Characterization of Monoliths

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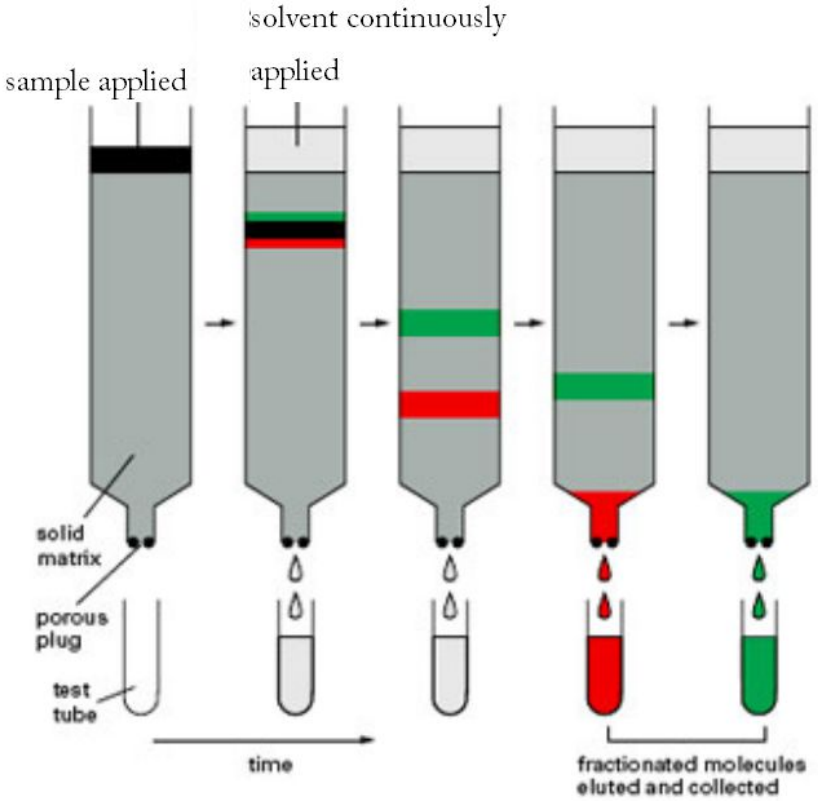
Chromatography

- a set of laboratory techniques for the separation of mixtures
- compounds have different physical and chemical interactions with solid matrix – stationary phase
- compounds travel at different speed – separation happens

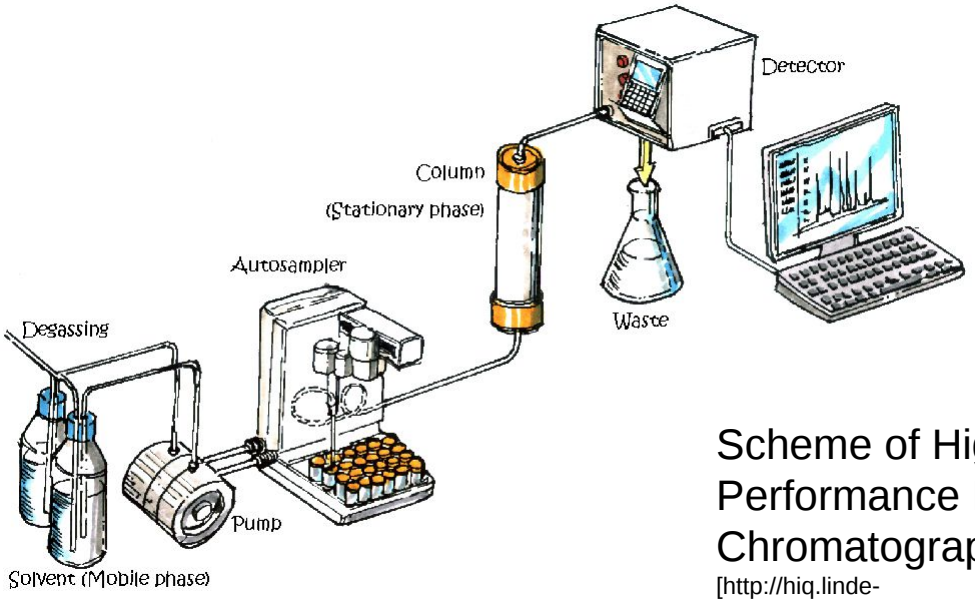


Scheme of separation of two different compounds using column chromatography.

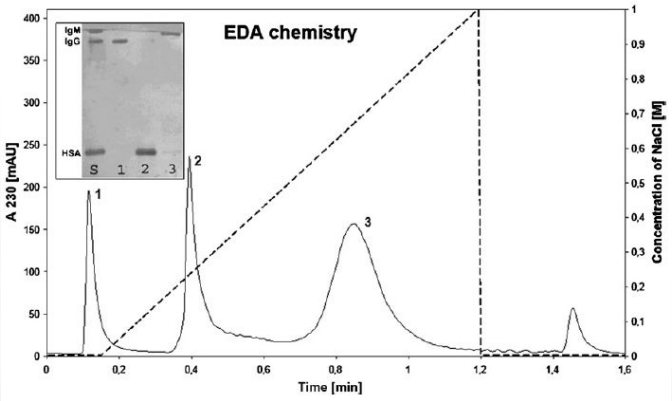
[http://talon.niagara.edu/~391s08/giacomini/PlasmidDNA&ProteinIsolation.html]

Chromatographic monolithic columns

- purification and separation of large biomolecules
  - large proteins
  - viruses
  - DNA



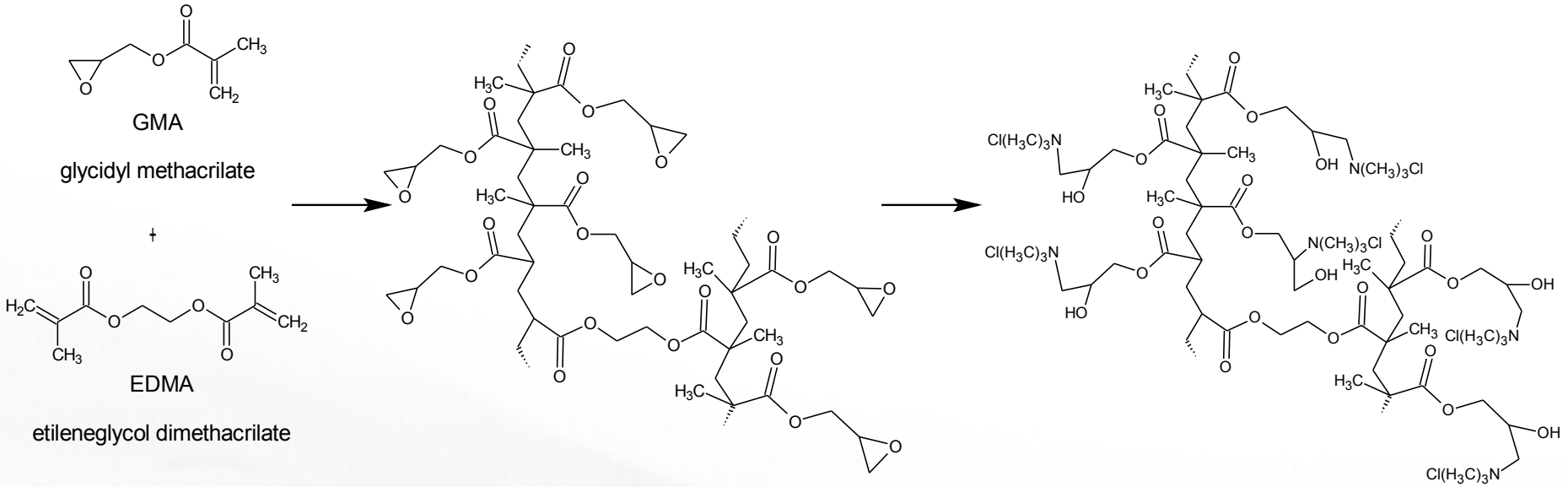
Scheme of High Performance Liquid Chromatograph- HPLC.
[http://hiq.linde-gas.com/international/web/lg/spg/like35lgspg.nsf/docbyalias/image_hplc]



Chromatogram presenting separation of three proteins (1 – IgG, 2 – HSA, 3- IgM) on CIM EDA monolithic column.
[Brne, P., Podgornik, A., Benčina, K., Gabor, B., Štrancar, A., Peterka, M. *Fast and efficient separation of IgM from IgG using short monolithic columns.* J. Chromatogr. A, 1144 (2007) 120-125.]

Convective Interaction Media - CIM® monoliths

- methacrylate polymer
- mechanically and chemically stable skeleton
- single piece of porous material
- average pore diameter = 1400nm



- 1. step: **polymerization** of two monomers
- 2. step: **modification**; example: epoxy group into quarternary amino group

Characterization

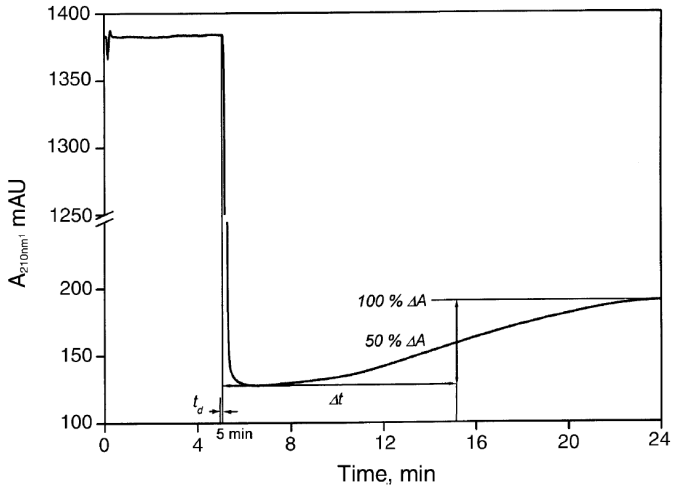
- chromatographic properties
  - type and amount of the active groups
  - amount of adsorbed molecules
- porosity
- pore size
- pore size distribution
- specific surface area
- chemical structure of ligands
- hydrophilicity of surface
- behavior of surface in mobile phases with different composition

SEM
TEM
HROM
AFM
BET
BJH
MIP
ISEC
(SS) NMR
GRAVIMETRIC METHODS
GAS PYCNOMETRY
X-RAY MCT
MRI
CLSM
TSD
FMS
CONFOCAL MICROSCOPY
IR SPECTROSCOPY
RAMAN SPECTROSCOPY
EPR
CONTACT ANGLE MEASUREMENTS
CHROMATOGRAPHIC METHODS

Ionic capacity

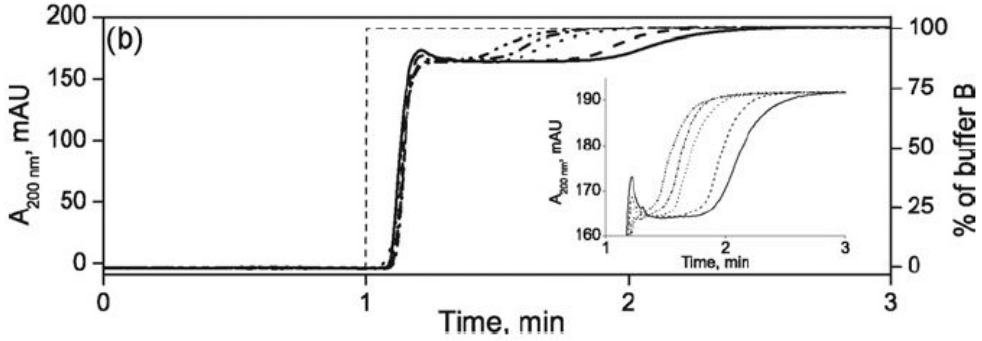
- amount and type (cation and anion, weak and strong) of ion-exchange groups
- observation of the pH profile during the step change between two buffer solutions varying in ionic strength but with the same pH
- duration of pH transient is proportional to the total ionic capacity (gravimetric determination)

Determination of the time of the pH transient for a CIM QA disk monolithic column.



[Lendero, N., Vidič, J., Brne, P., Podgornik, A., Štrancar, A. *Simple method for determining the amount of ion-exchange groups on chromatographic supports.* J. Chromatogr. A, 1065 (2005) 29-38.]

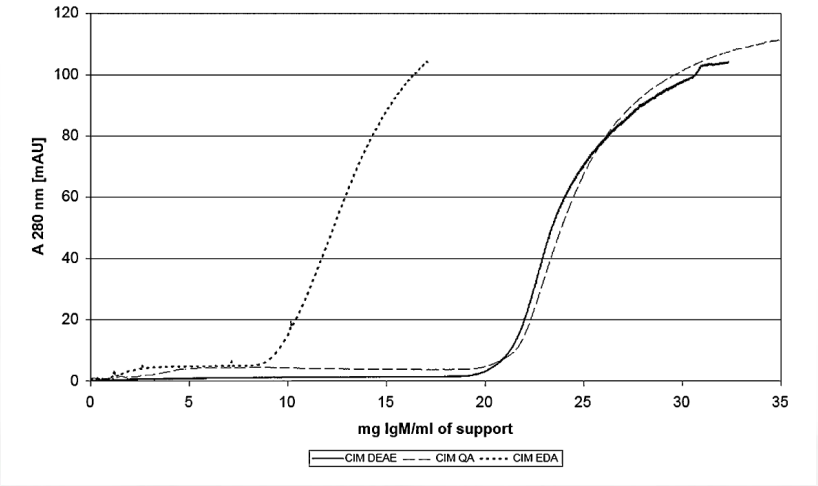
Absorbance profiles for CIM SO_3 columns with different amount of ion exchange groups.



[Lendero, N., Vidič, J., Brne, P., Frankovič, V., Štrancar, A., Podgornik, A. *Characterization of ion exchange stationary phases via pH trasition profiles*. J. Chromatogr. A, 1185 (2008) 59-70.]

DBC - Dynamic binding capacity

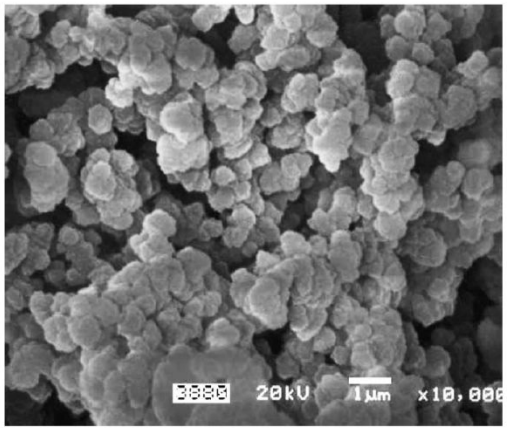
- very important information to end user
- amount of biomolecule that adsorbs on the column under certain conditions (buffer type, pH, flow rate,…)
- loading a column with solution of molecule – a sigmoidal breakthrough curve – DBC
- the driving force in the optimization of the productivity
- test proteins (BSA, lysozyme, IgG, IgM)



Breakthrough curve of standard IgM solution obtained on a CIM QA, CIM DEAE and CIM EDA monolithic columns.

[Brne, P., Podgornik, A., Benčina, K., Gabor, B., Štrancar, A., Peterka, M. *Fast and efficient separation of IgM from IgG using short monolithic columns.* J. Chromatogr. A, 1144 (2007) 120-125.]

SEM - Scanning Electron Microscopy

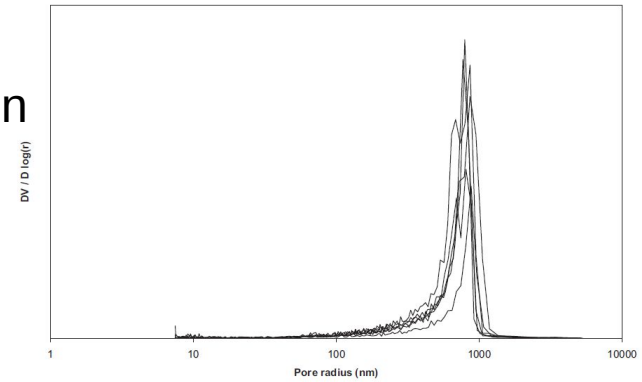


SEM picture representing the structure of CIM Epoxy monolith.

[Mihelič, I., Nemec, D., Podgornik, A., Koloini, T. *Pressure drop in CIM disk monolithic columns*. J. Chromatogr. A, 1065 (2005) 59-67.]

MIP – Mercury Intrusion Porosimetry

Determination of pore size distribution.



[Podgornik, A., Vidič, J., Jančar, J., Lendero, N., Frankovič, V., Štrancar, A. *Noninvasive Methods for Characterization of Large-Volume monolithic Chromatographic Columns*. Chem. Eng. Technol. 28 (2005) 1435-1441.]

- surface structure, connectivity of pores, the porosity and average pore diameter
- visible pores with r > 50 nm

- pore size, pore size distribution and specific surface area (calculated)
- several nanometers to micrometers pores

Disadvantages:

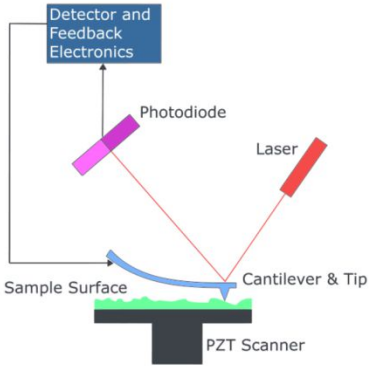
– **- dried samples –** alteration of the surface morphology

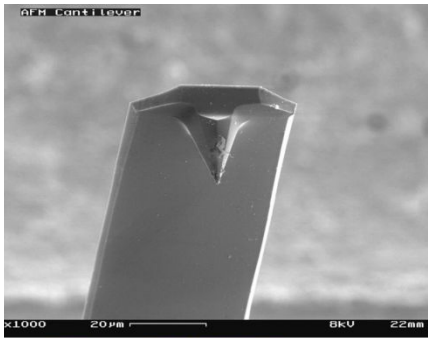
- coating metal film (Au, Pt) (SEM)

- destructive method – problematic sampling

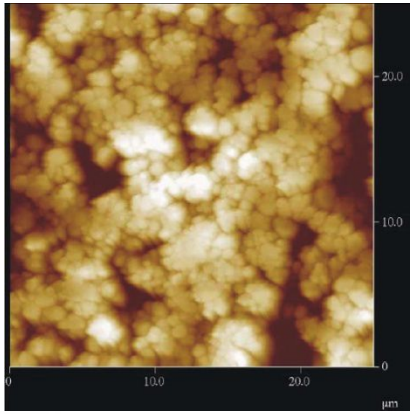
AFM - Atomic Force Microscopy

Block diagram of AFM.
[Wikipedia]





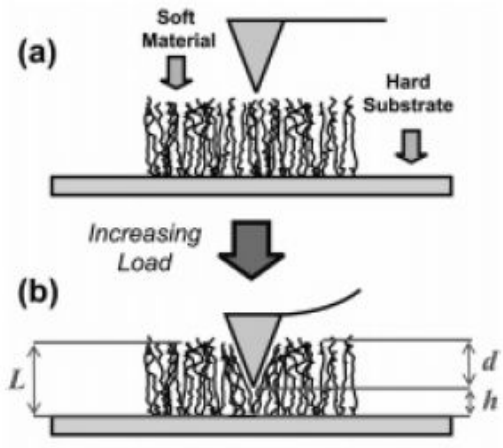
Electron micrograph of a used AFM cantilever.
[Wikipedia]



Topographic image of a 25µm x 25µm section of the polyacrylate monolith showed by AFM in air.

[Cabral, J., Bandilla, D., Skinner C. D. *Pore size characterization of monolith or electrochromatography via atomic force microscopy studies in air and liquid phase*. J. Chromatogr. A, 1108 (2006) 83-89.]

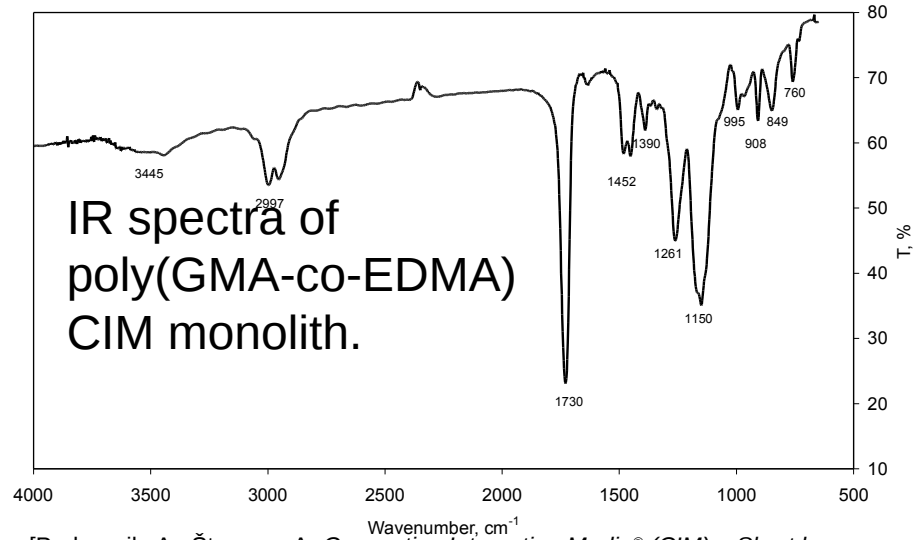
Simplified scheme depicting brush penetration.



[Azzaroni, O., Moya, S., Farhan, T., Brown, A. A., Huck, W. T. S. *Switching the properties of polyelectrolyte brushes via ‚hydrophobic collapse'.* Macromolecules, 38 (2005) 10192-10199.]

- fluid cell enables visualization of surface in solution
- measurement of the thickness of grafted layer on the surface
- no coating, destructive method

IR – Infrared spectroscopy



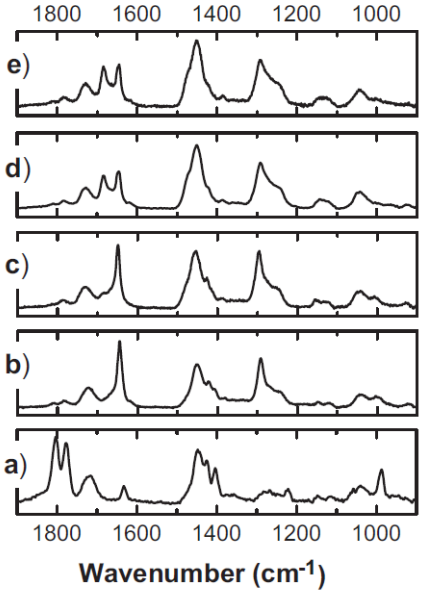
IR spectra of poly(GMA-co-EDMA) CIM monolith.

[Podgornik, A., Štrancar, A. *Convective Interaction Media® (CIM) – Short layer monolithic chromatographic stationary phase.* Biotechnology Annual Rev., 11 (2005) 281–333.]

- position, shape and intensity of peaks – molecular structure of the sample
- epoxy group: 908 and 849 cm^{-1}
- studies of influence of long-term exposure of CIM monoliths to 20% ethanol, acidic or alkali media
- efficiency of modification reactions

Raman spectroscopy

Raman spectra recorded in situ for poly(NAS-co-EDMA) (a) with pristine surface chemistry, (b) after reaction with allylamine, etc.



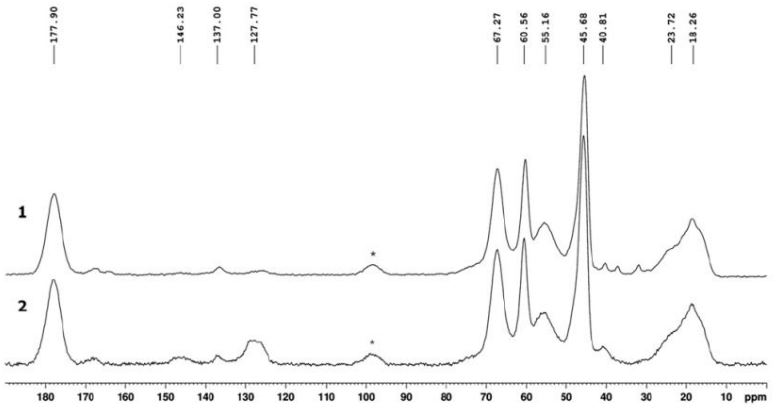
Tijunelyte, I., Babinot, J., Guerrouache, M., Valincius, G., Carbonnier, B. *Hydrophilic monolith with ethylene glycol-based grafts prepared via surface confined thiol-ene click photoaddition.* Polymer 53 (2012) 29-36.

- vibrational, rotational and other low-frequency modes in a system
- IR spectroscopy yields similar, but complementary information
- determination of chemical structure

Functional groups are determined, but on which carbon atom are they?

NMR - Nuclear Magnetic Resonance

- physical, chemical, electronic and structural information about molecules in solution and the solid state
- magnetic nuclei in a magnetic field absorb and re-emit electromagnetic radiation – NMR spectra
- the peaks of NMR spectra determine the structure of compounds
- distinguish among many atoms within a molecule which differ only in terms of their local chemical environment
  - efficiency of modification reactions
  - determination of chemical structure of polymer and ligands
  - behavior of water at hydrophilic/hydrophobic surfaces



SS NMR-^{13}C spectra of HEMA-GDMA copolymer without (1) and with polystyrene (2) in the polymeric matrix.

[Sinitsyna, E. S., Vlakh, E. G., Rober, M. Yu, Tennikova, T. B. *Hydrophilic methacrylate monoliths as platforms for protein microarray.* Polymer, 52 (2011) 2132-2140.]

Contact angle measurements

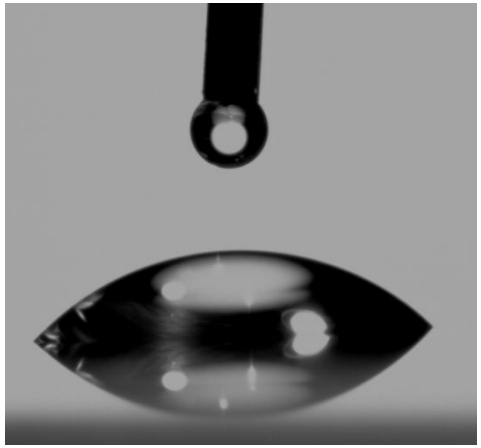
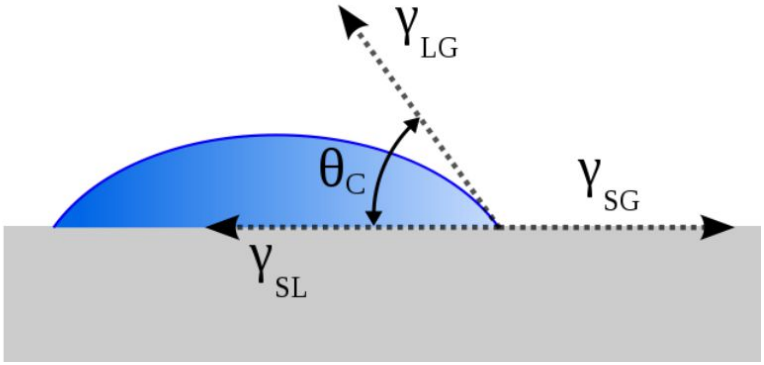


Image from a video contact angle device. Water drop on glass.

[Wikipedia]



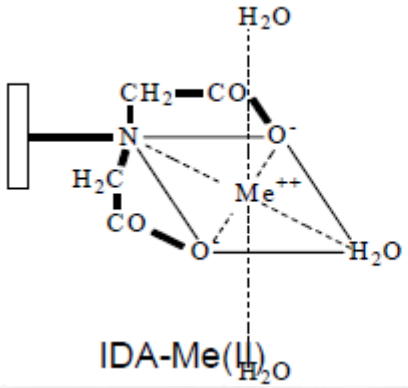
A contact angle of a liquid sample.

[Wikipedia]

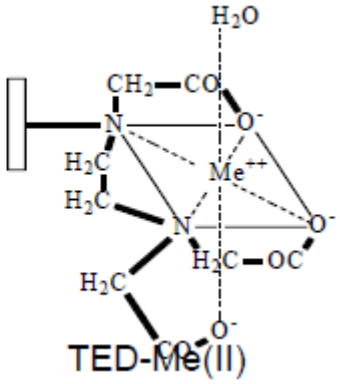
- the contact angle = angle at which a liquid/vapor interface meets a solid surface
- contact angle goniometer
- strongly hydrophilic solid – contact angle of 0° (0-30°)
- hydrophobic solid – higher contact angles (>90°)
- determination of hydrophilic/hydrophobic character of surface of various monoliths
- problem: porous material

XAS – X-ray absorption spectroscopy

- XAS - determination of the local geometric and/or electronic structure of matter
- IMAC = Immobilized Metal-Affinity Chromatography – covalently bound chelating compounds entrap metal ions – purification of proteins with exposed histidine tags
- neighborhood of metal ions on the surface: which atoms and how many are around metal ion; coordination structure
- problem: free beamlight



IDA-Me(II)



TED-Me(II)

Structures of chelators in coordinative complex with metal ions.

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