

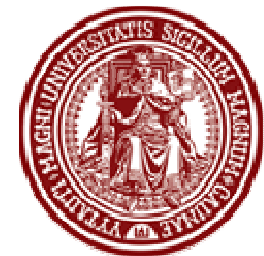
***INSTRUMENTINĖS ANALIZĖS METODŲ
VYSTYMAS IR JŲ TAIKYMAS
MOLEKULINEI BIOLOGINIŲ OBJEKTŲ,
SINTETINIŲ PRODUKTŲ IR APLINKOS
ANALIZEI
(F-08-03)***

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VDU KBS, Kaunas, 2012 02 06

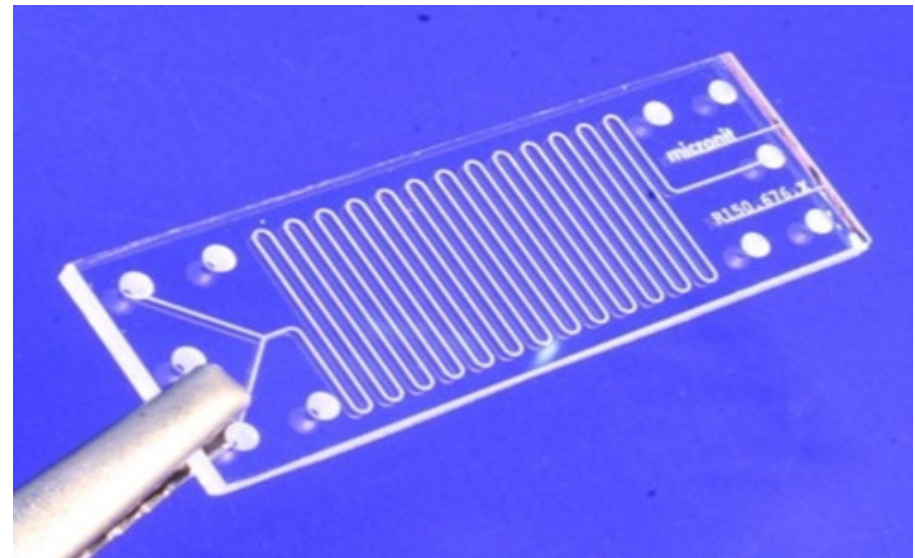


Dvi pagrindinės veiklos:

- **(I) fundamentiniai tyrimai analizės metodų vystymo srityje juos miniatiūrizuojant, integruojant ir keliant jų našumą**
- **(II) taikomieji moksliniai darbai diegiant ir panaudojant šiuolaikinę instrumentinę analizę molekuliniam tyrimams biomedicinos, pramonės ir aplinkotyros srityse.**

TRENDS IN ANALYSIS

- **minIATURIZTION**
- **INTEGRATION**



Continuous beds/Monoliths

Outline

1. Important dates: Two decades of success

1989 HPLC Polyacrylamide Cation Exchangers [S. Hjertén, J.-L. Liao, R. Zhang, J. Chromatogr. 473 (1989) 273].

2. Morphology: useful differences between continuous vs. particulate materials

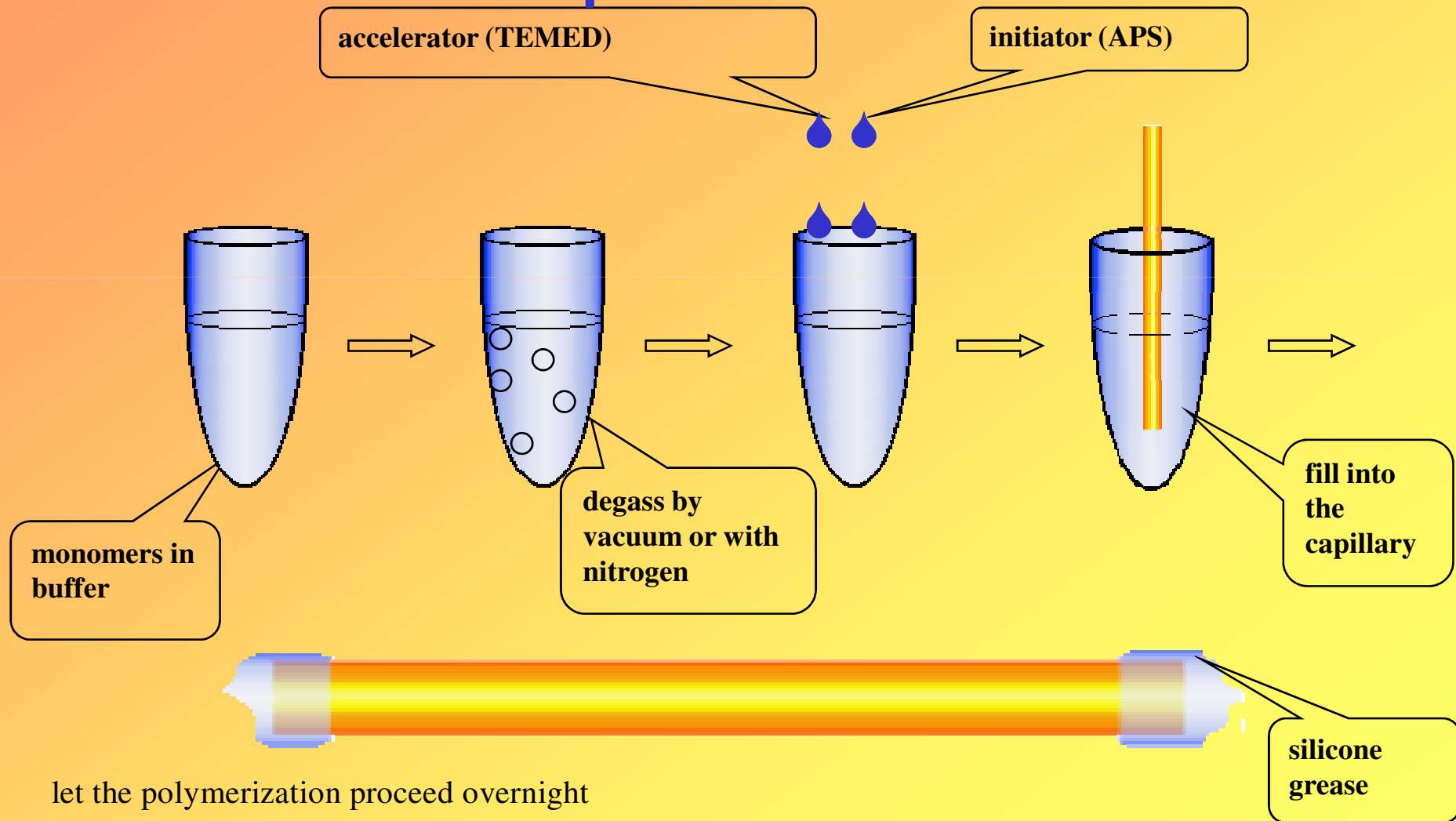
3. Unsurpassed flexibility in formats, chemistries and applications

- a) molecularly imprinted, chiral materials, etc.
- b) gradient beds
- c) SPME monoliths, coupling to CE
- d) restricted access materials (RAM)
- e) lab-on-a chip potentials



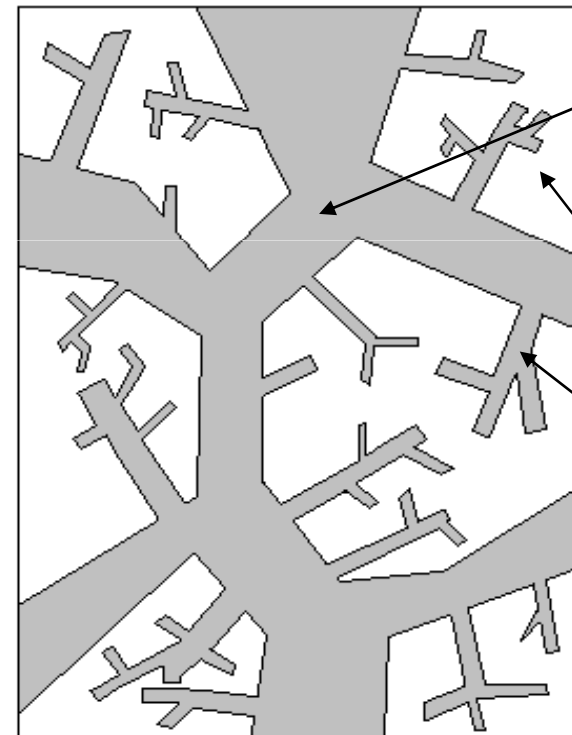
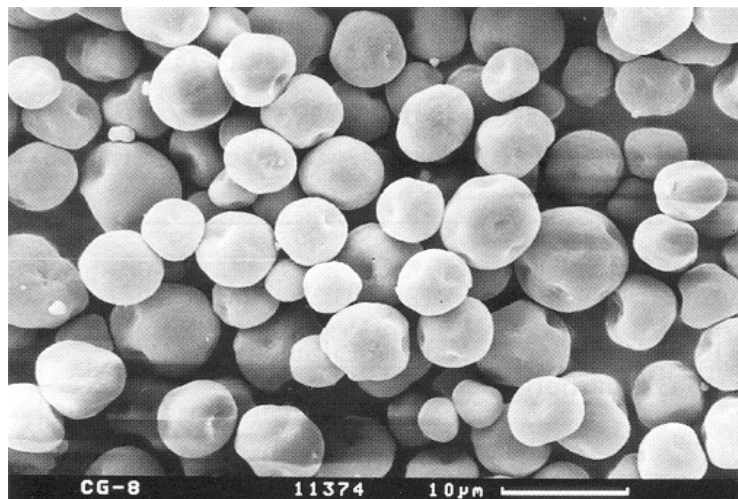
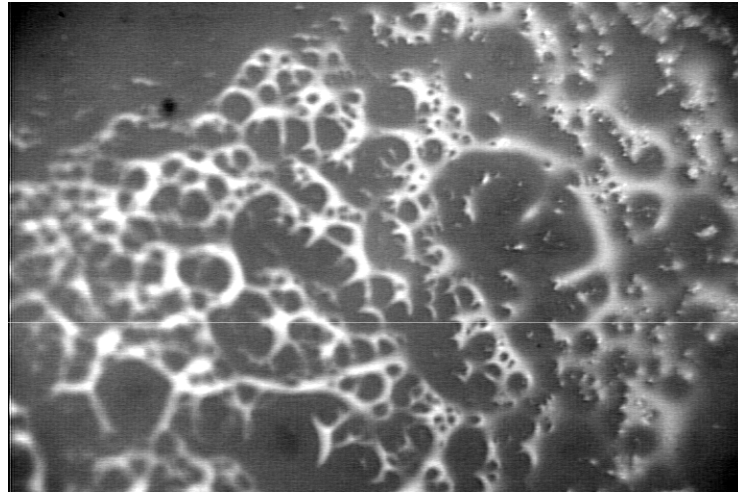
SIMPLE SYNTHESIS

Unlimited number of combinations and possibilities





NON-PARTICULATE vs. PARTICULATE PHASES



Transverse channels

Polymer matrix

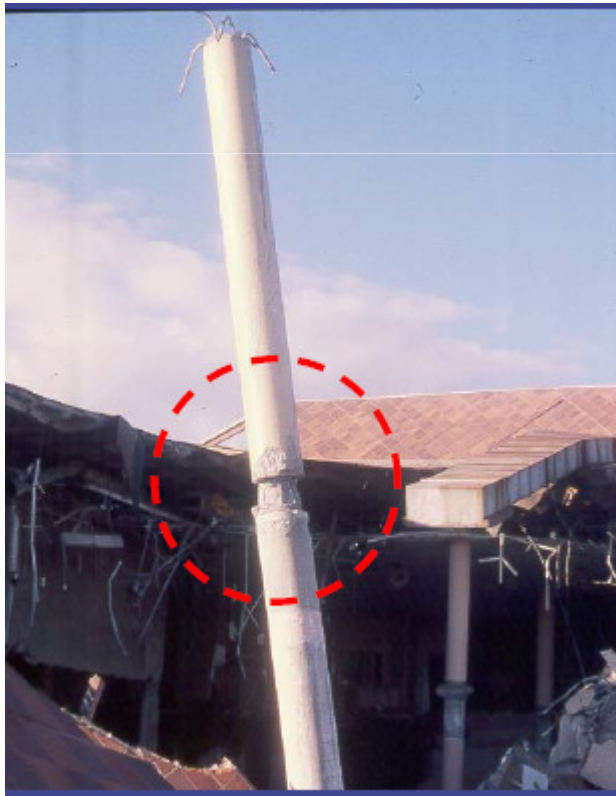
Pores in the matrix



ADVANTAGES OF CONTINUOUS BEDS (MONOLITHS)

- Synthesis - packing in **one – step**
- **No** supporting **frits** required
- **Stability** due to covalent attachment to the fused silica wall
- **Simple, cost-effective** procedure
- **Microformat-compatible**
- **High flow rate, low hydrodynamic resistance**
- **Low separation impedance**

Deformations and progressive collapse

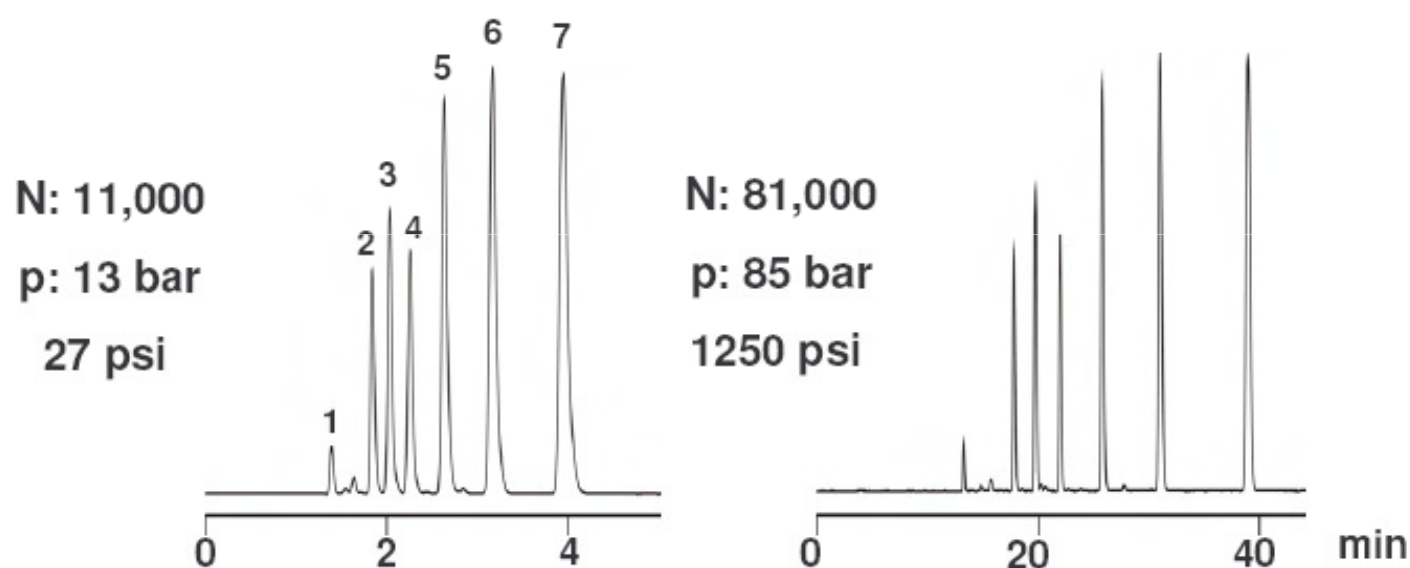


Low Pressure Drop Allows Columns to be Plumbed in Series

Chromolith™ Performance, 100- X 4.6mm

One Column

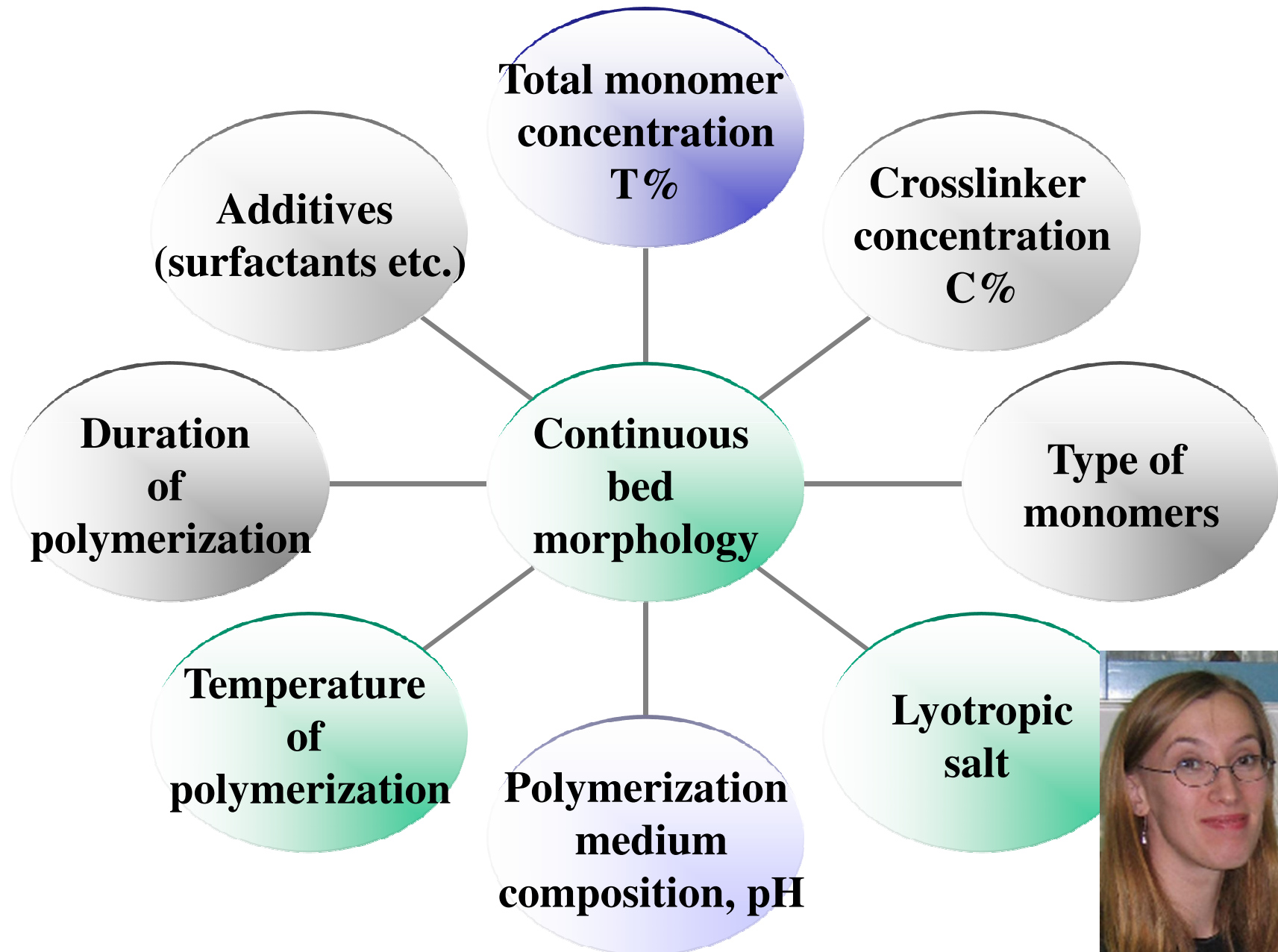
10 Columns = 1 meter



ACN/ water (80/ 20; v/v), 1mL/ min

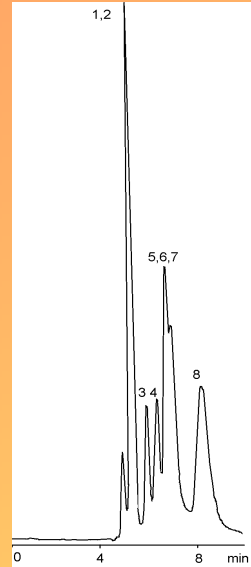
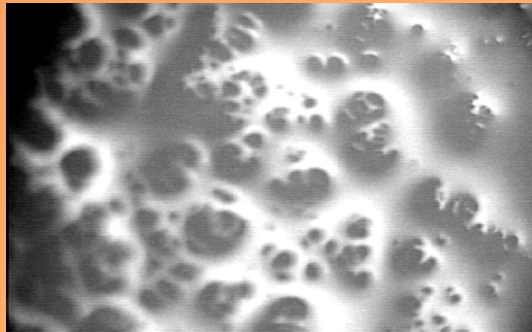
1. thiourea, 2. benzene, 3. toluene, 4. ethyl-, 5. propyl-, 6. butyl-, 7. pentylbenzene

Morphology regulation

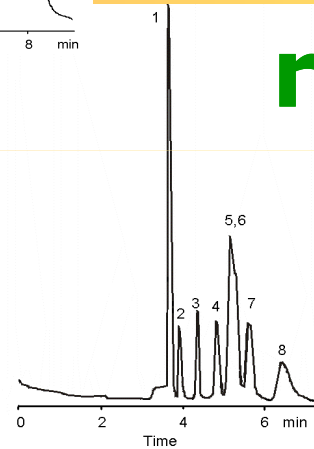
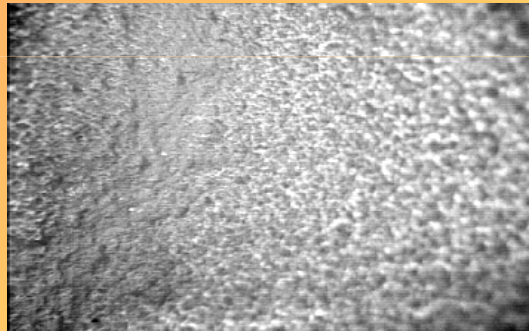


Effect of total comonomer concentration (%T) on morphology and resolution

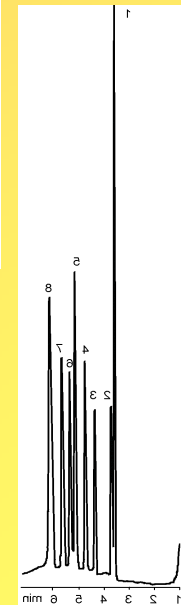
a

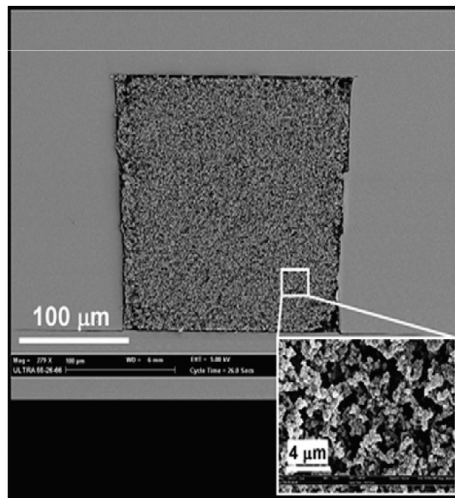
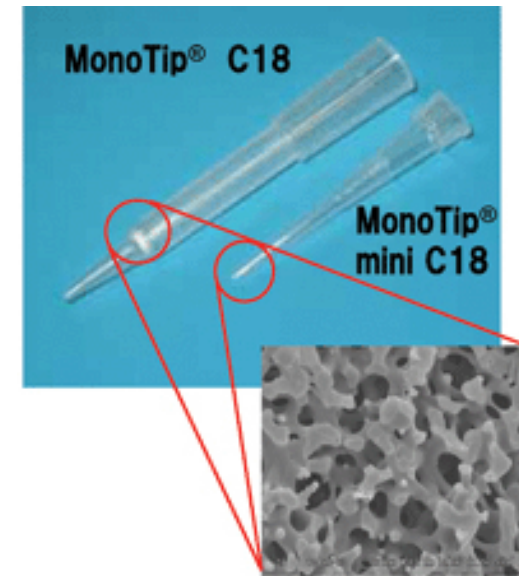
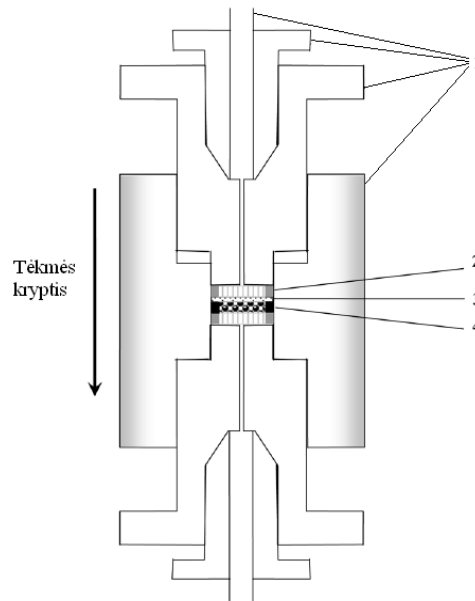


b



c





- **CIM monolith cylinder (BIA Separations, Liubliana, Slovenia) [1]**
- **Disc form monolith in the holder [2]**
- **GL Sciences pipet tip with the C₁₈ silica monolith;**
- **Polymeric monolith immobilized in the microchip[3].**

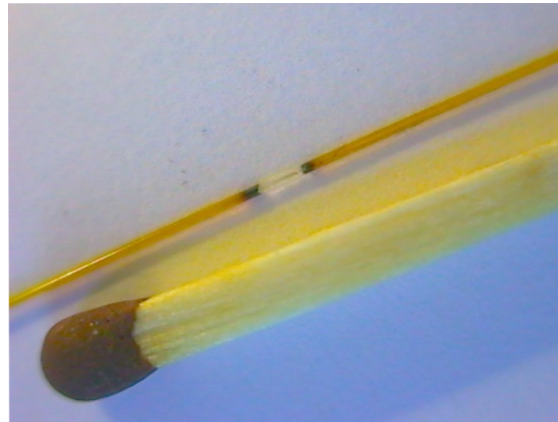
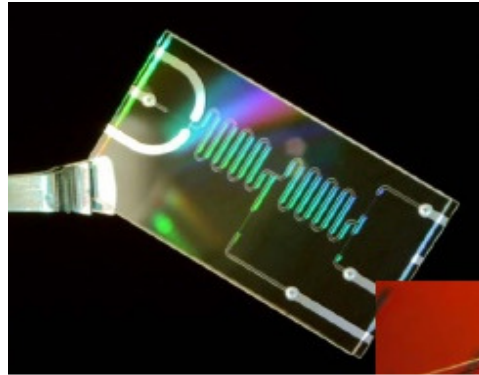
1.F. Svec, J. Krenkova **Rigid porous polymer monoliths as stationary phases and supports** // LC/GC LC column technology supplement April (2008) 2-8

2. R. Mallik, D. S. Hage **Affinity monolith chromatography** // Journal of Separation Science, 29 (2006) 1686 – 1704

3. A. Oriňák, G. Vering, H. F. Arlinghaus, J. T. Andersson, L. Halas, R. Oriňáková, L. Turčániová **New approaches to coupling TLC with TOF-SIMS** // Journal of Planar Chromatography, 18 (2005) 44-50

Various separation scale

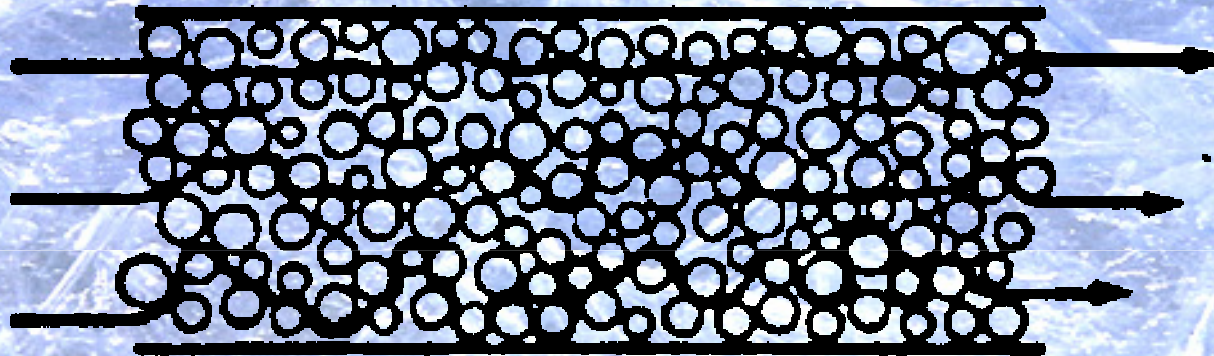
- Preparative
- Analytical HPLC
- Micro
- Nano scale
- SPE
- **SPME**



RP GELS FOR CEC

The Problem

Eddy diffusion



Main Task:

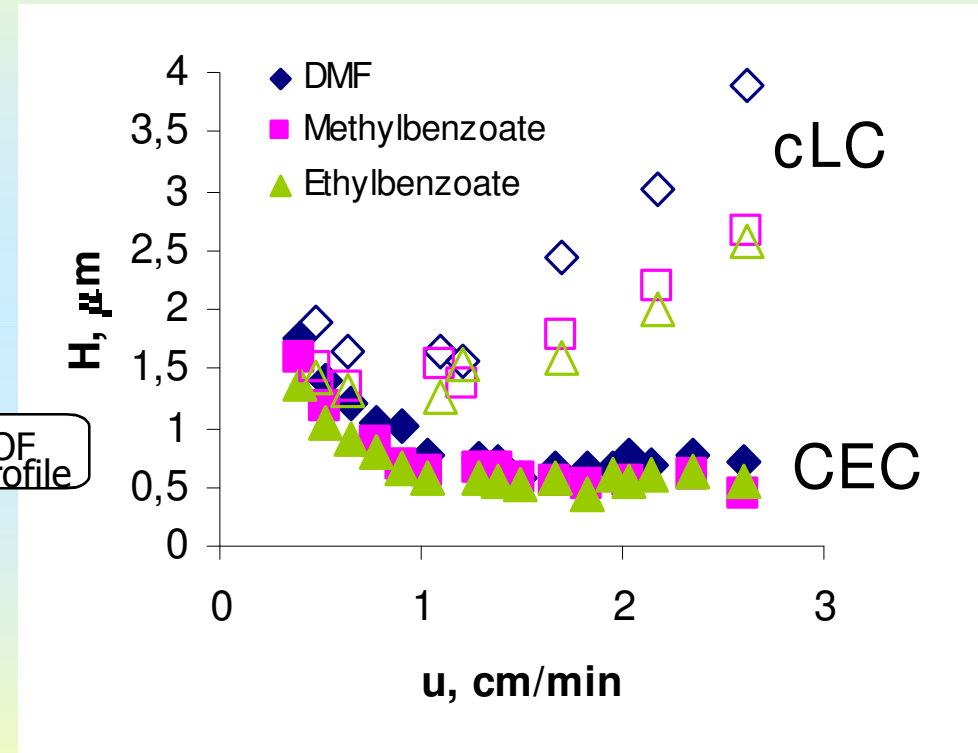
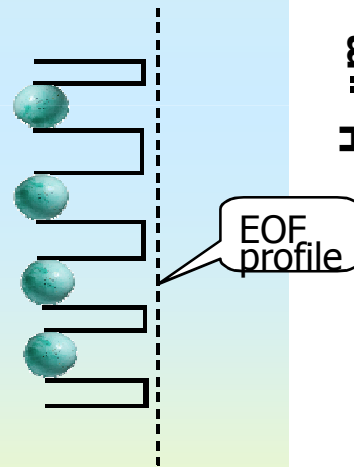
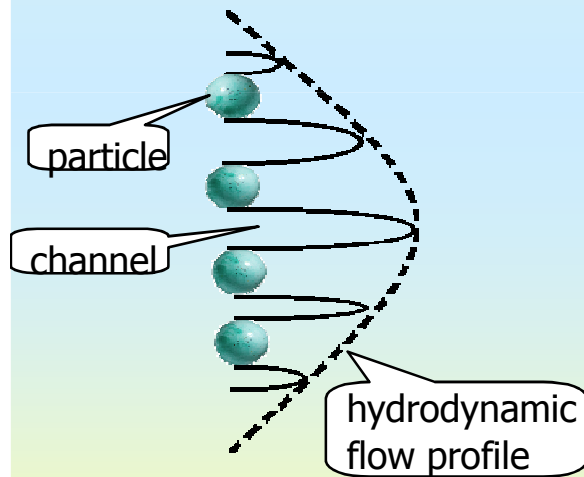
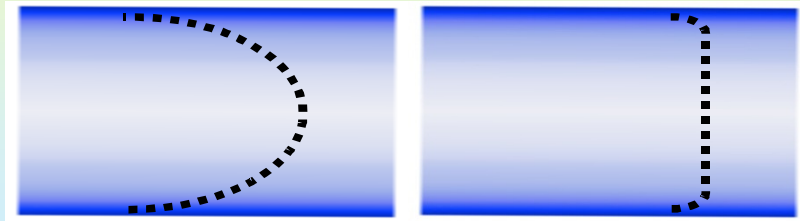
Development of RP thermal gels with zero Eddy diffusion and advanced mass transfer properties for CEC applications

RP
Gels

H/u curve: cLC vs. CEC

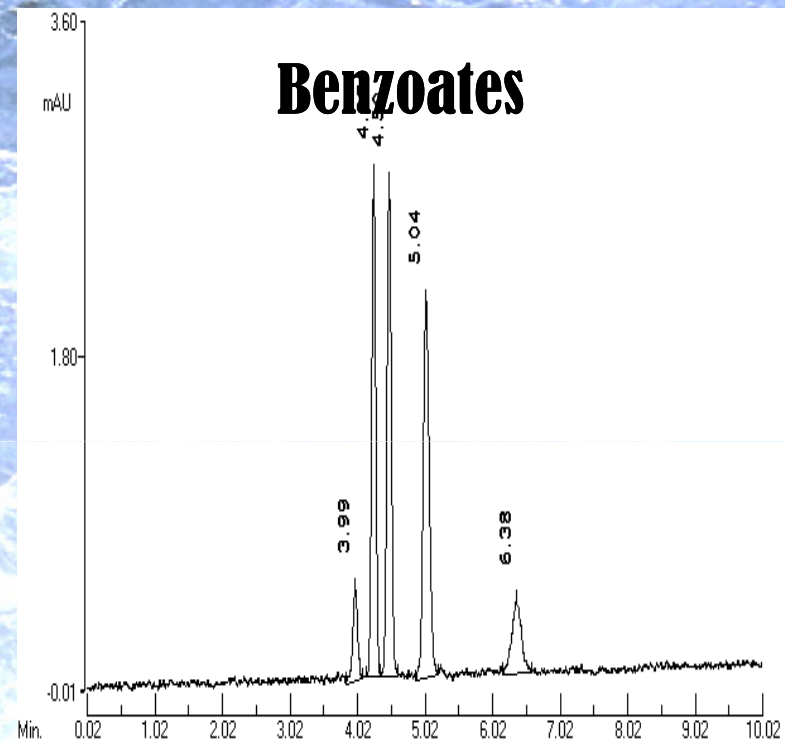
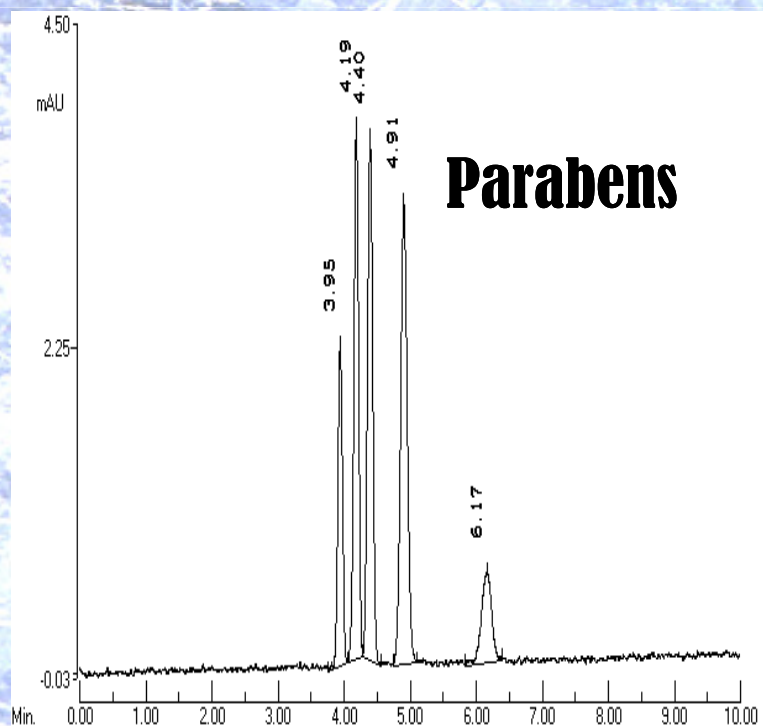
Laminar flow

Electroosmotic flow (EOF)



Reversed-phase

RP CEC WITH HOMOGENEOUS GELS

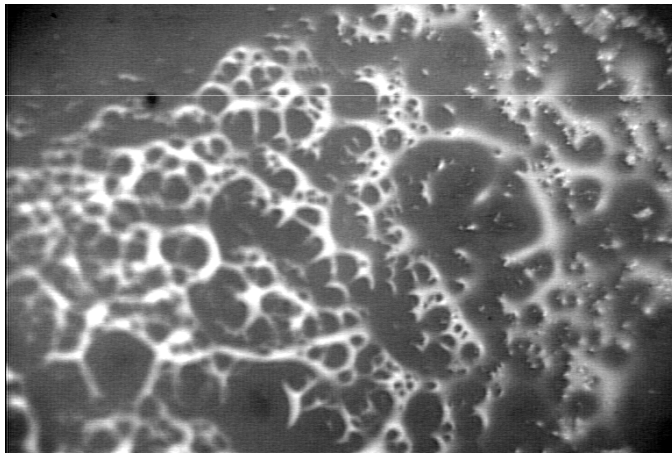


Agilent Technologies

RP

Diversity of the continuous beds morphology

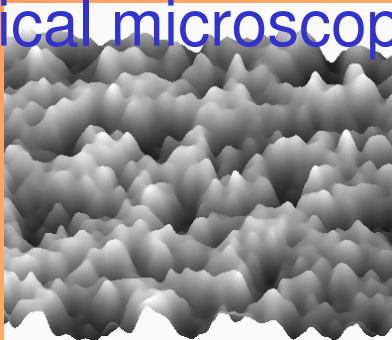
- from **Macroporous structures** (with perfusive channels)
- to Entirely **homogeneous gels** for CEC



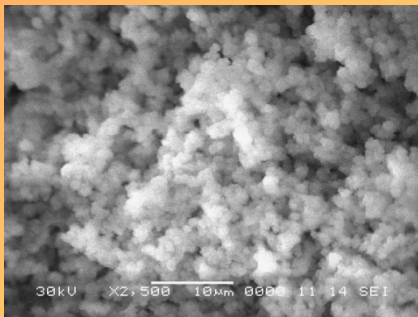
[A. Maruška, O. Kornyšova. *Homogeneous reversed-phase agarose thermogels for electrochromatography*. **J. Chromatogr. A.** 1044 (2004) 223-227]

Methodology: Morphology evaluation

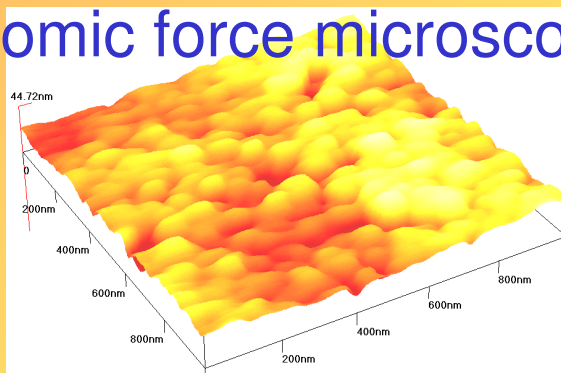
- **Microscopy:**
 - Scanning/confocal optical microscopy



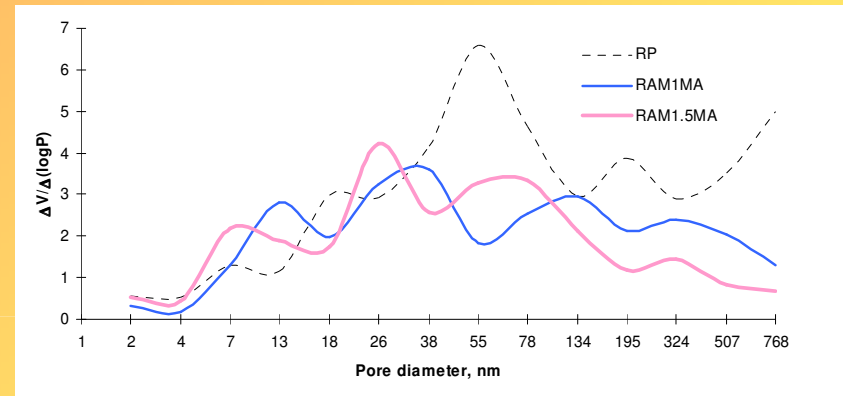
- Scanning electron microscopy



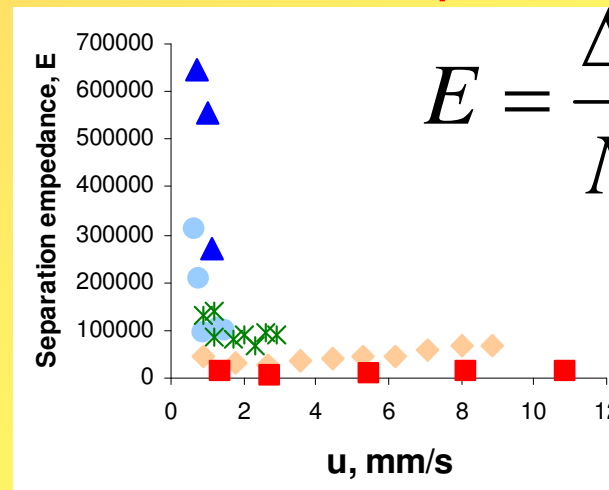
- Atomic force microscopy



- Inverse size exclusion Chromatographic Porosimetry



- Impedance factor, Electrokinetic porosity *etc.*



$$E = \frac{\Delta p t_0}{N^2 \eta}$$

Inverse size exclusion chromatographic porosimetry

Inversinė dydžio išskyrimo chromatografija (įvertinama porų dydis, tūris, kanalų tūris, polimerinio skeleto porėtumas)

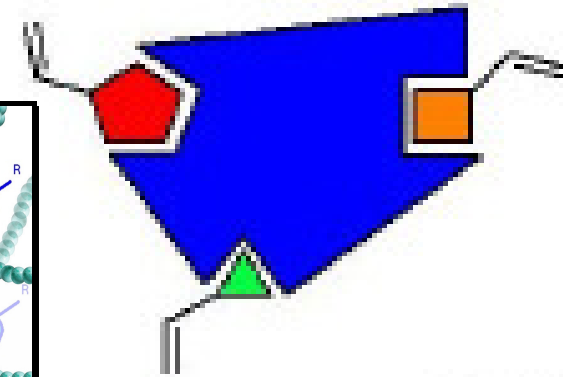
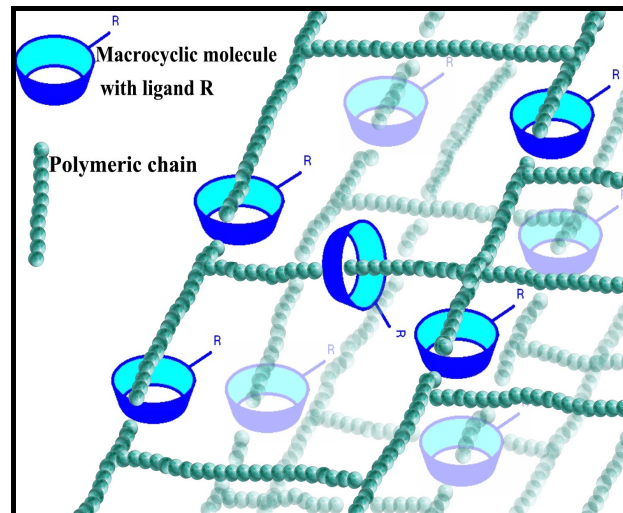
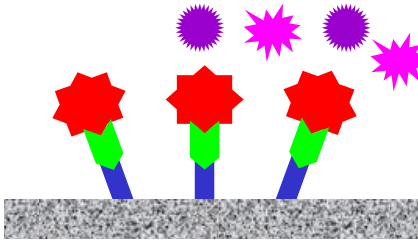


Kiti metodai:

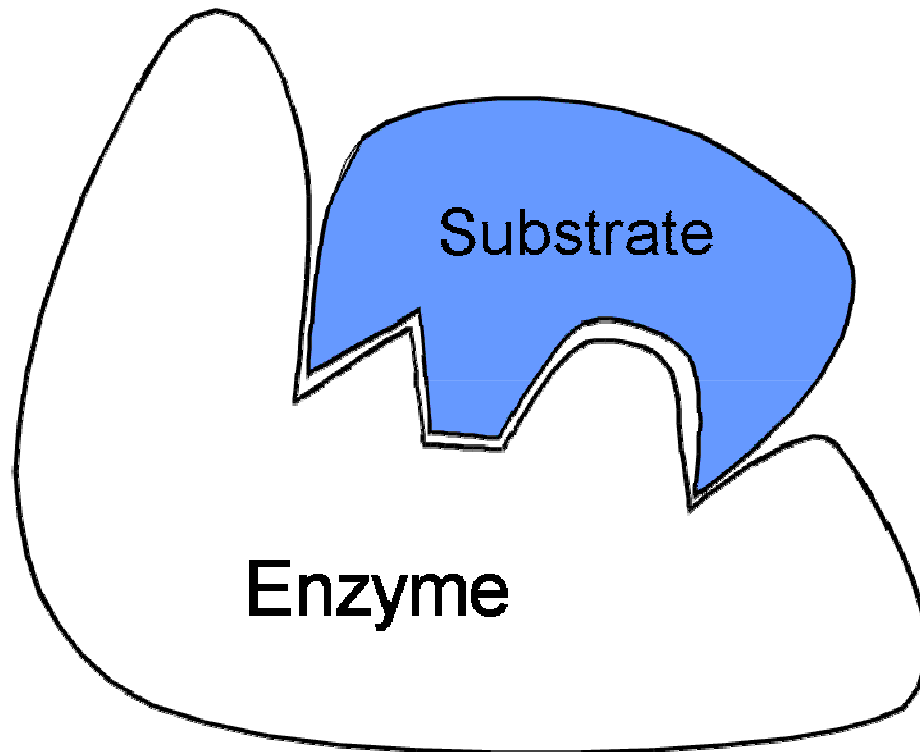
- **Mikroskopijos metodas** (įvertinama polimerinio sorbento paviršiaus struktūra)
- **Gyvsidabrio porometrijos metodas** (įvertinama sorbento poros ir kanalai, bei aklas ar pusiau susisiekiančias poras)

Different chromatographic modes

Reversed phase, normal phase, ion exchange, hydrophobic interaction, affinity, chiral separation, molecular recognition by imprinted polymers, ligand exchange, size exclusion, restricted access, immuno adsorption, other applications as sensors, bioreactors, 2D separations (particularly for 2ndD) in proteome, metabolome analysis etc.



Biological systems: molecular complexes



Fischer's Lock-and-Key concept in an enzyme-substrate complex

Non-covalent interactions caused by:

- hydrogen bonds
- ion pairing

Action of several weak non-covalent bonds leads to a very stable complex

Biotin-avidin binding:

- dissociation constant in femtomolar range
- binding energy ca. 90 kJ/mol at 25 °C

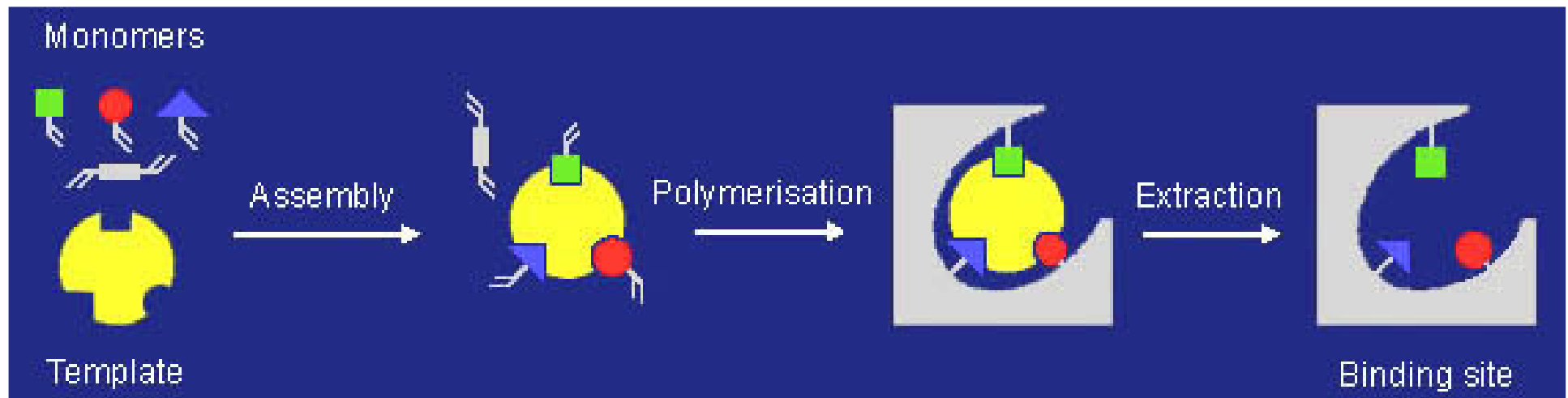
Development of Biomimetic Chemistry

Application of synthetic chemistry and modeling for:

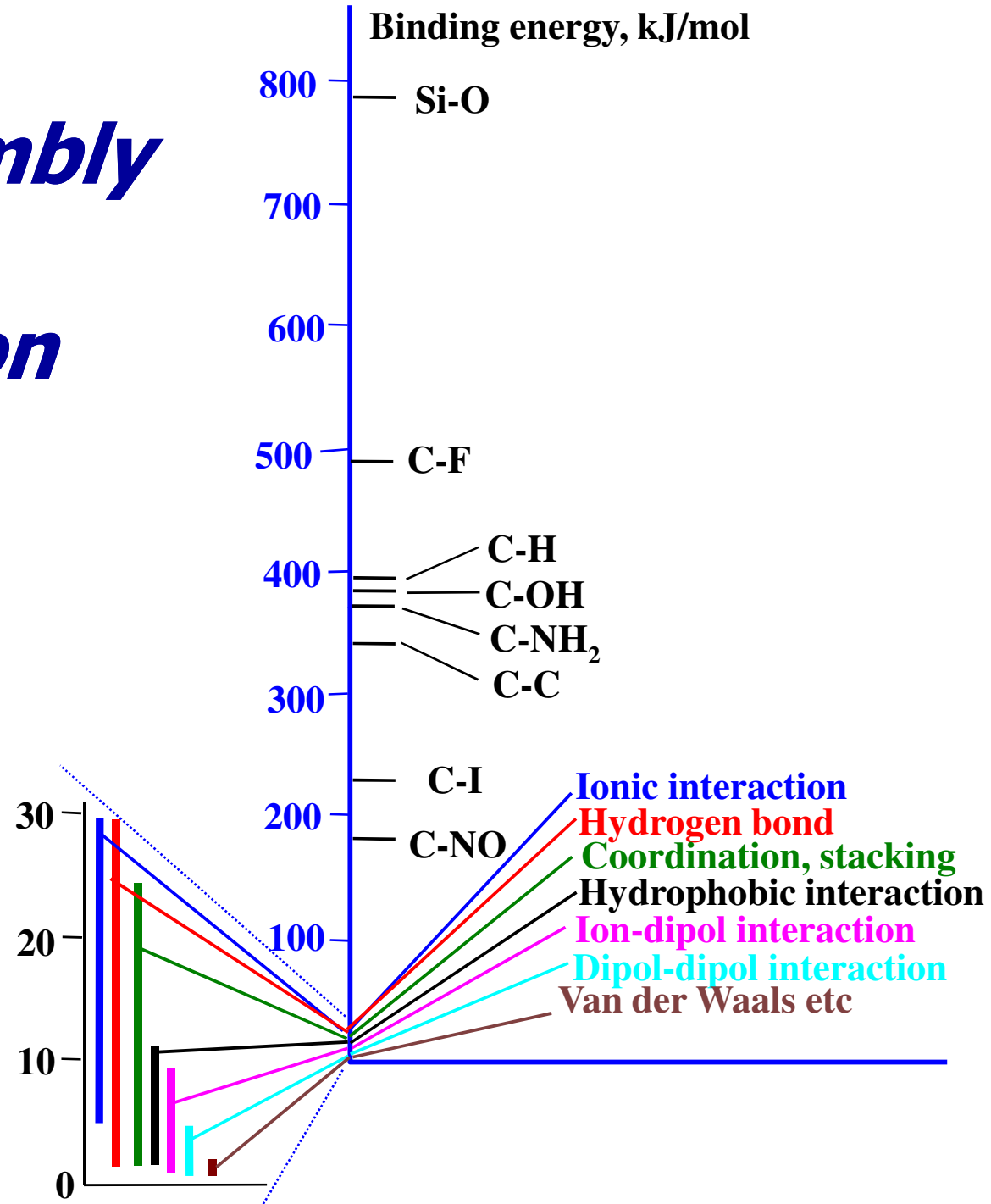
- molecular recognition
- ability to mimic natural binding phenomena

Nanoscale templating

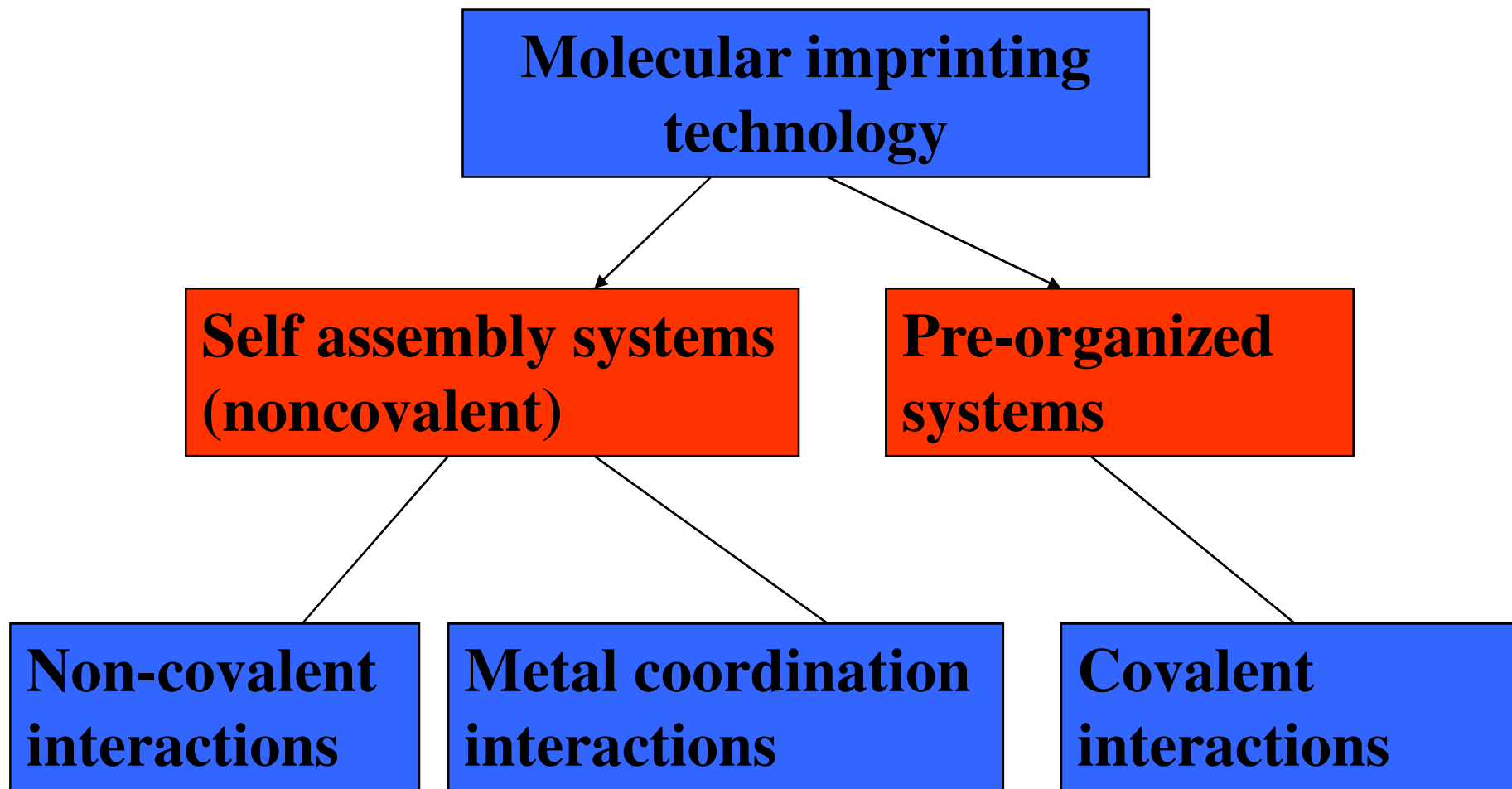
Making a molecular cast:



Self assembly and recognition forces



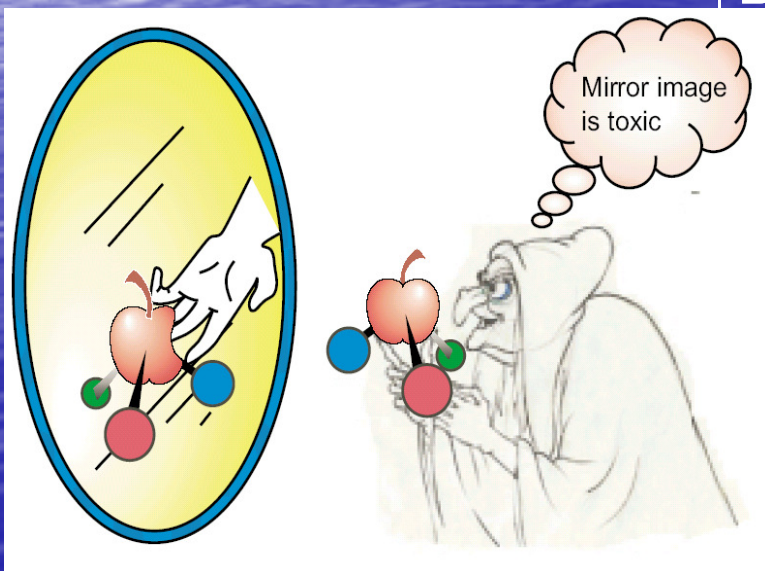
Principles of molecular imprinting



Chiral recognition is a prerequisite in biological systems

Chiral drug sales increased over 100 billions \$

Desired Effect	Side Effect
(R)-Thalidomide Hypnotic	(S)-Thalidomide Birth defect
(S)-Ketamine Analgesic/Hypnotic	(R)-Ketamine CNS Stimulation; hallucinogenic
Dextromethorphan Antitussive	Levomethorphan Narcotic
(R)-Terfenadine Antihistamine	(S)-Terfenadine Dizziness, Dry Mouth



FDA Guideline 1992

*Enantio*recognition studies

- Designing of enantioselective materials
- Investigation of the enantioseparation driving forces

AstraZeneca

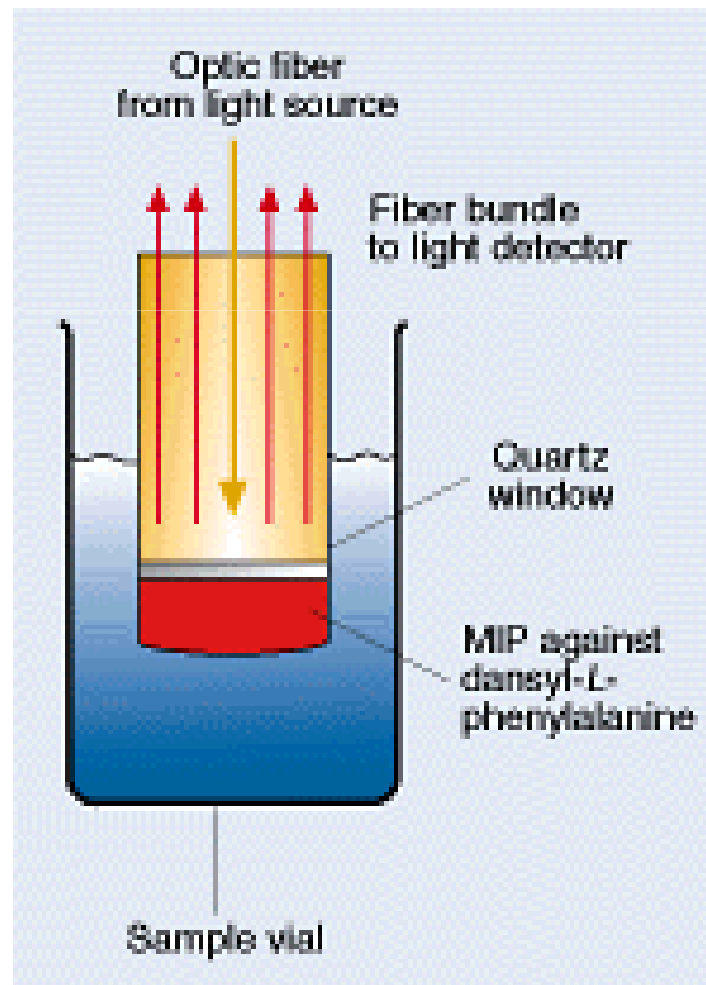


Enantio

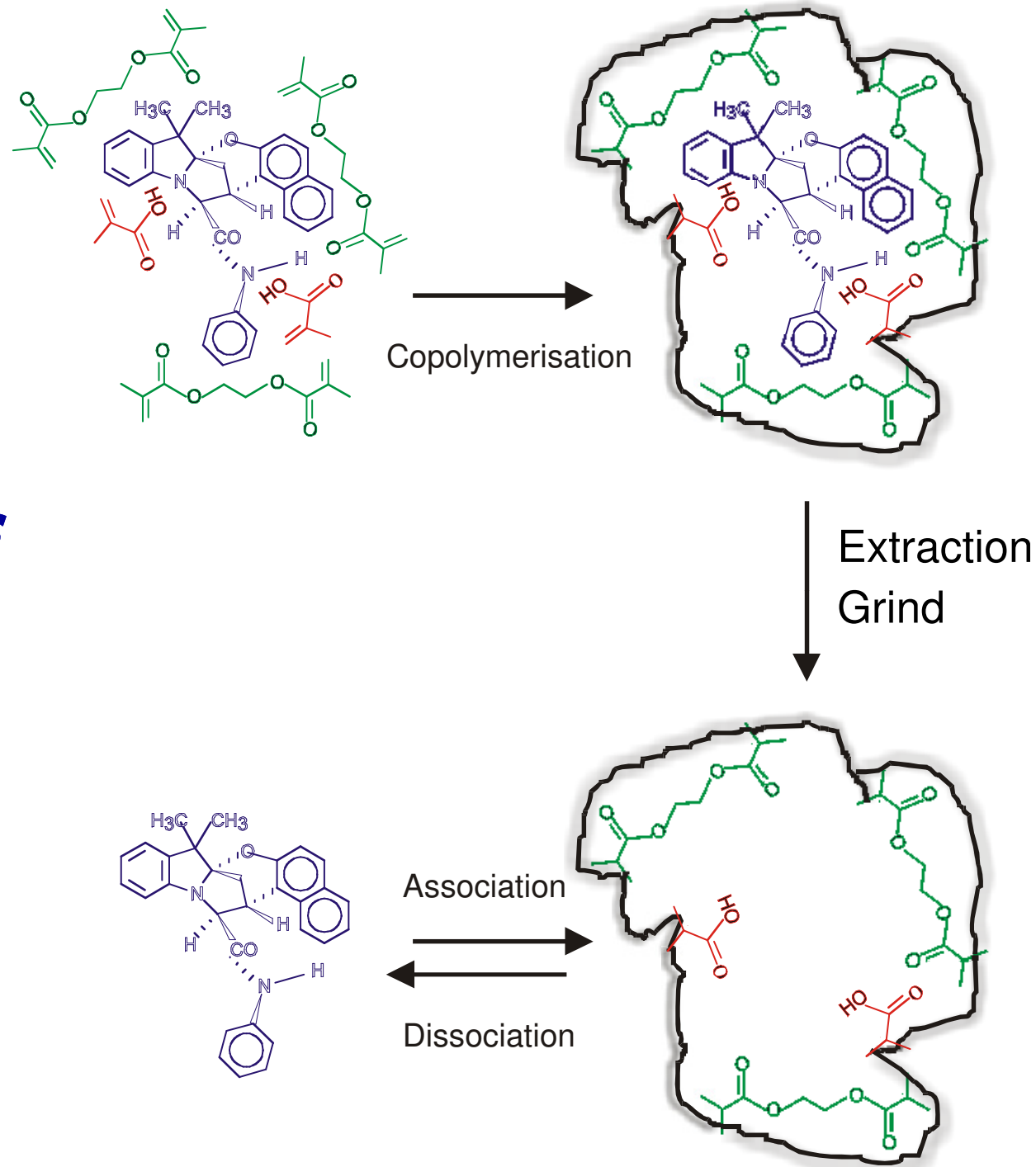
Advantages of molecularly imprinted polymers (MIPs) in comparison with natural receptors:

- The most generic and cost effective MIPs can be prepared for practically any compound
- Have similar affinity but often have better specificity
- Can work in organic solvents
- Stable at low/high pHs, pressure and temperature
- Compatible with microfabrication

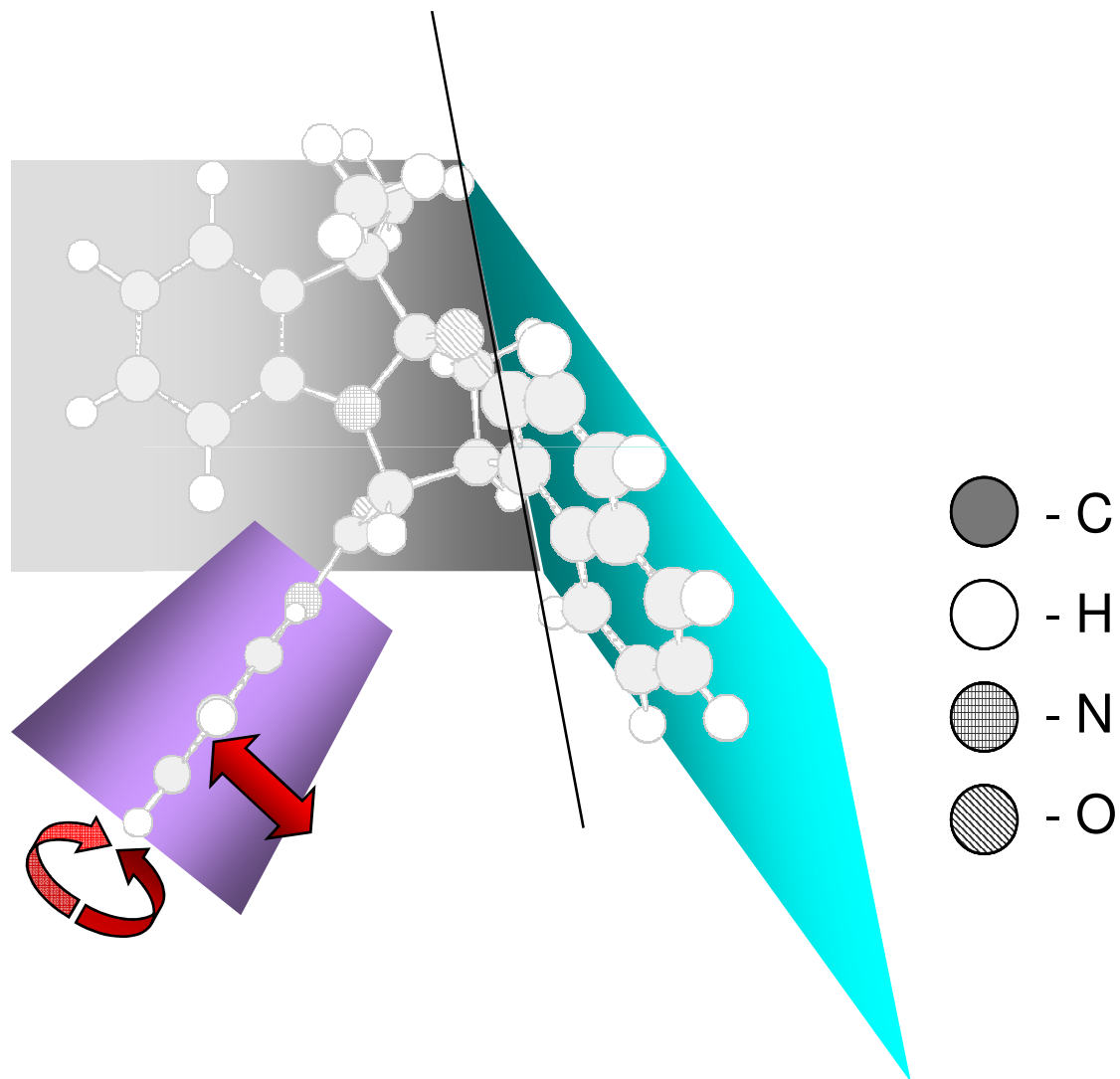
Biomimetic sensor in which an MIP selective for the fluorescently labeled amino acid dansyl-L-phenylalanine is applied as a layer on the tip of a fiber-optic sensing device



MIP synthesis



Imprinted chiral separation phase



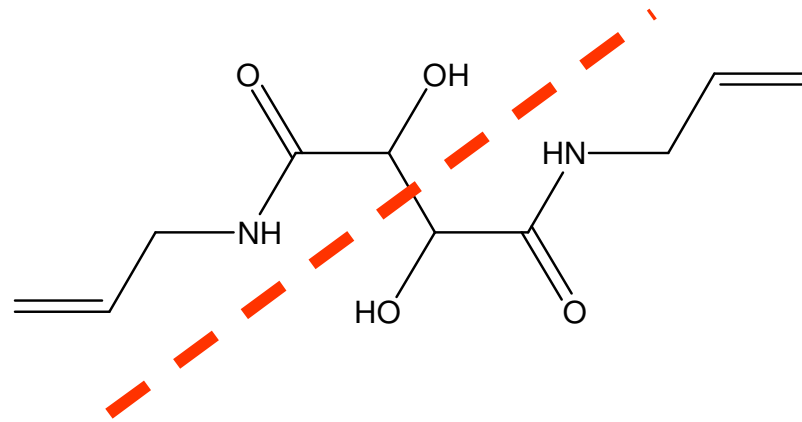
<i>Discrimination between different ligands</i>	$\Delta\Delta G$, kJ/mol
<i>trans</i> tert-butyl naphthyl → <i>trans</i> ethyl naphthyl	-0.263
<i>trans</i> ethyl naphthyl → <i>trans</i> phenyl naphthyl	-1.587
<i>trans</i> tert-butyl naphthyl → <i>trans</i> phenyl naphthyl	-1.850
<i>trans</i> phenyl naphthyl → <i>trans</i> 4-bromophenyl naphthyl	-0.299
<i>trans</i> phenyl R2 = H, R3 = Br → <i>trans</i> phenyl naphthyl	-1.305
<i>cis</i> ethyl naphthyl → <i>cis</i> methyl naphthyl	-0.150
<i>Enantiorecognition</i>	
<i>trans</i> (-/+) phenyl naphthyl	-0.855
<i>trans</i> (-/+) tert-butyl naphthyl	-0.717
<i>trans</i> (-/+) ethyl naphthyl	-0.711
<i>cis</i> (-/+) methyl naphthyl	-0.748
<i>Discrimination for geometric isomers</i>	
<i>cis</i> phenyl naphthyl → <i>trans</i> phenyl naphthyl	-0.256
<i>cis</i> ethyl naphthyl → <i>trans</i> ethyl naphthyl	-0.076

Active sites accessibility and homogeneity problem in MIPs





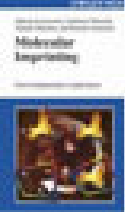

- Plausible solution is to use *controlled cleavage* of the monolithic polymer
- Synthesis of surface imprinted monoliths

Cleavage of N,N'-diallyltartardiamide (DATD) crosslinker

Cleavage of crosslinker with periodic acid gives a possibility to increase the porosity and accessibility of the polymeric skeleton



Books on Imprinting

<p>B. Sellergren (ed) Molecularly Imprinted Polymers Man-Made Mimics of Antibodies and their Application in Analytical Chemistry</p>		<p>R.M. Bartsch, M. Maeda (eds) Molecular and Ionic Recognition with Imprinted Polymers</p>	
<p>F. Diederich, P. J. Stang (eds) Templated Organic Synthesis</p>		<p>E. Reid, H. M Hill, I. D. Wilson (eds) Drug Development Asay Approaches: Including Molecular Imprinting and Biomarkers</p>	
<p>T. Takeuchi, H. Asanuma, M. Komiyama (eds) Molecular Imprinting – From Fundamentals to Applications</p>		<p>K. J. Shea, M. Yan, M.J. Roberts (eds) Molecularly Imprinted Materials – Sensors and Other Devices</p>	

Imprinting Research Groups



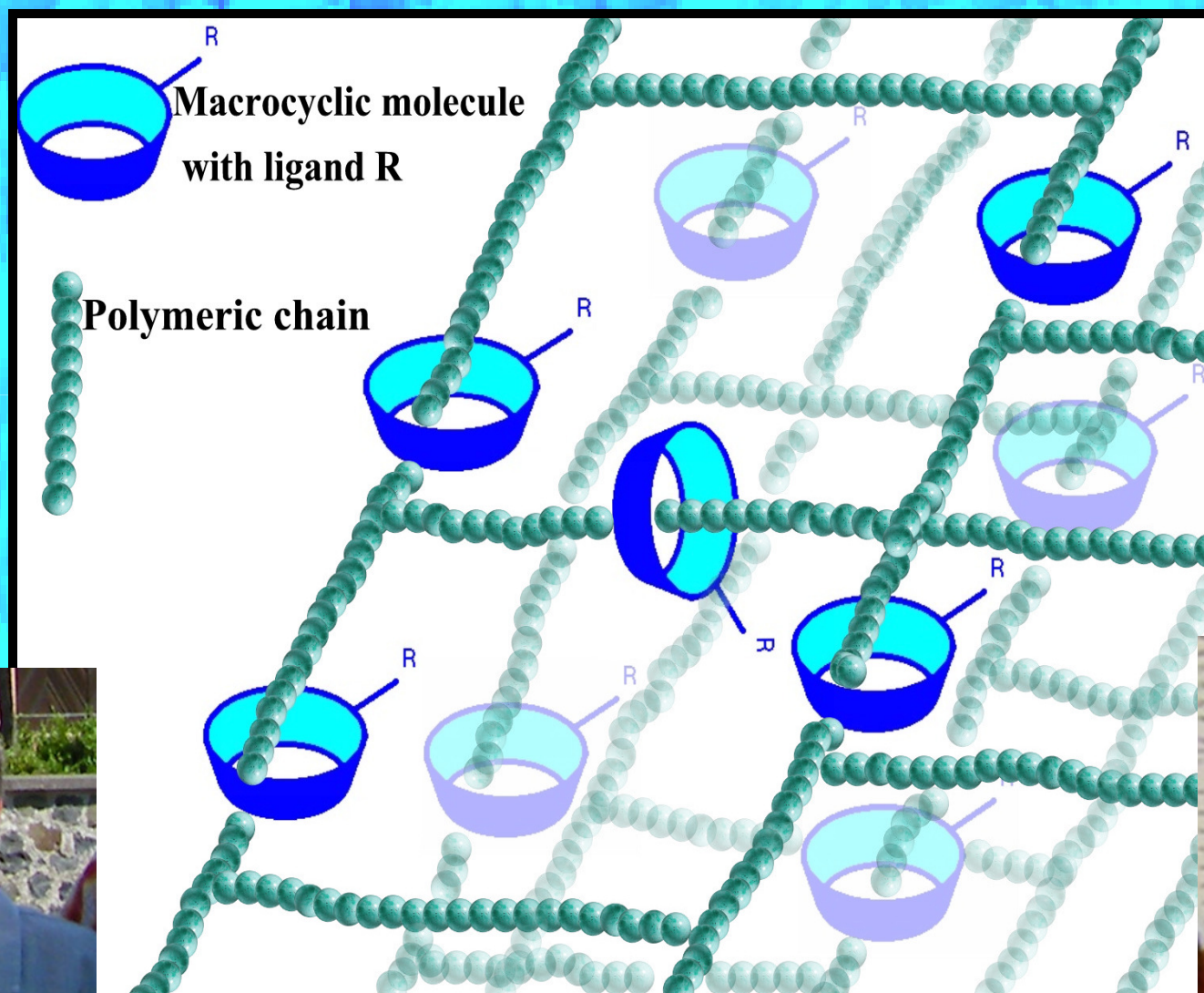
Ref. <http://www.smi.tu-berlin.de/groups.htm>



***CHEMISTRY WITHOUT
CHEMICAL REACTION?***

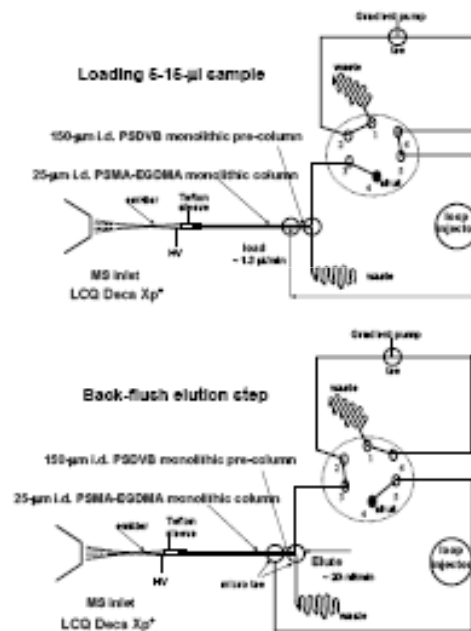
SELF ASSEMBLY of the mechanically interlocked structures

POLYROTAXANE STRUCTURE

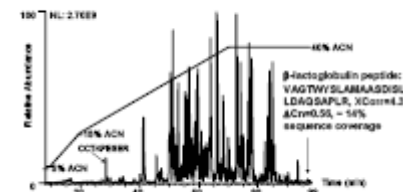


Proteome analysis

2. An Automated Tandem Monolithic Column Nano-HPLC/MS System



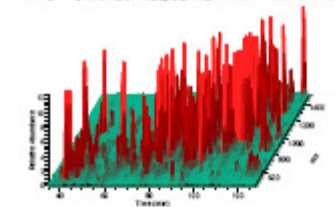
Nano-HPLC/MS Analysis of a Mixture with 10⁴ Concentration Dynamic Range



Tandem monolithic column nano-HPLC/MS analysis of 100-fmol BSA tryptic digest and 10-fmol β -actin tryptic digest

Nano-HPLC/MS Analysis of a SDS-PAGE Protein Section

UNSL_0000_00000000_00000000_00000000_0000_0000_0000



Total protein (10,000 cells, LCM) amount = 2-µg applied to SDS-PAGE. In-gel tryptic digestion of protein section in 35-55 kD range.

Protein and Peptide Identification

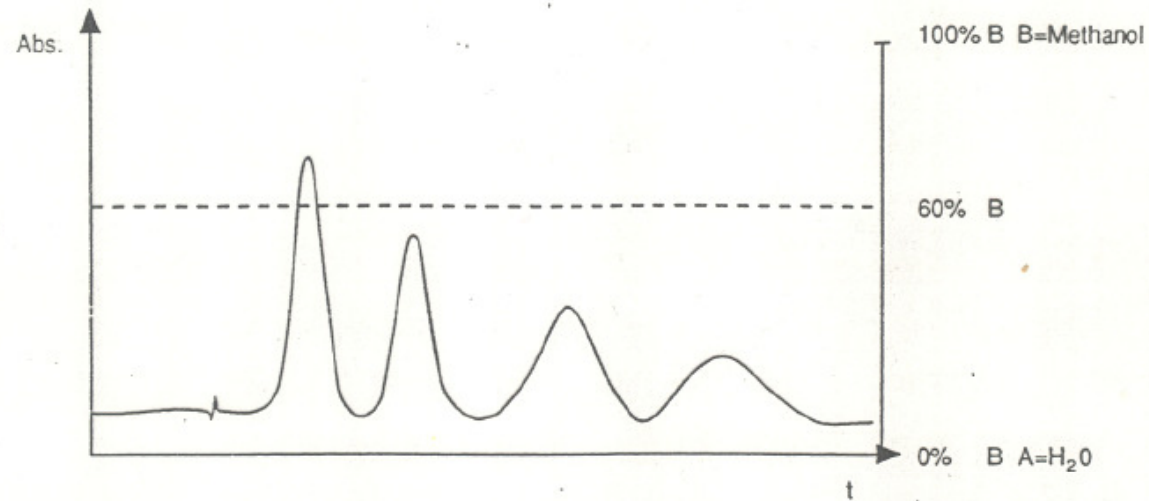
SEQUEST search: Filter: Xcorr \geq 2.0, 2.5, 3.76 for +1, +2, +3 ions, respectively; $\Delta Cn \geq$ 0.1; no (M+H)⁺ peptides, 76 proteins identified.

Ultra-Narrow Bore Monolithic Column Technology for Proteome Research

Jian Zhang¹; Lawrence L. Licklider¹; Christina I. Orazine¹; Carla Waggett²; Gary Valaskovic²; Barry L. Karger¹

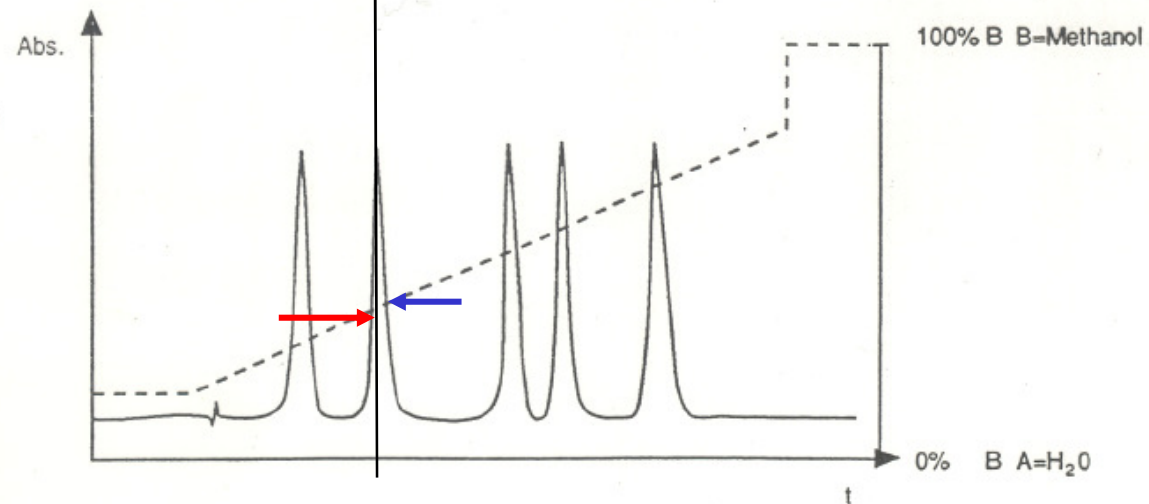
Mobile phase gradient and isocratic elution

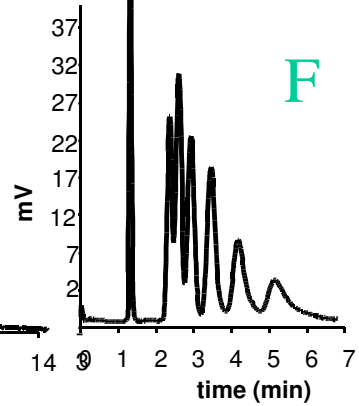
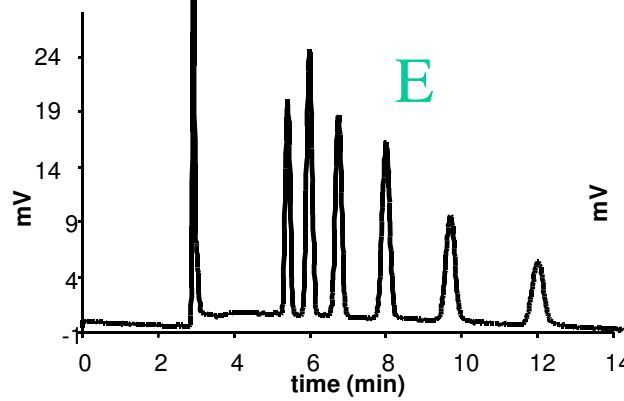
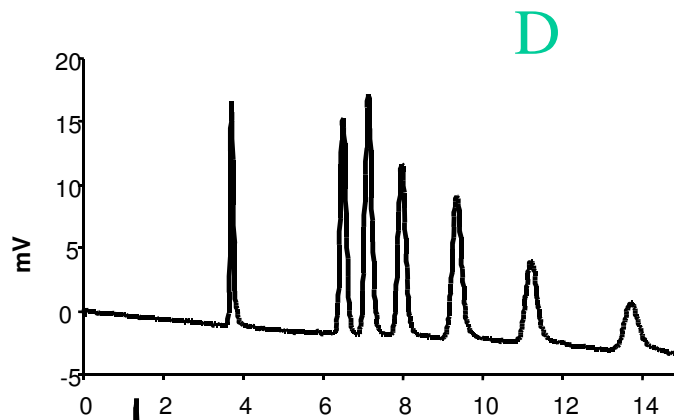
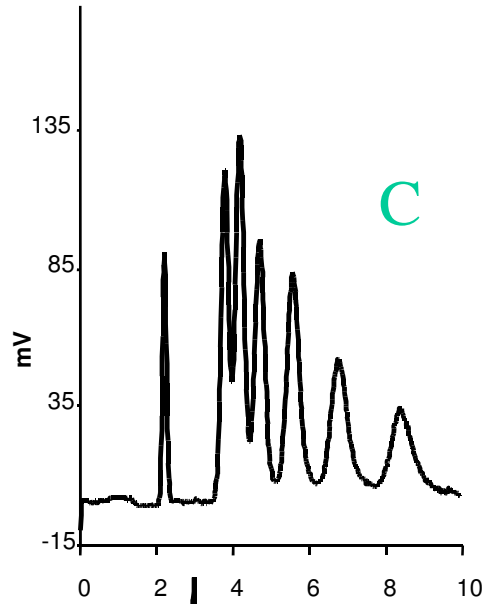
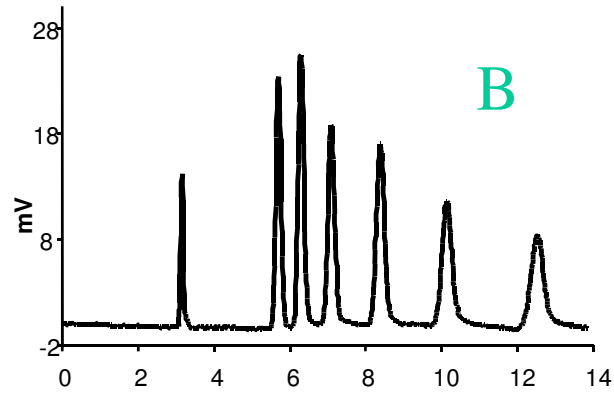
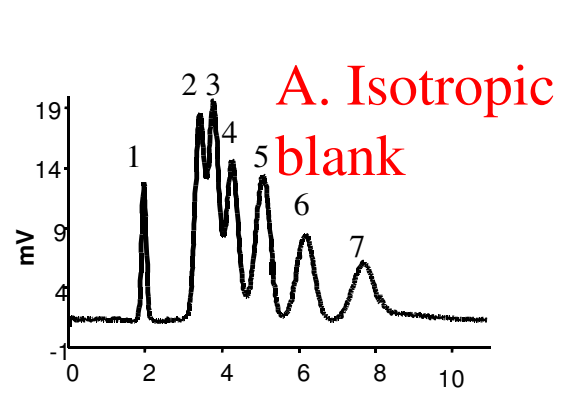
● Isocratic Elution



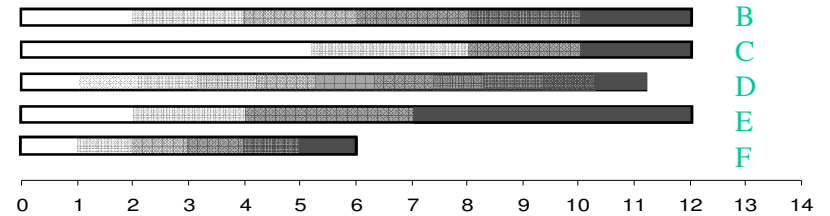
● Gradient Elution

Focusing effect

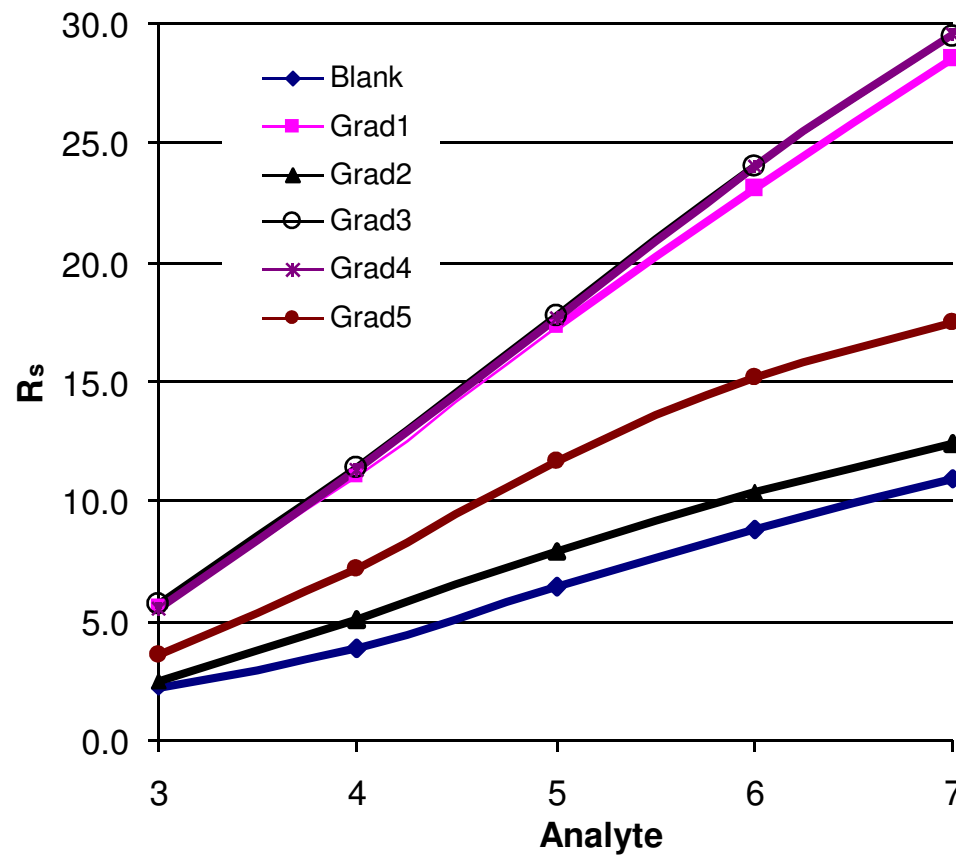




Hydrophobic monomer concentration



Comparison of resolution for gradient and isotropic capillary columns

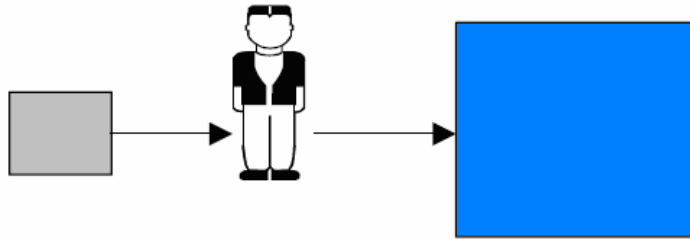


[Maruška A., Rocco A., Kornyšova O., Fanali S. *J. Biochem. Biophys. Methods* 70 (1) (2007) 47-55]

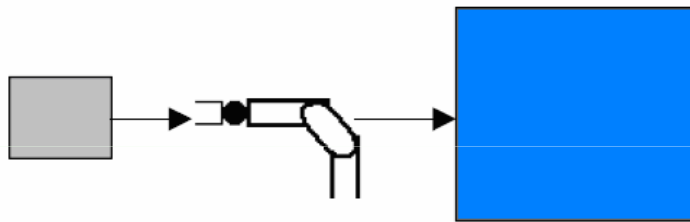
Coupled methods

KFME

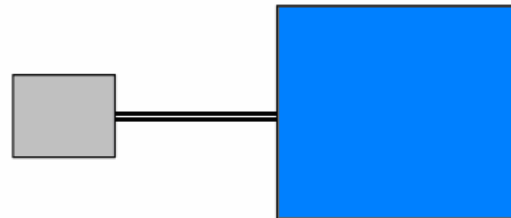
KE



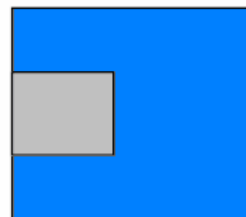
Netiesioginis
(rankinis)



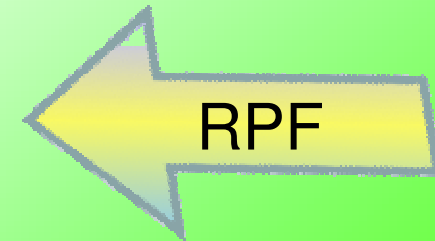
Netiesioginis
(automatizuotas)



Nuoseklusis



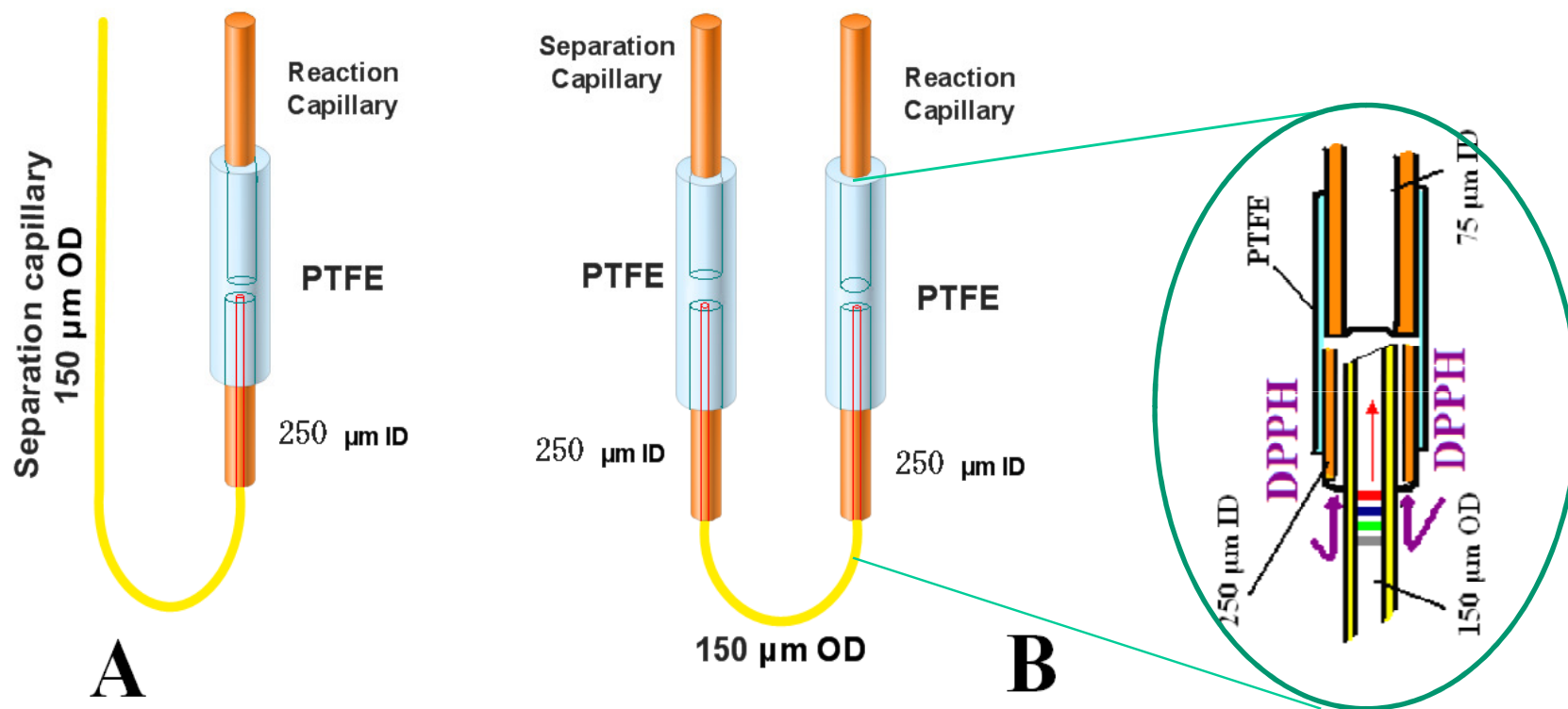
Tiesioginis



On-line Coupling of CE to Radical Scavenging Reaction Detection

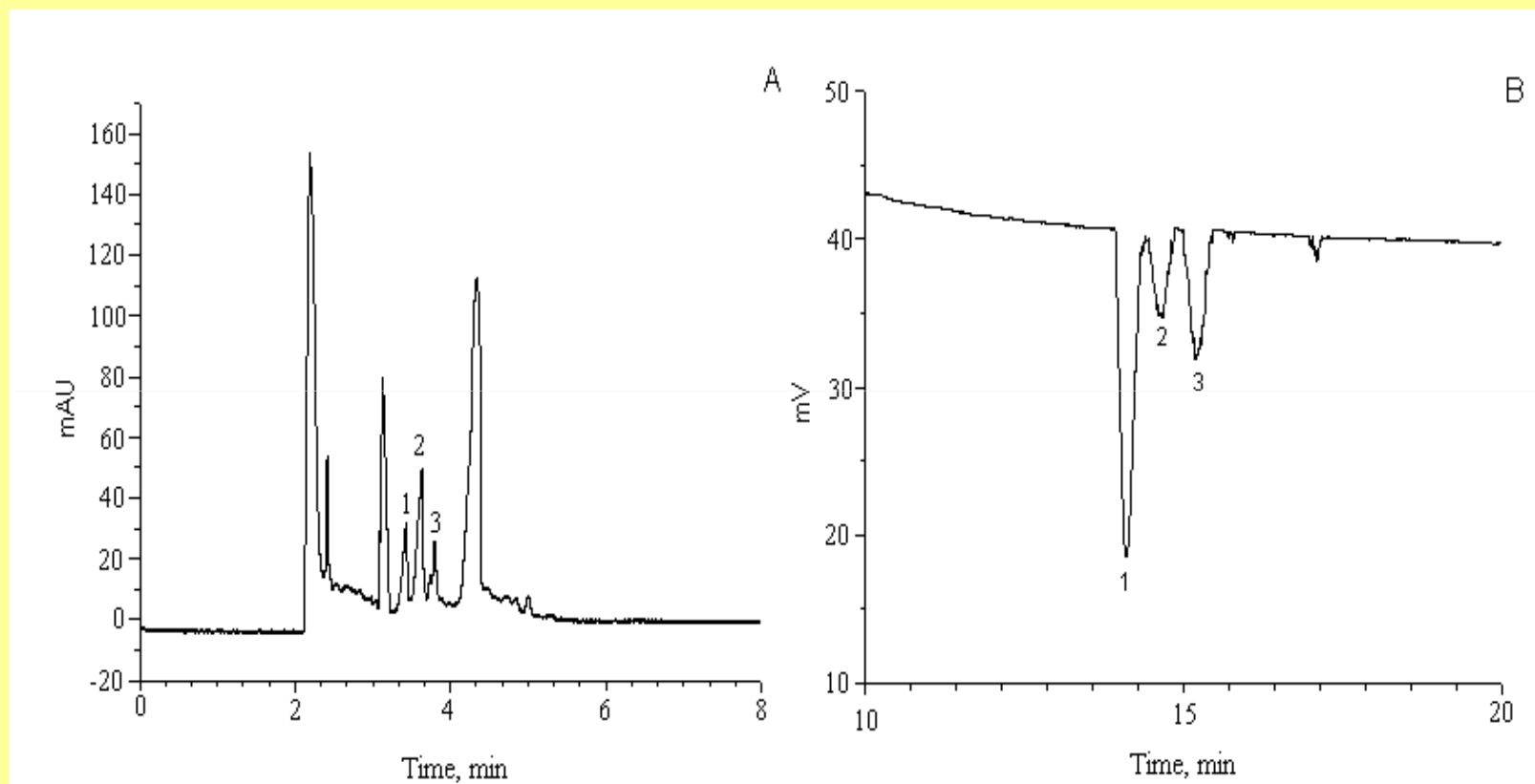


Connection of Separation and Reaction Capillaries



- A** – thin 150 μm OD separation capillary bind into 250 μm ID capillary 1 cm length sleeve.
- B** – Standard 50 μm ID separation capillary and 75 μm ID reaction capillary connected with thin 150 μm OD and 50 μm ID capillary link.

ON-LINE CE-RADICAL SVACENGING DETECTION



Electropherogram of the separated compounds of honey bee propolis aqueous extract obtained at UV wavelength 200 nm. (B) The DPPH• reaction detection signal profile at 517nm. Detector coupled on-line to the CE system. DPPH Reagent concentration of 50 μ g/ml. Peaks 1- ferulic acid; 2 - trans-p-coumaric acid; 3 - caffeic acid.

Sample clean-up a nightmare?

- Additional labour,
matter and
instrumental
needs

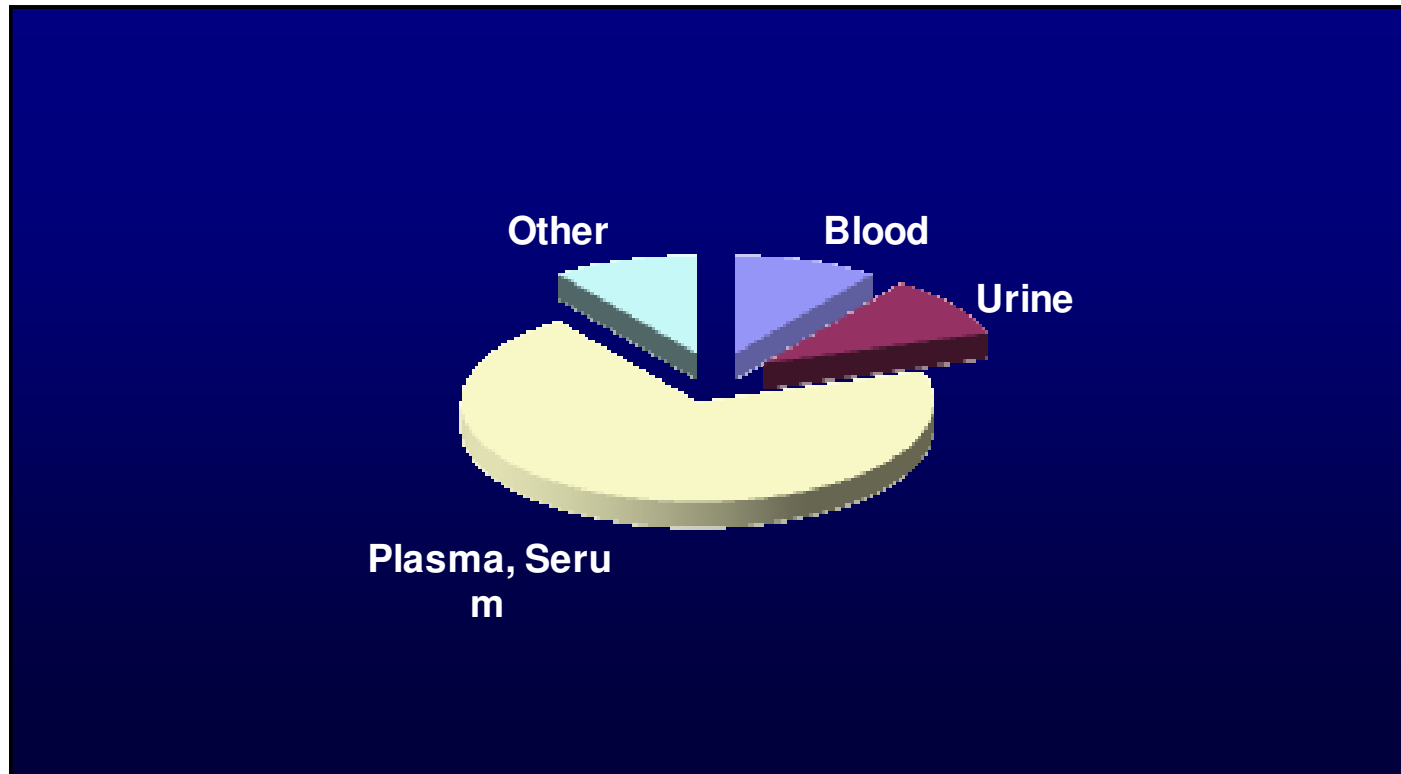
- Plus 60% time
consumption

- Plus 30% error





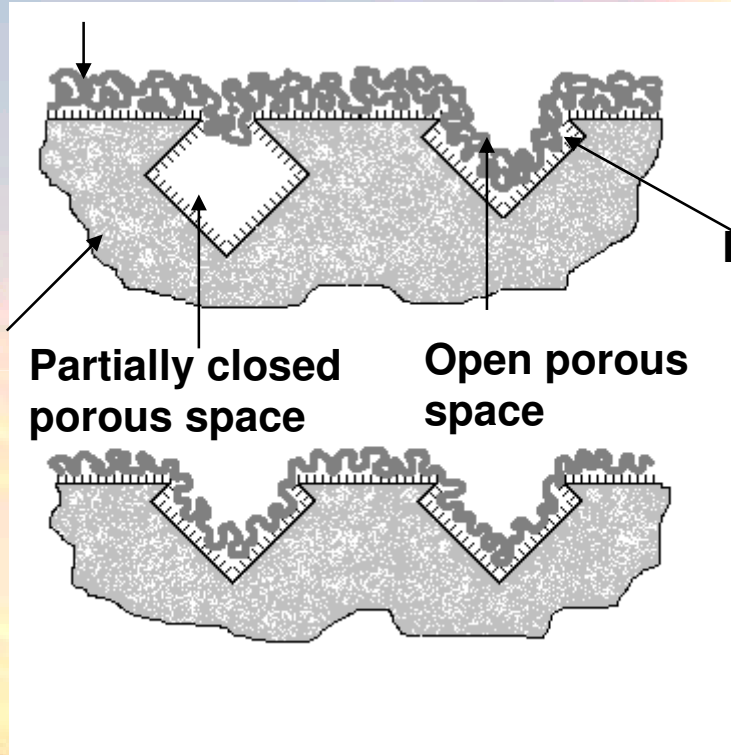
Proteinaceous Biological Samples



TOPOCHEMICAL ARCHITECTURE OF RAMs

Hydrophilic polymer shield

Polymer matrix



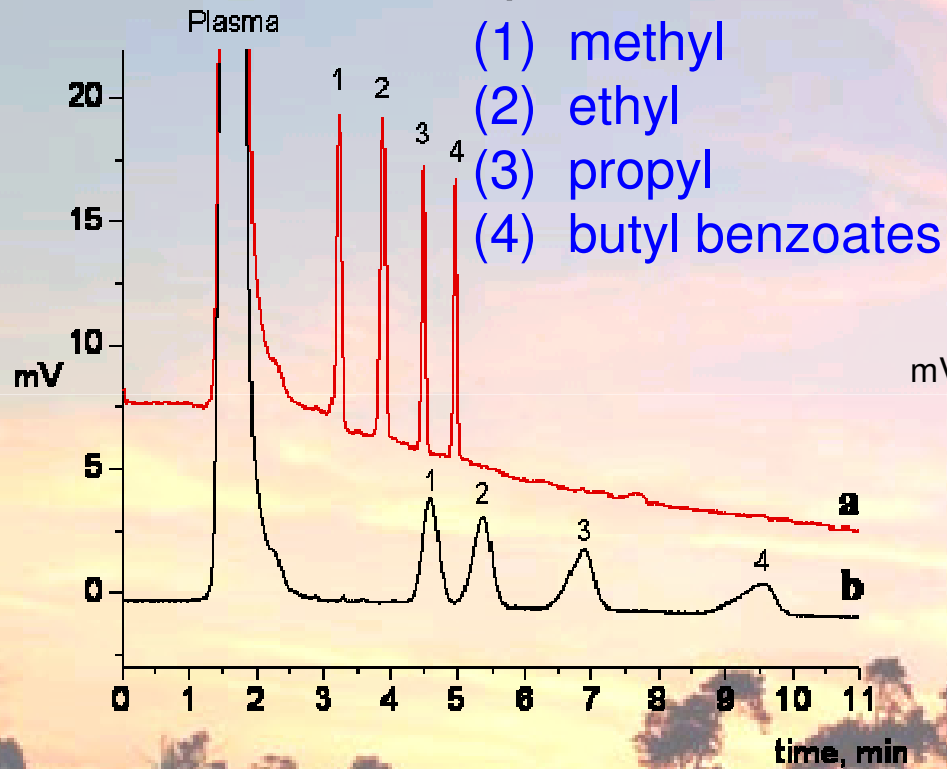
Hydrophobic active sites

Partially closed morphology

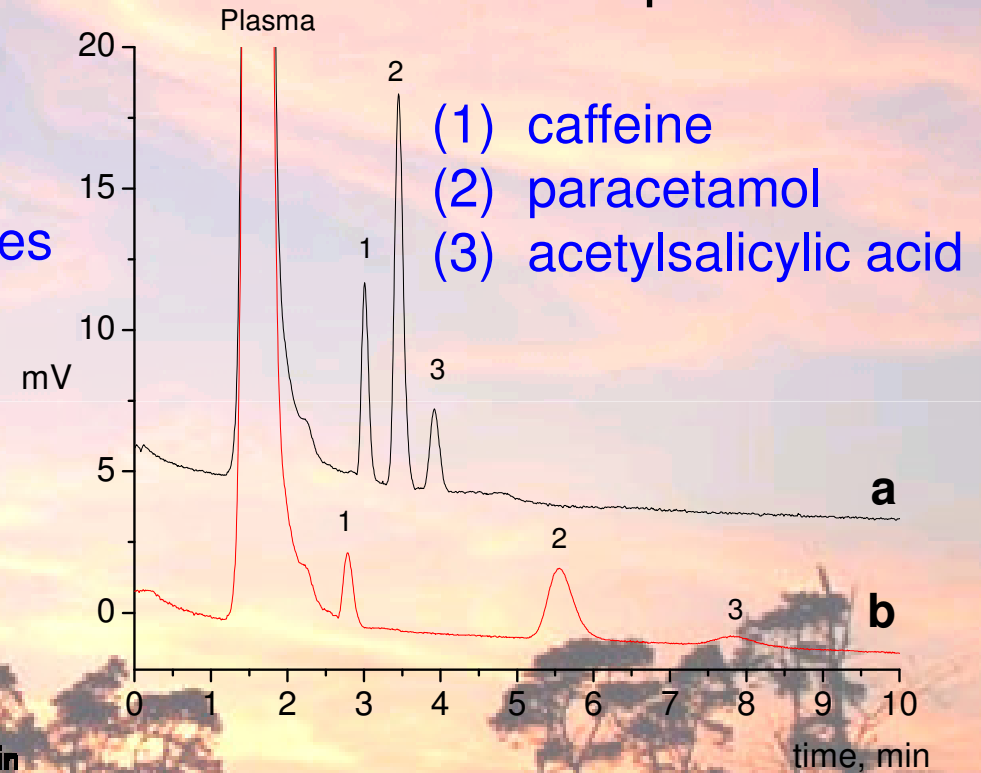
Open morphology

DIRECT INJECTION OF BIOFLUIDS

Benzoates in plasma



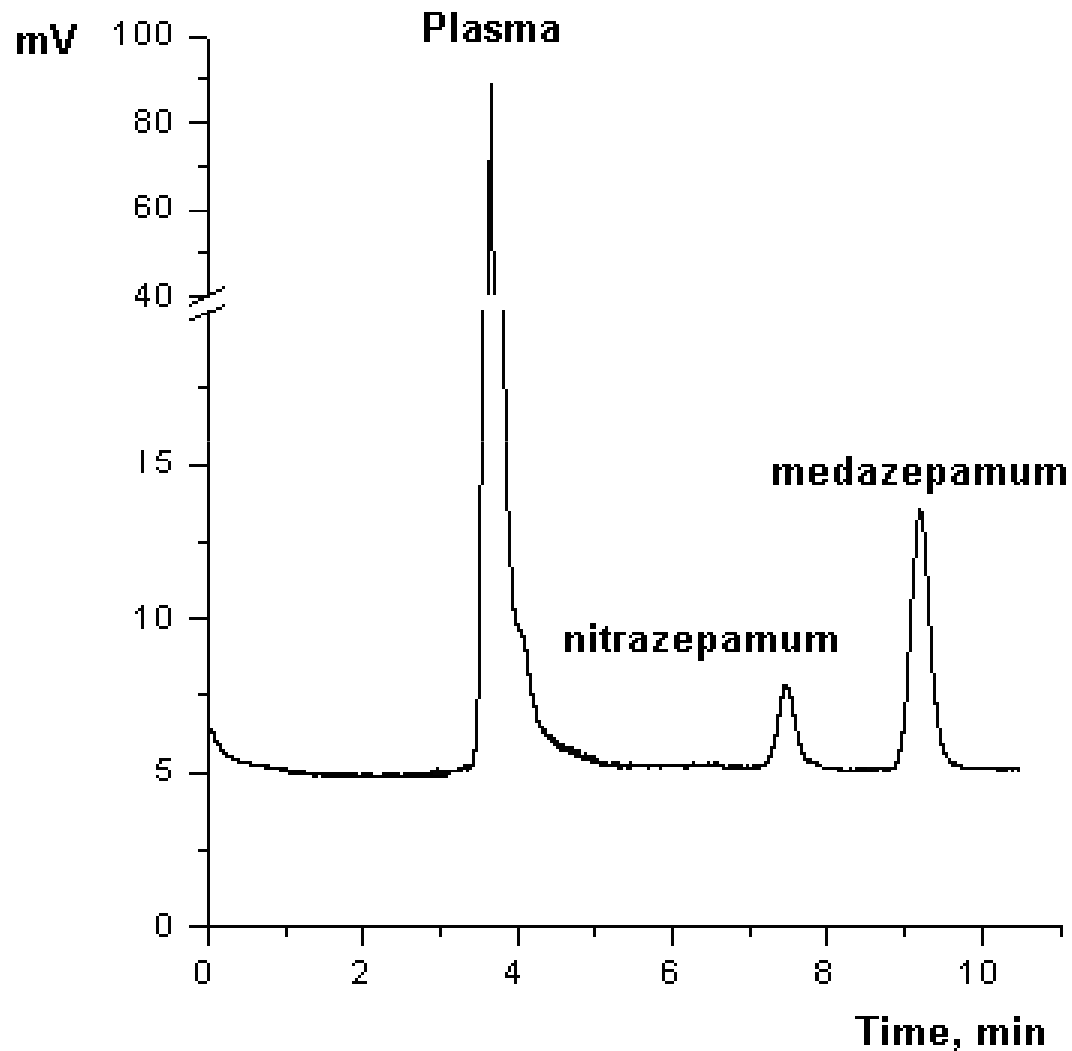
Citramonum P in plasma





DIRECT INJECTION OF BIOFLUIDS

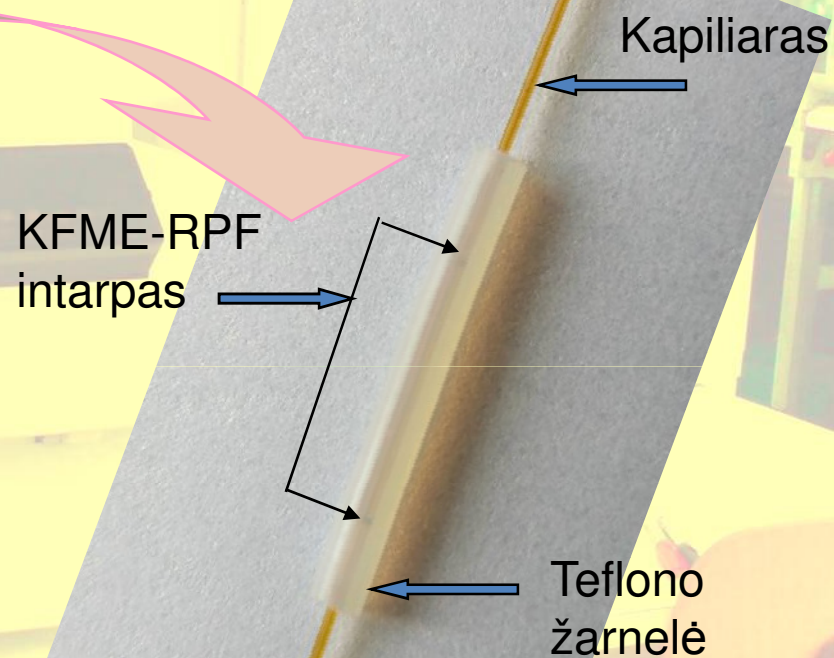
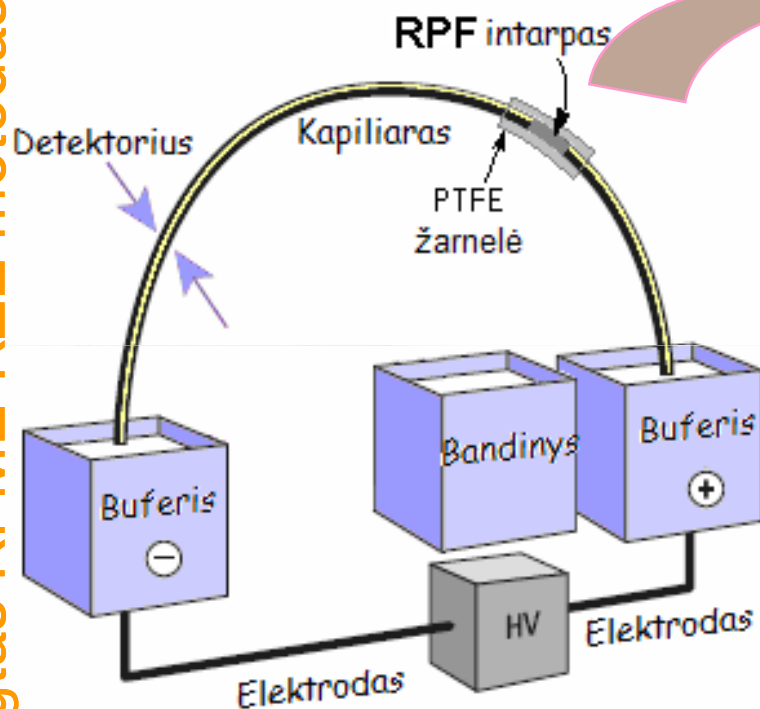
Benzodiazepines in plasma



LOD: nitrazepamum (2.2 $\mu\text{g}/\text{ml}$) and medazepamum (1.8 $\mu\text{g}/\text{ml}$)

COUPLED METHODS: SPME-CZE

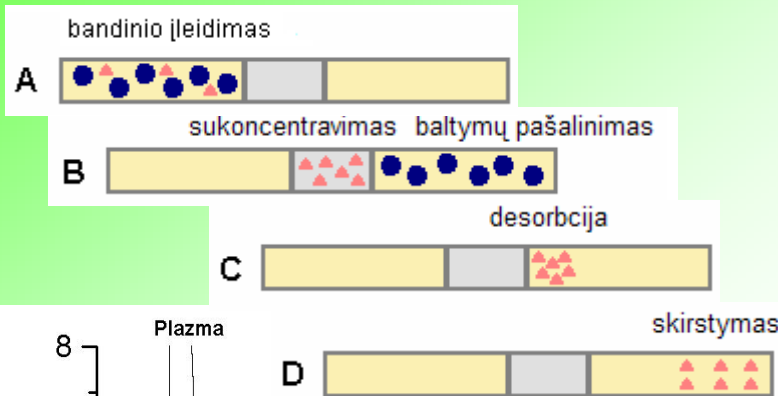
Apjungtas KFME-KZE metodas



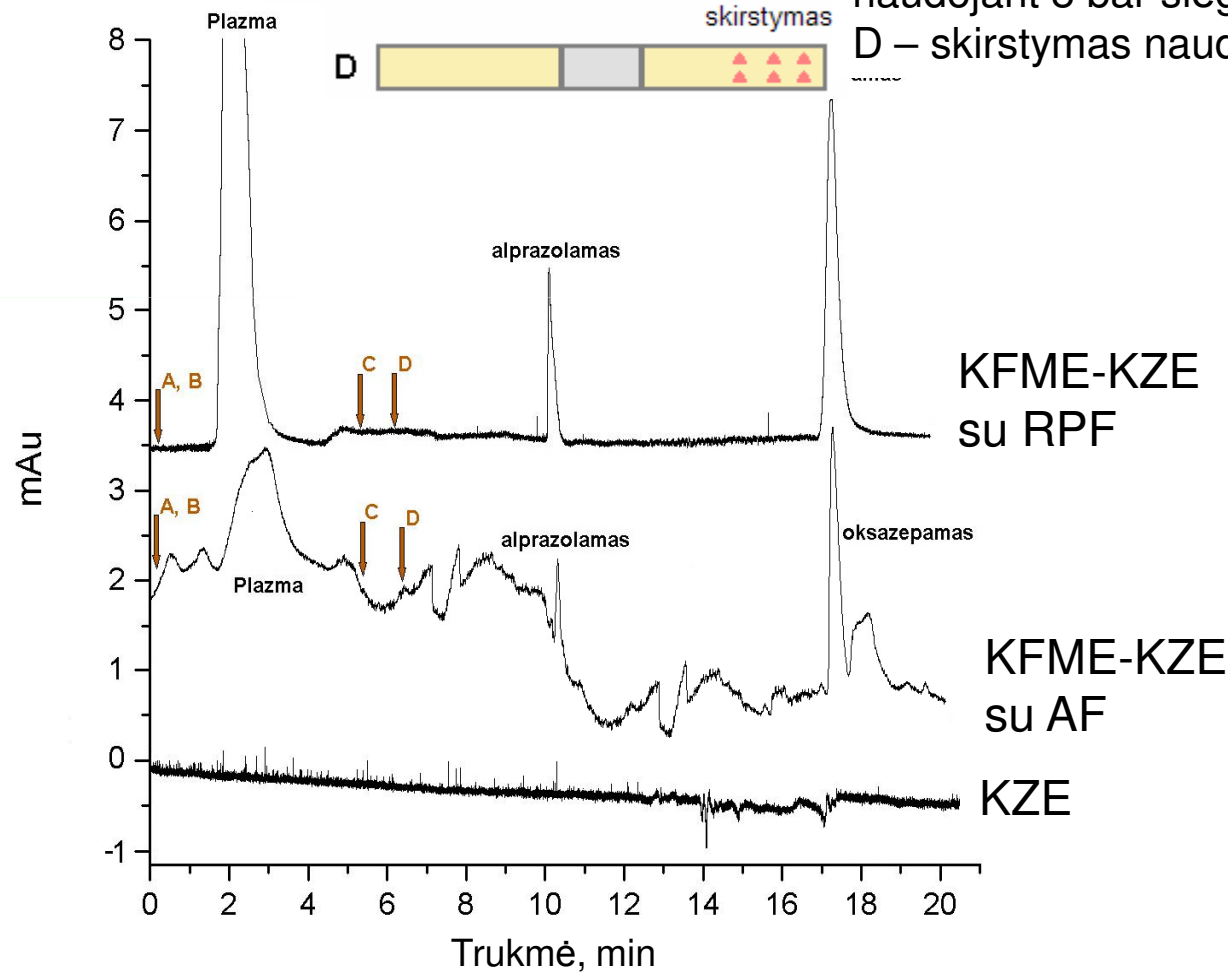
Apjungta KFME ir KE atlikta naudojant Hewlett-Packard HP^{3D} kapiliarinės elektroforezės sistemą su diodų matricos detektoriumi (Agilent Technologies, Vokietija)

COUPLED METHODS: SPME-KZE

Apjungtas KFME-KZE metodas

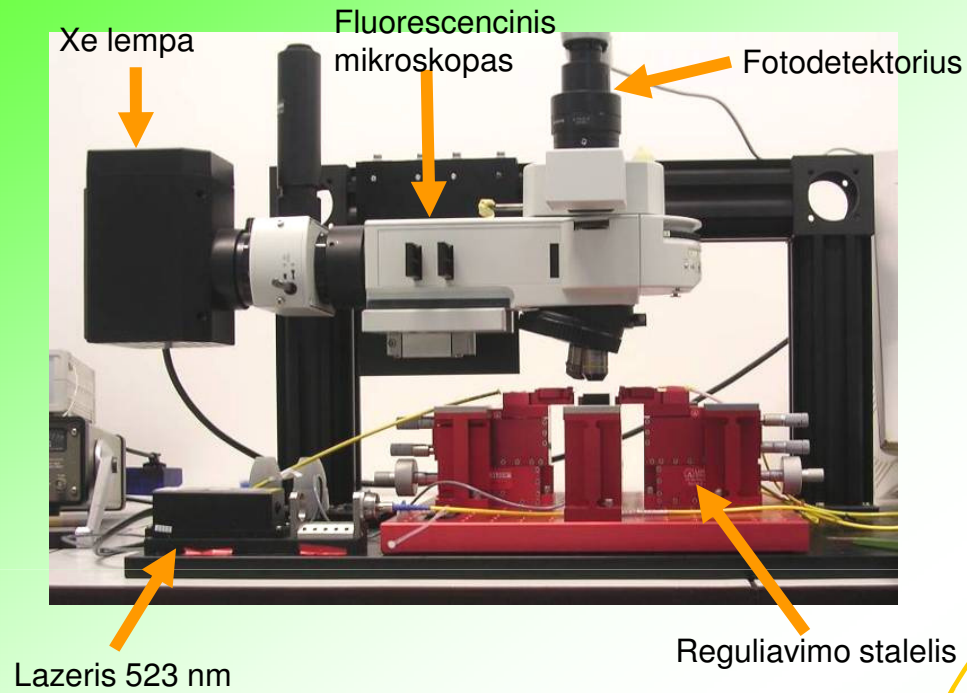


A, B – bandinio įleidimas ir sukonzentravimas naudojant 8 bar slėgį 1,5 min,
C - analičių desorbpcija 80 tūrio % metanoliu naudojant 8 bar slėgį 1 min,
D – skirstymas naudojant 30 kv įtampą.

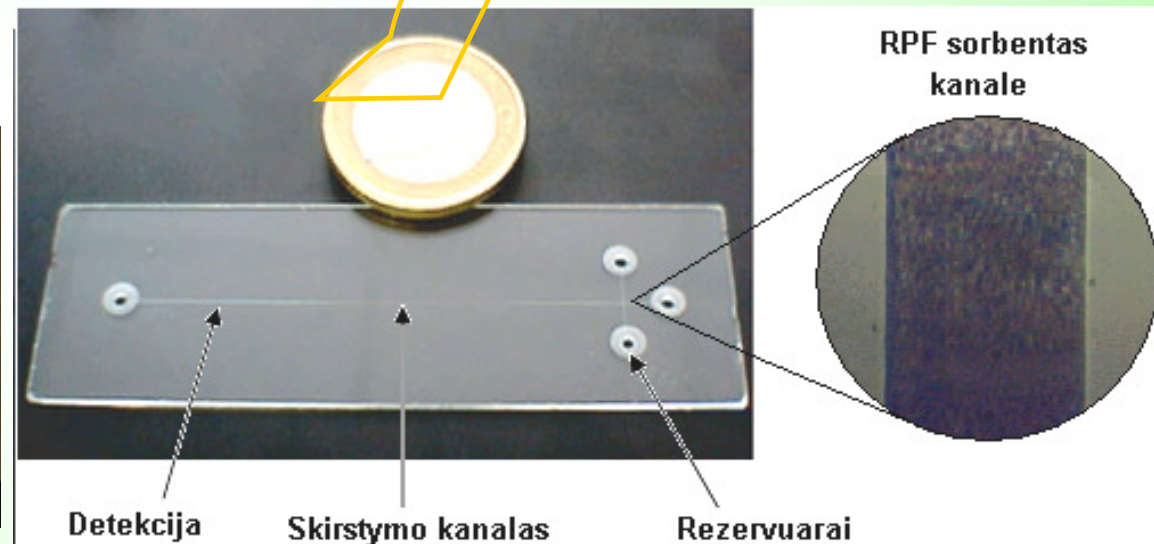
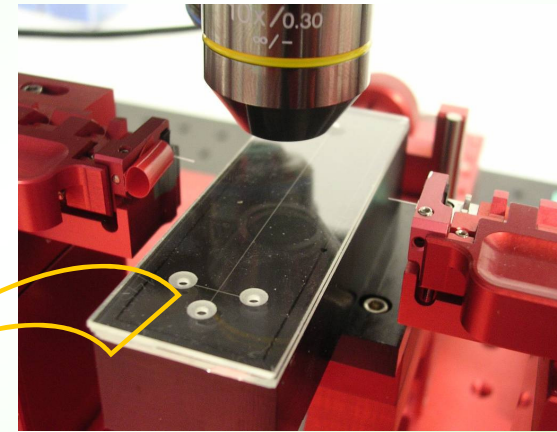


Bandinys: jaučio plazma sumaišyta su alprozolamu (35,6 ng/ml), oksazepamu (84,4 ng/ml).
Buferis: 5 mM fosfatinis su 0,5% ACN, pH 2,3.
Bandinio tūris: 12,7 µl.

Laboratorija mikroluste



Mikrolaboratorija (dydis 7×2 cm)

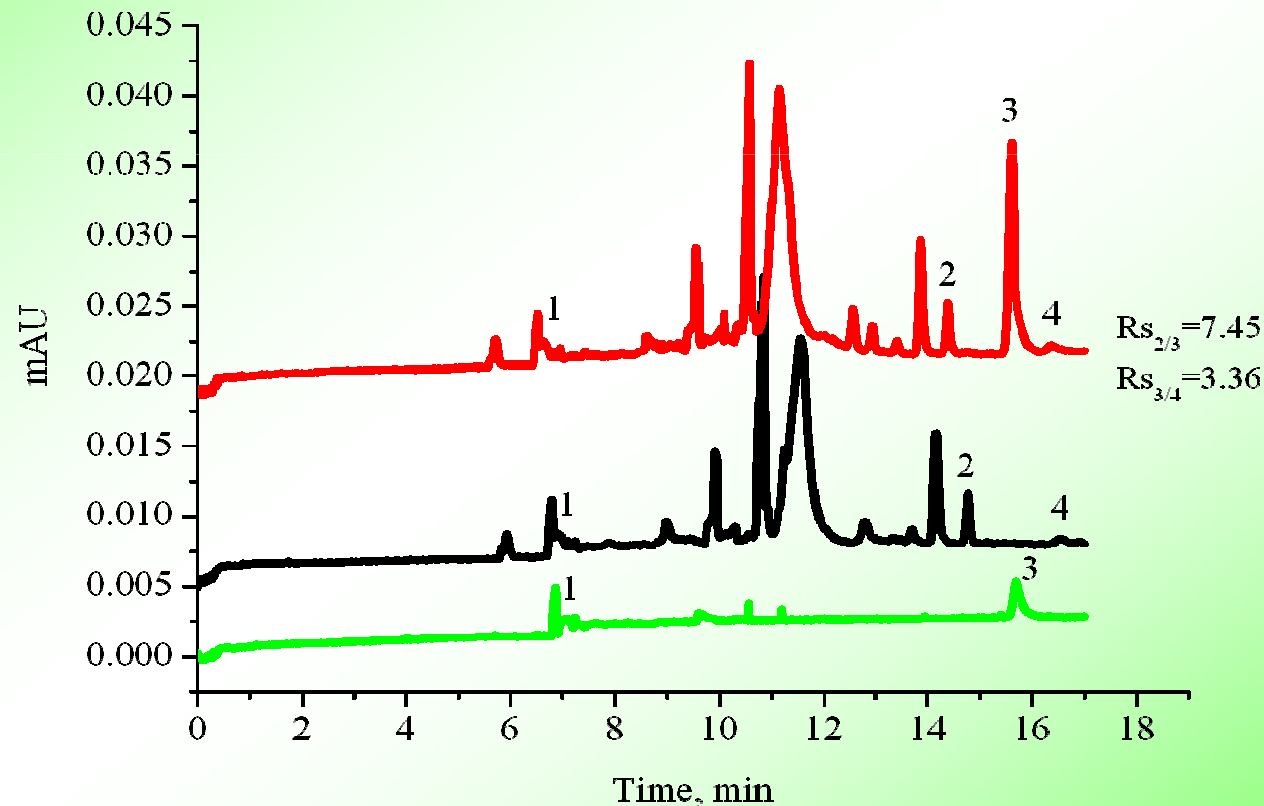


5-MINOLEVULINIC ACID DETERMINATION IN HUMAN DERMIS BY MEANS OF CAPILLARY ELECTROPHORESIS

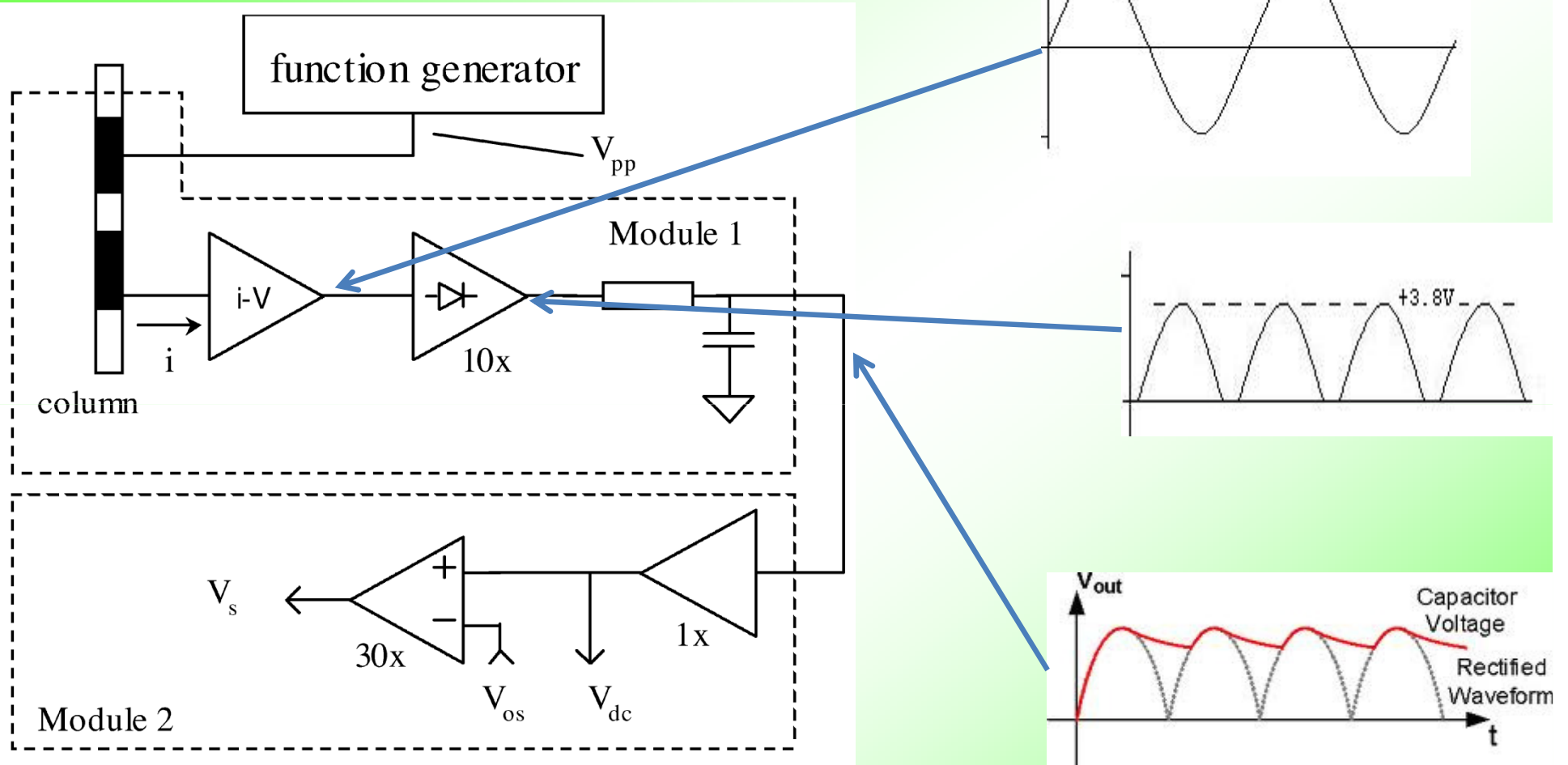
PhD student Tomas Drevinskas

- 1- Electroosmotic flow
- 2- Unknown compound from dermis
- 3- ALA peak
- 4- Traces of unknown compound

— Blank dermis extract
— Blank dermis extract spiked with ALA
— ALA solution



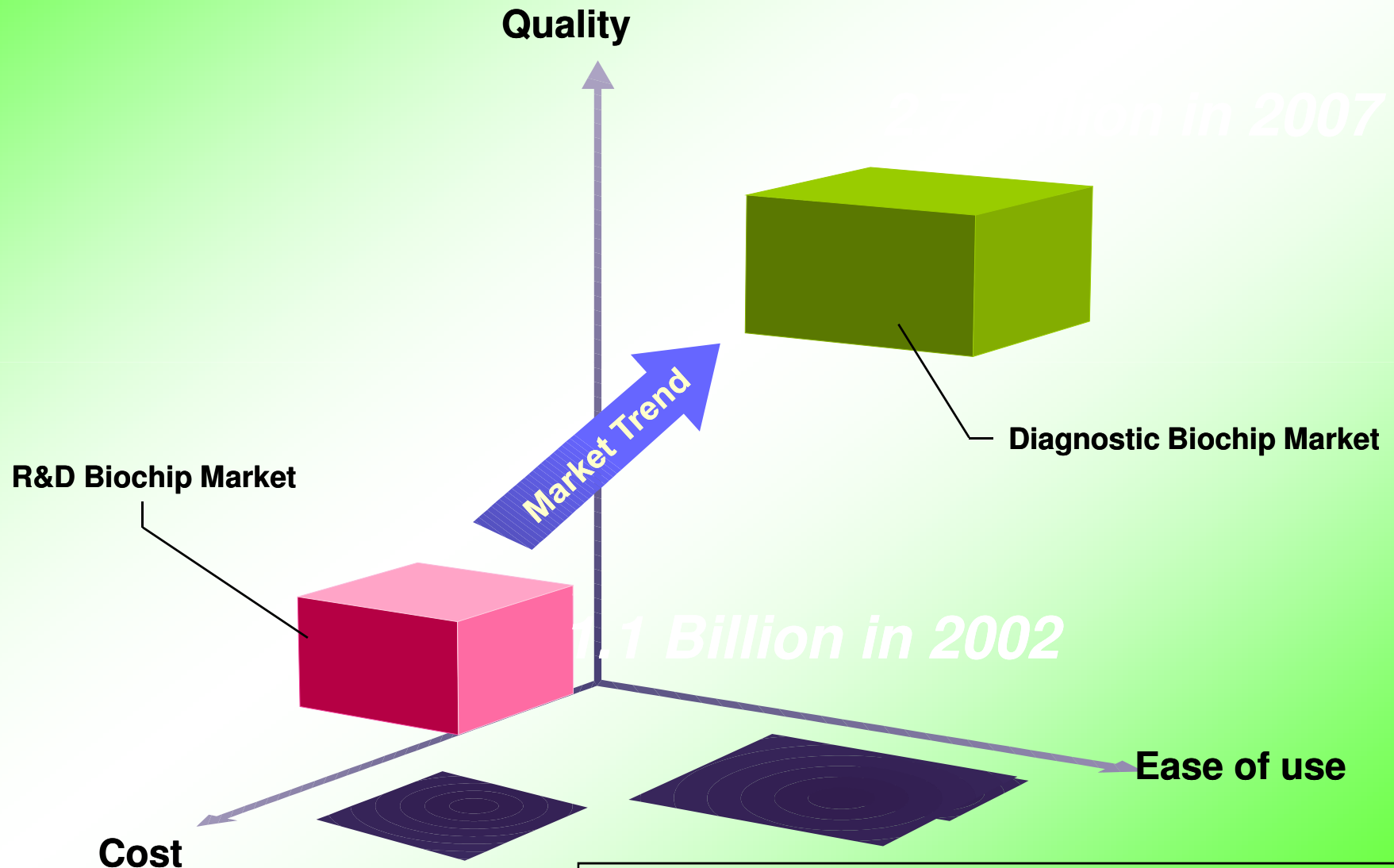
C4D detection mechanism



*Jose et al., Contactless conductivity detection for capillary electrophoresis hardware improvements and optimization of the input-signal amplitude and frequency, J. Chromatography A, 2001



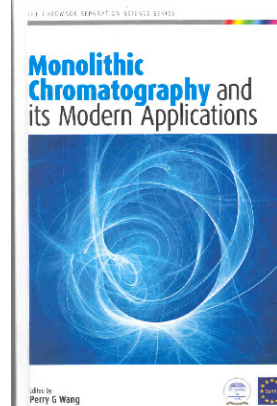
Biochip Market Overview



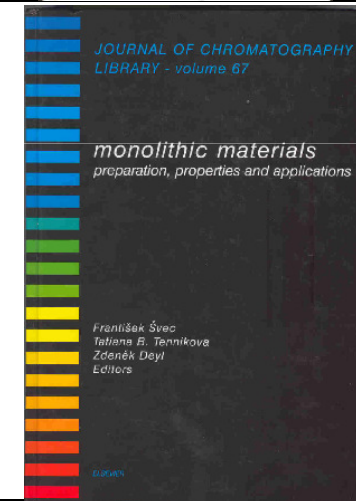
http://www.fuji-keizai.com/e/report/us_biochip_e.html

Books on Monoliths

Perry G Wang (ed)
Monolithic Chromatography and its Modern Applications
ILMPublications



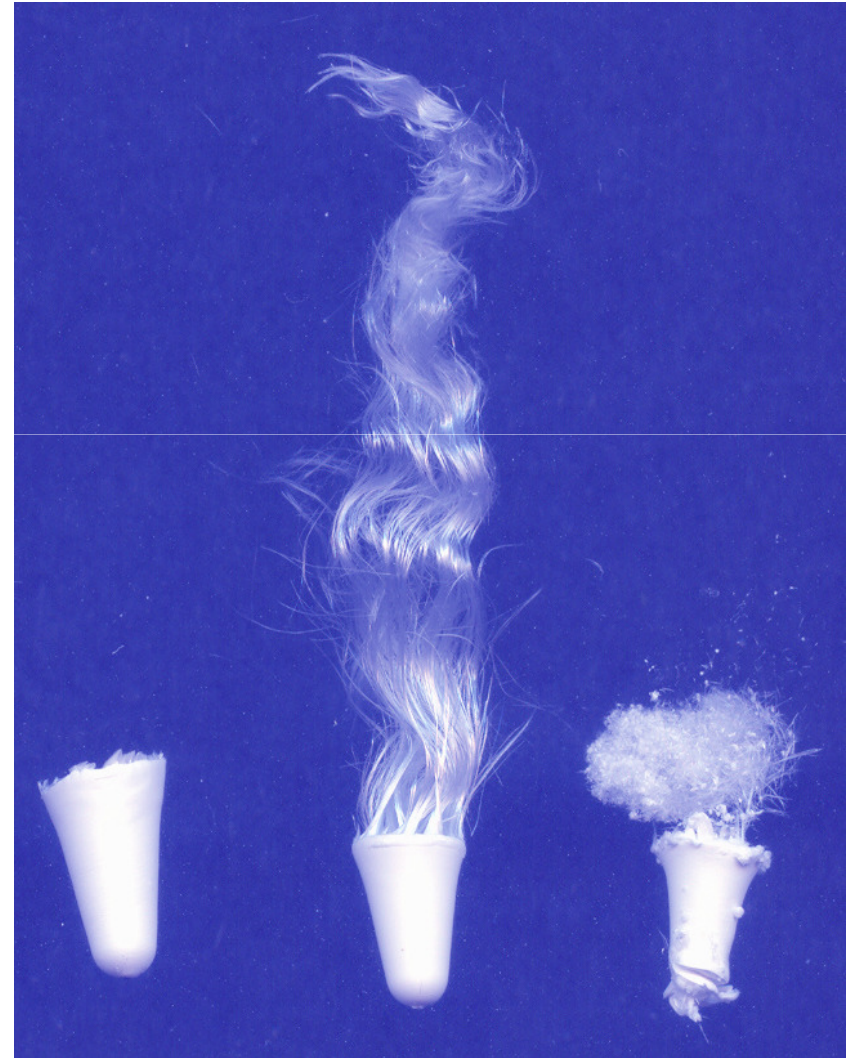
F. Švec, T.B. Tennikova, Z. Deyl (eds)
Monolithic Material Preparation, Properties and Applications
ELSEVIER



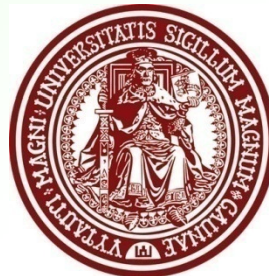
Journal of Biochemical and Biophysical Methods
(Sp. Iss. Monolithic Materials (Continuous Beds) and their Applications). *ELSEVIER* ISSN 0165-022X
Guest Editor A. Maruska



tentacle type flexible polymeric filaments formation



LVMSF projekts
**Vaistinės augalinės žaliavos kokybės
gerinimas, naudojant augalų
biotechnologijas**

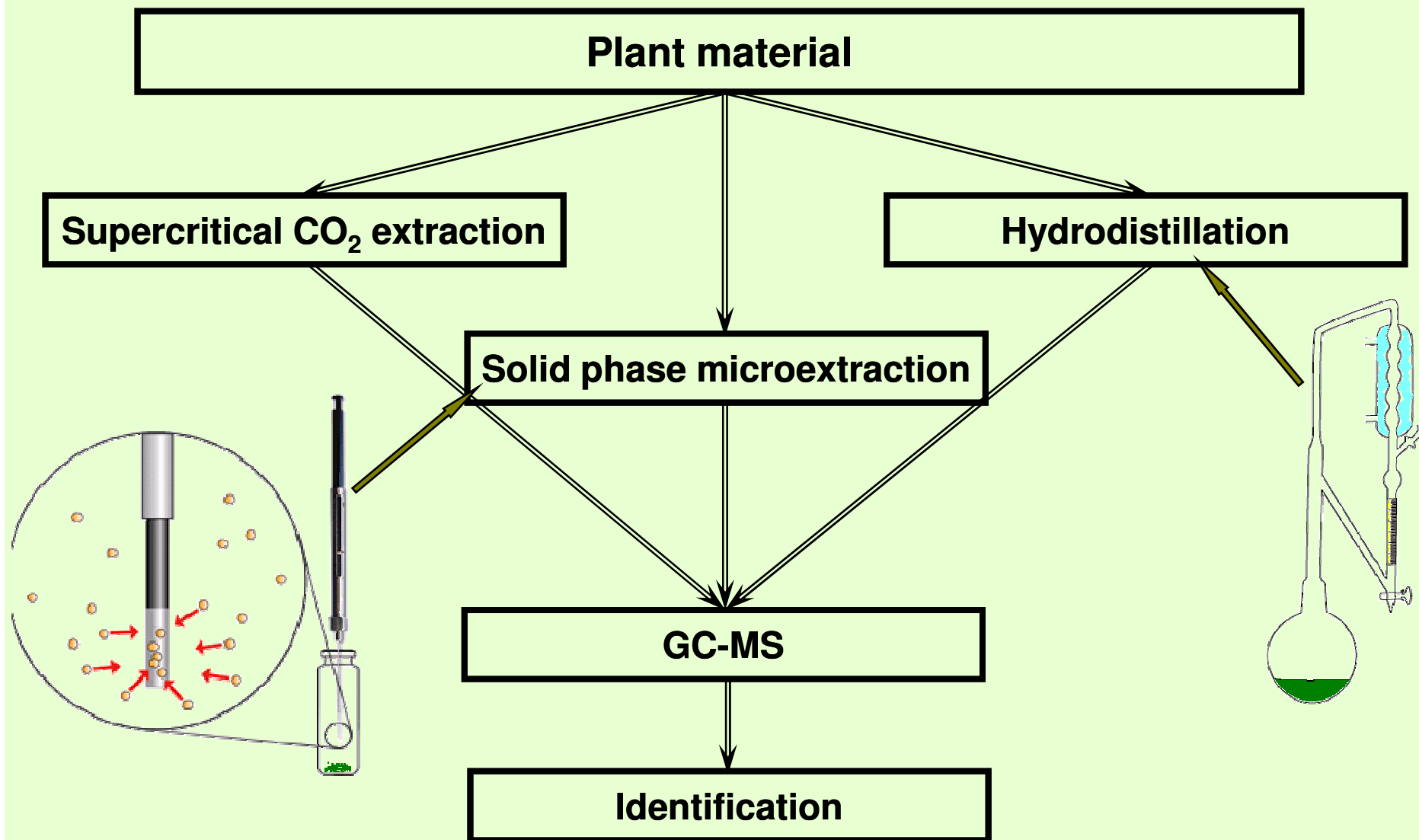


Registravimo Nr. N-07008, Sutarties Nr. N-14/2008

Pramoninės biotechnologijos plėtros programa

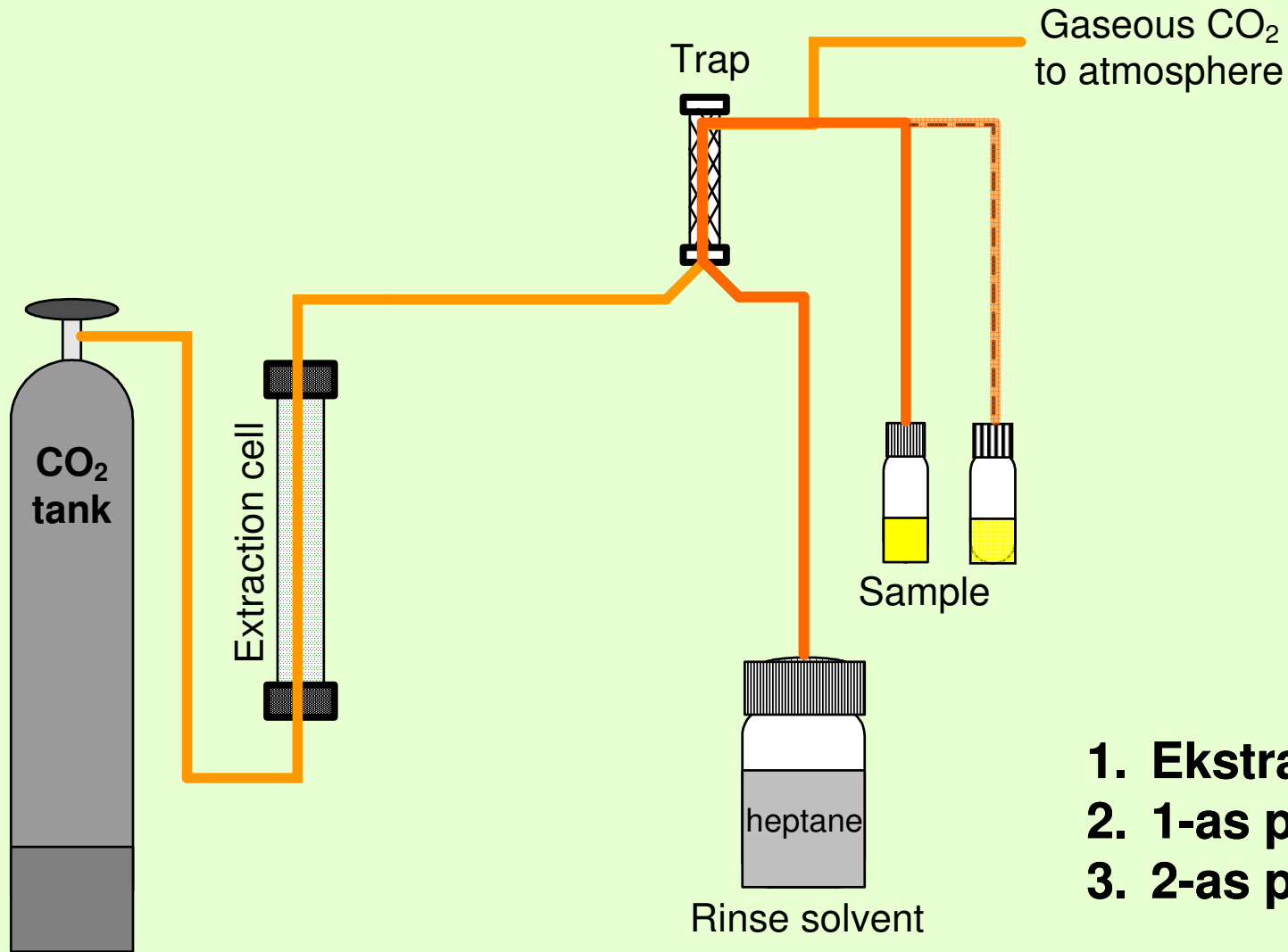


Eterinių aliejų ekstrakcijai



Eterinių aliejų ekstraktijai

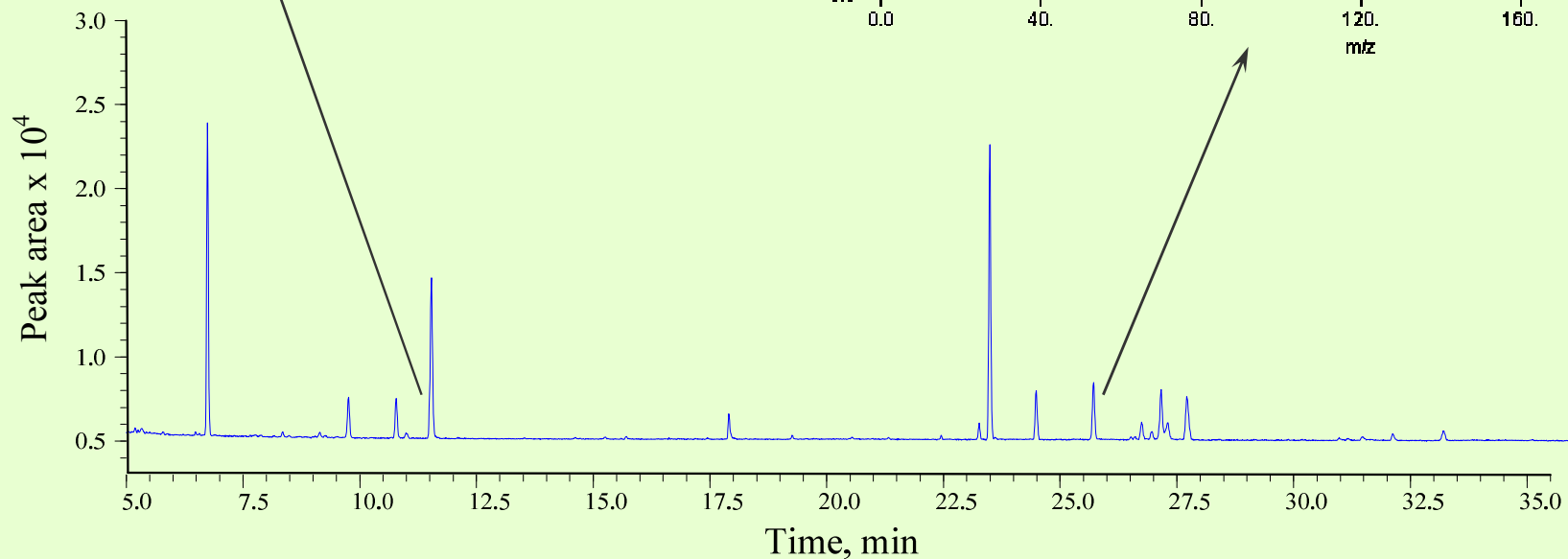
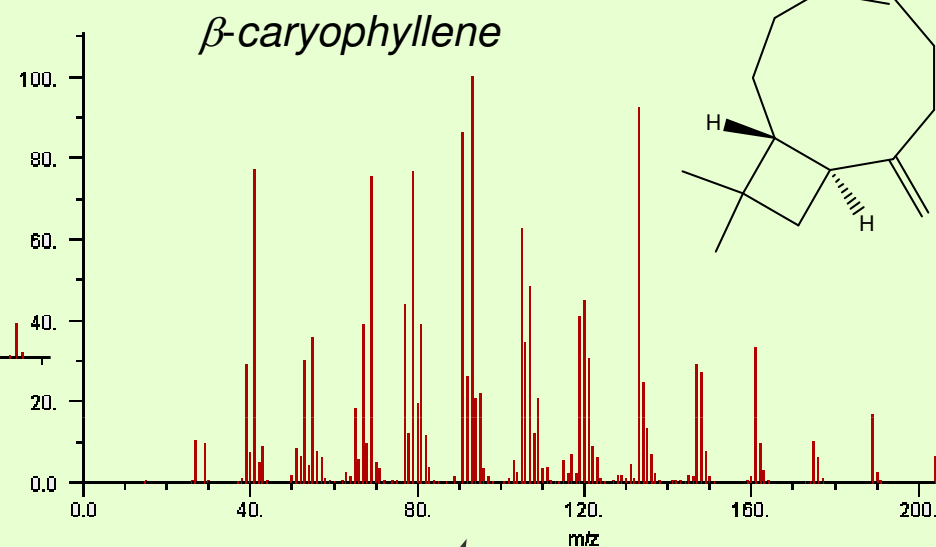
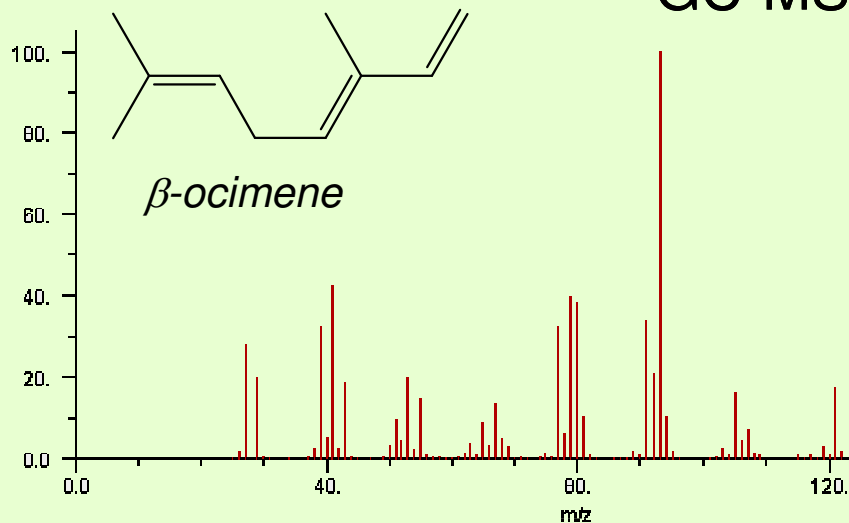
Superkritinių skysčių (CO₂) ekstraktorius



- 1. Ekstrakcija**
- 2. 1-as praplovimas**
- 3. 2-as praplovimas**

Eterinių aliejų ekstrakcijai

GC-MS analizė



Bidens tripartita dujų chromatograma

Nelakių junginių analizė

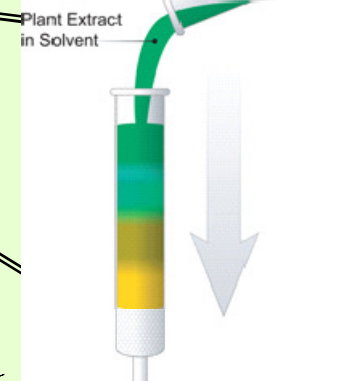
Plant material collected at different vegetation periods

Extraction with MeOH/H₂O



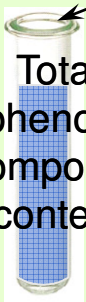
Purification

Solid phase extraction



Spectroscopic methods

Total phenolic compound content



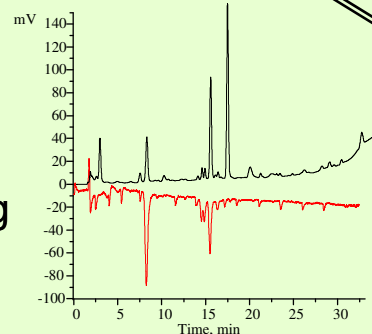
Total flavonoid content



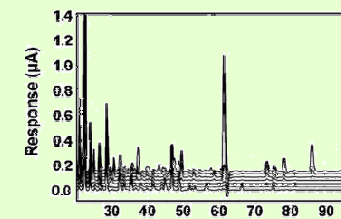
DPPH radical scavenging



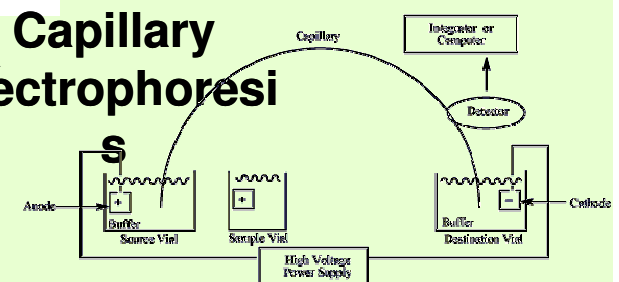
HPLC-DPPH



HPLC-ECD

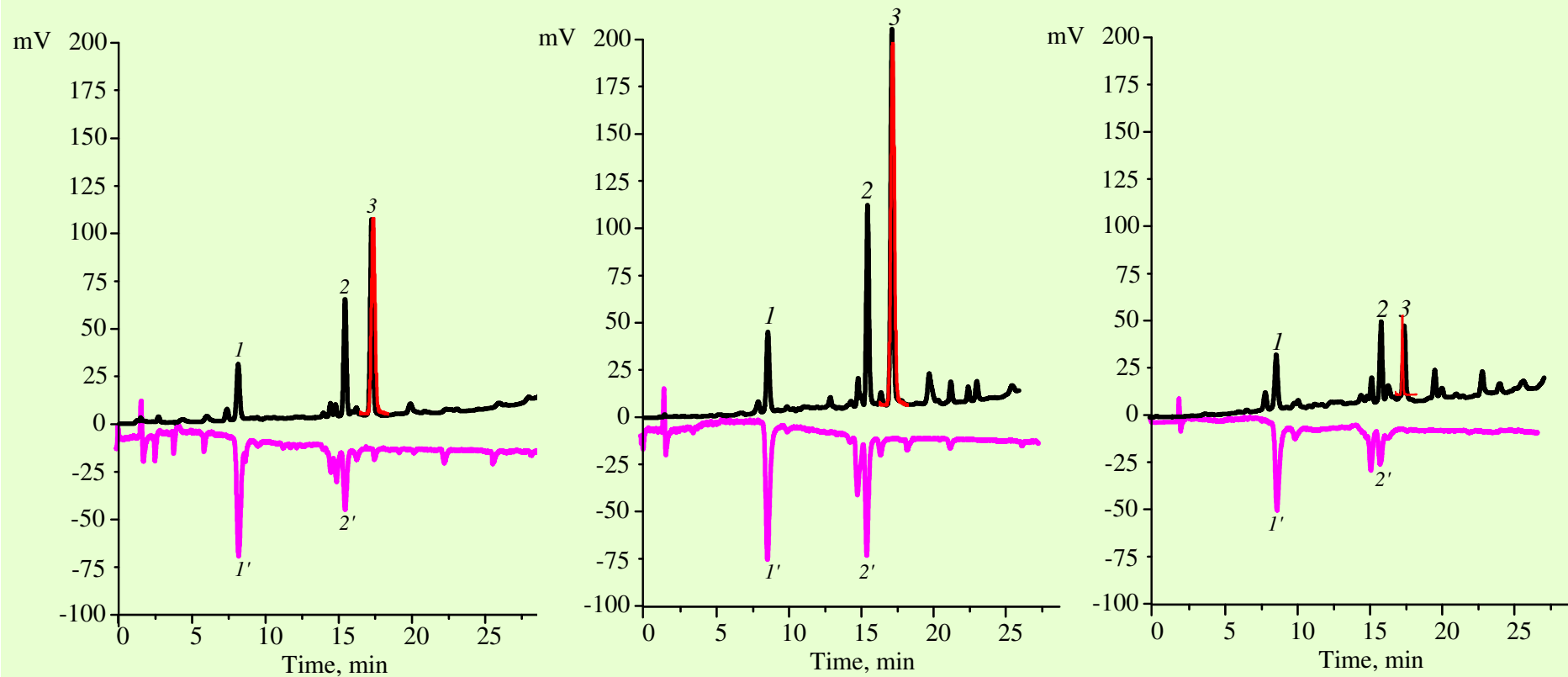


Capillary electrophoresis



Nelakių junginių analizė

ESC-DPPH analizė



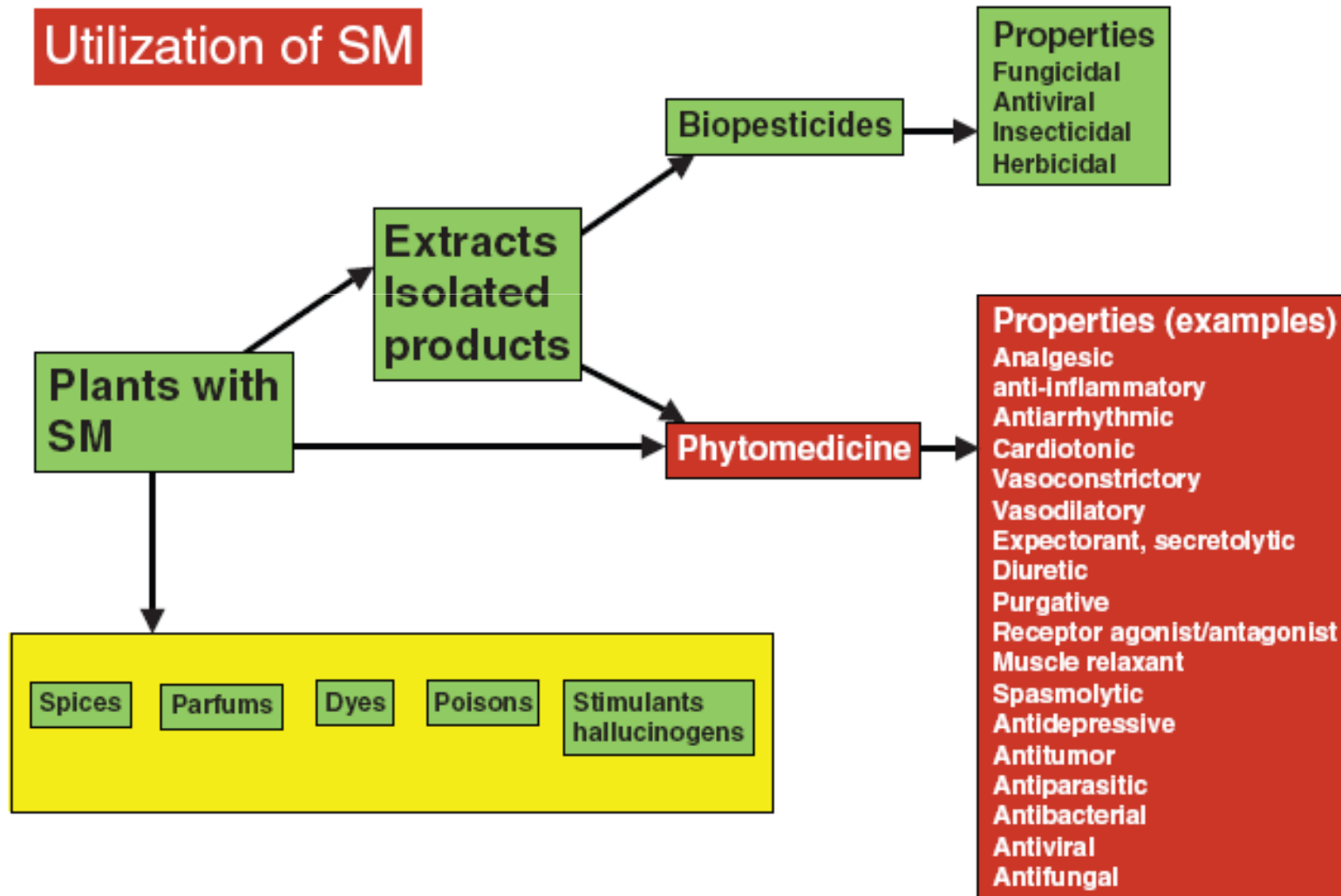
Growing period

Buds development period

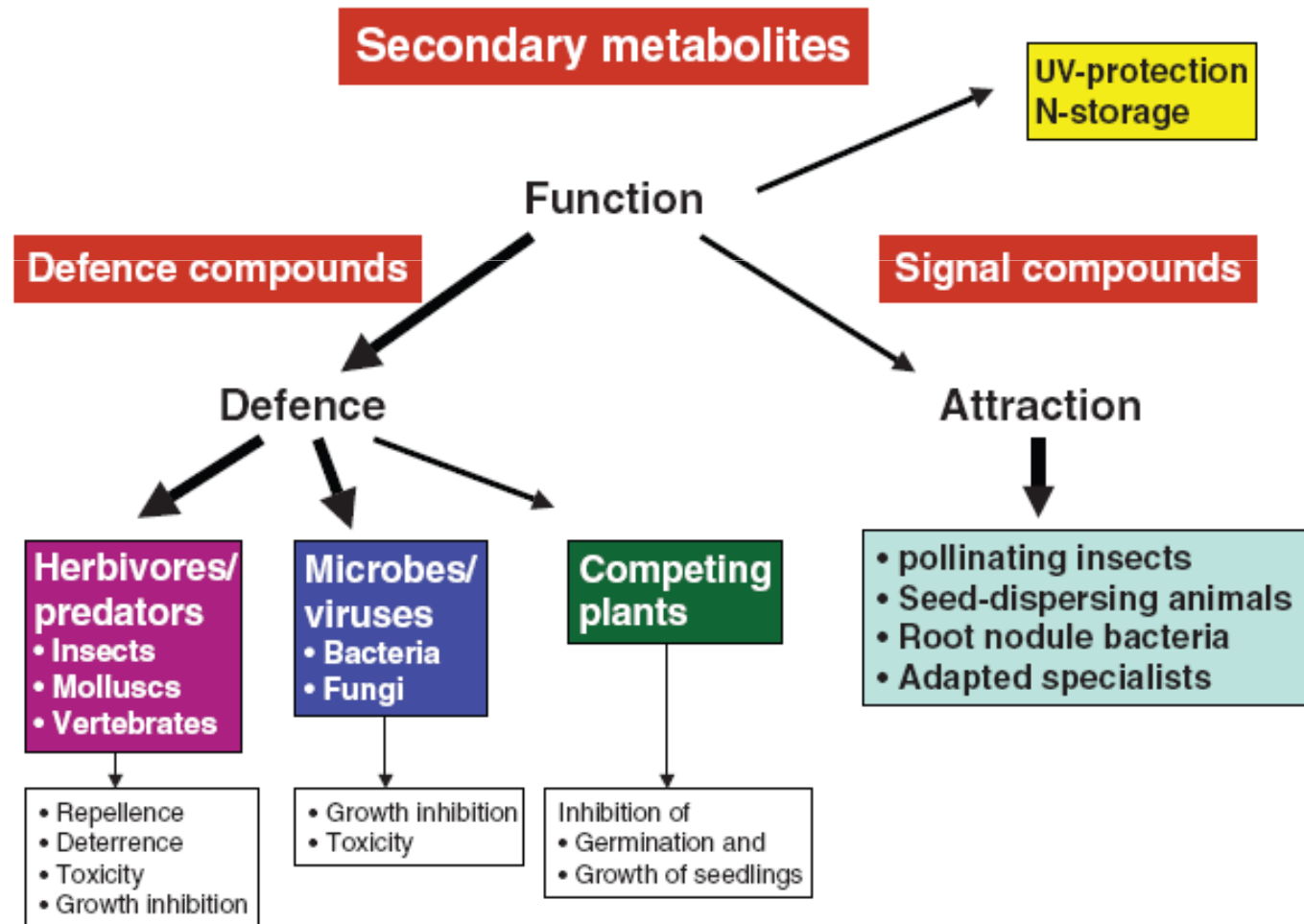
The end of flowering period

S. marianum L. ESC-DPPH chromatogramos skirtingais vegetacijos periodais

Antrinių metabolitų



Antrinių metabolitų ekologinės ir fiziologinės funkcijos



Tyrimų schema

Diploidinė forma



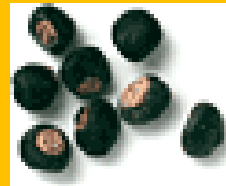
Biologiškai aktyvių junginių
kokybinė ir kiekybinė
analizė

Diploidinių
formų
atranka

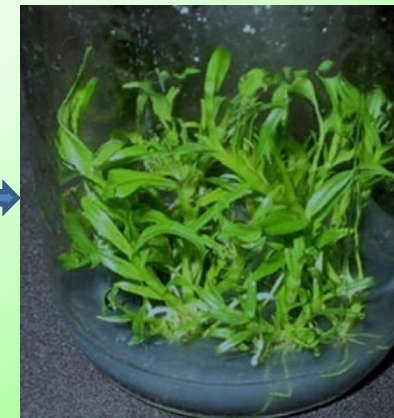


Sėklų
daiginimas

Poliploidinių
formų
indukcija



Poliploidinė forma



Orizalino poveikyje kaip poliploidinės formos pagal morfologiją atrinkti **vaistinės medetkos** augalai ir kontrolinis diploidinis augalas



Poliploidas A



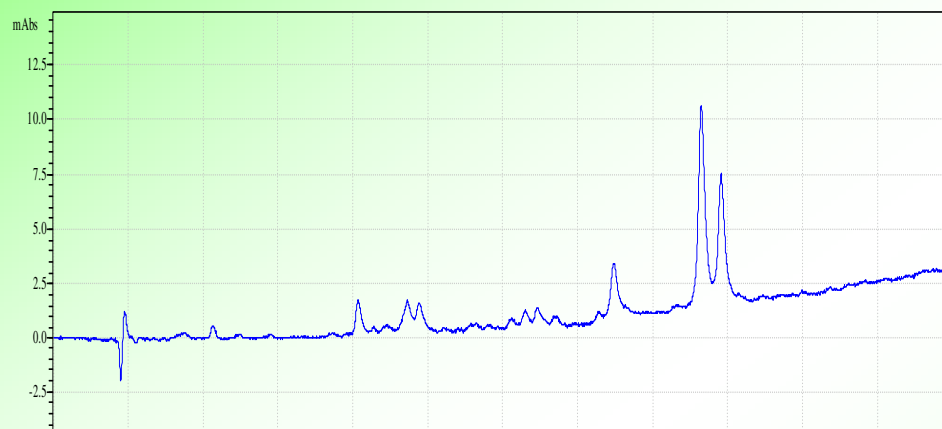
Poliploidas B



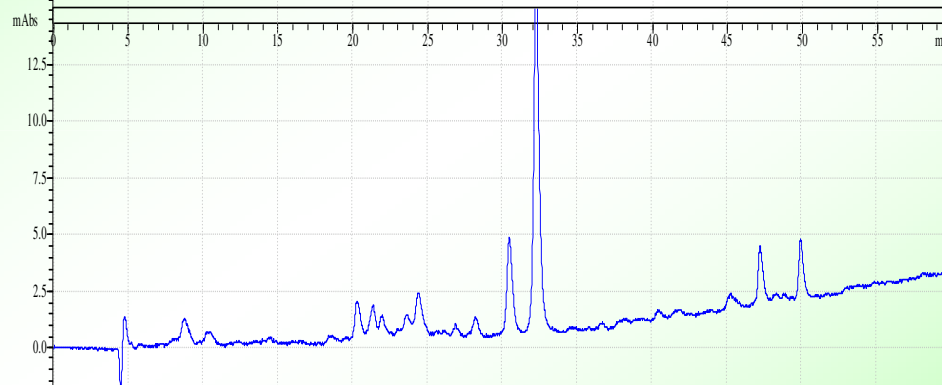
Kontrolinis diploidinis augalas

Triskiaučio lakišiaus (*Bidens tripartita* L.) chromatogramos

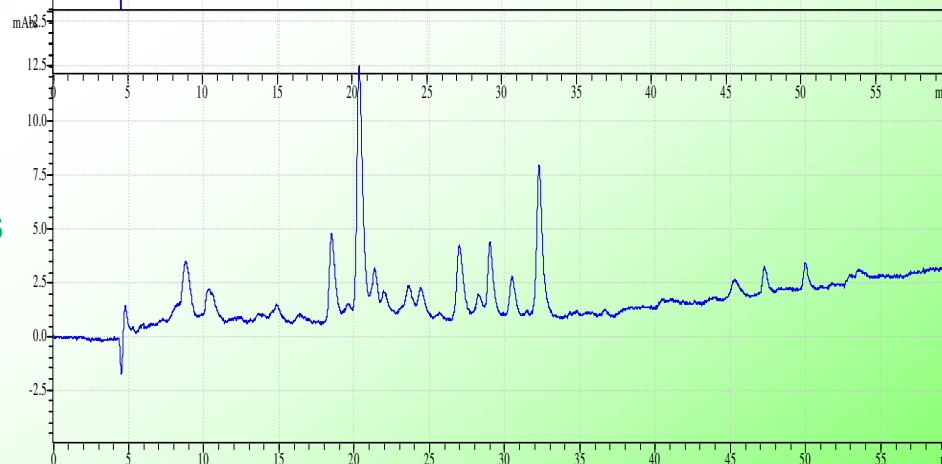
Kontrolė



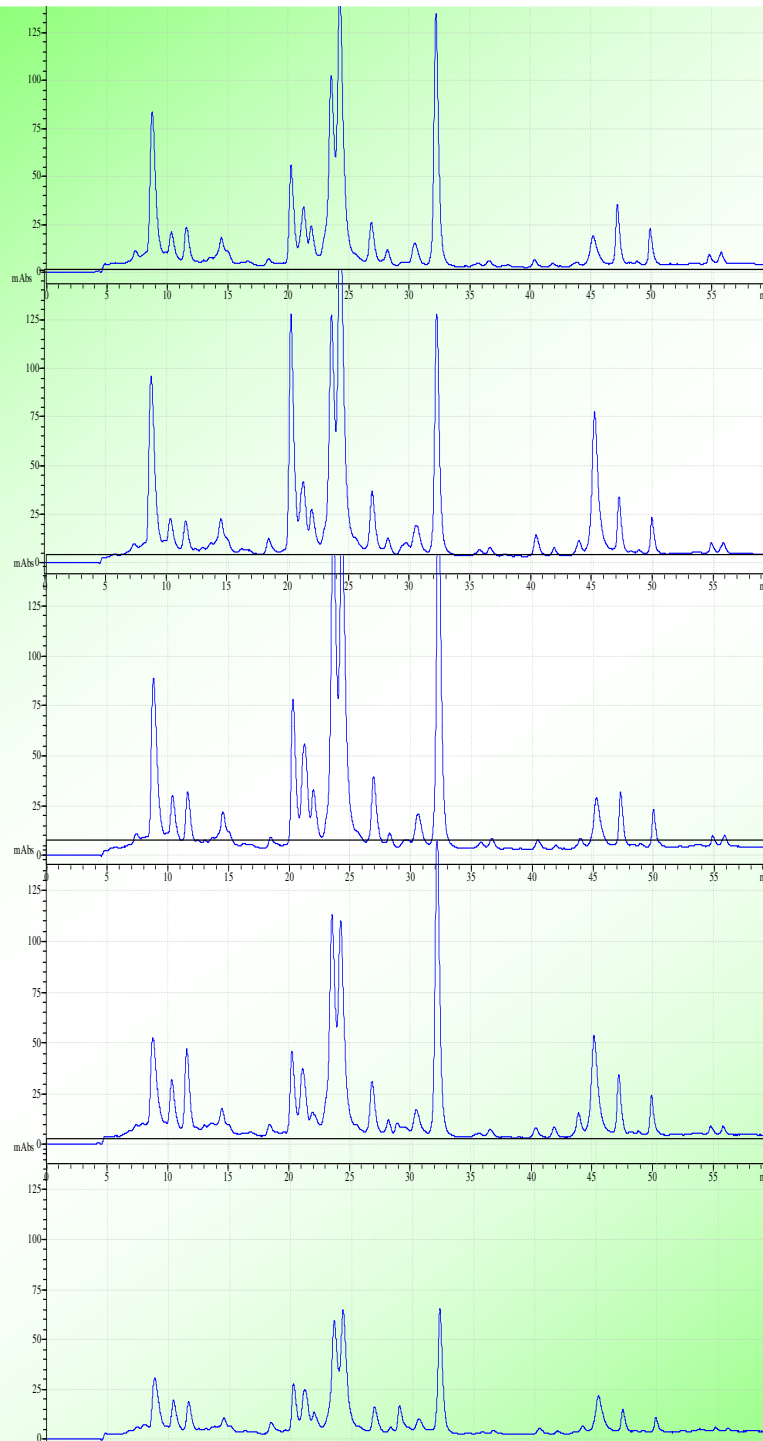
Poliploidas
2 klonas



Poliploidas
6 klonas



**Triskiaučio lakišiaus
chromatografinio
profilio dinamika
skirtingais vegetacijos
periodais**



Intensyvus augimas

Butonizacija

Žydėjimo pradžia

Žydėjimo pabaiga

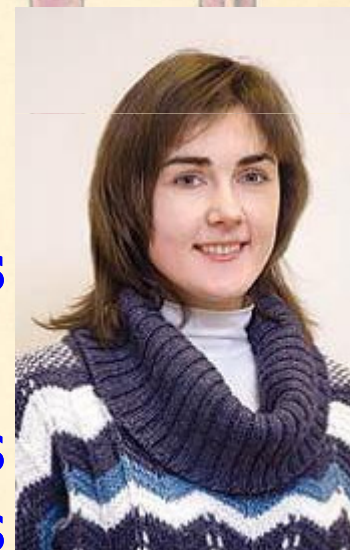
**Vaisių nokimas-lapų
nykimo pradžia**

Vilma Kaškonienė

XXX, 1. *142. Compositae.*
P. Carduinae!
**2009-2011 podoktorantūros
stažuotė:**

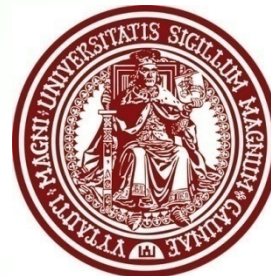
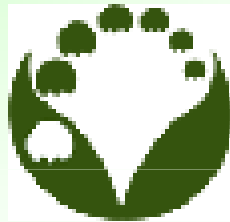
**VAISTINIŲ AUGALŲ ANTRINIŲ METABOLITŲ
ANALIZĖ PANAUDOJANT DUJŲ IR SKYSČIŲ
CHROMATOGRAFIJOS METODUS SU MASIŲ
SPEKTROMETRIJA**

- eterinių aliejų ekstrakcija **superkritiniais skysčiais** ir jų analizę naudojant GC-MS;
- nelakių junginių analizė spektrofotometriniais metodais ir skysčių chromatografijos su įvairiais detektoriais būdu.



LVMSF projekts

**Padidējusios bioloģinēs vērtēs ir saugesnīu maīsto
produktu kūrimas taikant augalinēs žaliavos
kietafazē fermentacīju bakteriocīnos
produkuojančiomis piēno rūgštīs bakterijomis**



Nacionalinē mokslo programa: „Sveikas ir saugus maistas“
(Nr. SVE-409/2011). 2011 10 01–2013 12 31., VDU, KTU,
LSMU, UAB „Fazer kepyklos“,

2011 metų klasterio publikacijos

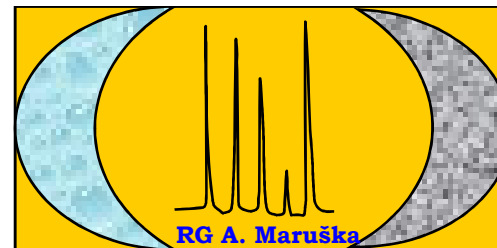
- mokslo straipsniai leidiniuose, referuojamuose ir turinčiuose citavimo indeksą Mokslinės informacijos instituto duomenų bazėje „ISI Web of Science“: **4**
- mokslo straipsniai kituose leidiniuose, referuojamuose Mokslinės informacijos instituto duomenų bazėje „ISI Web of Science“: **3**
- mokslo straipsniai kituose recenzuojamuose periodiniuose, tęstiniuose arba vienkartinuose mokslo leidiniuose: **2**
- padaryta **18** mokslinių pranešimų klasterio tematika.

Postdoc'ai, doktorantai ir magistrantai klasterio darbe

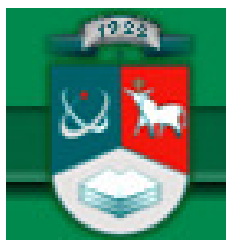
- Mokslininkas stažuotojas: **1 + 1**(LSMU Veterinarijos akademija)
- Doktorantai: **6 + 1** LSMU (apgynė) + **1** (LAMMC *Mišky institutas*)
- Magistrantai: **25**, iš jų **5** apgynė magistro darbą.

Acknowledgements

To the Research Group members,
Cooperation partners,
Foundations and Sponsoring
companies



LIH



VMU KBG



AstraZeneca



Agilent Technologies



WAGENINGEN UNIVERSITY
WAGENINGEN UR

***Welcome to the 7th
NoSSS International
Conference in
Stockholm, Sweden,
August, 2013***

