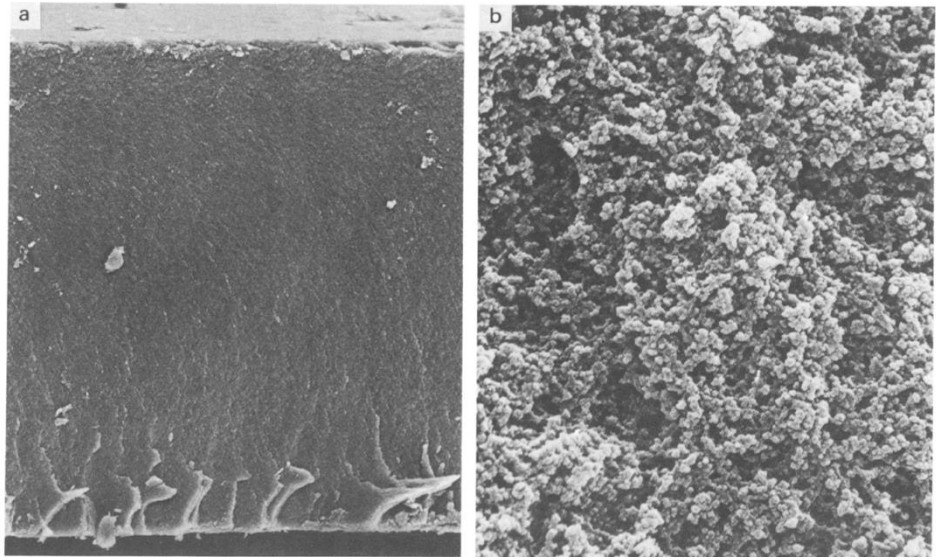
~~Pro separace vysokomolekulárních látek je potřeba velmi krátká kolona (B.G. Belenkii)~~



Glycidyl metakrylát

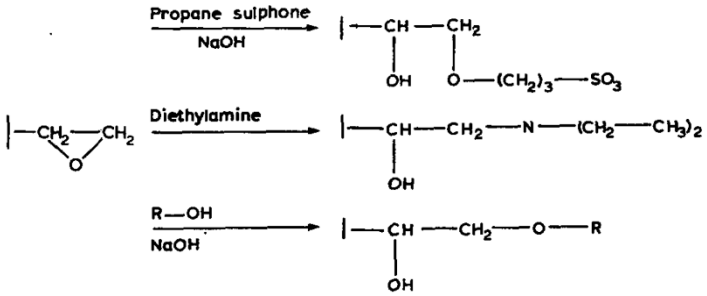


Ethylen dimetakrylát



800x 5400x

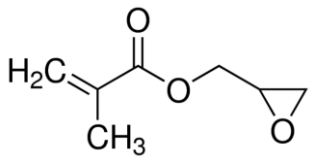
Reaktivní povrch



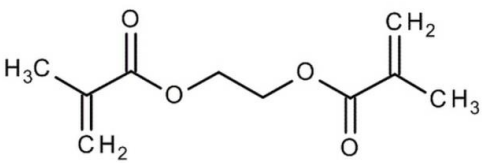
J. Chromatogr. 555 (1991) 97-107.

MEMBRÁNOVÁ CHROMATOGRAFIE

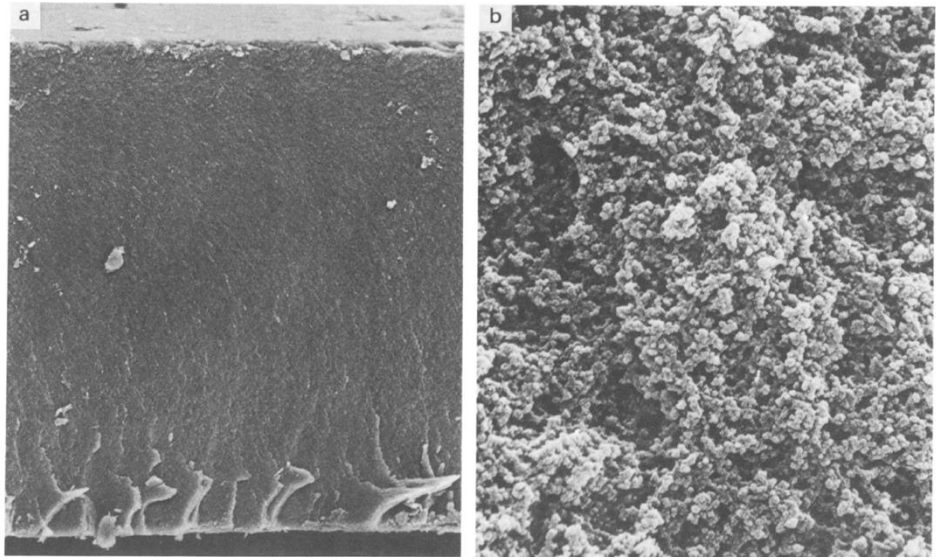
~~Pro separace vysokomolekulárních látek je potřeba velmi krátká kolona (B.G. Belenkii)~~



Glycidyl metakrylát

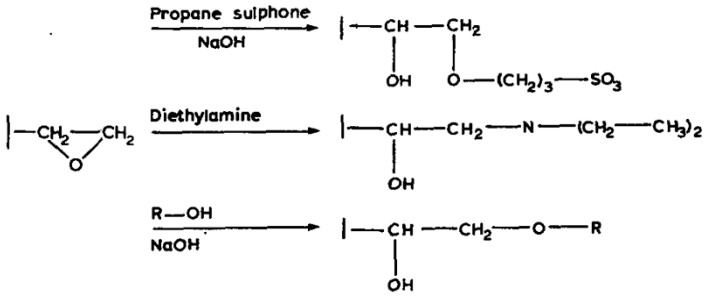


Ethylen dimetakrylát



800x 5400x

Reaktivní povrch

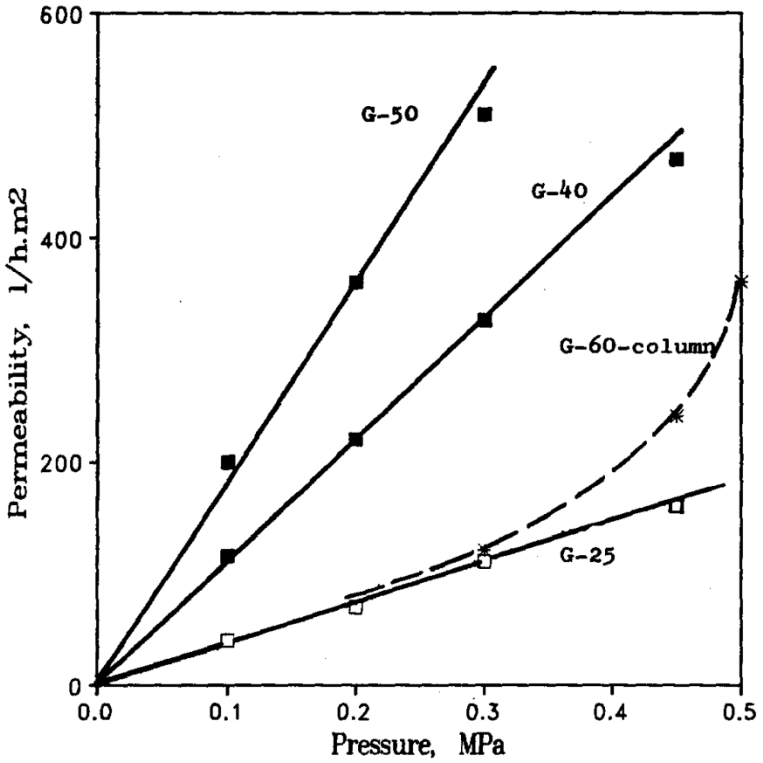


J. Chromatogr. 555 (1991) 97-107.

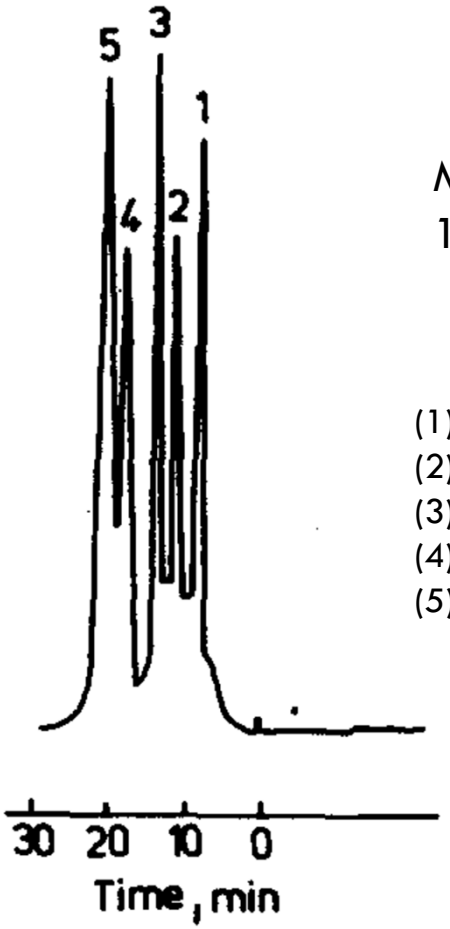
MEMBRÁNOVÁ CHROMATOGRAFIE

Vliv složení polymerační směsi

(rostoucí koncentrace GMA)



Separace bílkovin

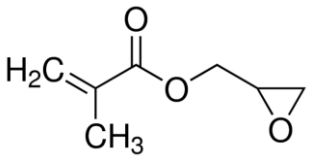


Membrána
1 x 20 mm

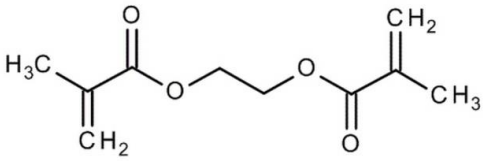
(1) Myoglobin
(2) Ovalbumin
(3) Ribonucleáza A
(4) Lysozym
(5) Chymotrypsinogen

J. Chromatogr. 555 (1991) 97-107.

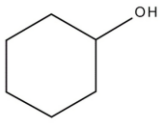
POLYMERNÍ MONOLITY



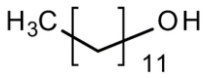
Glycidyl metakrylát



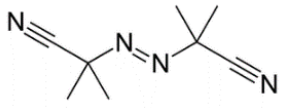
Ethylen dimetakrylát



Cyklohexanol



Dodekanol

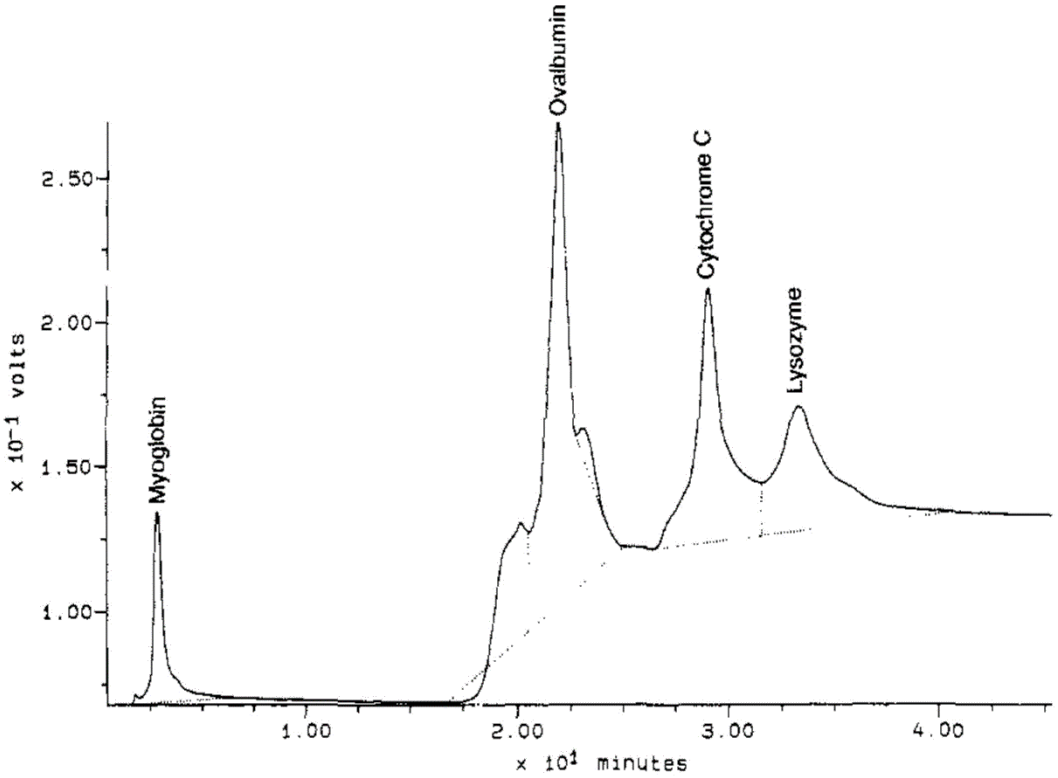


Azobisisobutyronitril

6 h při 70 °C
30 x 8 mm

Chromatografie iontové výměny

modifikováno (diethylamino)hydroxypropylem



Anal. Chem. 64 (1992) 820-822.

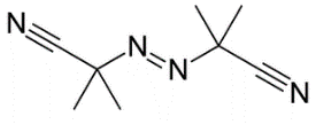
RADIKÁLOVÁ POLYMERACE

RADIKÁLOVÁ POLYMERACE

Fáze polymerace

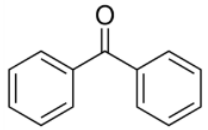
1) Iniciace
2) Propagace
3) Terminace

Azobisisobutyronitril

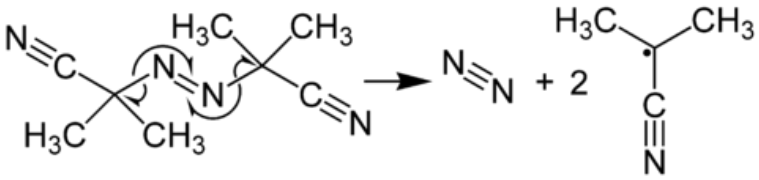


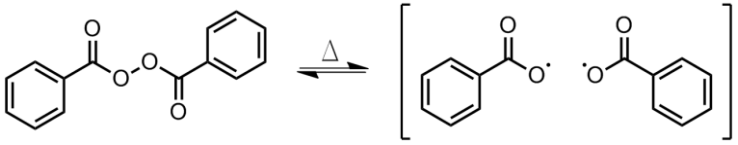
Teplotou iniciovaná polymerace

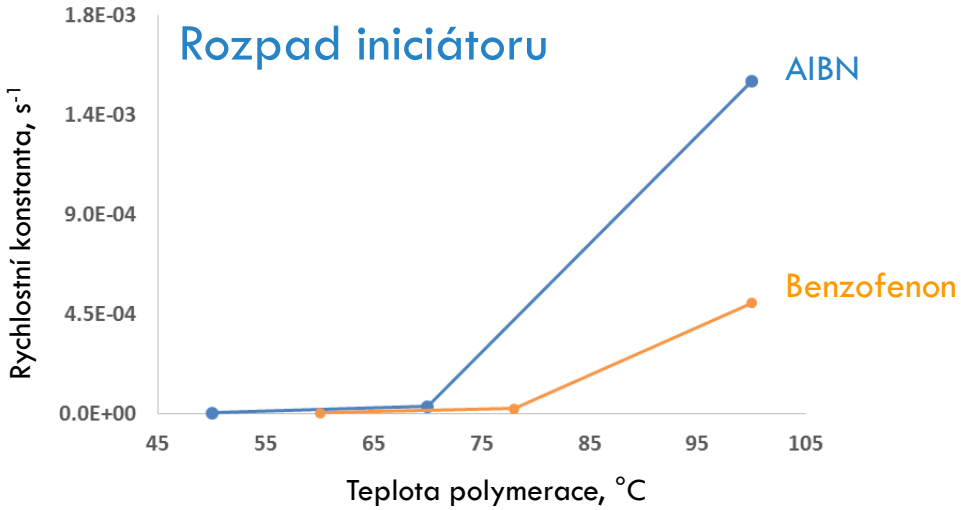
Benzofenon



UV zářením iniciovaná polymerace







POLYMERNÍ MONOLITY

Modifikace vnitřní stěny

↓

Kapilára

↓

Iniciace

UV, T, …

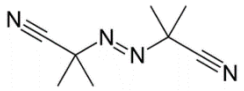
↓

Monolitická kapilární kolona

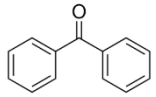
Polymerační směs

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

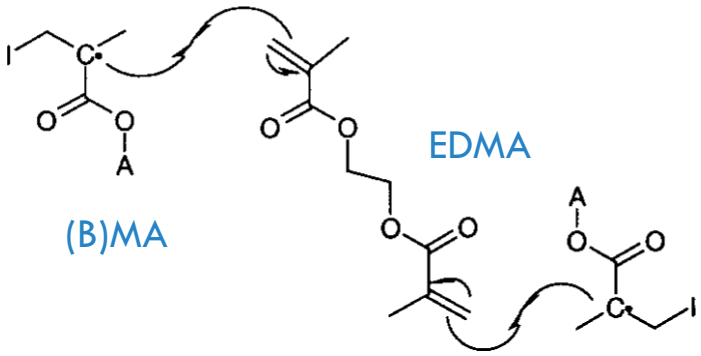
azobisisobutyronitril



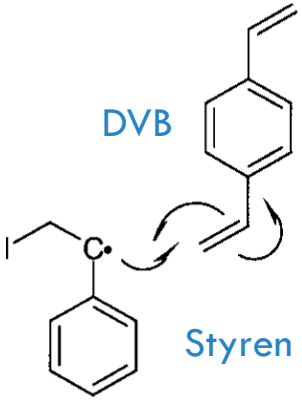
benzofenon



Polymetakryláty

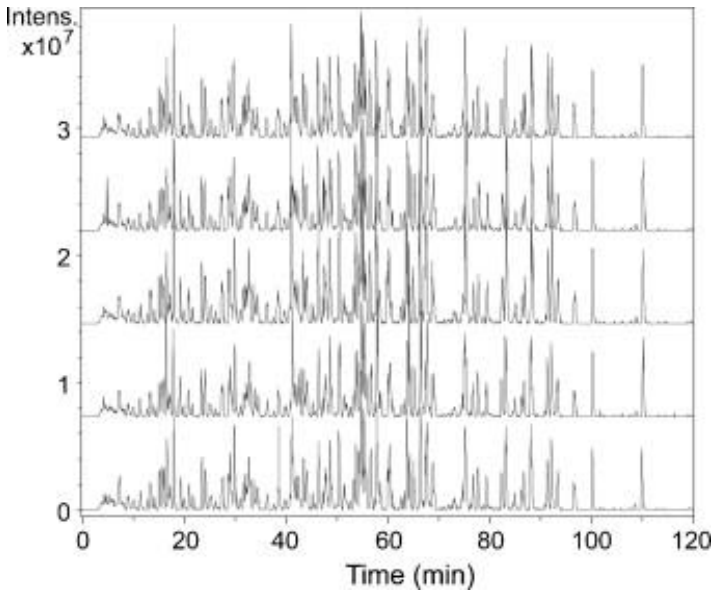


Styren-divinylbenzen



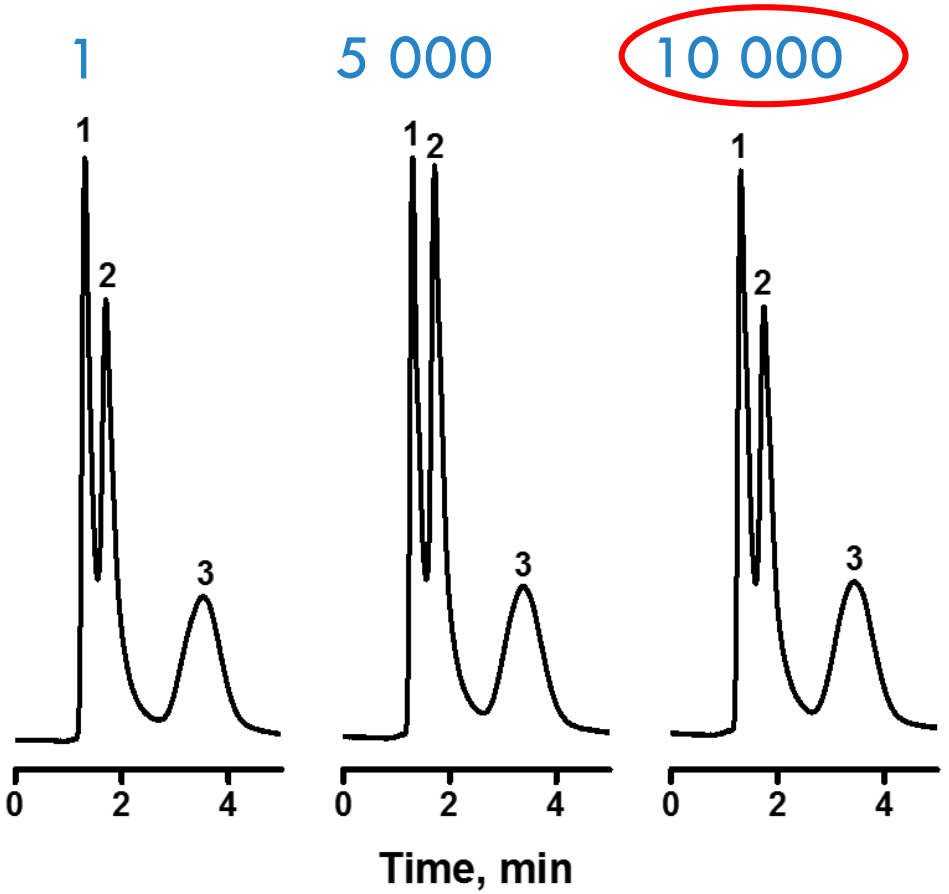
ROBUSTNOST MONOLITŮ

Digest šesti proteinů, monolit 250 mm



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

Izokratická separace malých molekul



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

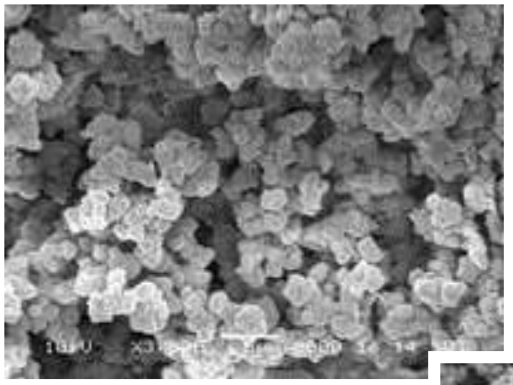
Stabilní a reprodukované výsledky

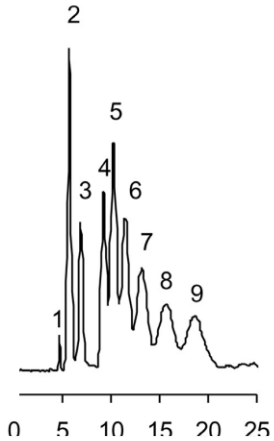
(stále ale pouze výzkumné laboratoře)

KONTROLA MORFOLOGIE

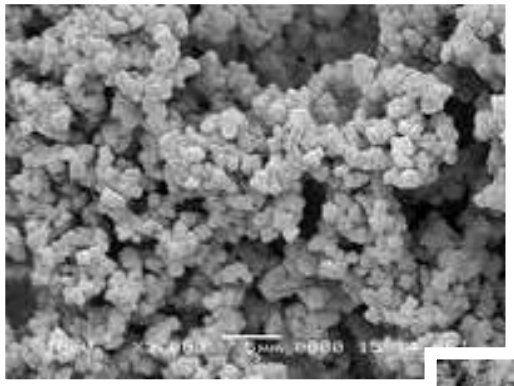
Vliv koncentrace 1,4-butandiolu v polymerační směsi

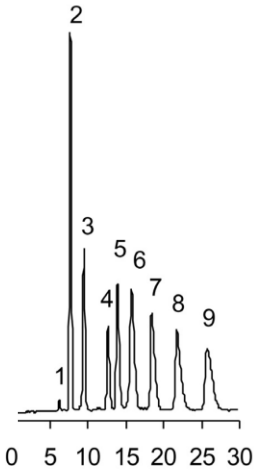
30%



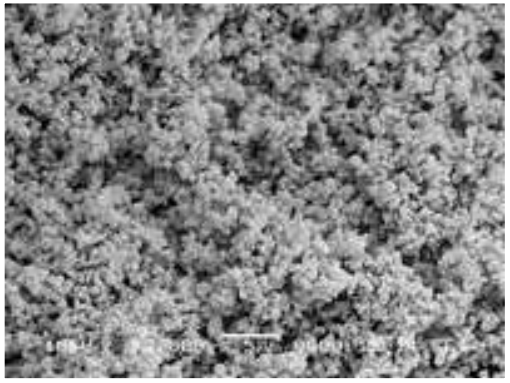


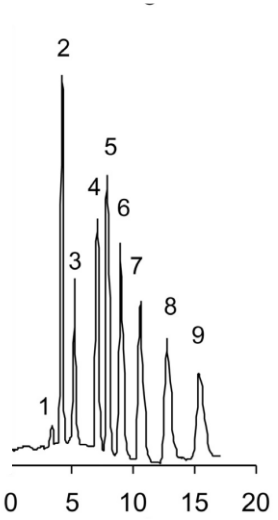
29%





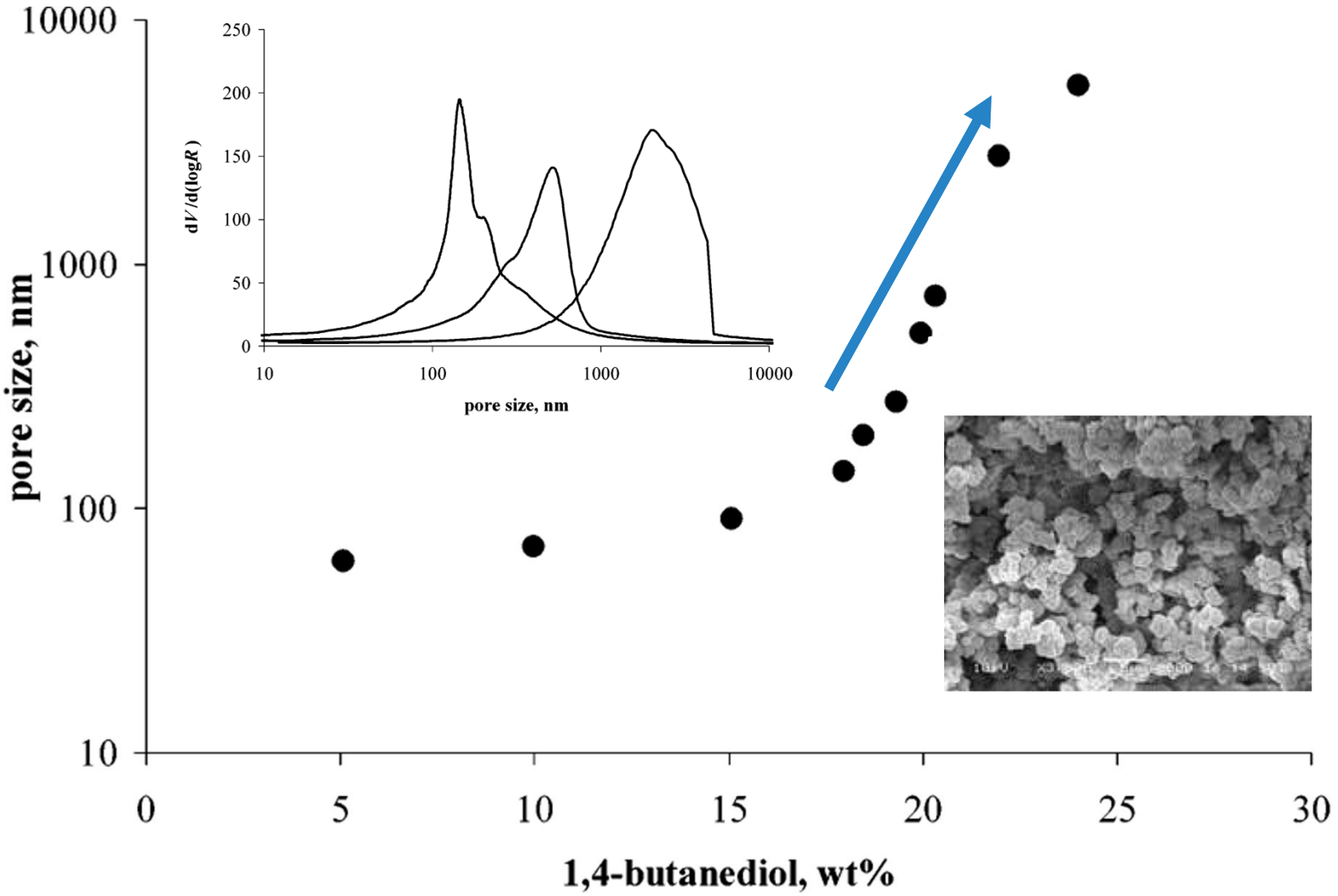
28%





J. Sep. Sci. 26 (2003) 1005 – 1016.

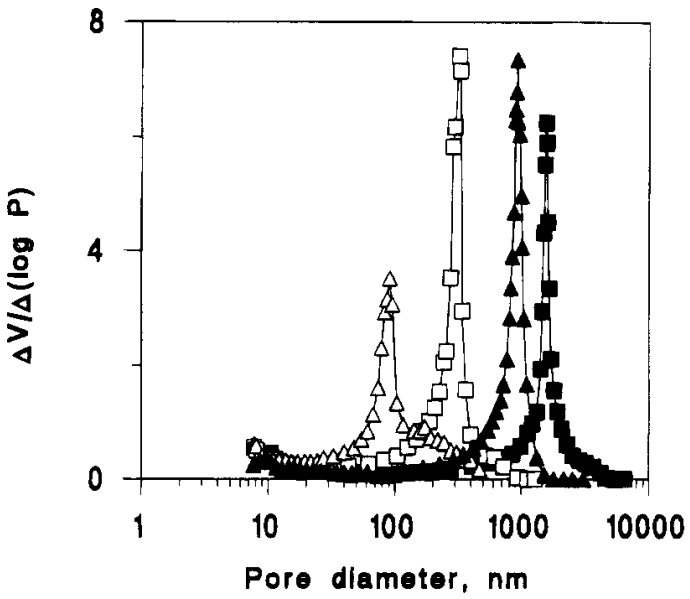
DOMINANTNÍ PRŮTOČNÉ PÓRY



Anal. Chem. 77 (2005) 7342-7347.

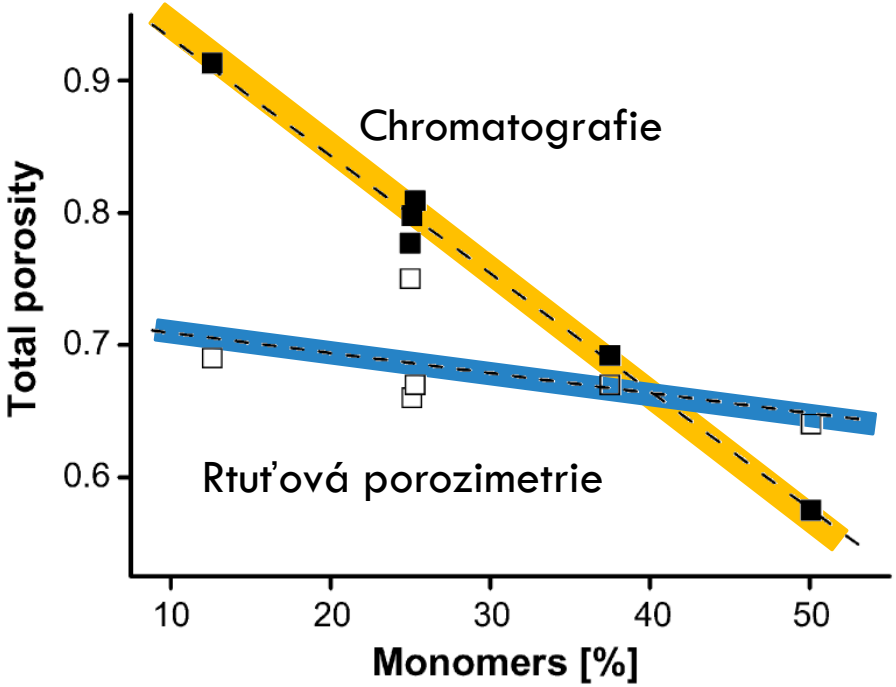
PORÉZNÍ VLASTNOSTI

Rtuťová porozimetrie



Chem. Mater. 7 (1995) 707.

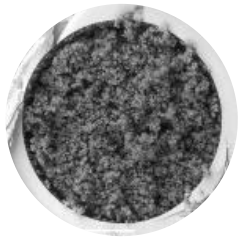
Chromatografická porozimetrie



Suchý *vs.* nabobtnaný stav

Porozita, aktivní povrch, distribuce pórů

vs.



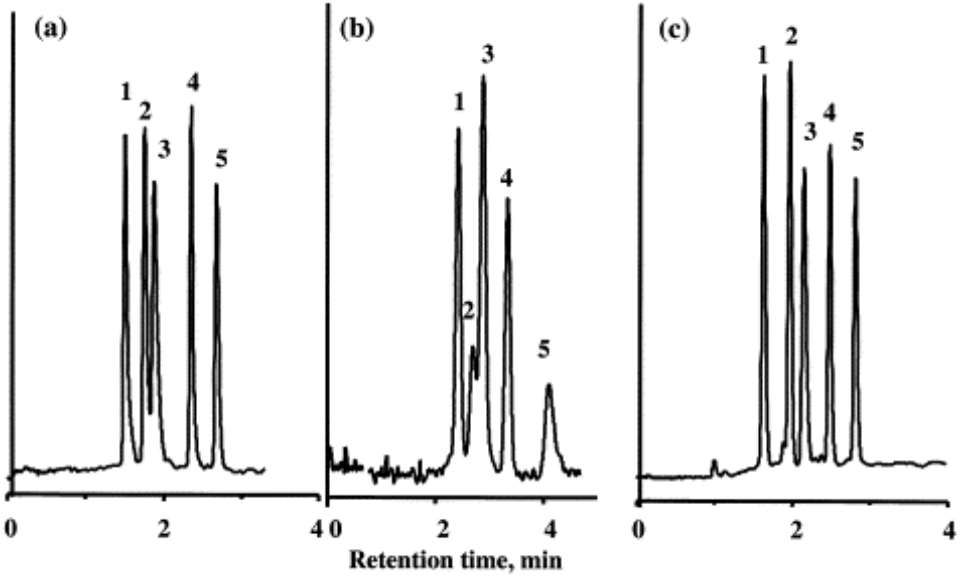
J. Chromatogr. A 1182 (2008) 161 – 168.

MAKROPORÉZNÍ MONOLITY

Monolit
50 x 4.6 mm

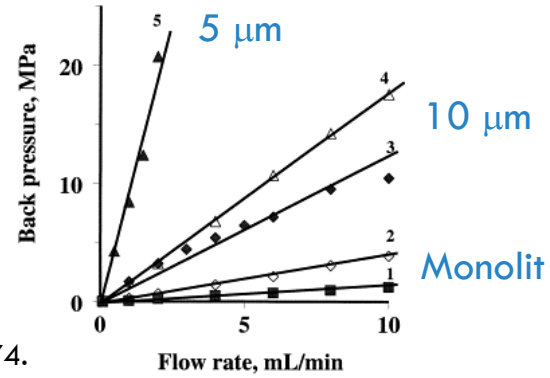
Polymer
150 x 4.6 mm, 10 μm

C8 Silikagel
150 x 4.6 mm, 5 μm



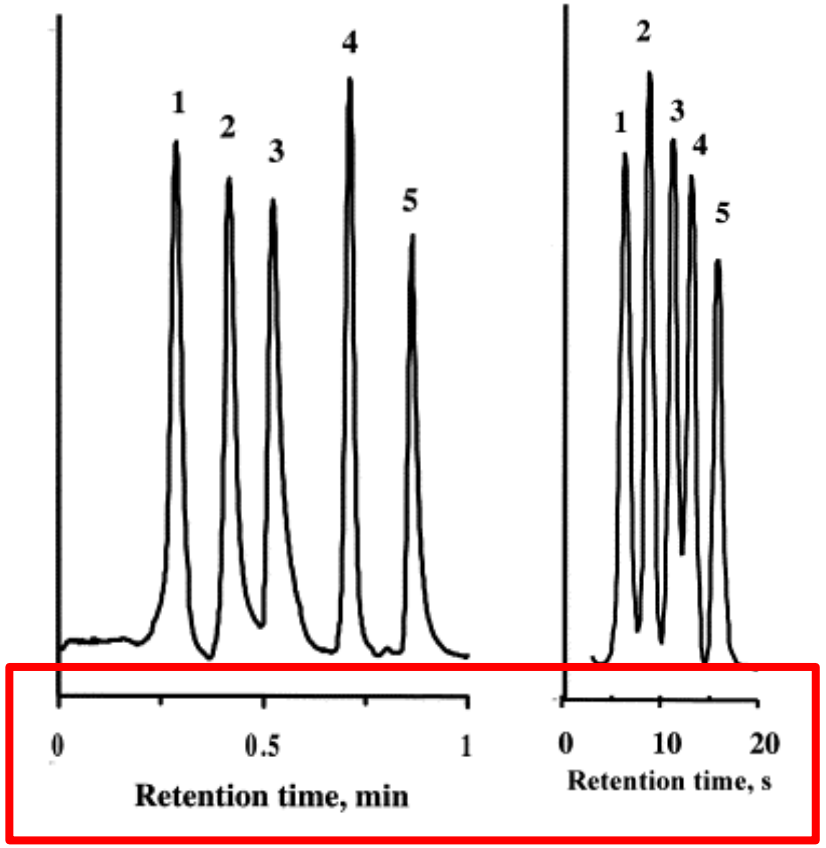
Nízký pracovní tlak

Rychlý průtok mobilní fáze



Peptidy

Proteiny

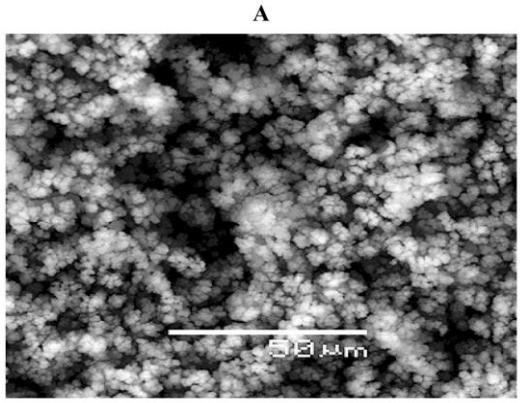


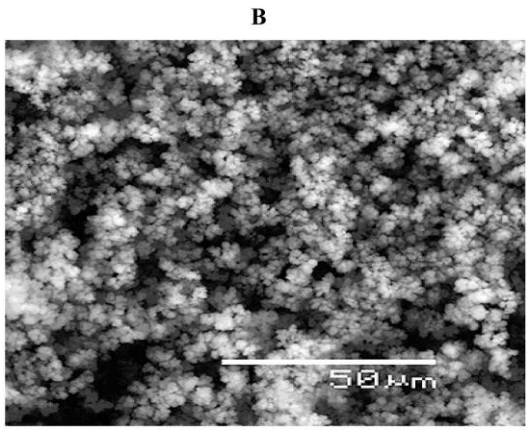
Rychlé gradientové separace

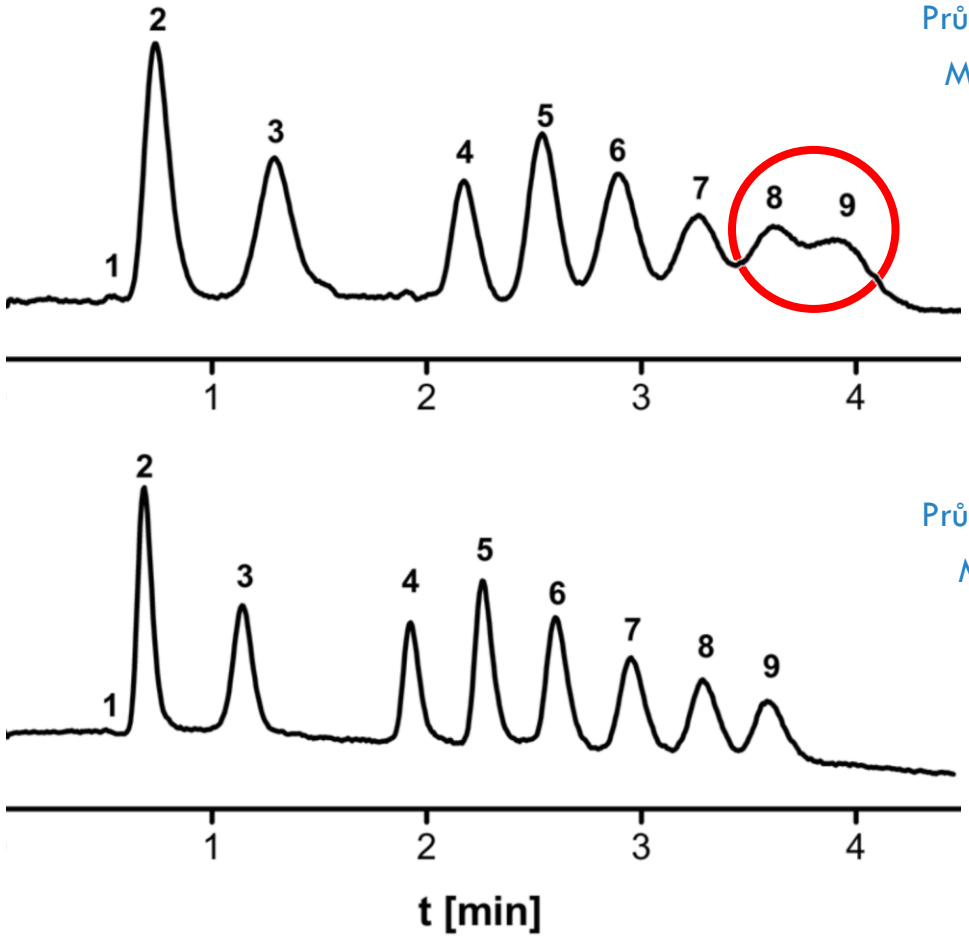
J. Chromatogr. A 865 (1999) 169–174.

VLIV MALÝCH PÓRŮ

Gradientová eluce malých molekul







Průtočné póry 54.0%

Mezopzóry12.0%

Průtočné póry 61.9%

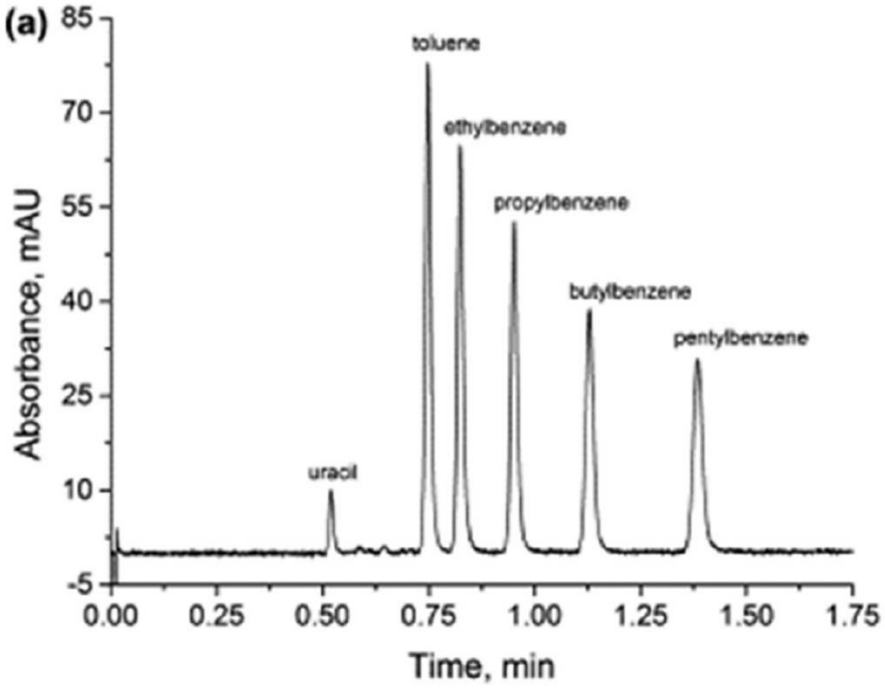
Mezopóry 7.6%

J. Sep. Sci. 29 (2006) 1064 – 1073.

MONOLITICKÉ STACIONÁRNÍ FÁZE

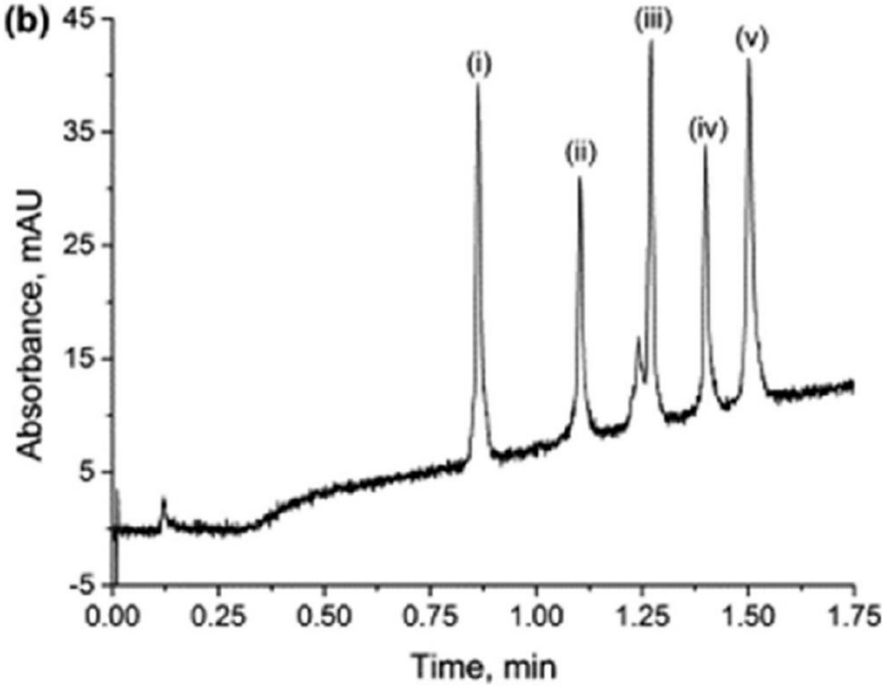
Anorganické monolity

malé molekuly



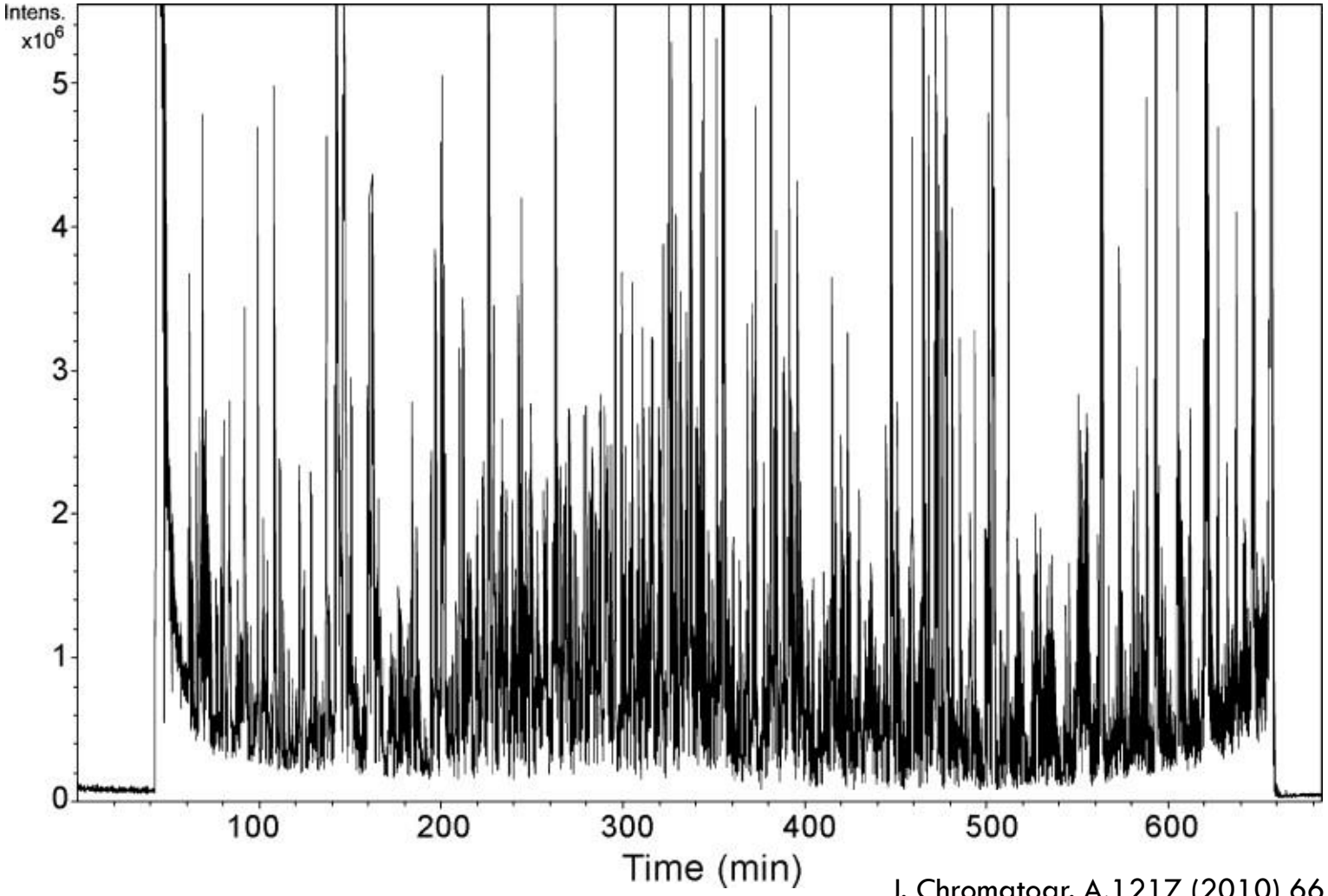
Organické monolity

velké molekuly



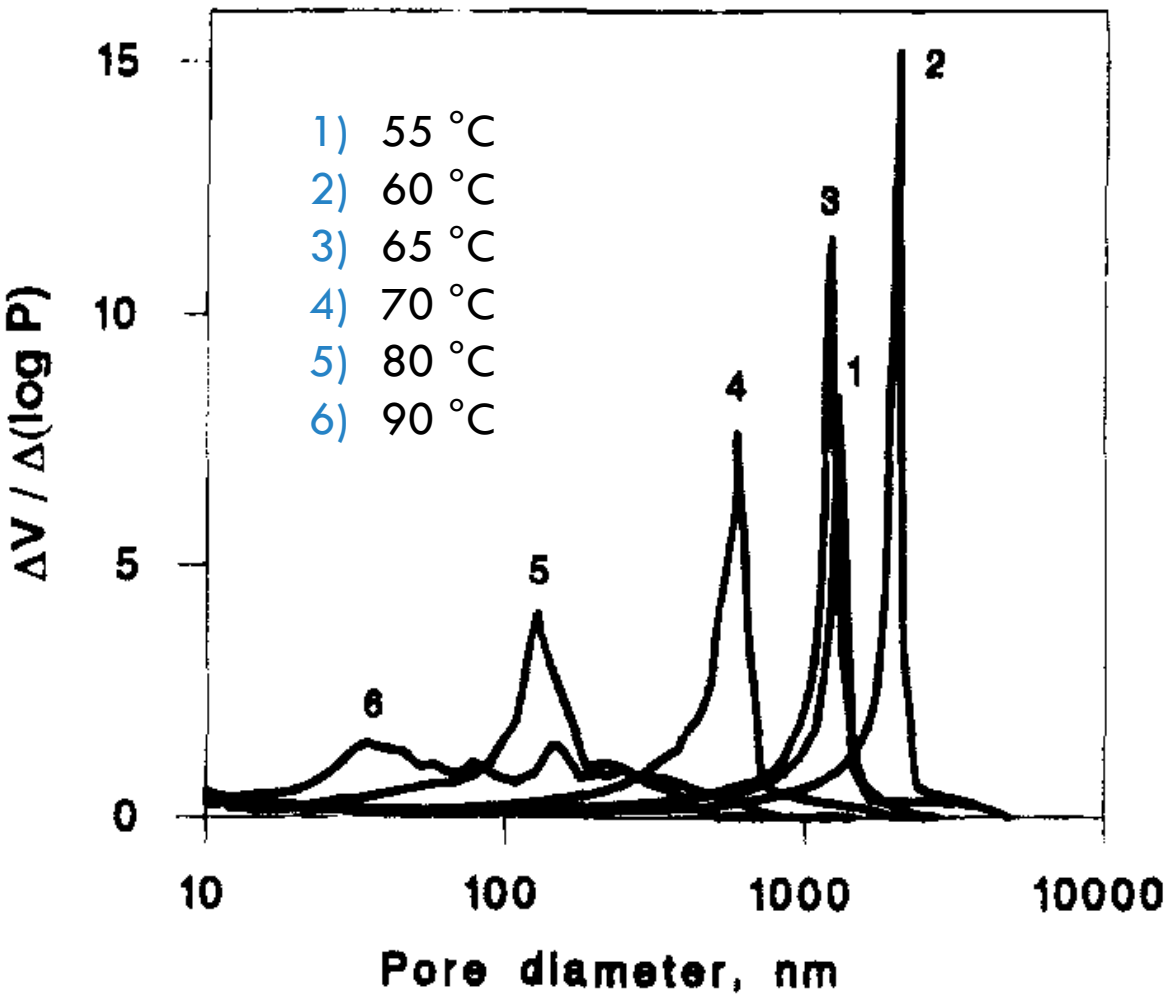
E. COLI DIGEST

1 m monolit, píková kapacita 1038



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

TEPLOTA POLYMERACE



Vyšší teplota

↓

Víc radikálů

↓

Víc center polymerace

↓

„Hustší“ monolit

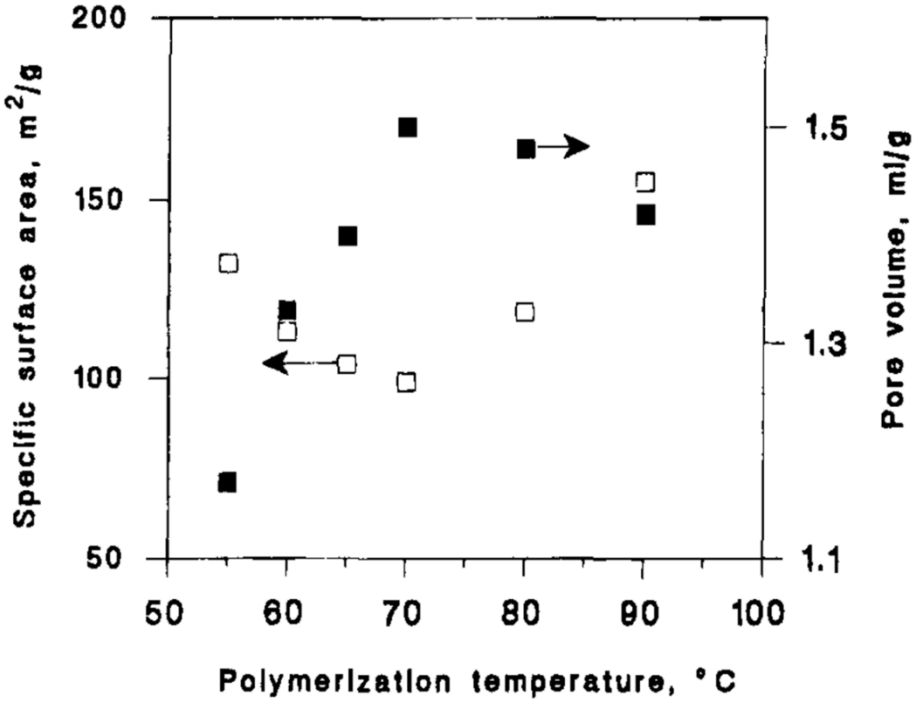
↓

Menší póry

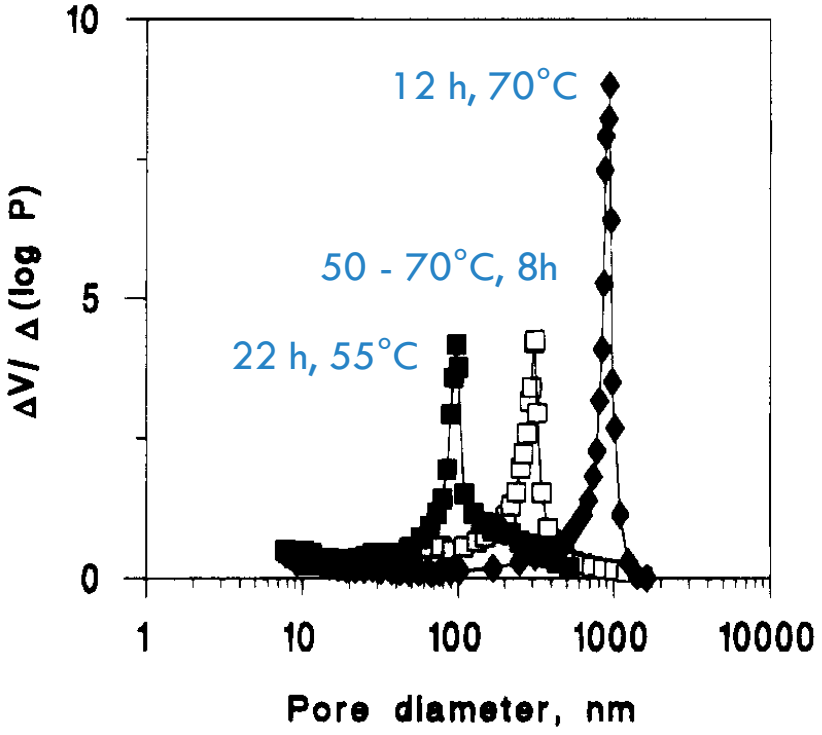
Chem. Mater. 1995, 7, 707-715.

TEPLOTA POLYMERACE

Povrch & Objem pórů



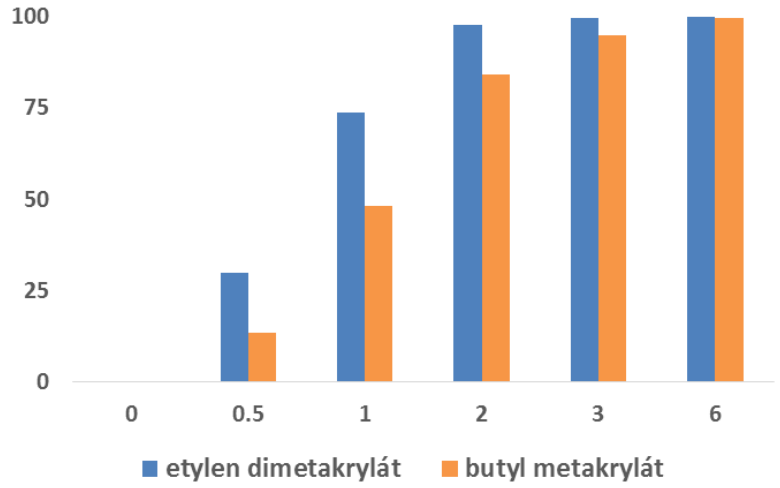
Distribuce pórů

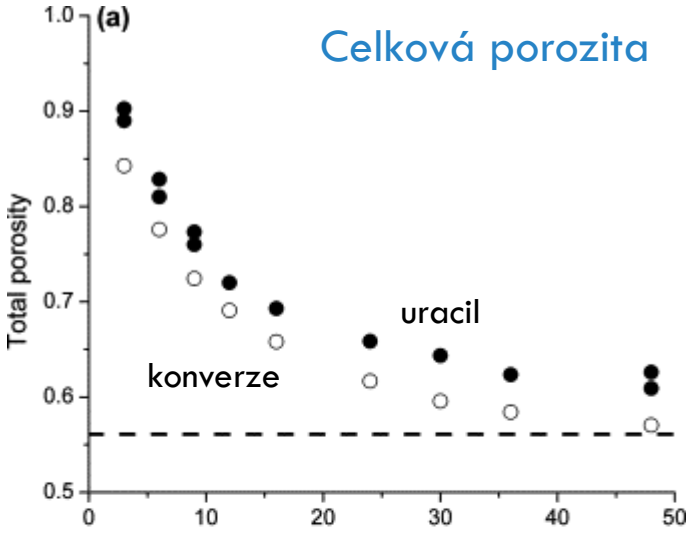


Macromolecules 28 (1995) 7580 – 7582.

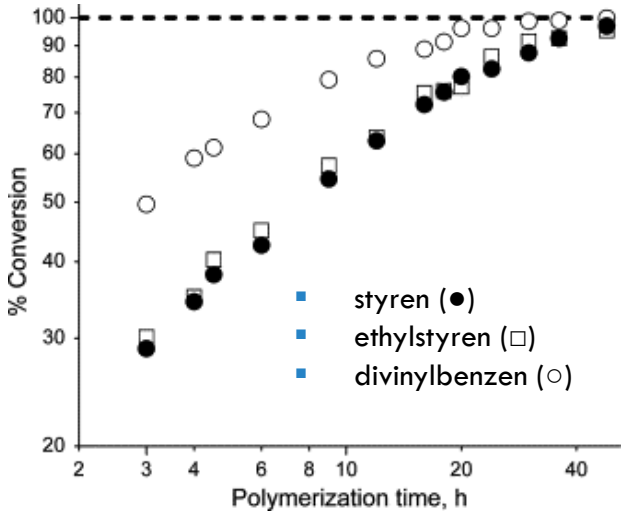
Chem. Mater. 1995, 7, 707-715.

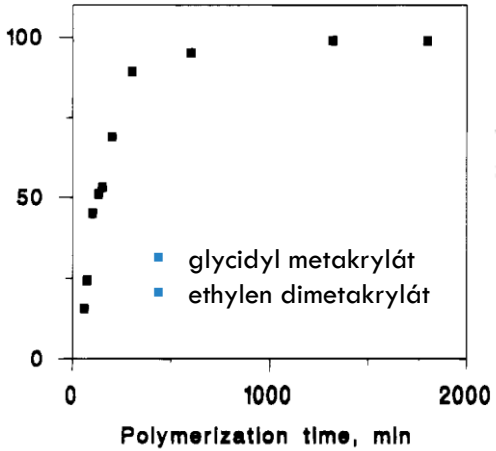
KONVERZE POLYMERAČNÍ REAKCE

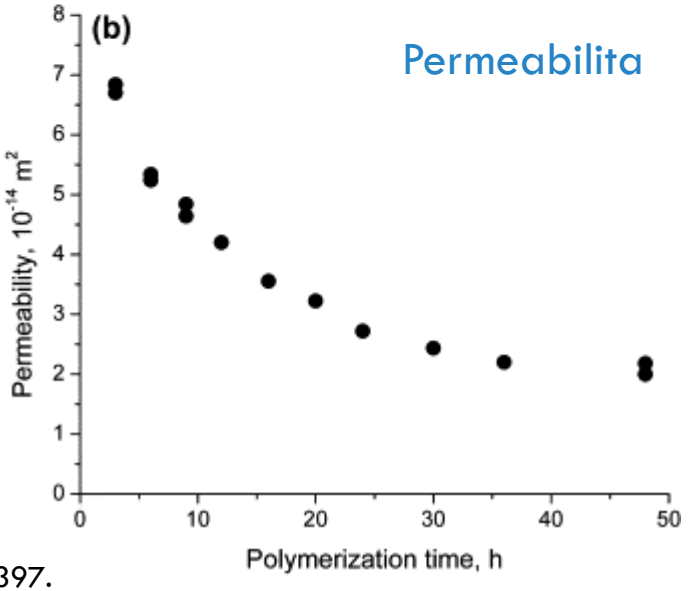




Celková porozita







Permeabilita

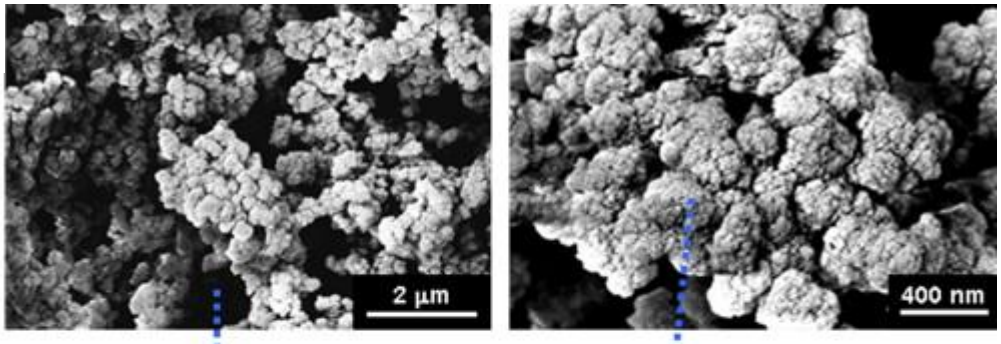
J. Chromatogr. A 1217 (2010) 7514 – 7522. | J. Chromatogr. A 1217 (2010) 5389 – 5397.

KONVERZE POLYMERAČNÍ REAKCE

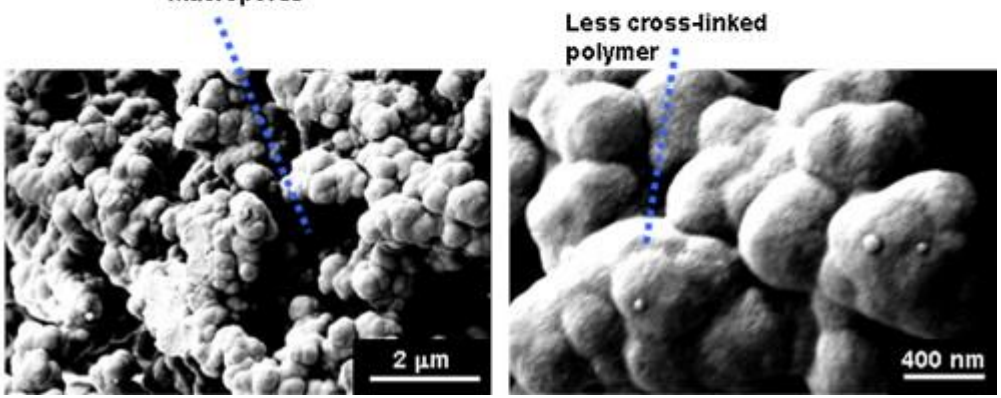
Morfologie a kvalita separace

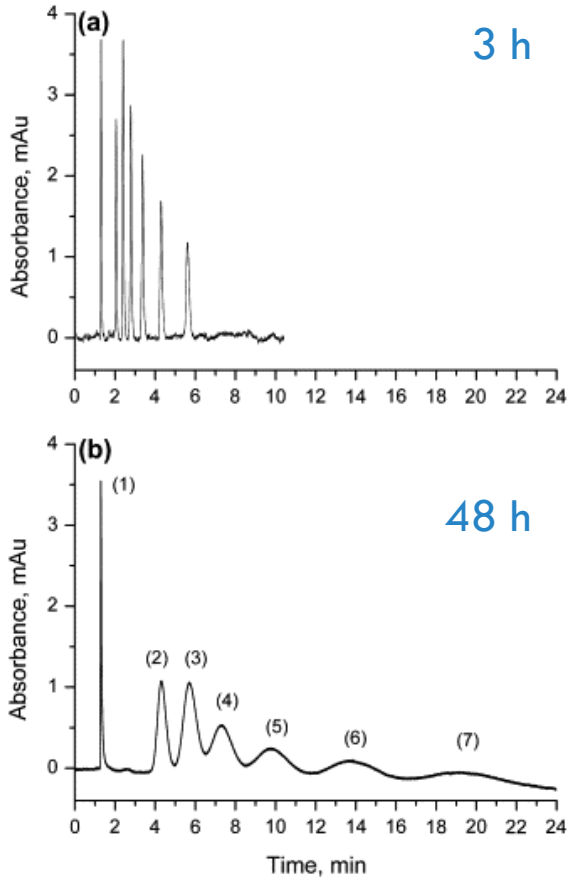
poly(styren-co-divinylbenzen)

3 h



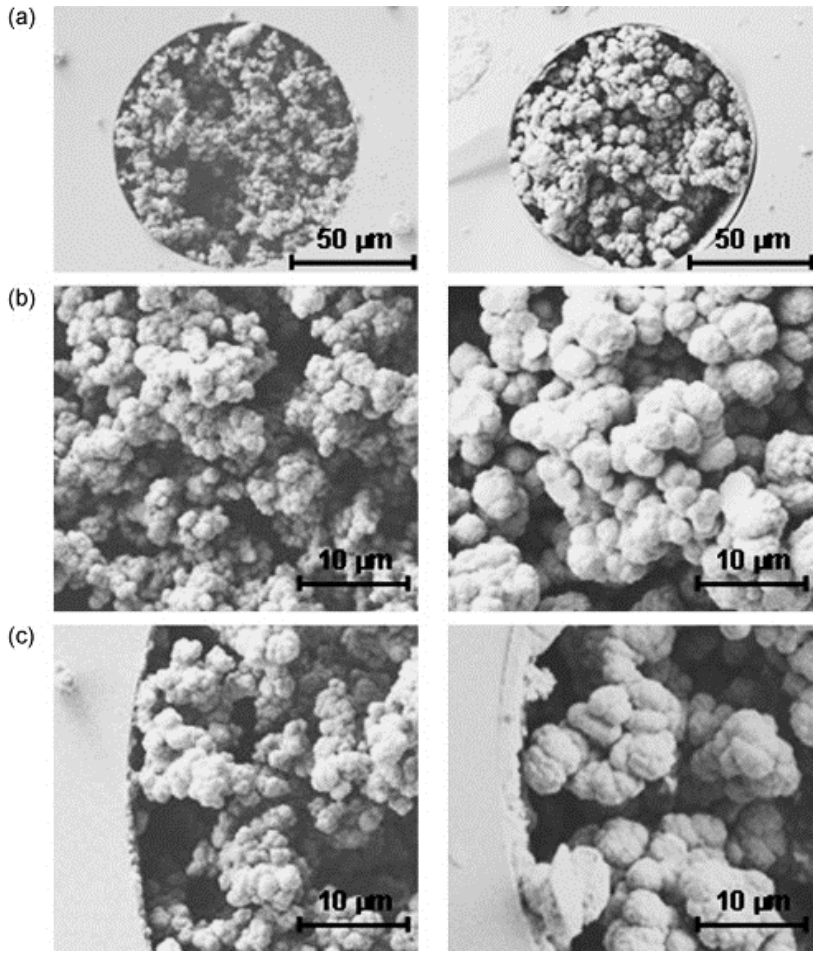
48 h



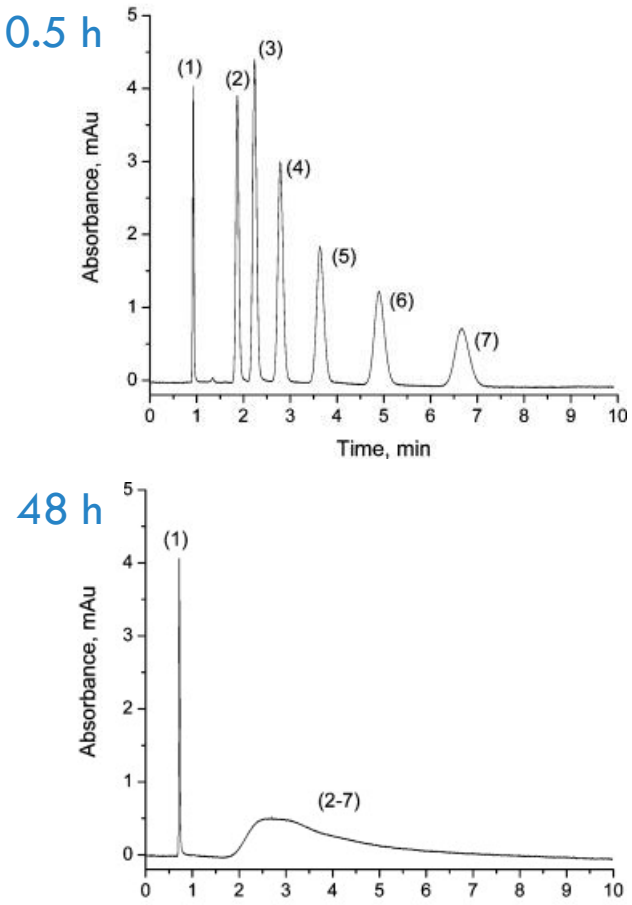


J. Chromatogr. A 1217 (2010) 7514 – 7522. | J. Chromatogr. A 1217 (2010) 5389 – 5397.

KONVERZE POLYMERAČNÍ REAKCE



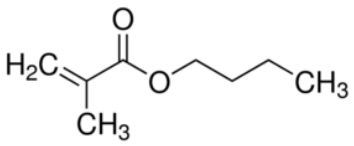
poly(butyl metakrylát-co-ethylen dimetakrylát)



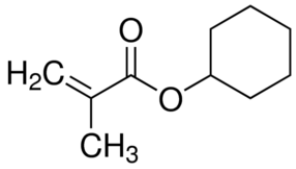
J. Chromatogr. A 1217 (2010) 7514 – 7522. | J. Chromatogr. A 1217 (2010) 5389 – 5397.

FUNKČNÍ MONOMER

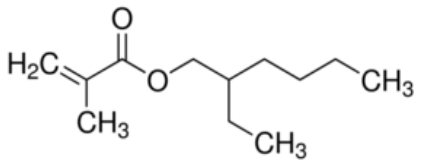
Butyl metakrylát (BMA)

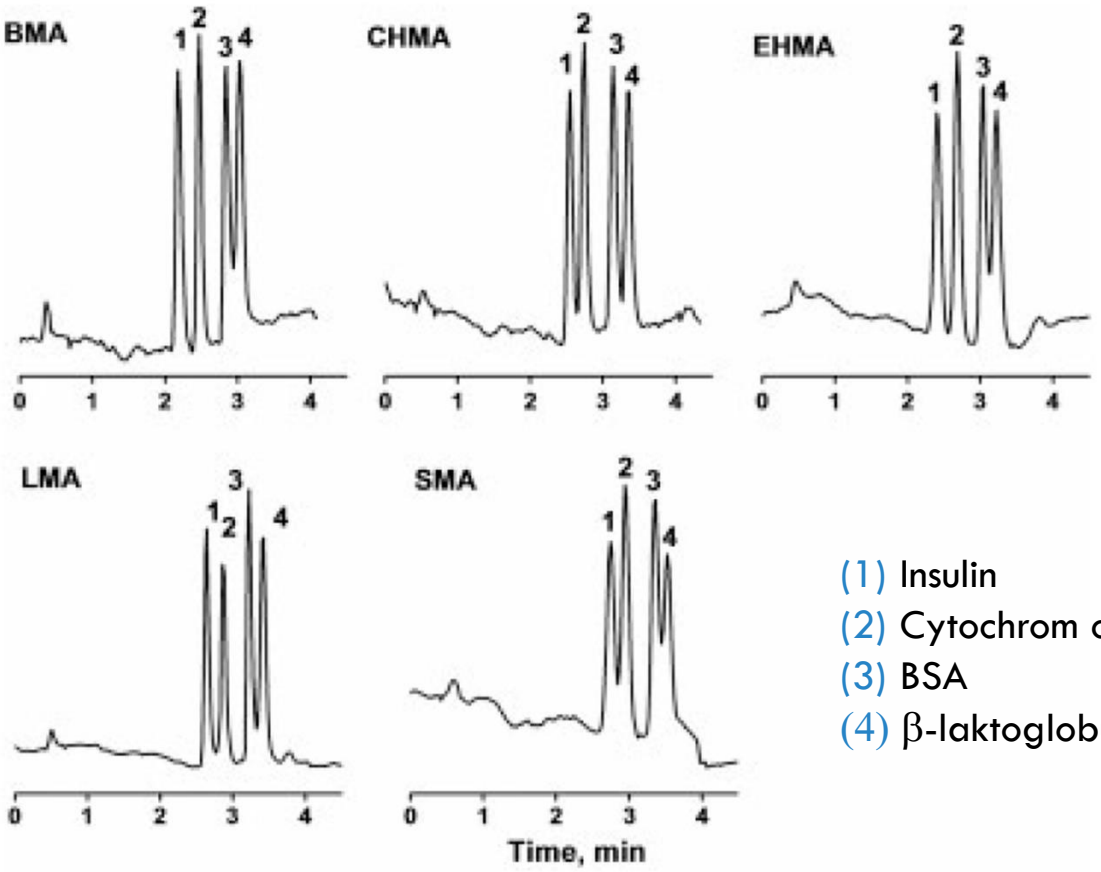


Cyklohexyl metakrylát (CHMA)



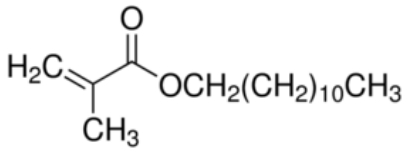
Ethylhexyl metakrylát (EHMA)



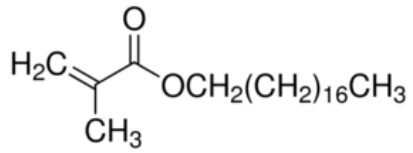


(1) Insulin
(2) Cytochrom c
(3) BSA
(4) β-laktoglobulin.

Lauryl metakrylát (LMA)



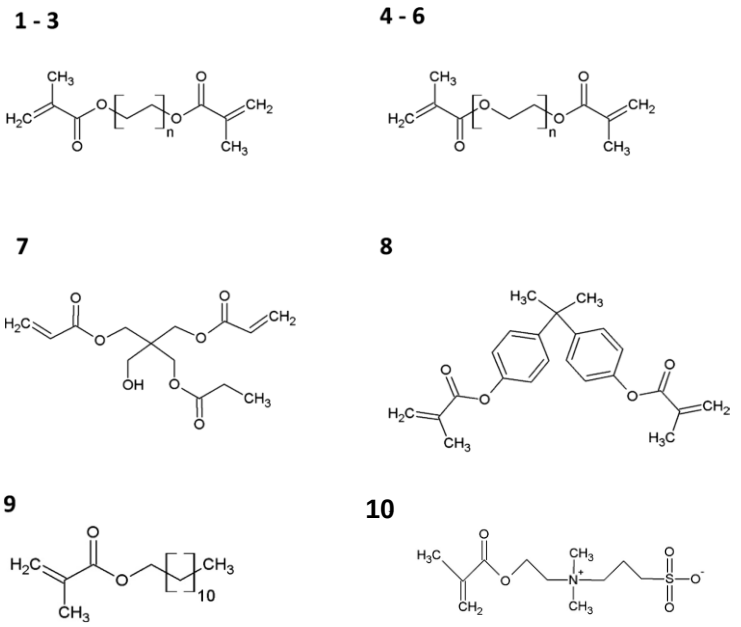
Stearyl metakrylát (SMA)

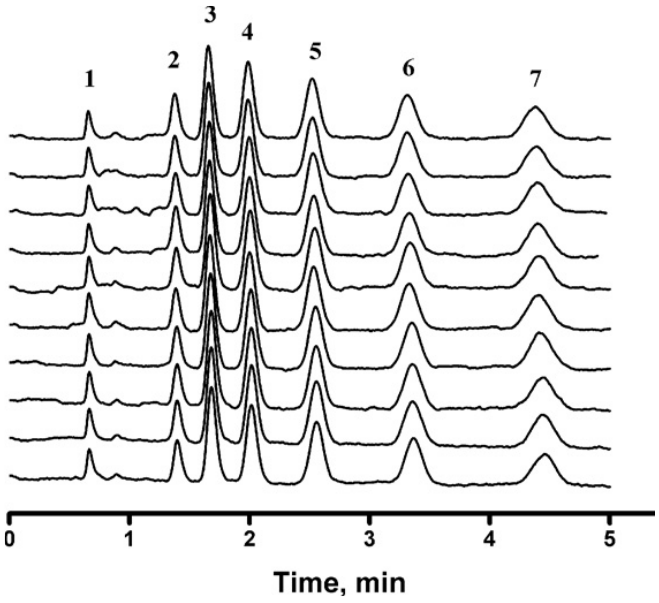


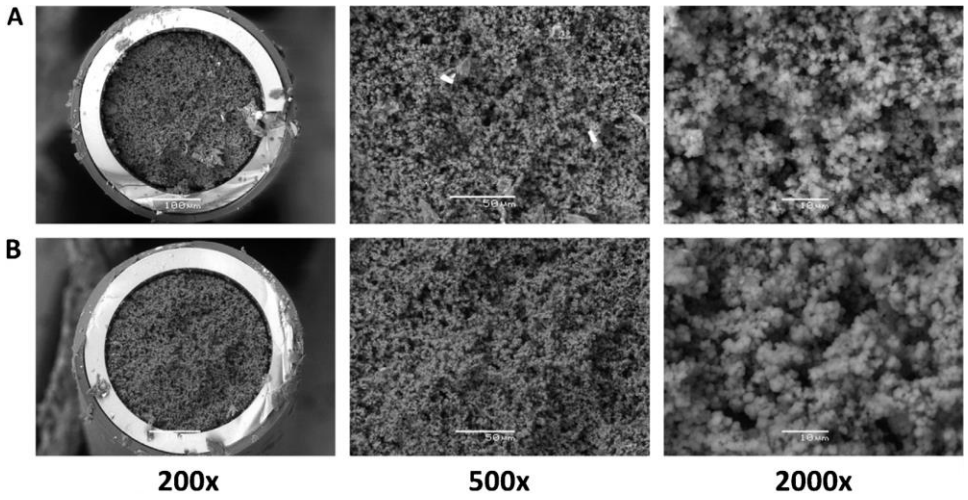
Nutnost (neustálé) optimalizace složení směsi

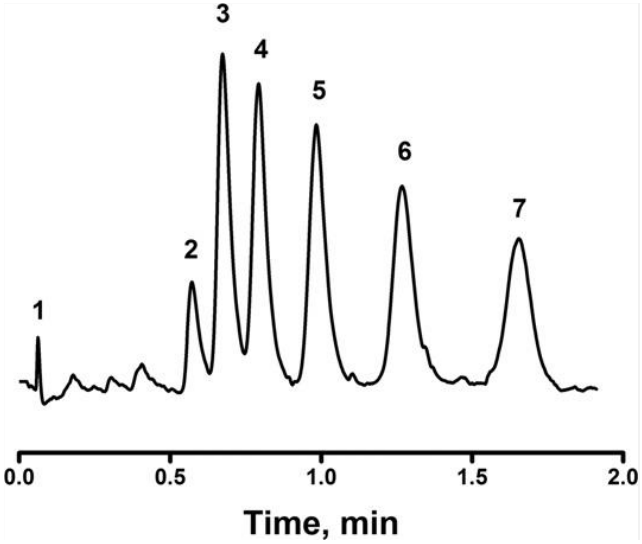
J. Sep. Sci. 34 (2011) 2054 – 2062.

SÍŤUJÍCÍ MONOMER





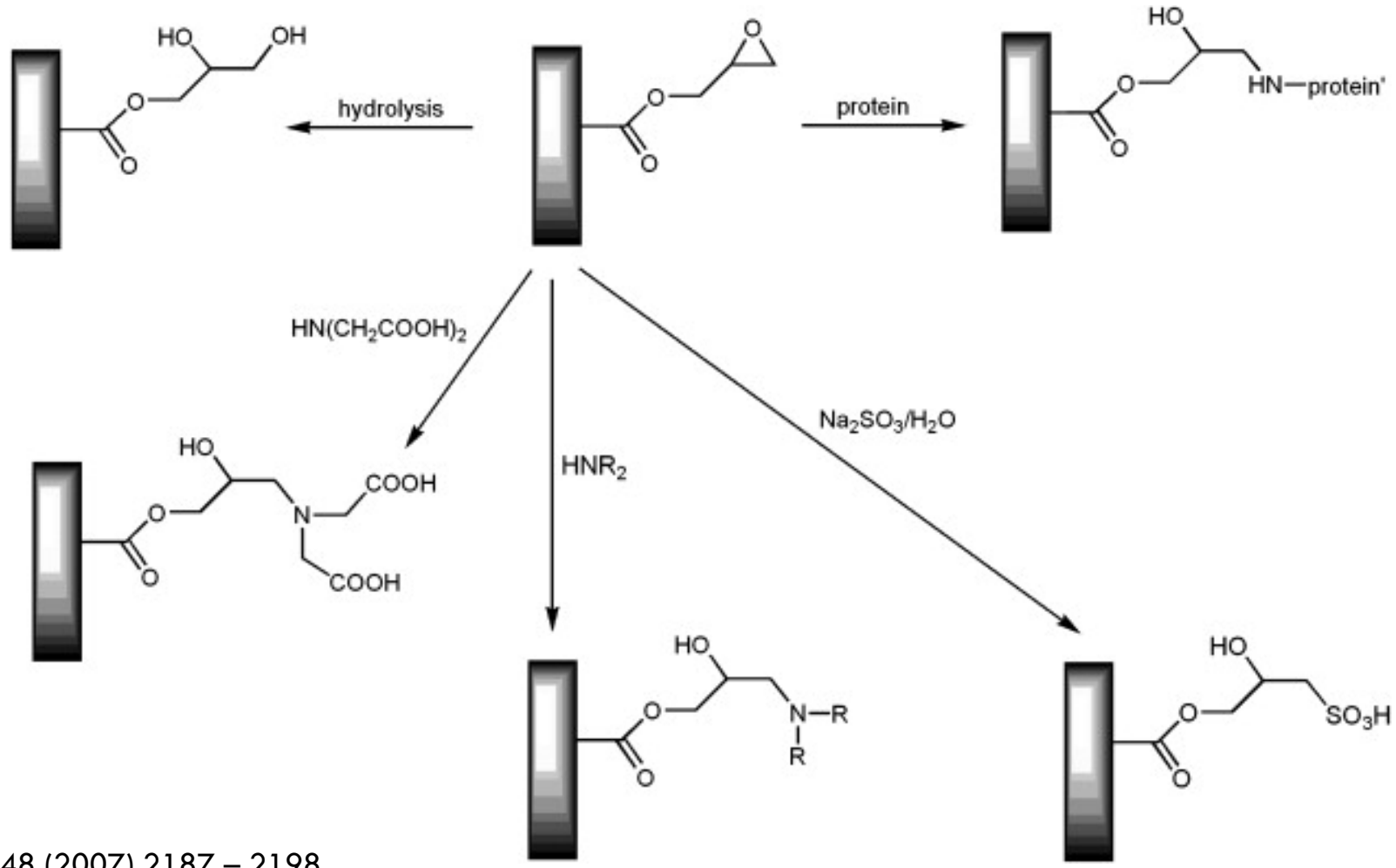




J. Chromatogr. A 1289 (2013) 47 – 57., J. Chromatogr. A 1274 (2013) 97 – 106.

MODIFIKACE POVRCHU

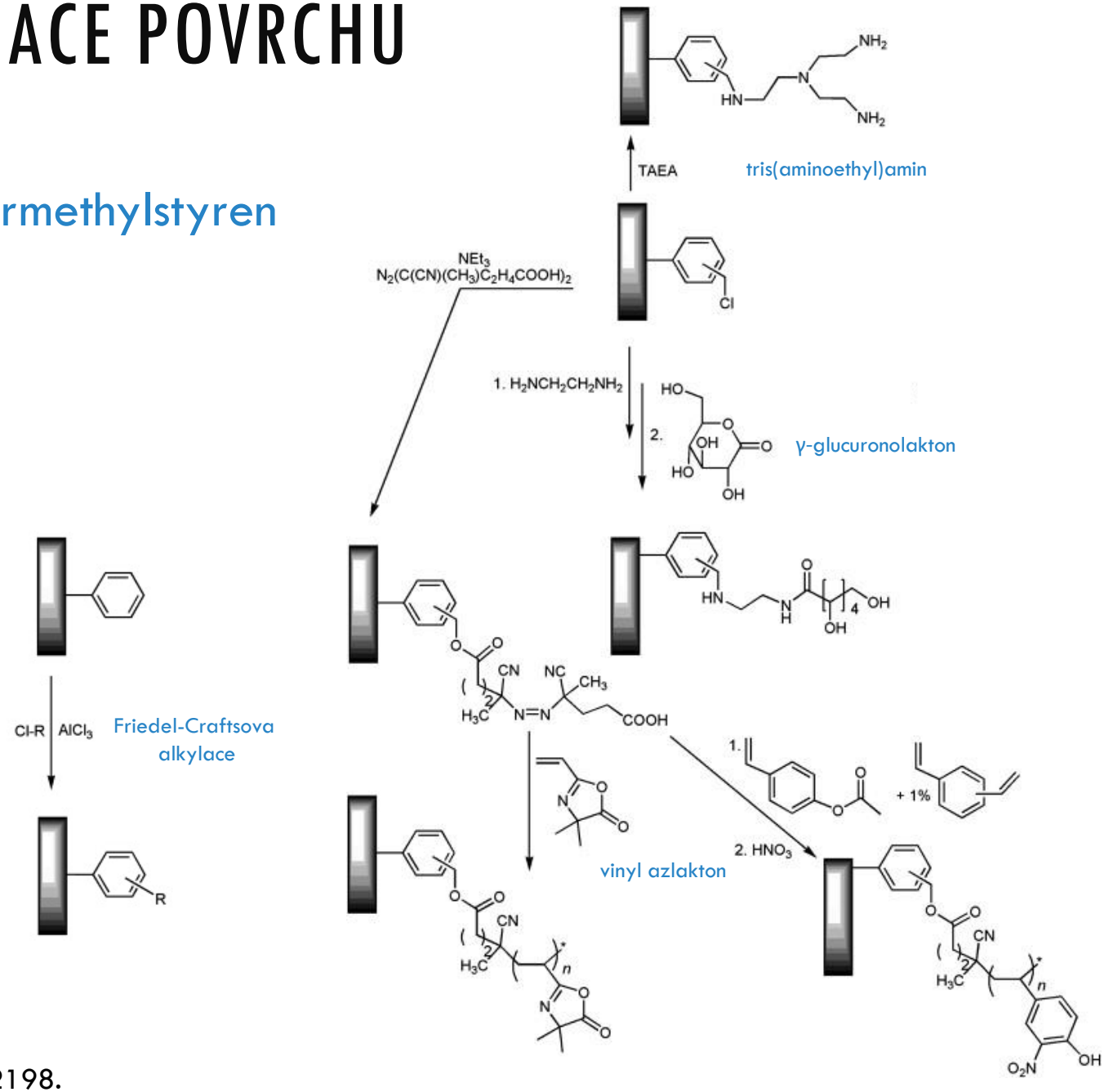
Glycidyl metakrylát



Polymer 48 (2007) 2187 – 2198.

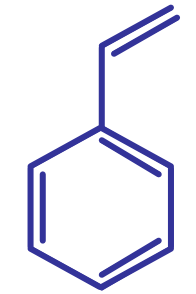
MODIFIKACE POVRCHU

Styren & Chlormethylstyren

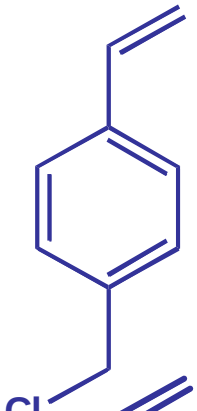


Polymer 48 (2007) 2187 – 2198.

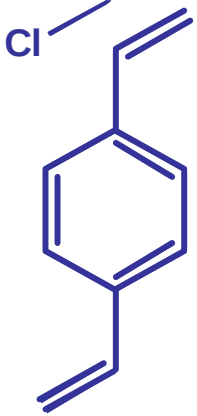
OBECNÝ MONOLIT



Styrene (ST)



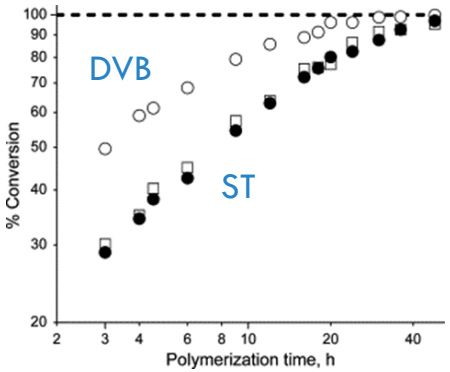
Chloromethyl-
styrene (CMS)

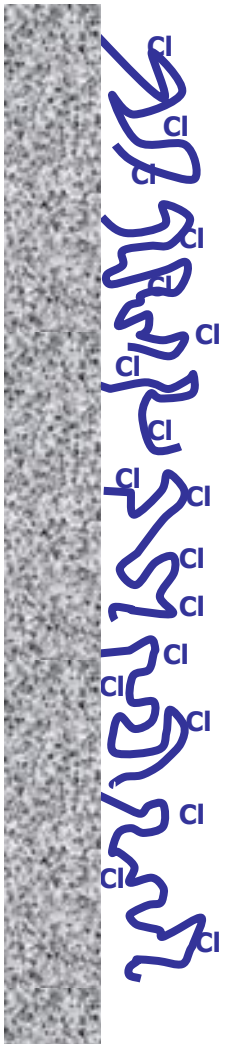


Divinylbenzene (DVB)

Toluene (TOL)
Dodecanol (DOD)
AIBN

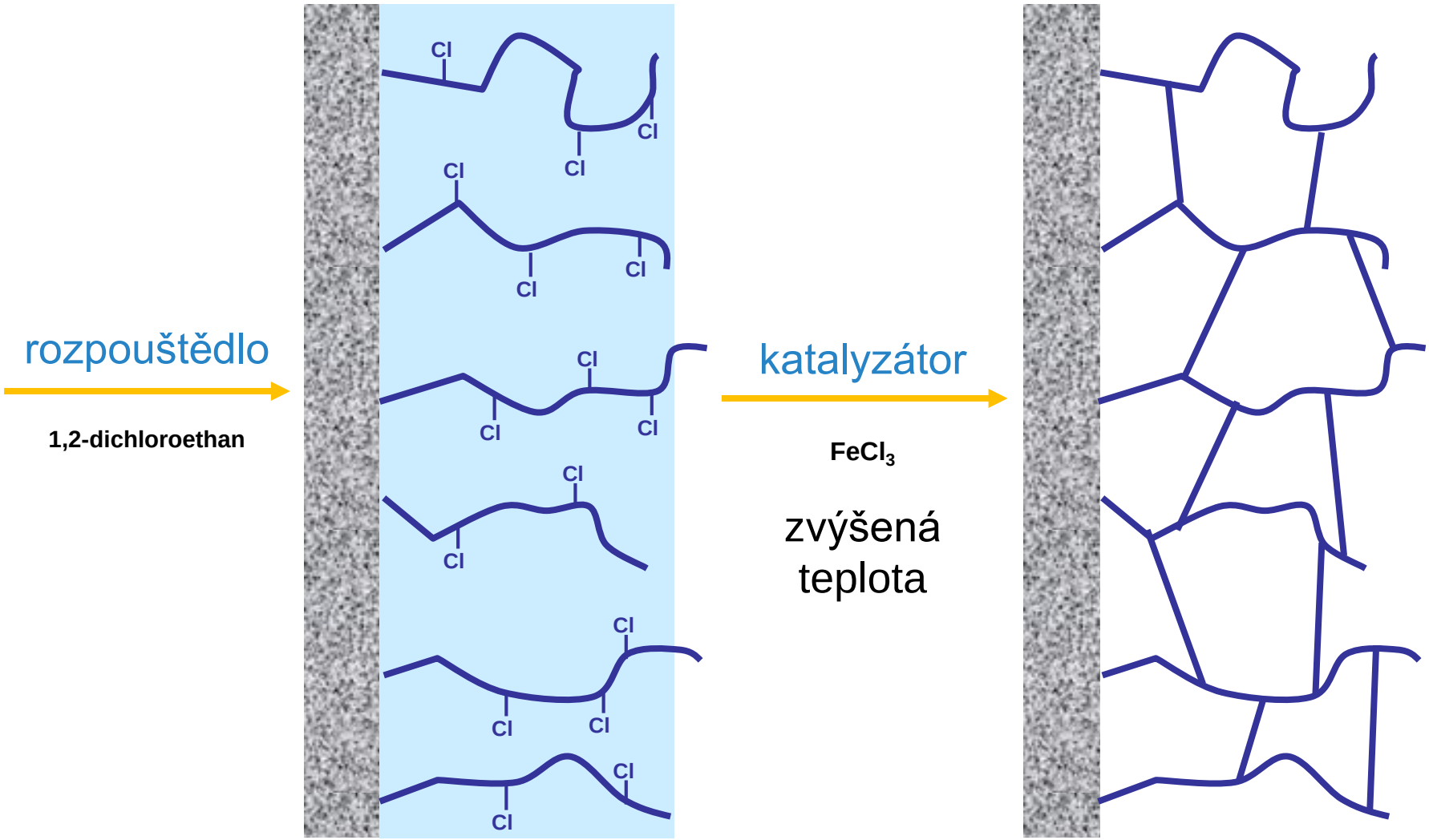
70 °C, 20 h



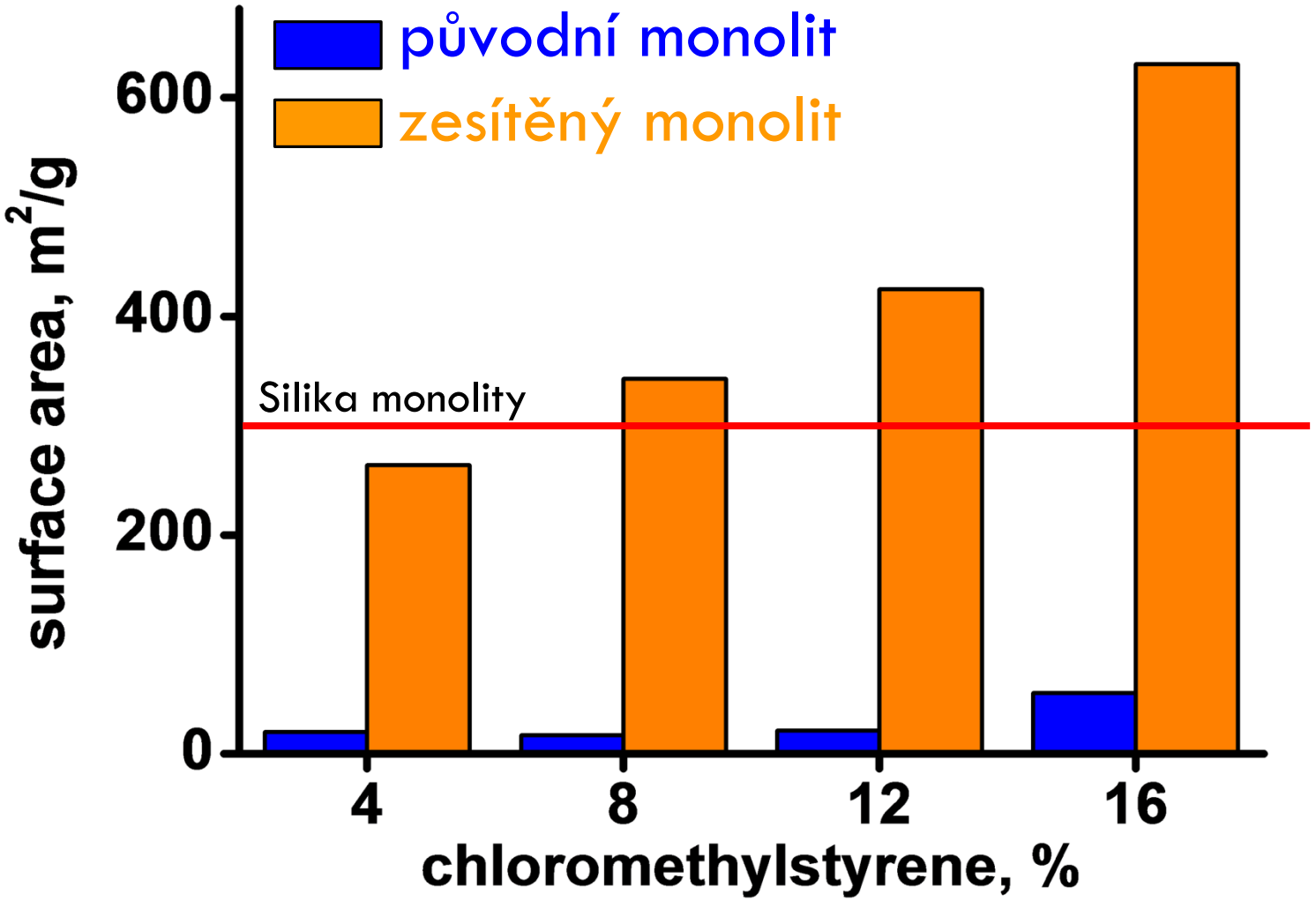


Anal. Chem. 82 (2010) 1621 – 1623. | *J. Chromatogr. A* 1217 (2010) 8212 – 8221.

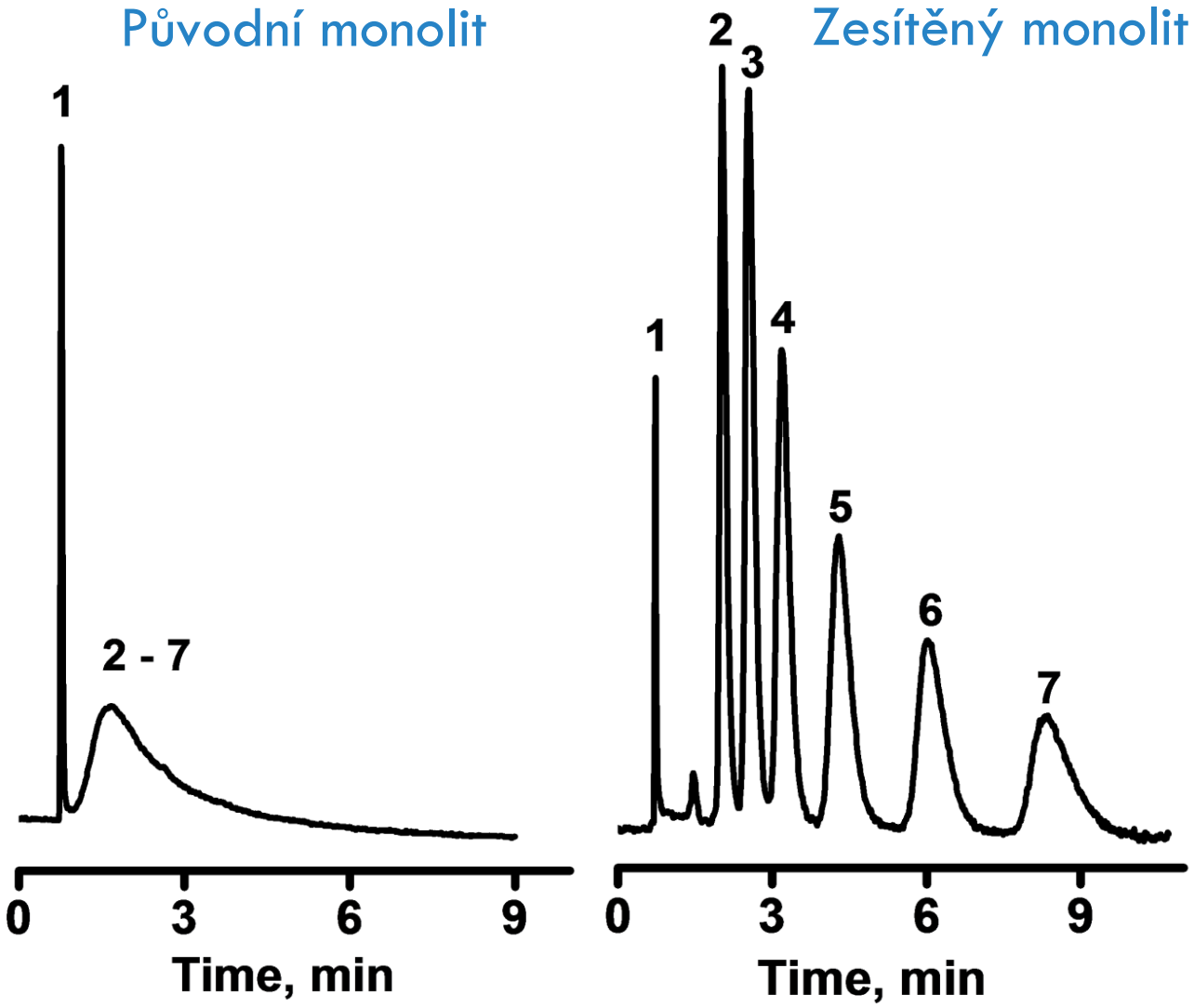
ZESÍTĚNÍ (HYPERCROSSLINKING)



ZVÝŠENÍ AKTIVNÍHO POVRCHU



ZLEPŠENÍ SELEKTIVITY

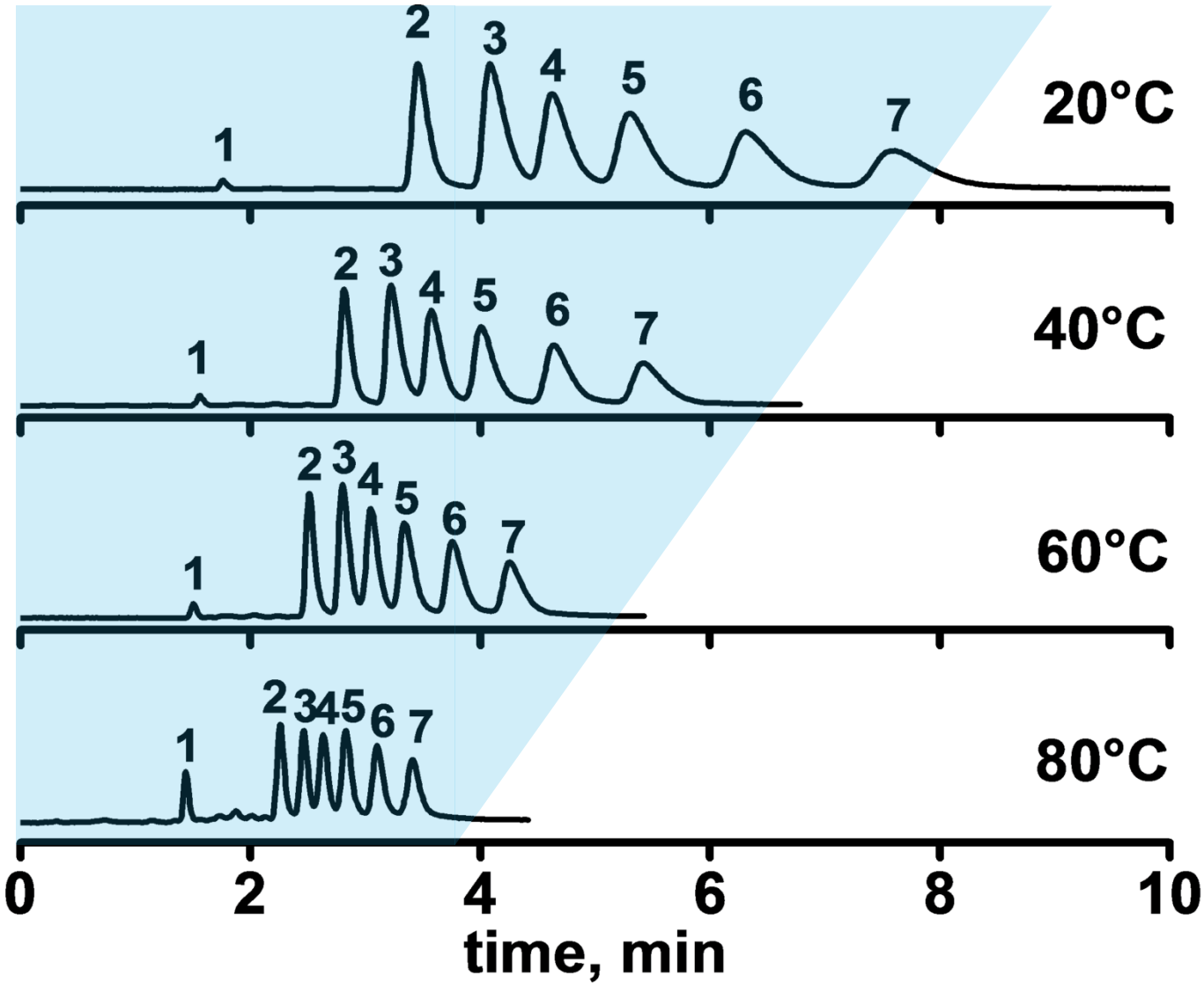


Kolona 170 × 100 mm,
80% ACN, 1.5 μL/min,
16.4 MPa, 254 nm

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

J. Chromatogr. A 1217 (2010) 8212 – 8221.

TEPLOTA ANALÝZY



Čas analýzy

8 min

6 min

5 min

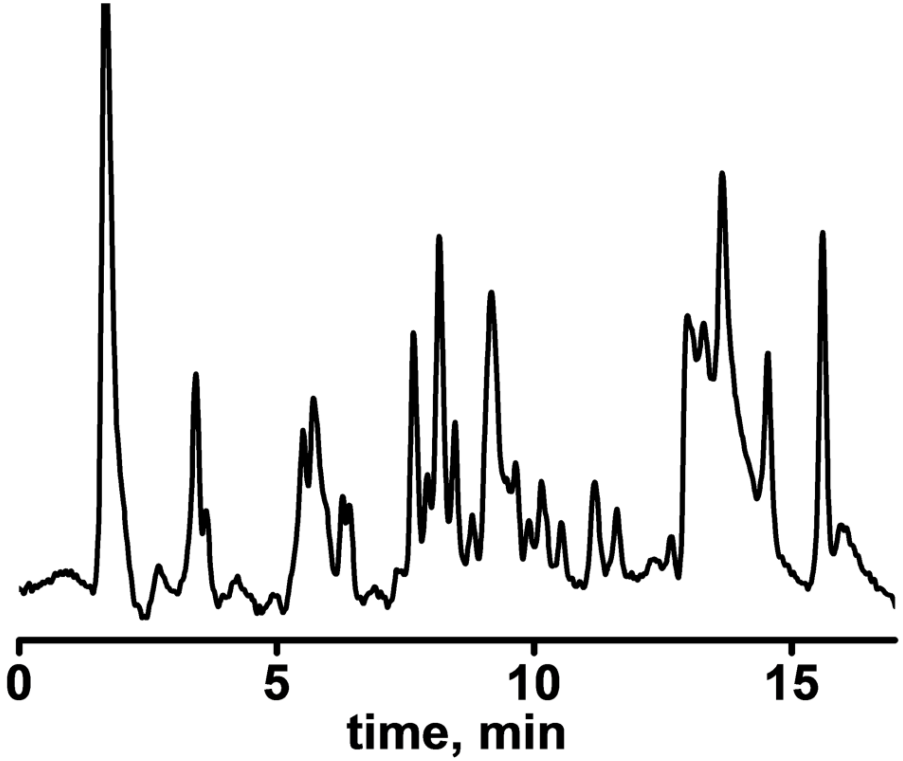
3.5 min

Mobilní fáze 20% vody, 20% THF a 60% ACN, průtok 0.5 µL/min. UV detekce při 254 nm.

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

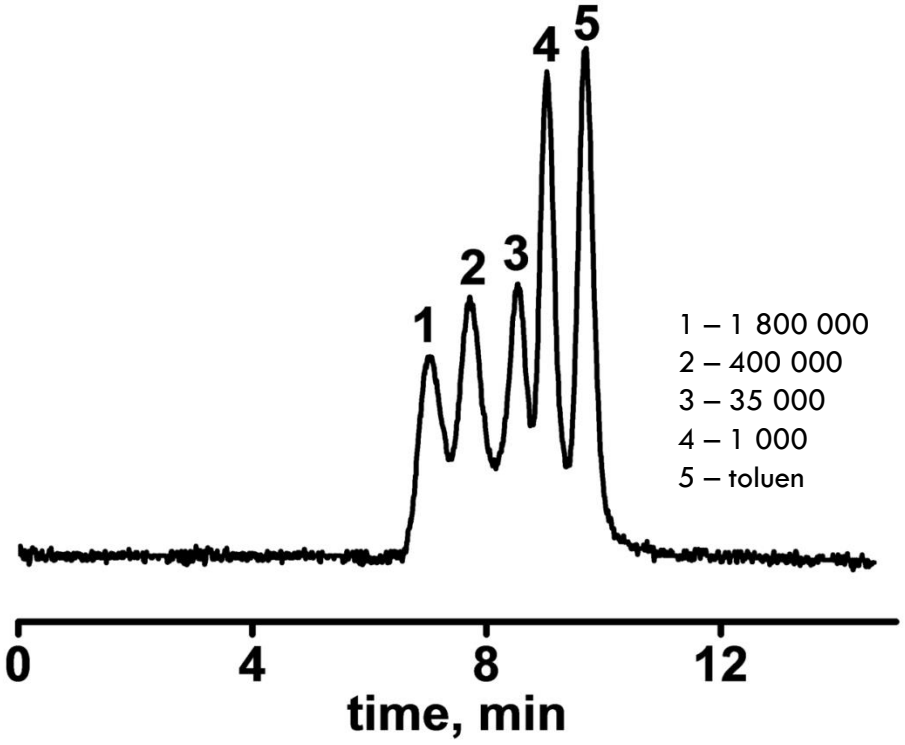
UNIVERZÁLNÍ KOLONA(?)

Gradientová eluce tryptic digestu (Cytochrome C)



Column modified for 2 h at 90°C, length 130 mm, flow rate 0.5 μL/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

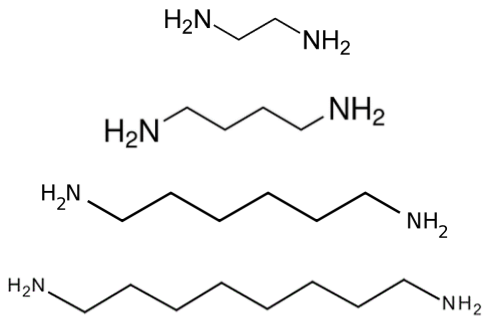


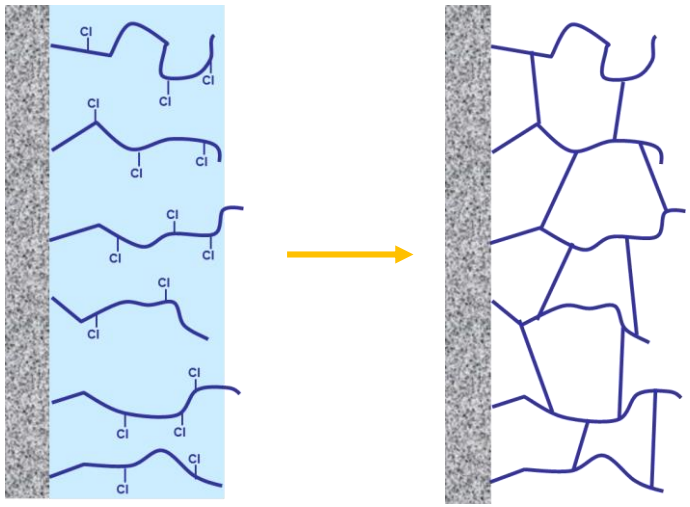
Hypercrosslinked column modified for 2 h at 80°C, length 670 mm (320 + 350 mm), flow rate 0.5 mL/min, pressure 205 bar, 100% THF as mobile phase, polystyrene standards and toluene

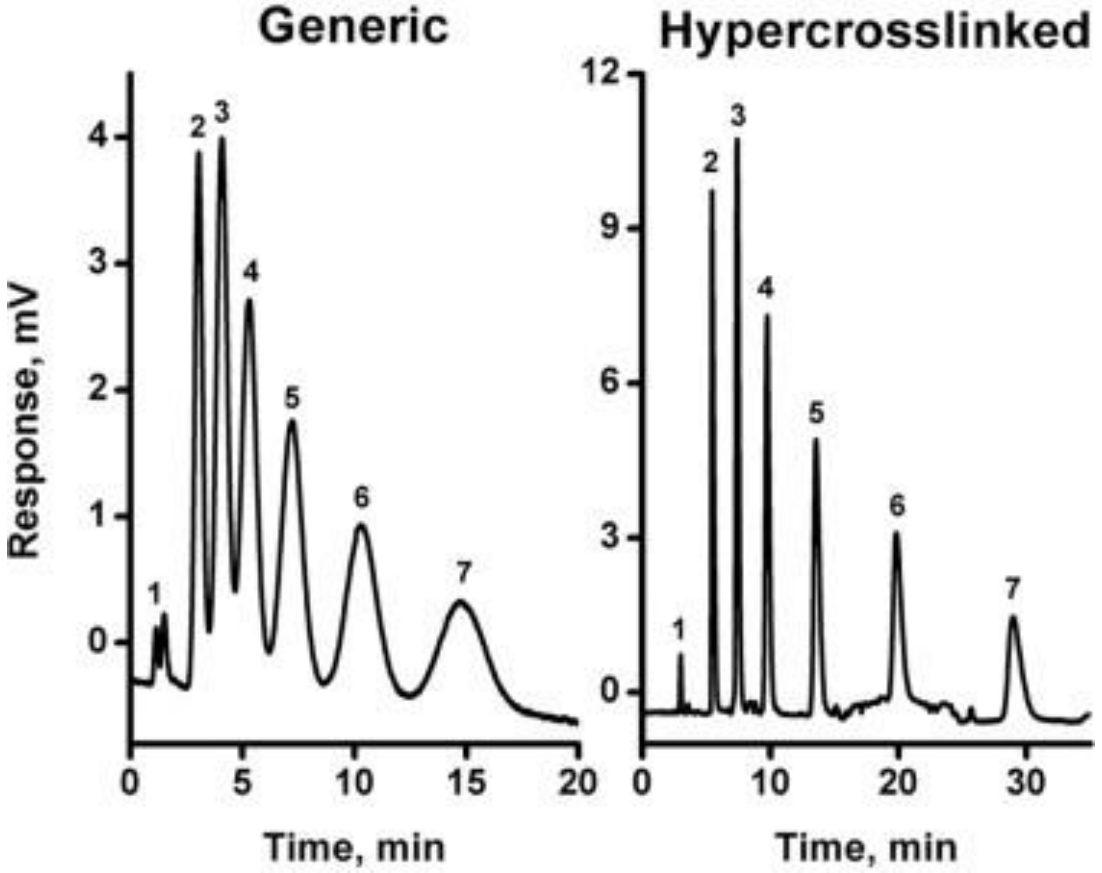
J. Chromatogr. A 1217 (2010) 8212 – 8221.

ZESÍTĚNÍ NUKLEOFILNÍ SUBSTITUCÍ

Diaminoalkany



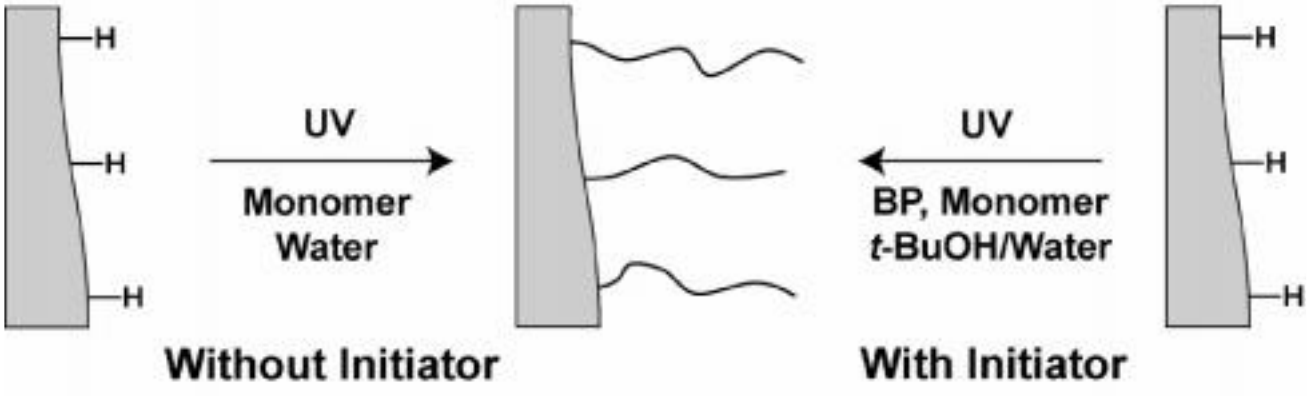




J. Chromatogr. A 1388 (2015) 151–157.

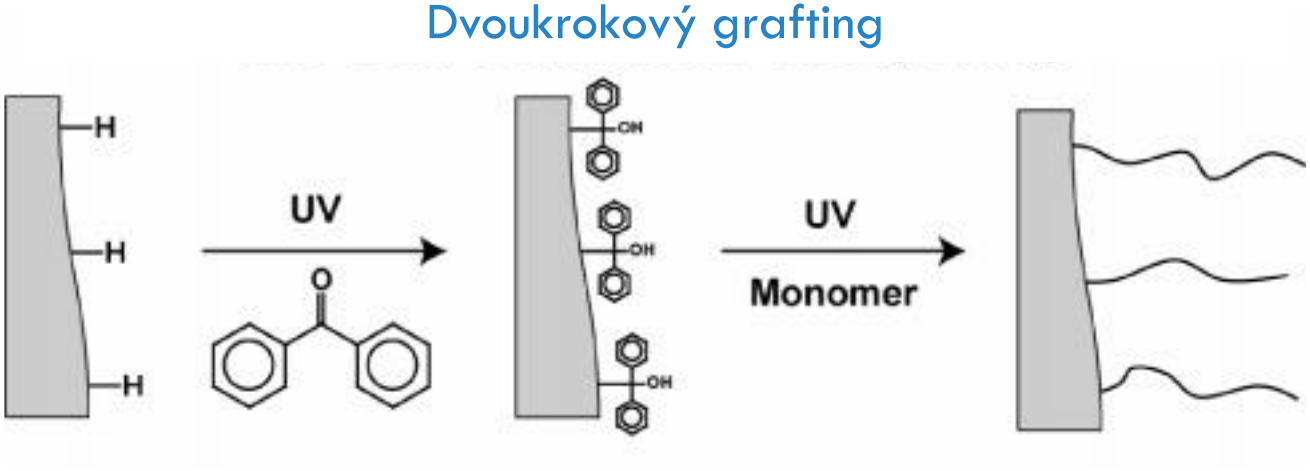
ROUBOVÁNÍ (GRAFTING)

Jednokrokový grafting



- Horší kontrola modifikace
- Možnost ucpání pórů

Dvoukrokový grafting



- Cílená modifikace
- Maximální pokrytí povrchu

Chem. Mater. 18 (2006) 5950 - 5957.

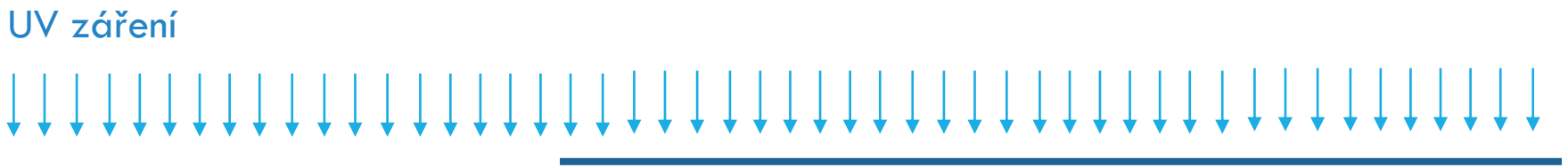
Nutné UV transparentní monomery (metakryláty)

DVOUKROKOVÝ GRAFTING

Původní monolit

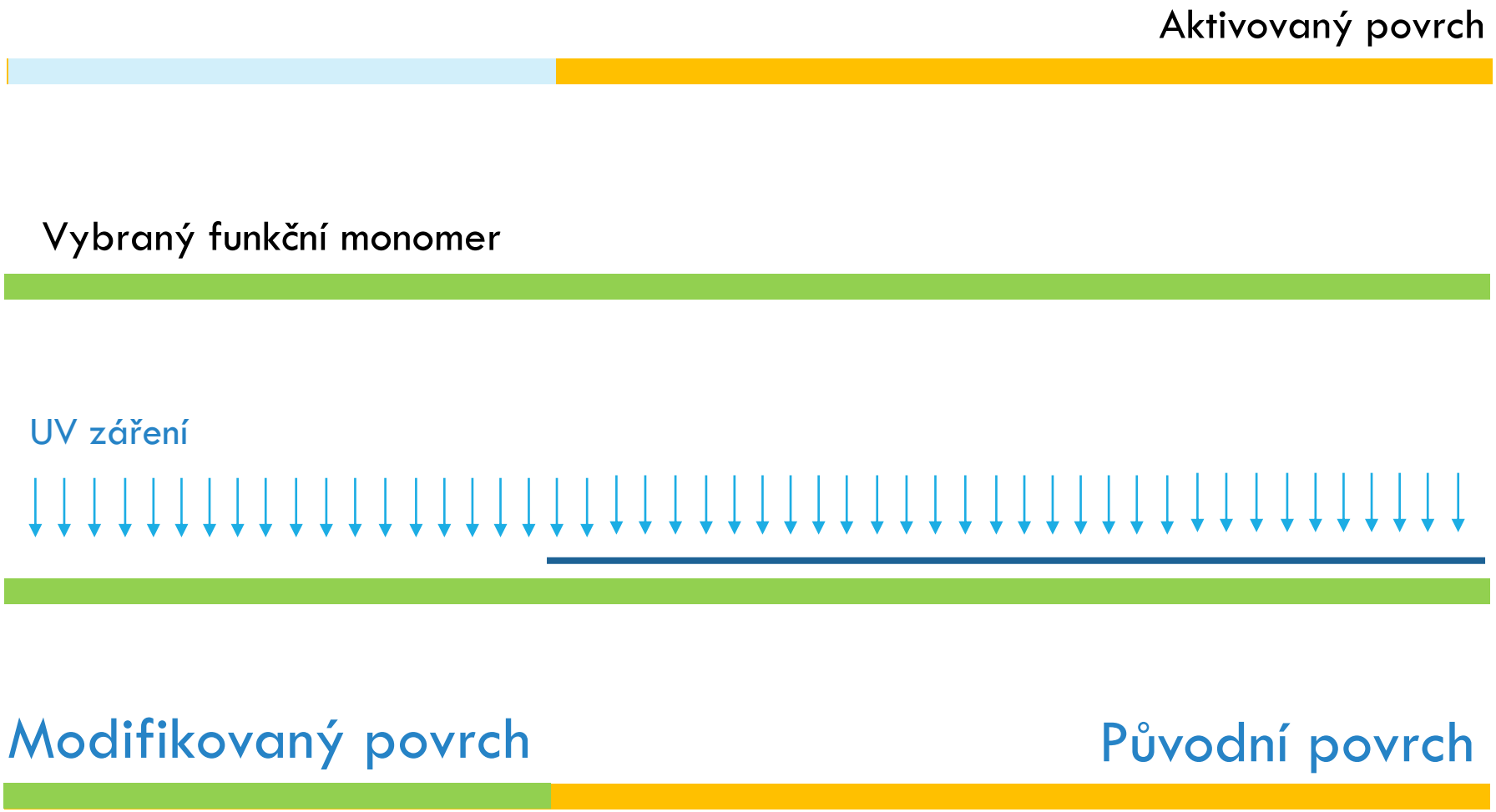
Benzofenon

Původní monolit

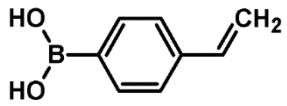


Aktivovaný povrch

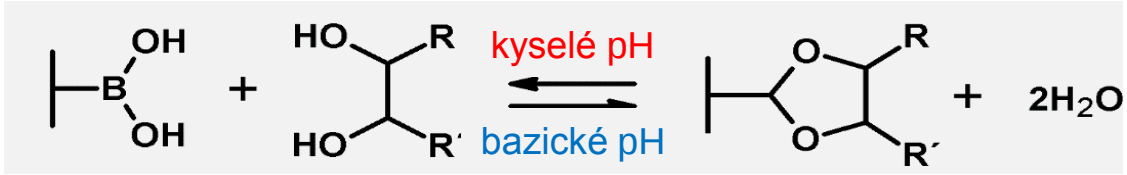
DVOUKROKOVÝ GRAFTING

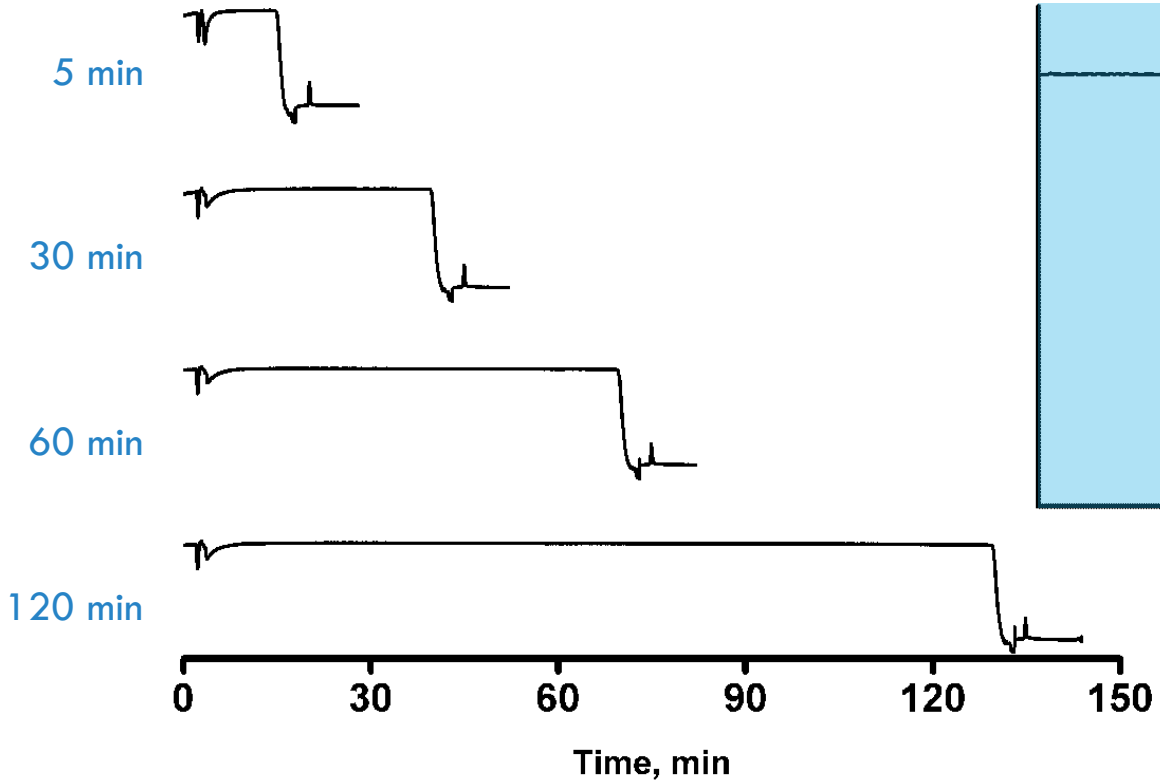


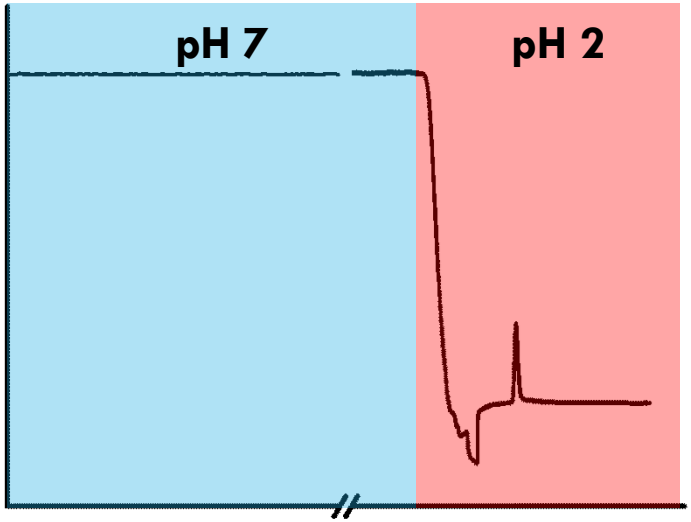
KONTROLOVANÝ ZÁCHYT ANALYTŮ



4-vinylphenyl kyselina boritá

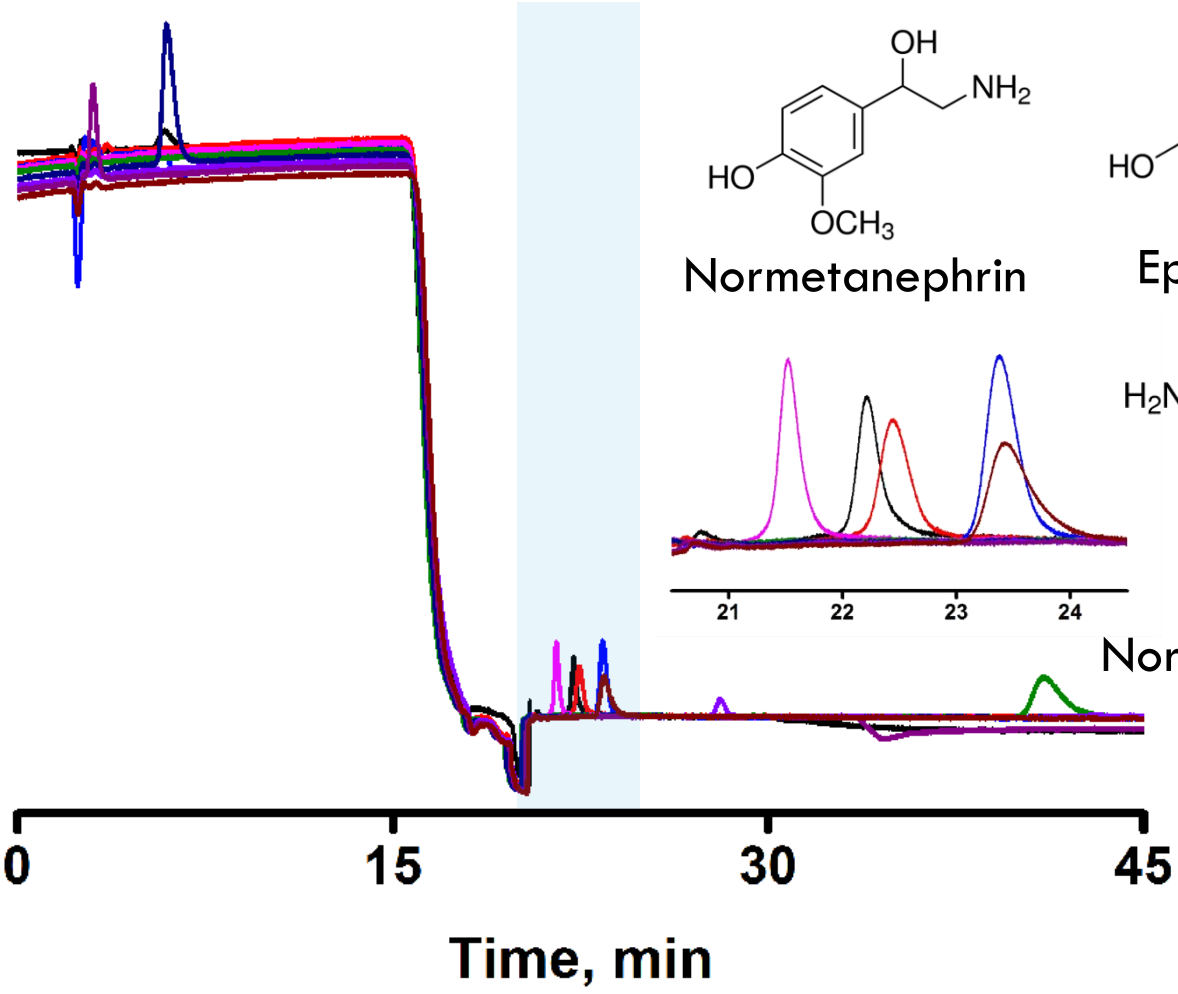




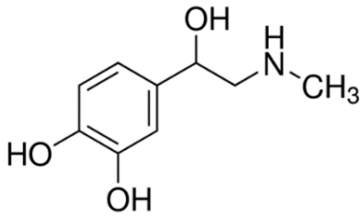


pH *7* – 5 mM HEPES | **pH 2** – 2% acetonitril + 0.1% TFA, dopamin, 0.35 μl/min

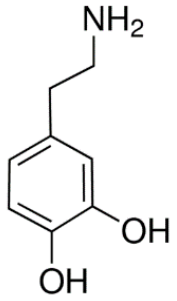
EXTRAKCE A ANALÝZA NEUROTRANSMITERŮ



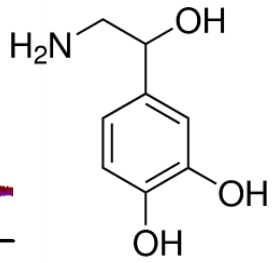
Normetanephrin



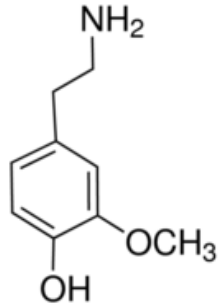
Epinephrin



Dopamin



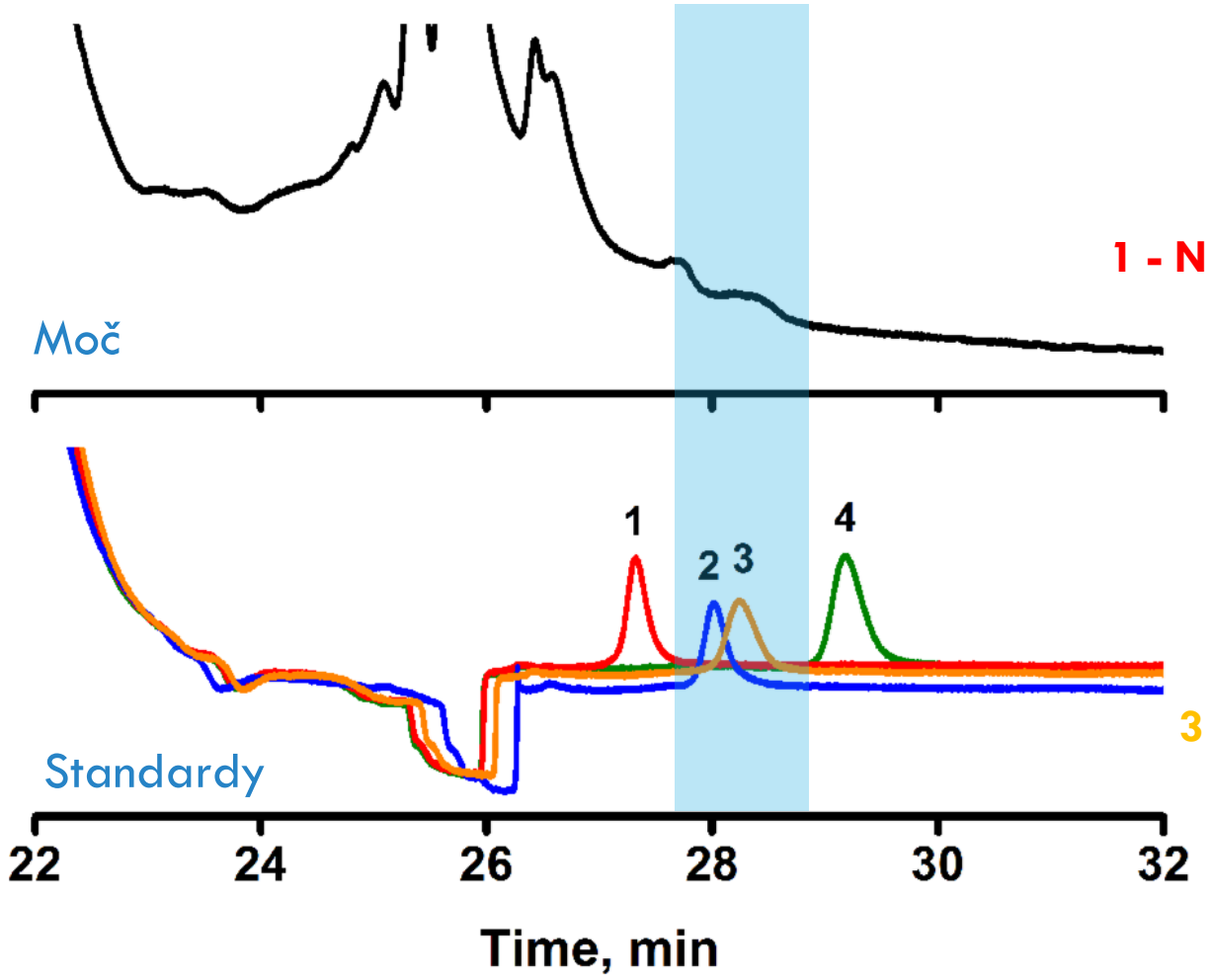
Norepinephrin

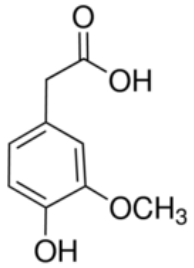


Methoxytyramin

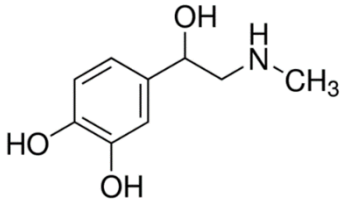
pH 7 – 5 mM HEPES | **pH 2** – 2% acetonitril + 0.1% TFA, dopamin, 0.35 μl/min

STANOVENÍ NEUROTRANSMITERŮ V MOČI

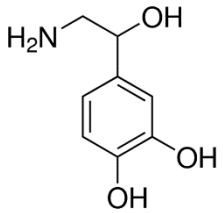




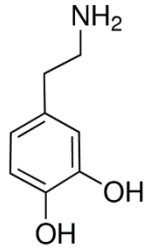
1 - Normetanefrin



2 - Epinefrin

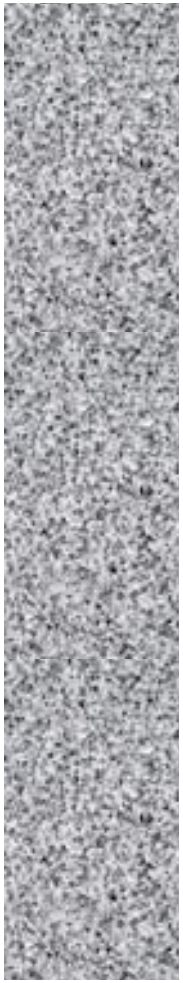


3 - Norepinefrin



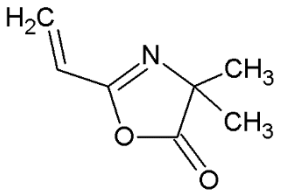
4 - Dopamin

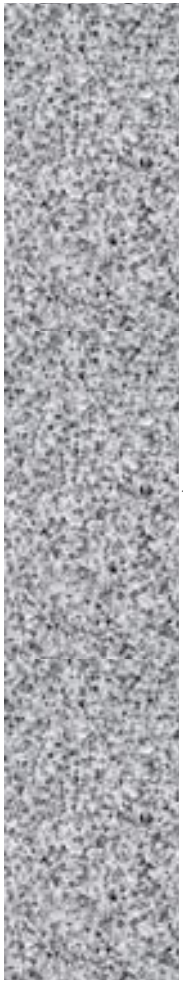
ENZYMAZTICKÉ REAKTORY (IMERS)



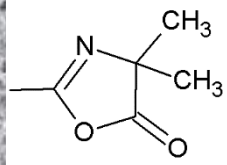
Monolit

Dvoukrokový grafting (vinyl azlacton)

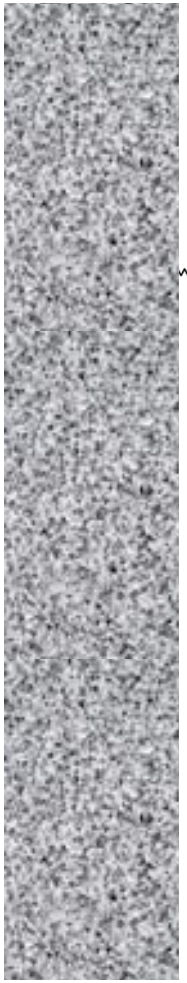


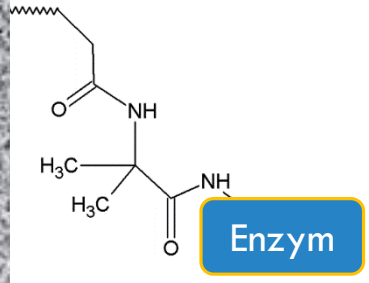


Aktivovaný monolit



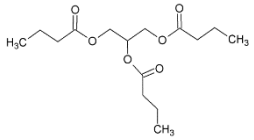
Immobilizace enzymu





Immobilizovaný Monolitický Enzymatický Reaktor

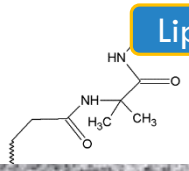
PŘÍPRAVA A CHARAKTERIZACE

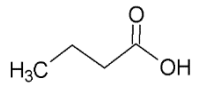


Tributyrin

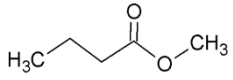
Metanol

Lipáza

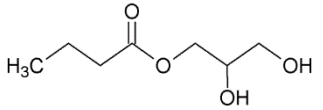




Kyselina máselná

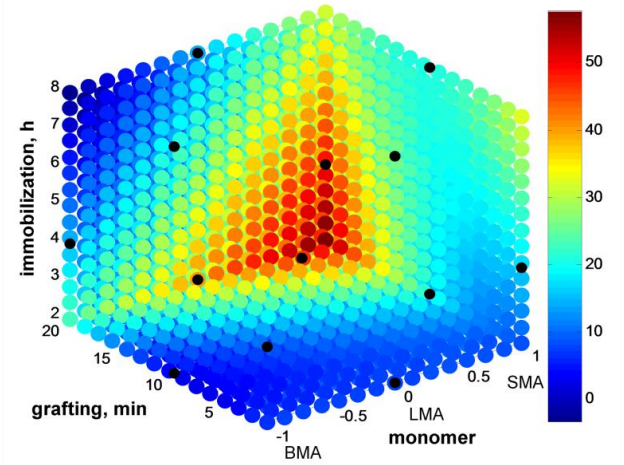


Methylbutyrát

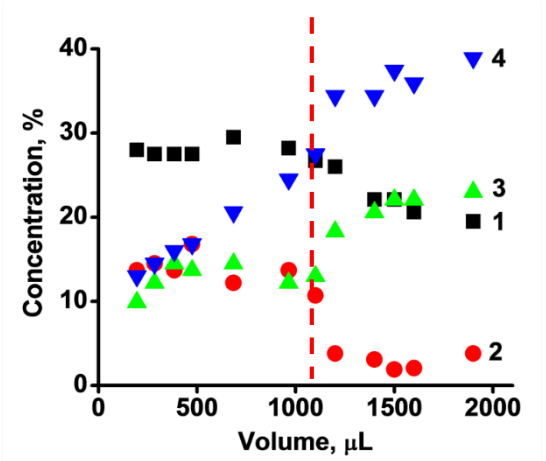


Dihydroxypropylbutyrát

Optimalizace reakčních podmínek



Stabilita reaktoru

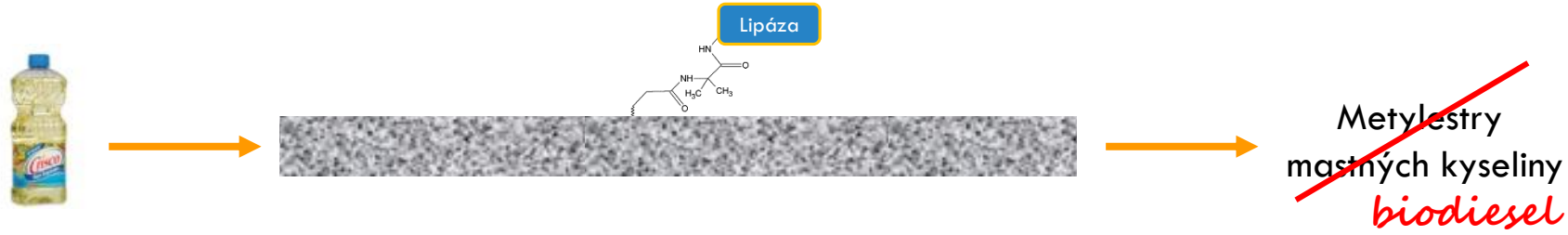


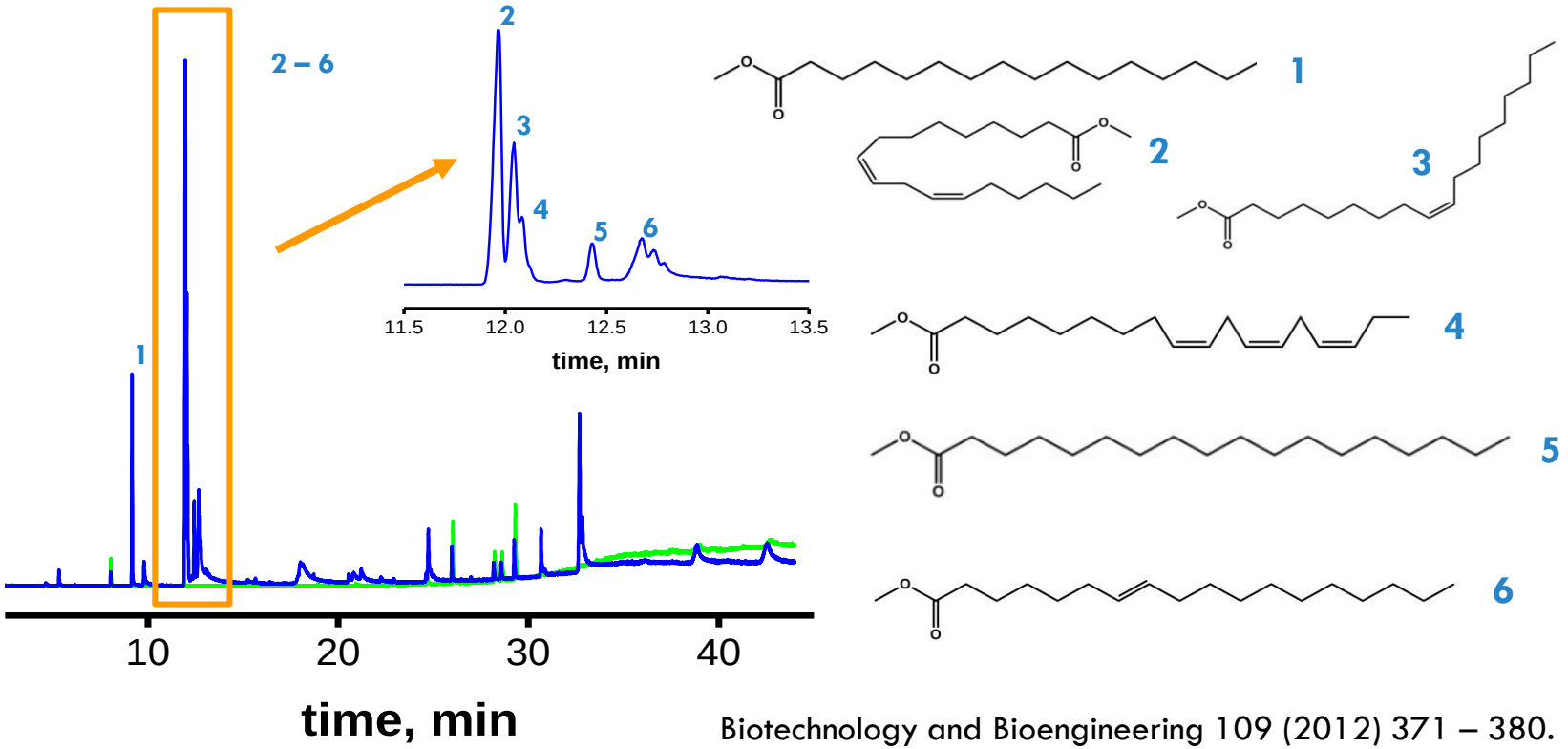
Enzymatický reaktor pracující v čistě organickém prostředí

- Stearyl metakrylát
- 20 min VAZ
- 2 h lipase

Biotechnology and Bioengineering 109 (2012) 371 – 380.

„SYNTÉZA“ BIODISELU

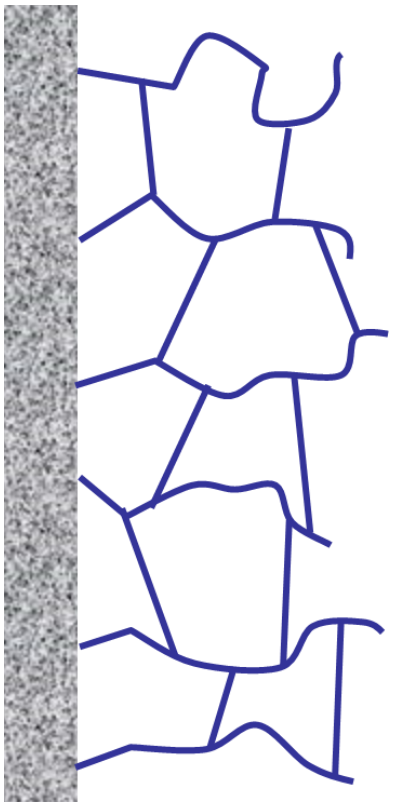


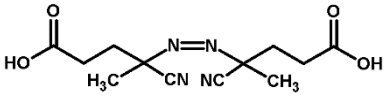


Biotechnology and Bioengineering 109 (2012) 371 – 380.

TEPLOTNÍ GRAFTING

Zesítěný monolit

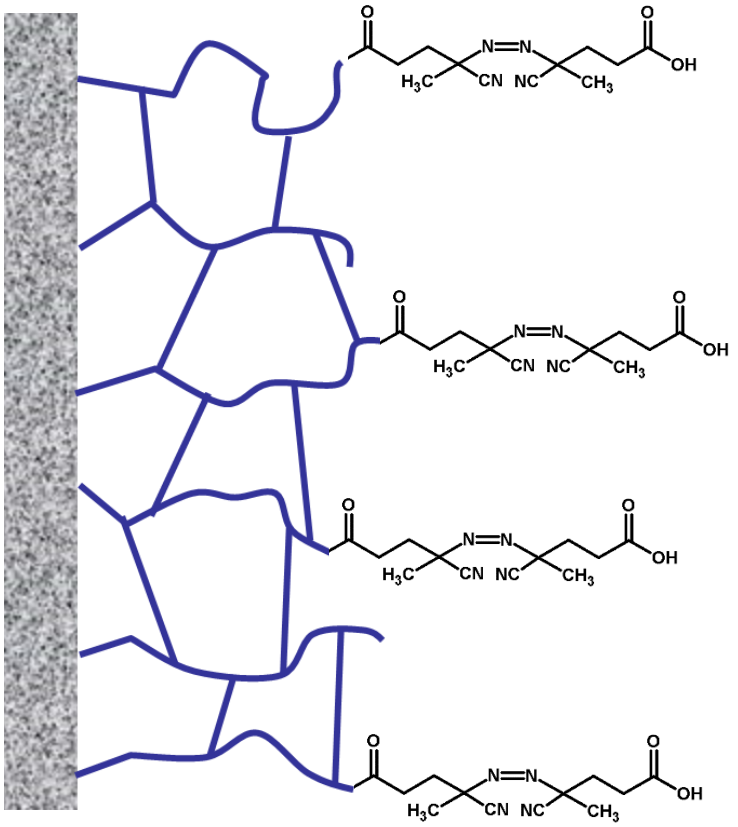




ACVA

TEA
DMF
laboratorní teplota
1 – 20 h

Aktivovaný povrch



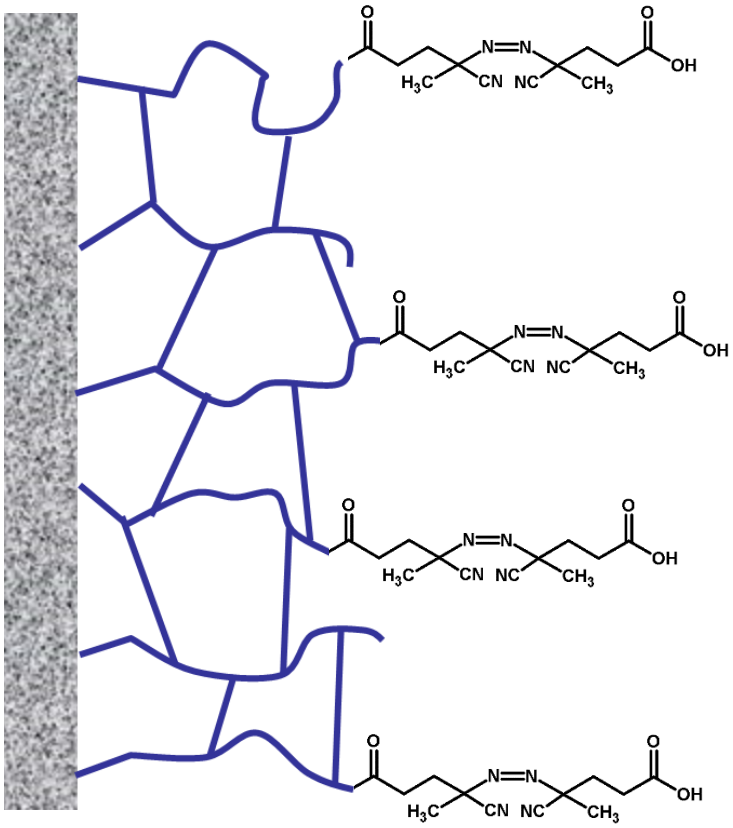
TEA – triethylamin, DMF - dimythylformamid

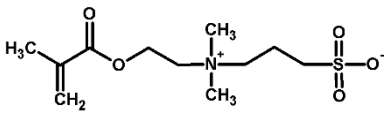
J. Sep. Sci. 2013, 36, 2806 – 2812.

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

TEPLOTNÍ GRAFTING

Aktivovaný povrch

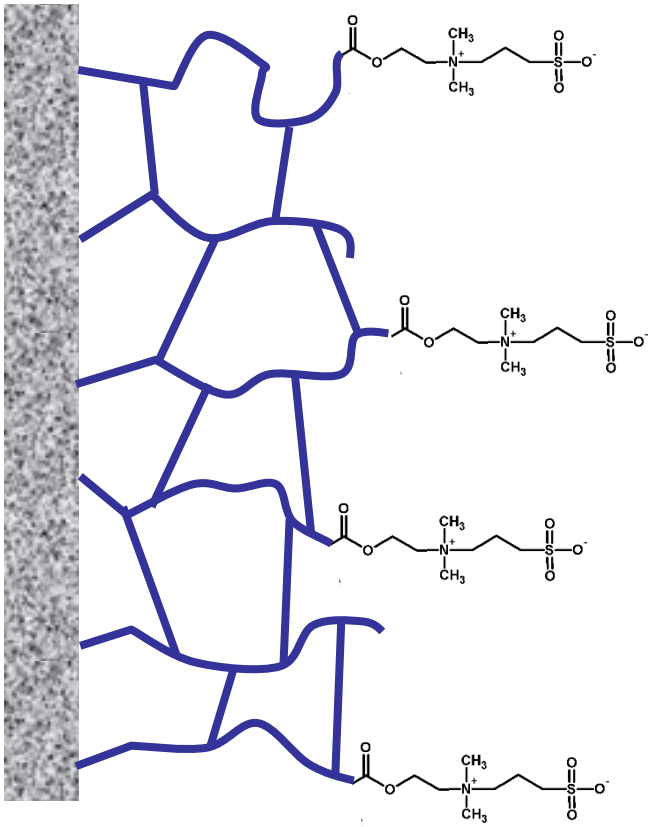




MEDSA

Metanol
50 – 90°C
1 – 24 h

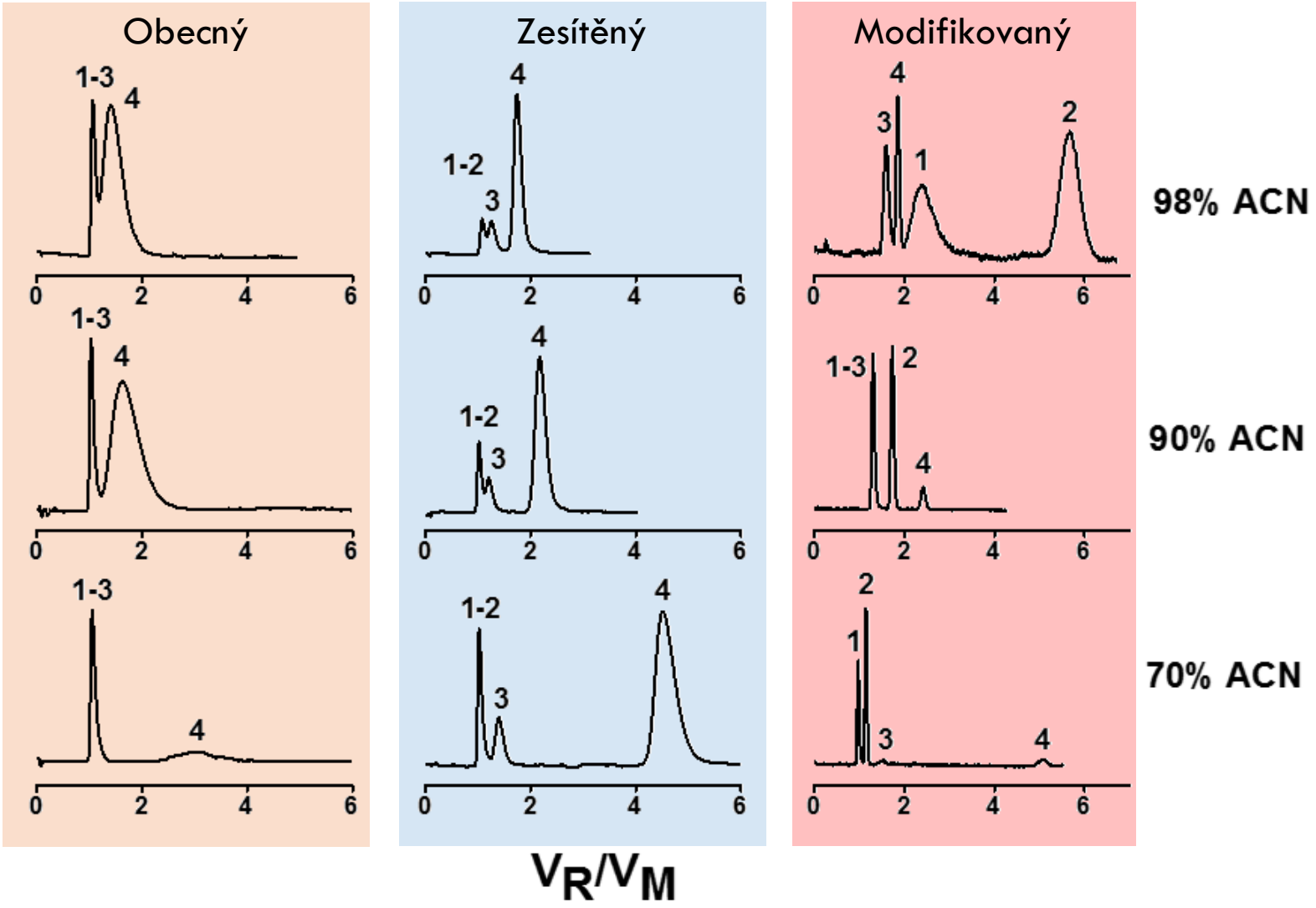
Modifikovaný povrch



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

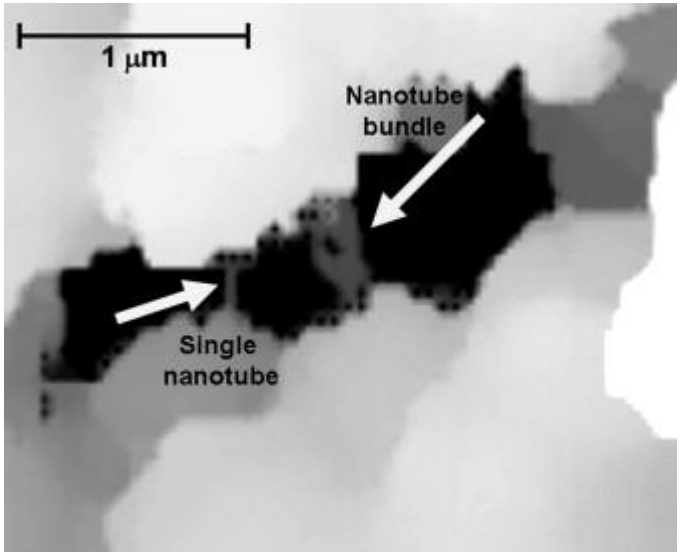
J. Sep. Sci. 2013, 36, 2806 – 2812.

DUÁLNÍ RETENČNÍ MECHANISMUS

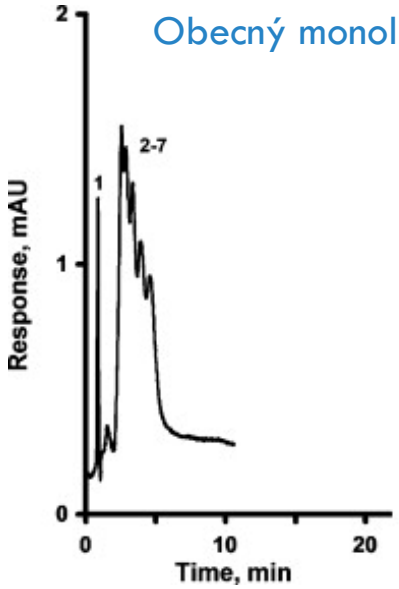


Uracil (1), thiomočovina (2), fenol (3) a toluen (4)

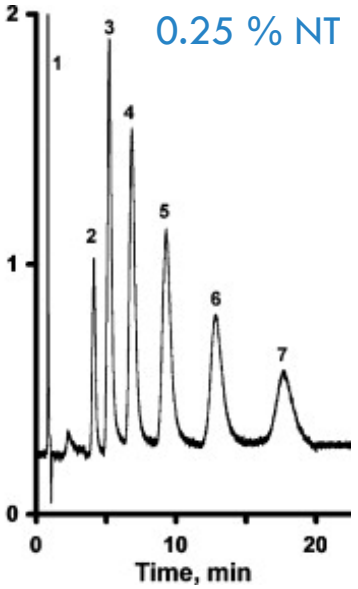
MODIFIKACE NANOČÁSTICEMI



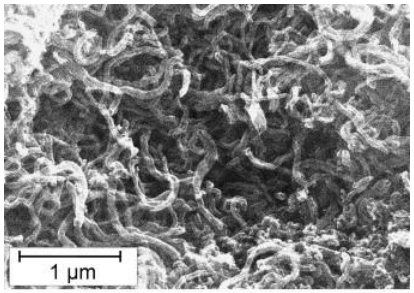
Obecný monolit



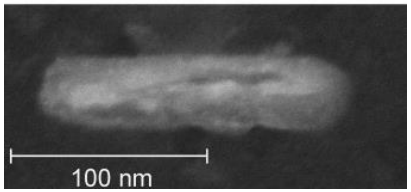
0.25 % NT

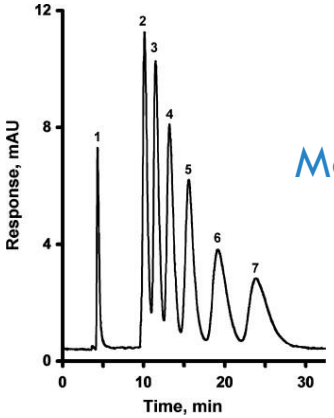


„Směs“ nanotrubiček



„Nařezané“ NT

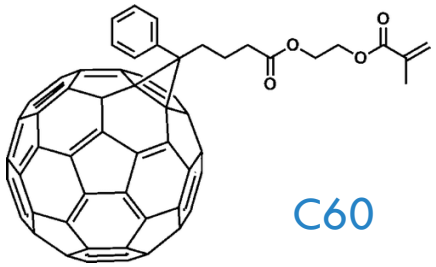




Modifikovaný povrch

J. Chromatogr. A1218 (2011) 2546 – 2552

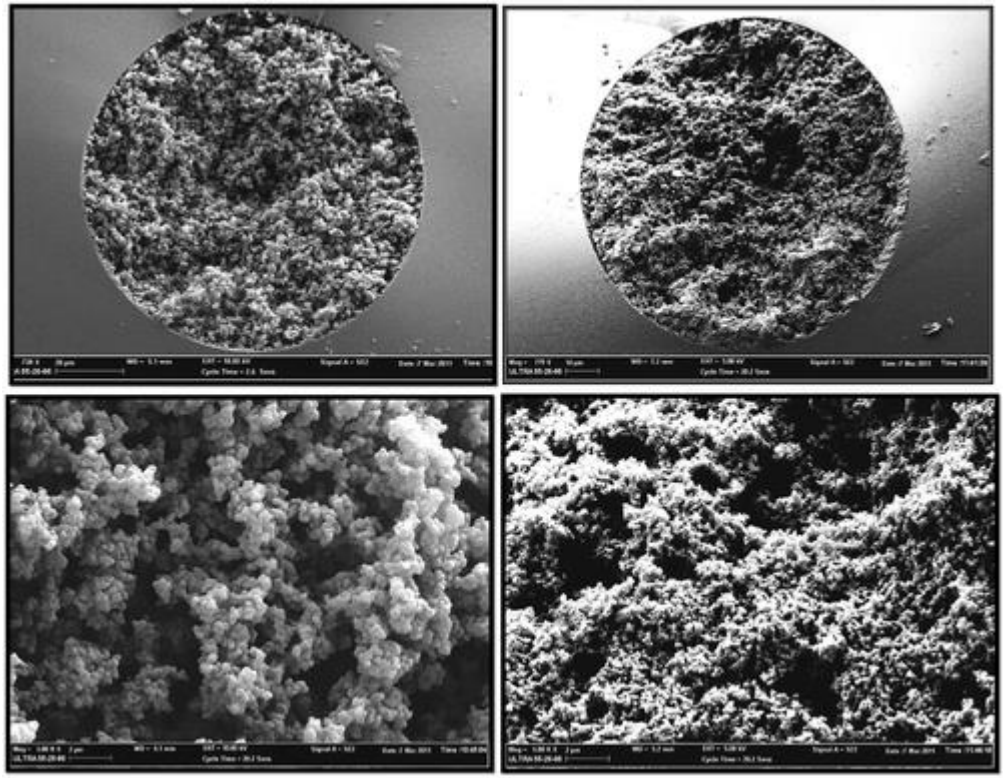
MODIFIKACE NANOČÁSTICEMI

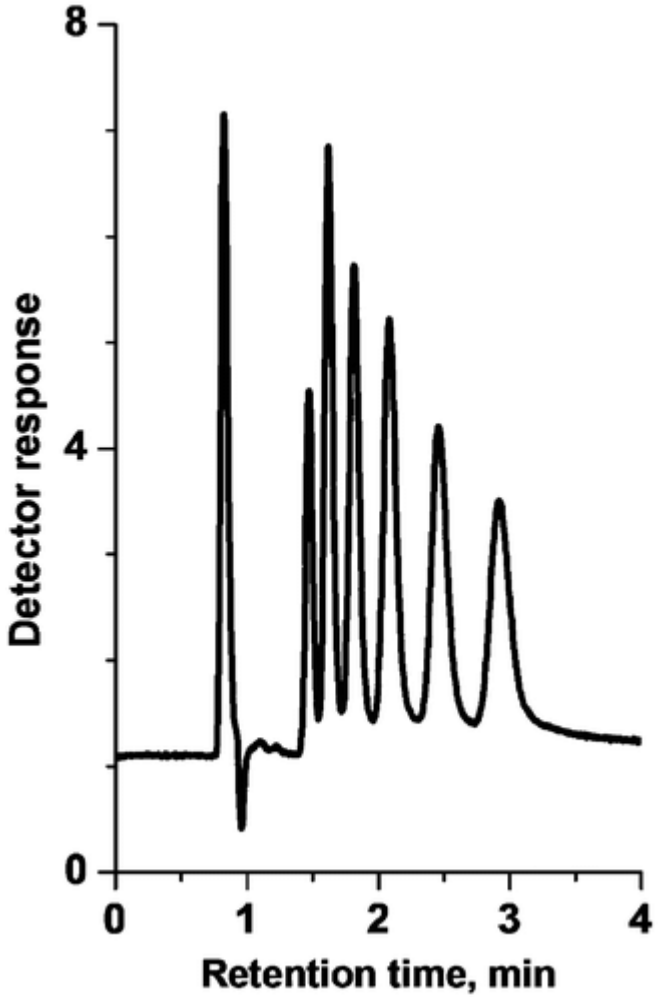


C60

0 %

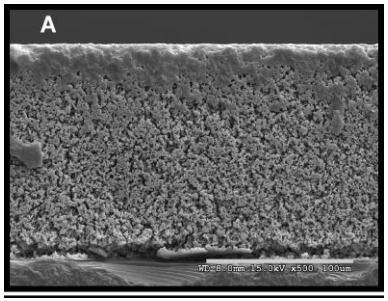
1 %

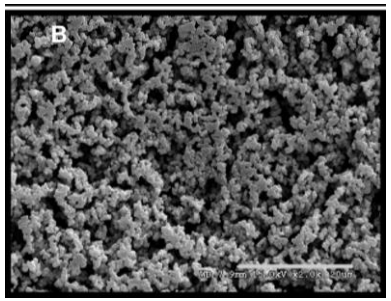


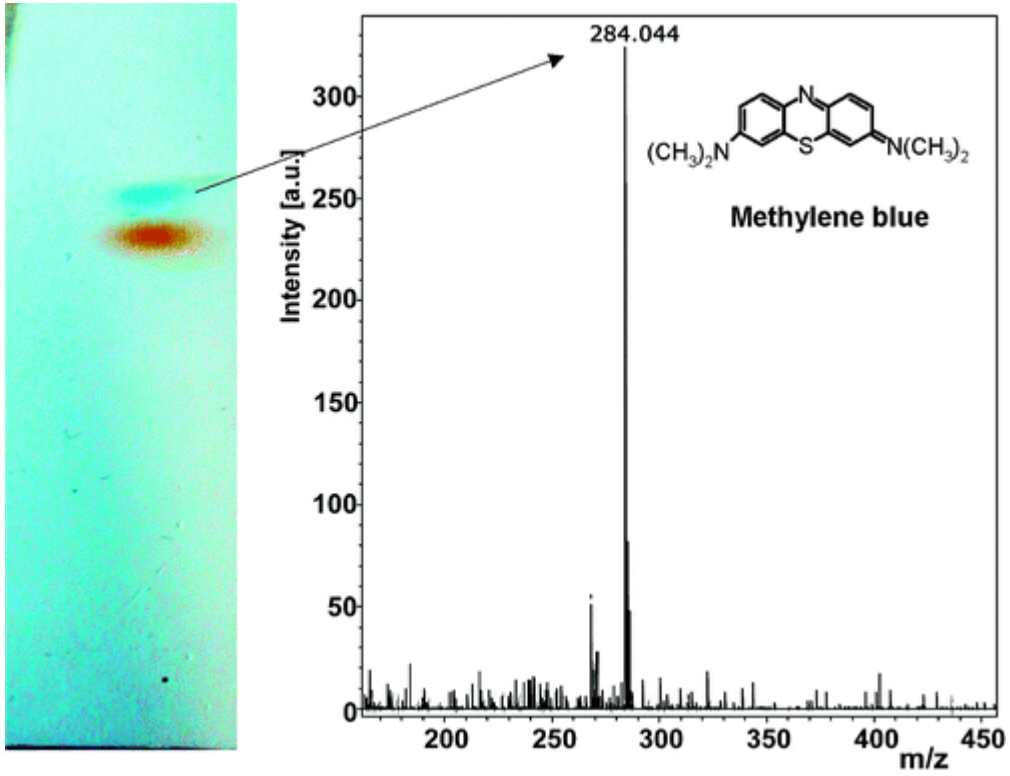


Anal. Chem., 83 (2011) 9478 – 9484.

TENKOVRSTEVNÁ CHROMATOGRAFIE







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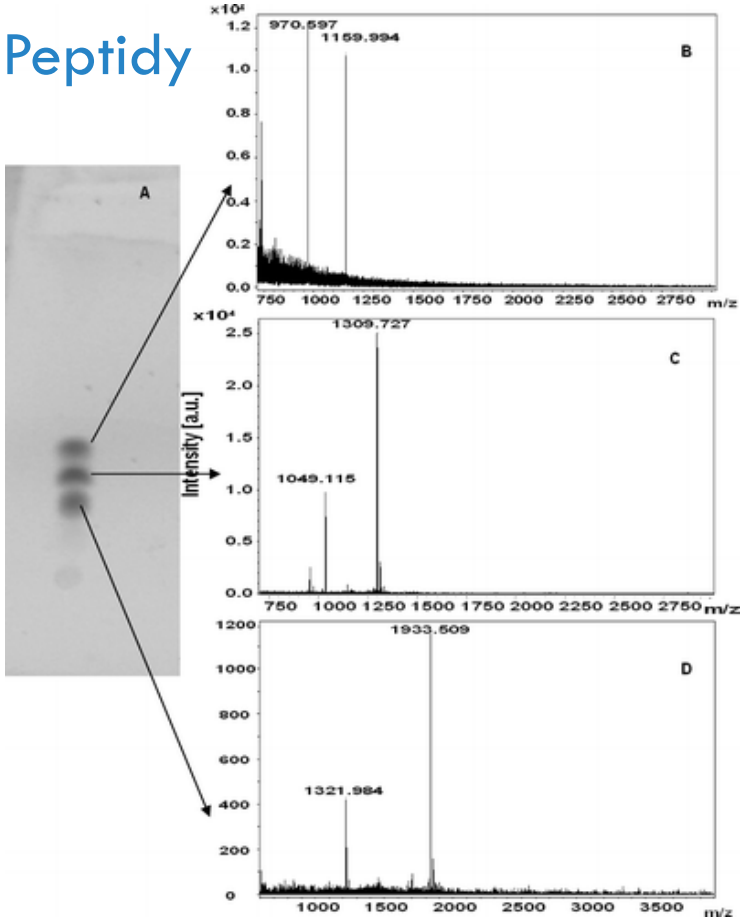
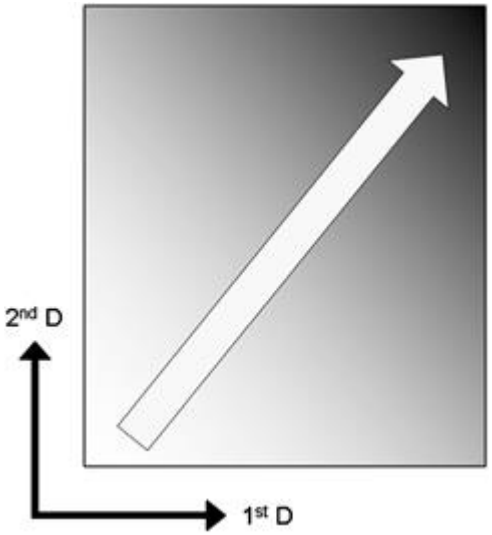
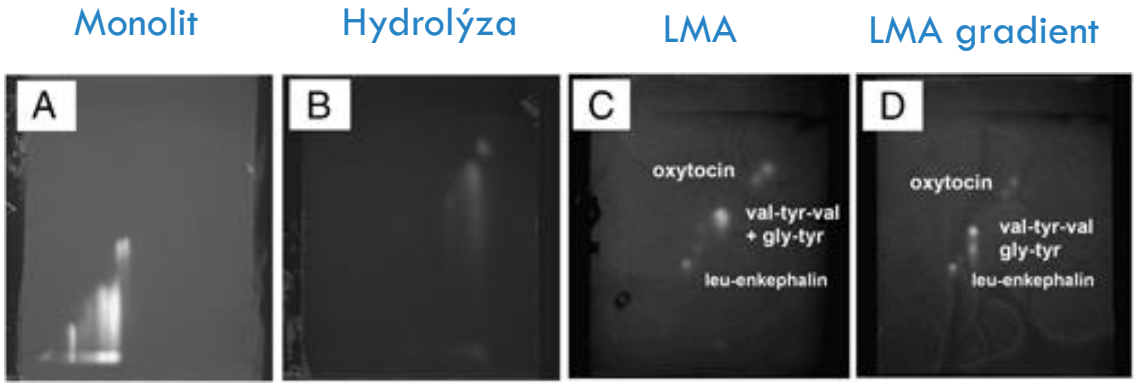
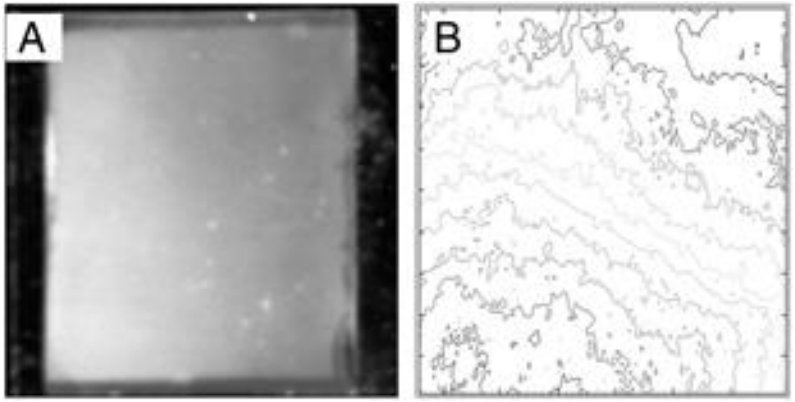


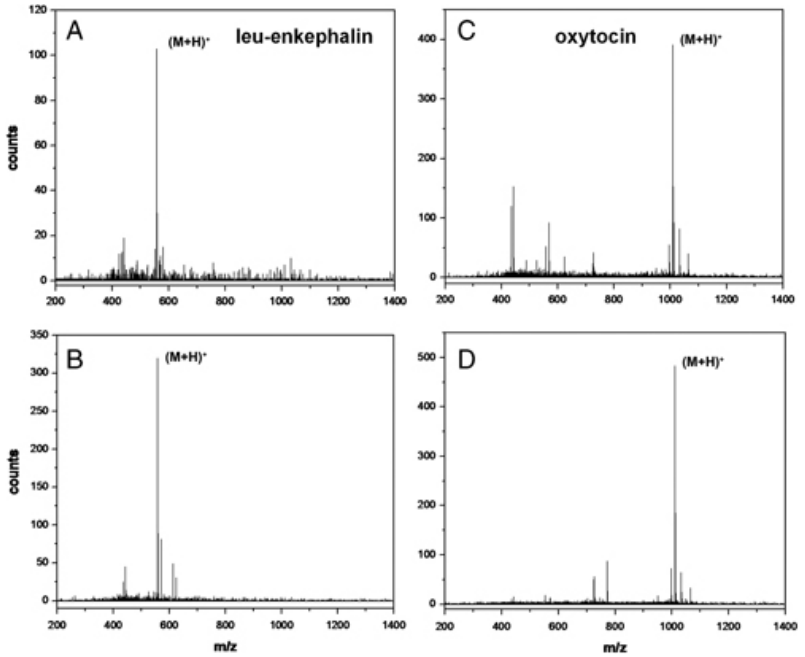
Anal. Chem. 79 (2007) 486 – 493.

TENKOVRSTEVNÁ 2D CHROMATOGRAFIE









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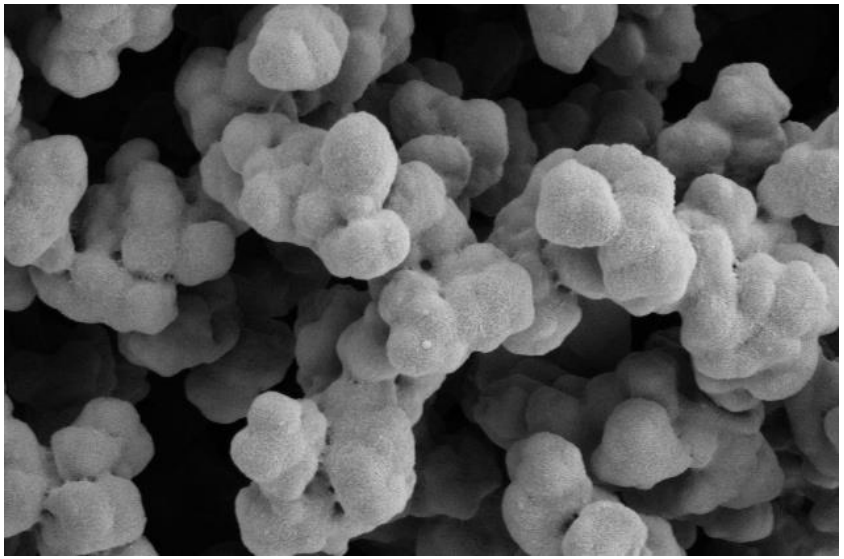
POLYMERNÍ MONOLITY

- 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