Monolithic chromatographic materials

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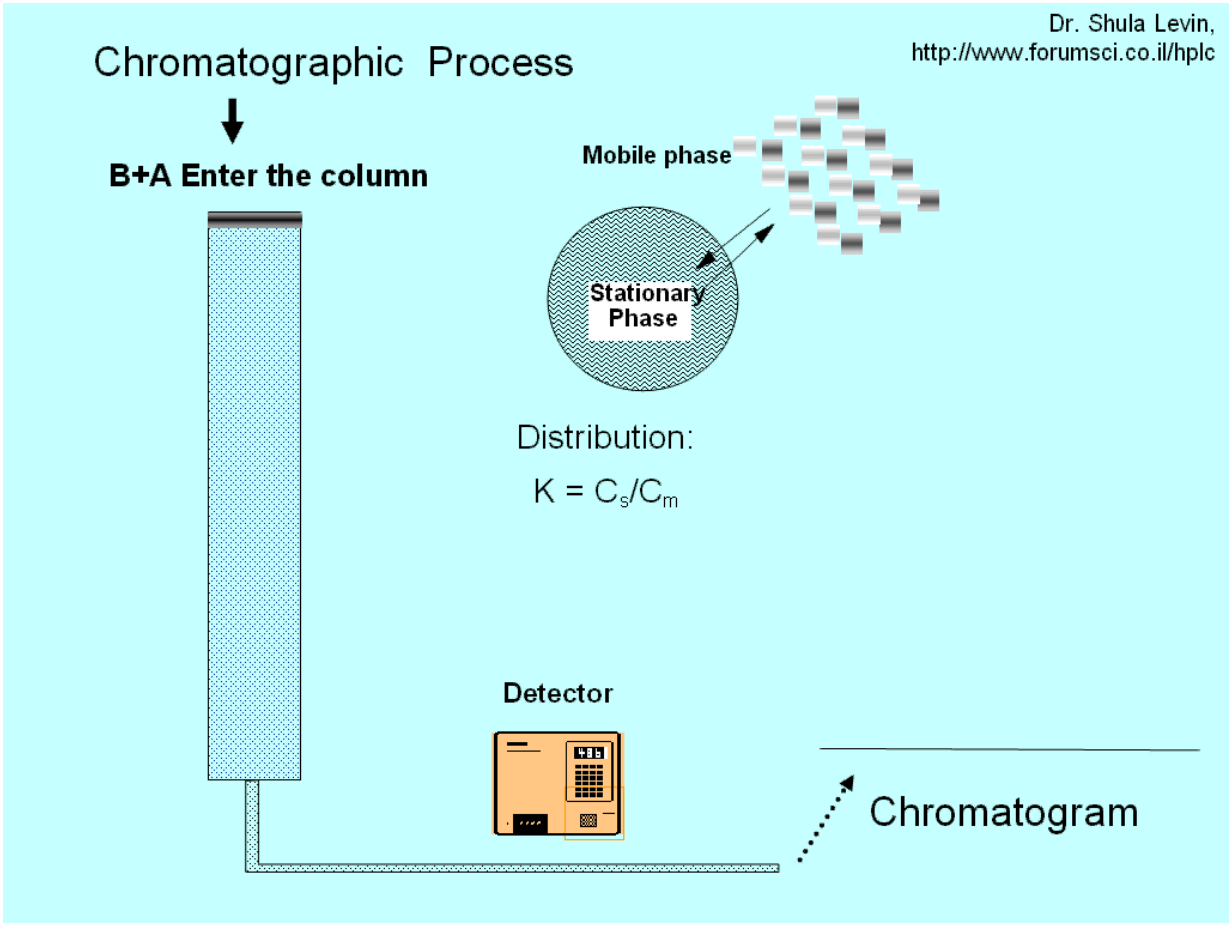
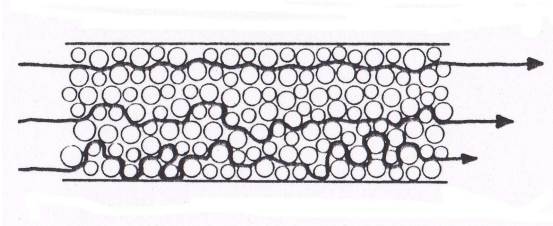
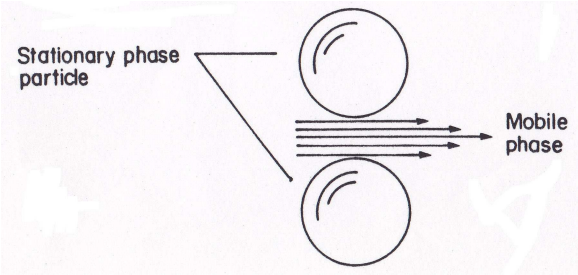
Chromatography

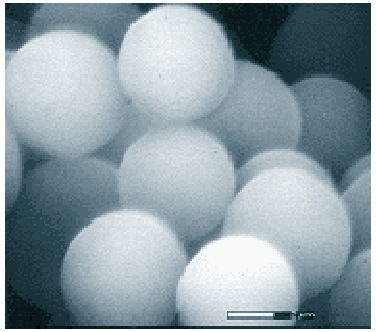
a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase) while the other (mobile phase) moves in definite direction.







Stationary phases in HPLC



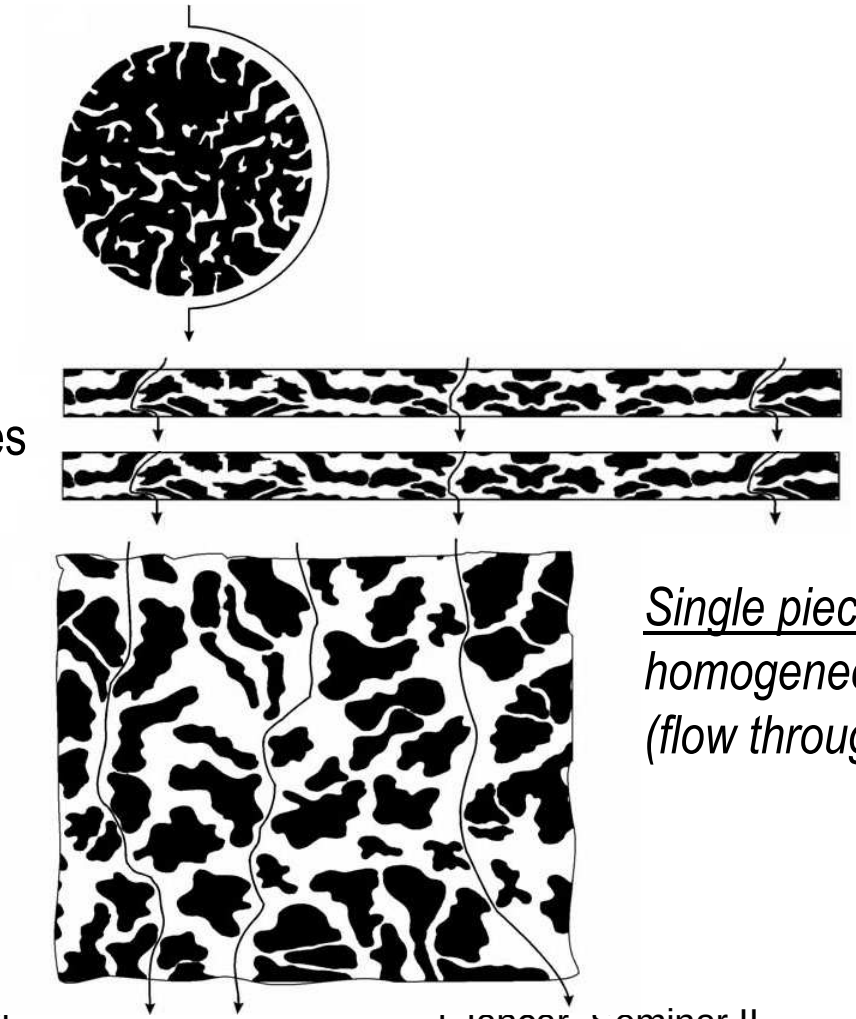
Cogent 4µm Spherical Silica

All conventional stationary phases in HPLC comes in the form of particles

What are monoliths?

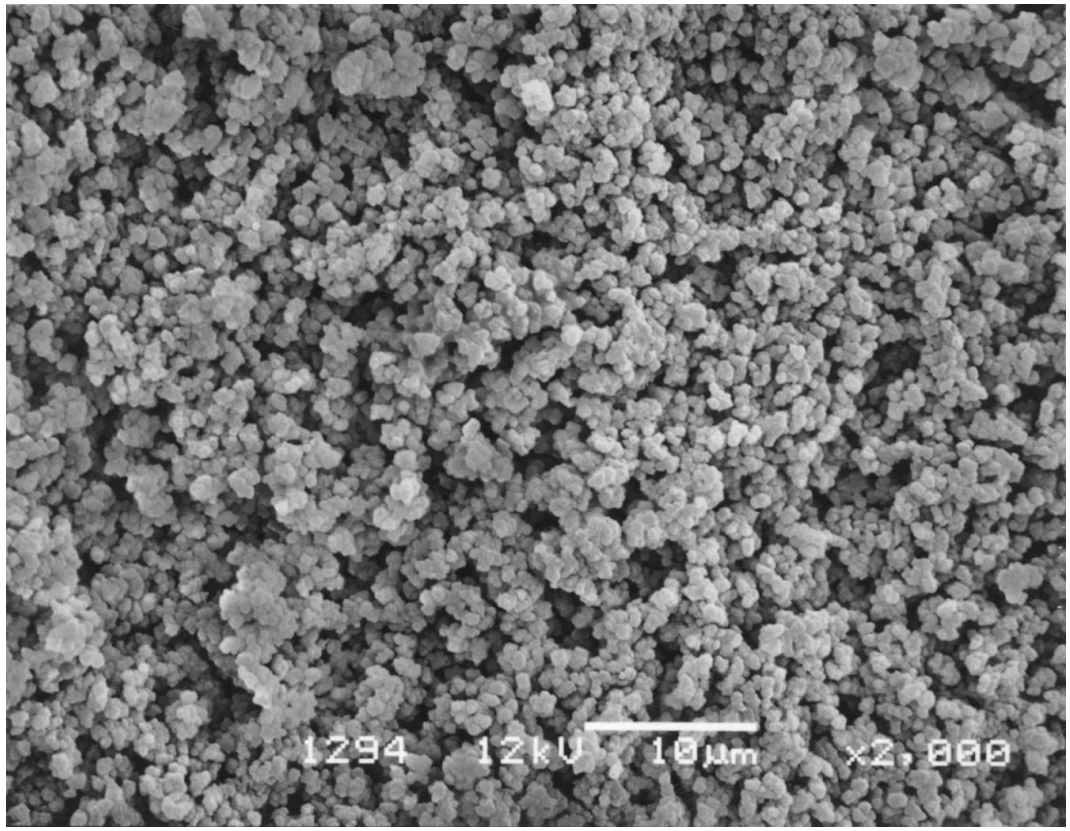
Putting things into perspective:

- Particles
- Membranes
- Monoliths



<u>Single piece (continuous) units</u> with a homogeneous open pore structure (flow through channels)

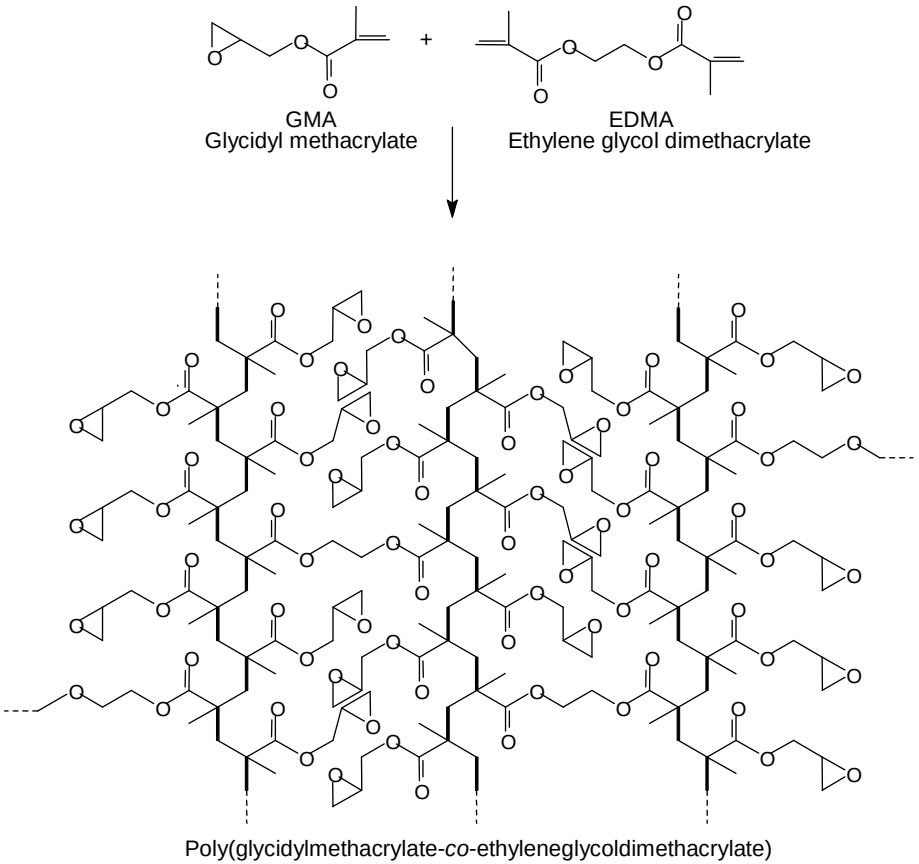
Methacrylate monolith structure – network of highly interconnected channels

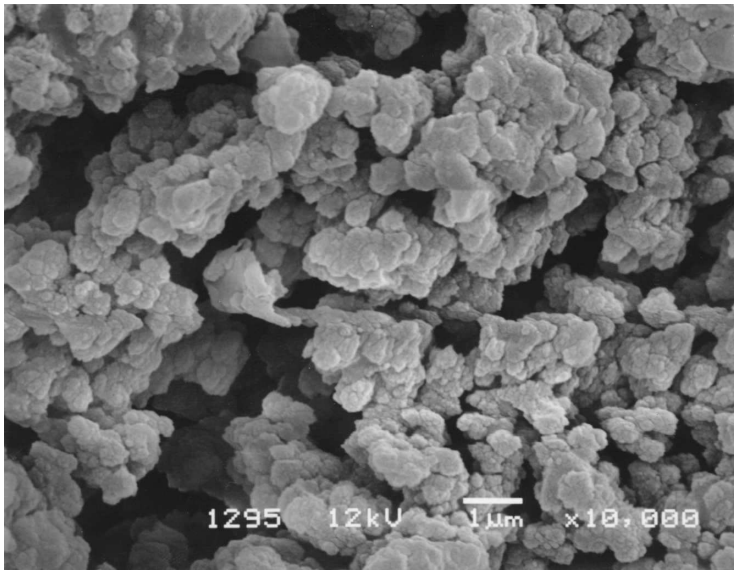


SEM of GMA/EDMA monolith

GMA/EDMA monoliths

Preparation of methacrylate monolith

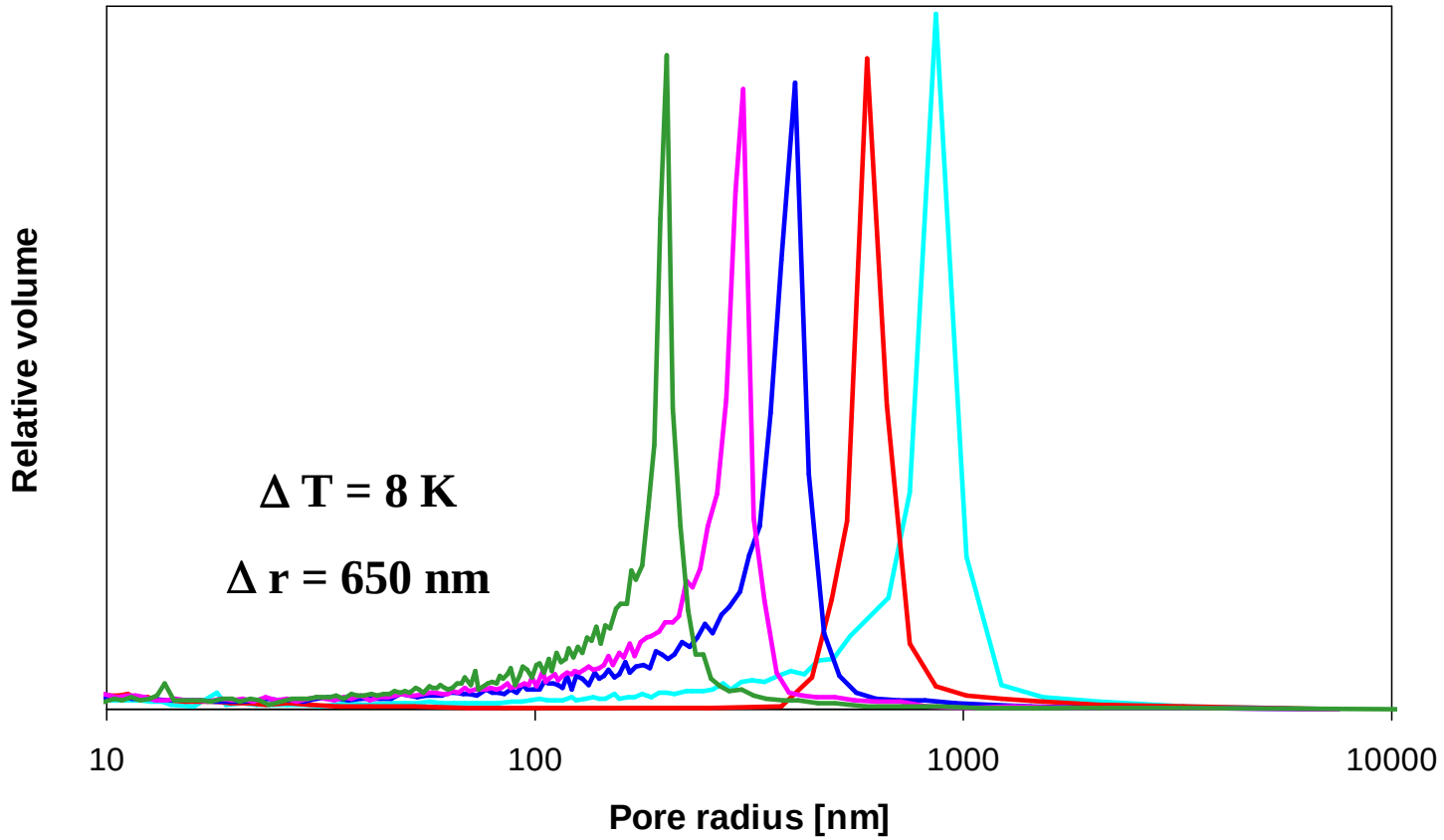




CIM supports are porous rigid monolithic polymers with:

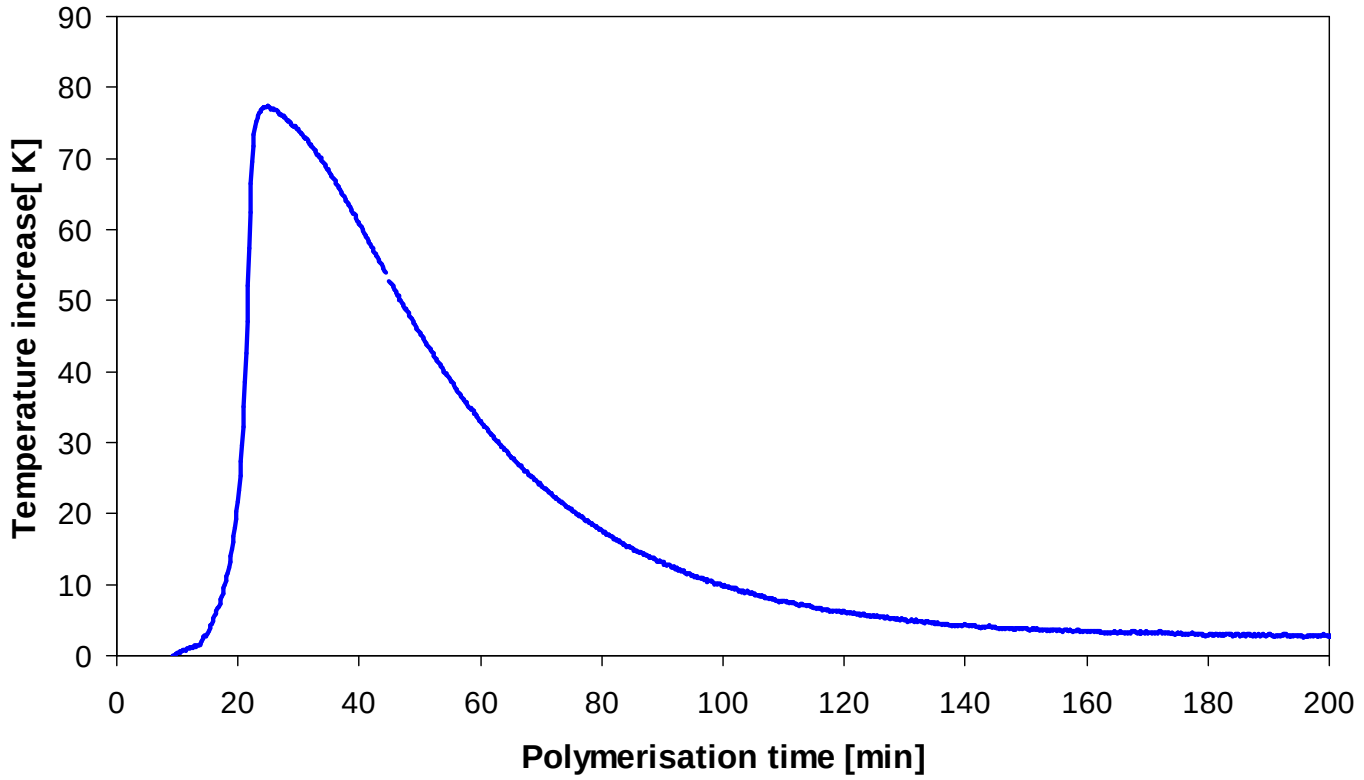
- Methacrylate matrix (well proven & biocompatible)
- High porosity (over 60 %)
- Flow-through pores (channels) having large diameter (> 1 µm)
- Uniform pore connectivity in 3D (homogeneous structure).

Effect of the polymerization temperature on pore size distribution



Štrancar et al., Advances in Biochemical Engineering / Biotechnology, Vol. 76: R. Freitag (Ed.), Modern Advances in Chromatography, Springer-Verlag, Heidelberg, 2002, 49.

Temperature increase in the polymerization mixture during polymerization



50 mm SS cylindrical mold, T was monitored in center of cylinder

Why monoliths?

Typical advantages over classical particle supports:

- **Faster separation runs**
  - mass transfer based on convection rather than diffusion
  - lower back pressure
- **Higher binding capacity for large biomolecules**
  - larger pores - accessible internal surface
  - flow unaffected binding capacity
- **Simple to use**
  - no column packing
  - no air bubble hassles
- **Absence of dead volume**
  - no stagnant zones
  - no peak broadening

Low pressure drop in monoliths

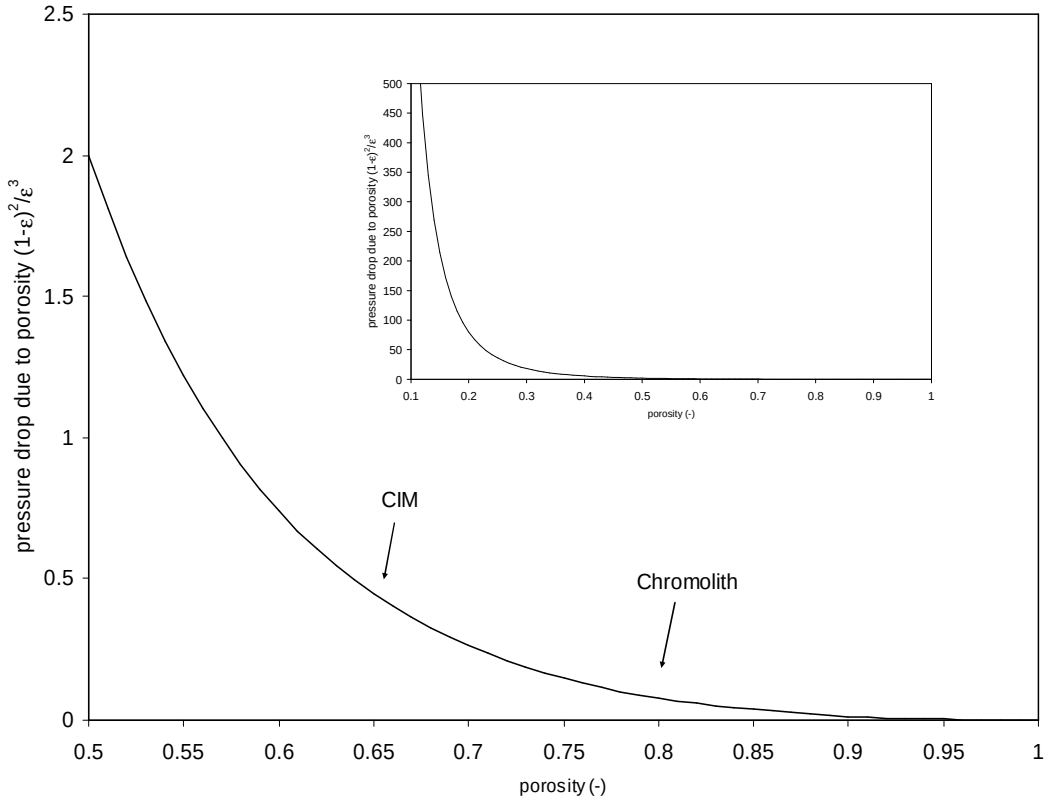
Low pressure drop of the monoliths is mainly result of extremely high porosity. In addition, for structures exhibiting parallel connectivity, pressure drop might be further reduced.

Therefore, high throughput can be achieved at low pressure drop resulting in lower equipment cost.

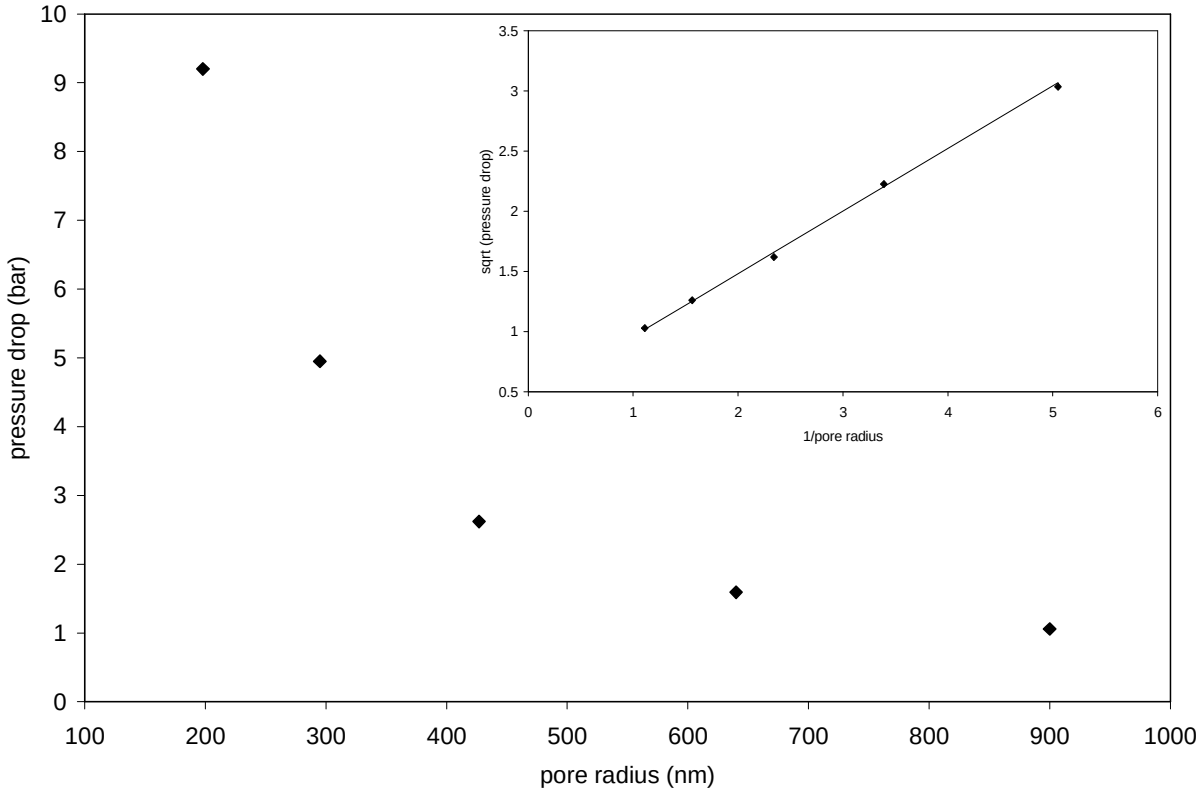
Effect of porosity on the pressure drop

$$\Delta P = \frac{150 \cdot \eta \cdot L \cdot v_0}{d_p^2} \cdot \frac{(1-\varepsilon)^2}{\varepsilon^3}$$

Blake-Kozeny equation

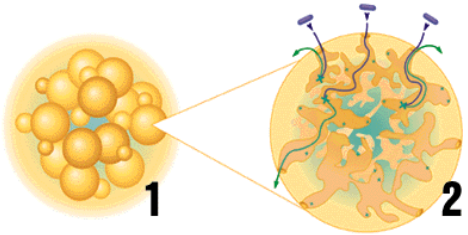


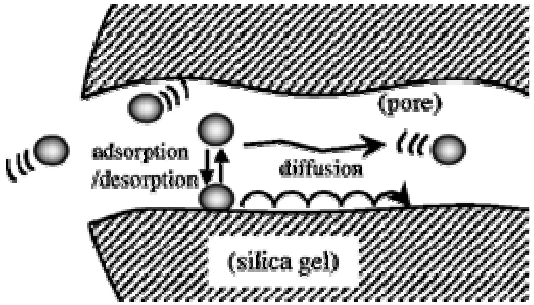
Effect of pore radius on the pressure drop of methacrylate monoliths



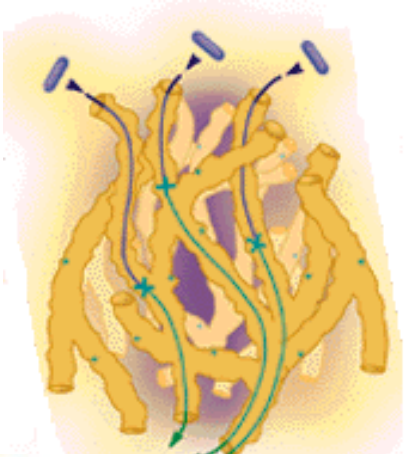
From: Barut et al. in F. Švec, Z. Deyl, T.B. Tennikova (Editors), Monolithic Materials, Elsevier, Amsterdam, 2003, p. 51.

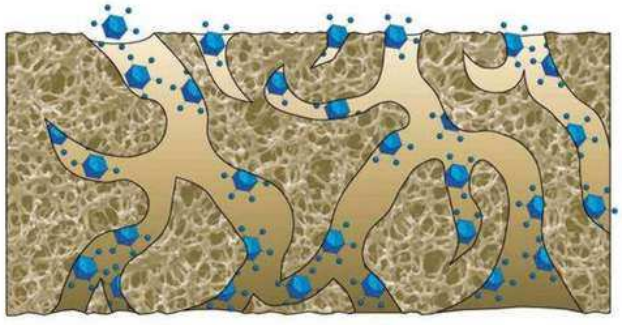
Comparison of mass transfere within particles and monoliths





Transport mechanism in particles.
Botlneck is pore diffusin





Transport mechanism in monoliths.
(cenvectiv media)
No pore diffusion

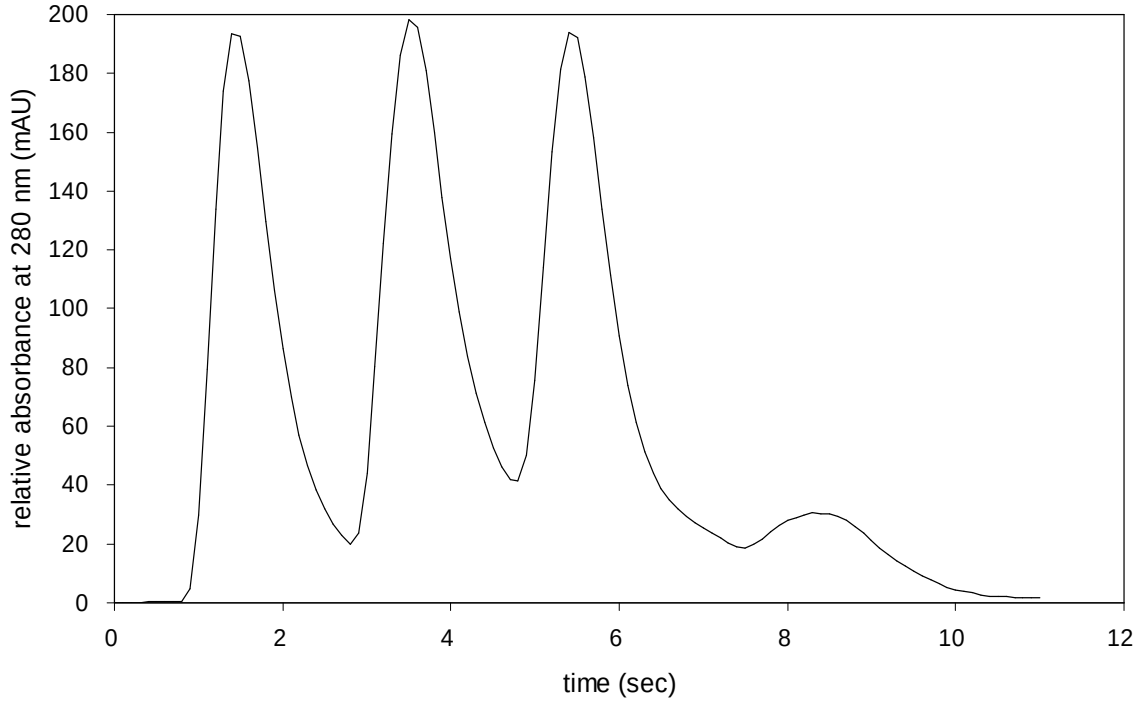
Diffusivity of the molecules

molecule	MW	D_e (cm^2/s)
H^+	1 Da	1×10^{-4}
NaCl	58 Da	1.4×10^{-5}
hemoglobin	64 kDa	7×10^{-7}
BSA	66 kDa	6.1×10^{-7}
urease	482 kDa	3.5×10^{-7}
cucumber mosaic virus (CMV)	6 000 kDa	1.2×10^{-7}
tobbaco mosaic virus (TMV)	40 000 kDa	5×10^{-8}
DNA	4.4 kbp	1.9×10^{-8}
DNA	33 kbp	4×10^{-9}

$$\langle t \rangle = \frac{d^2}{D_e}$$

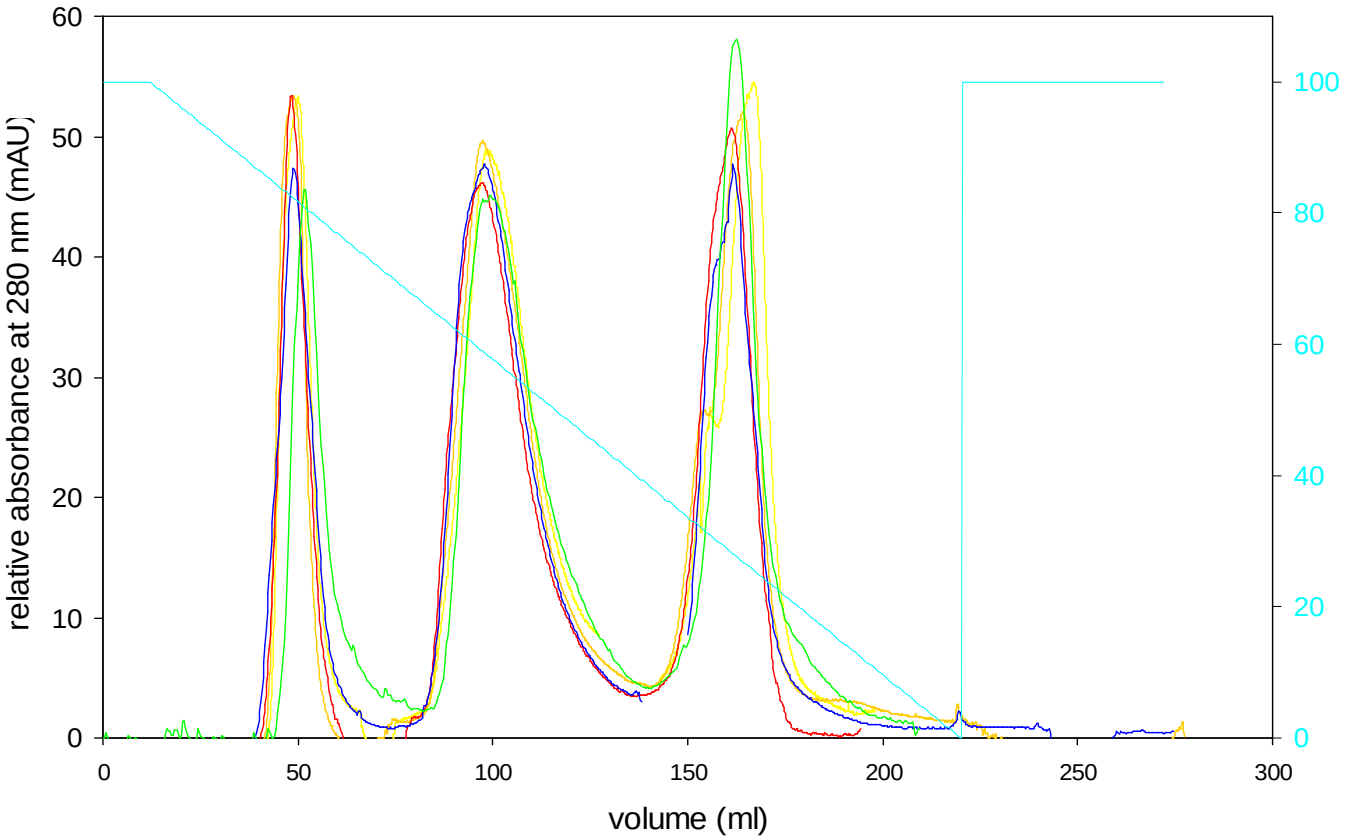
If a pore diameter is 2 μm and molecule diffusion is 1×10^{-8} cm^2/s, than the time for the molecule to reach pore wall is 4 s. The shortest monolithic columns have the length of 3 mm. To give to molecule enough time to reach the pore surface maximal flow rate is 5 ml/min. On the other hand, if a distance of 3 mm should be passed by diffusion, required time would be 9×10^6 s or approximately 3.5 months.

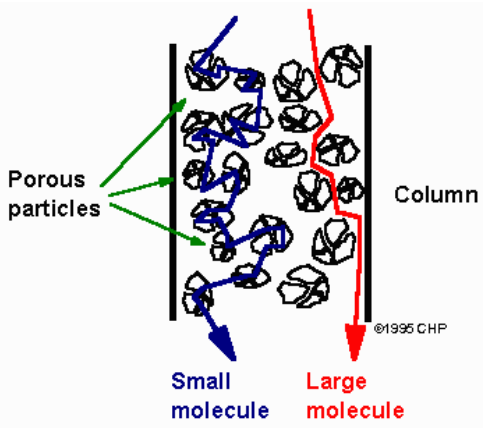
Extremely fast gradient separation of proteins using a monoliths



Štrancar et al., Anal. Chem. 68 (1996) 3483 .

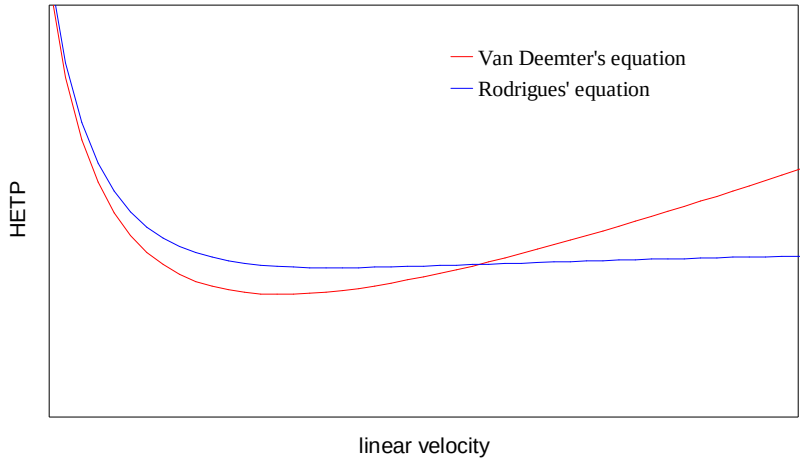
Flow independent binding resolution





Gradient separation of three proteins using CIM® DEAE disk monolithic column at different flow rates - normalized to elution volume

Effect of linear velocity on HPLC column efficiency



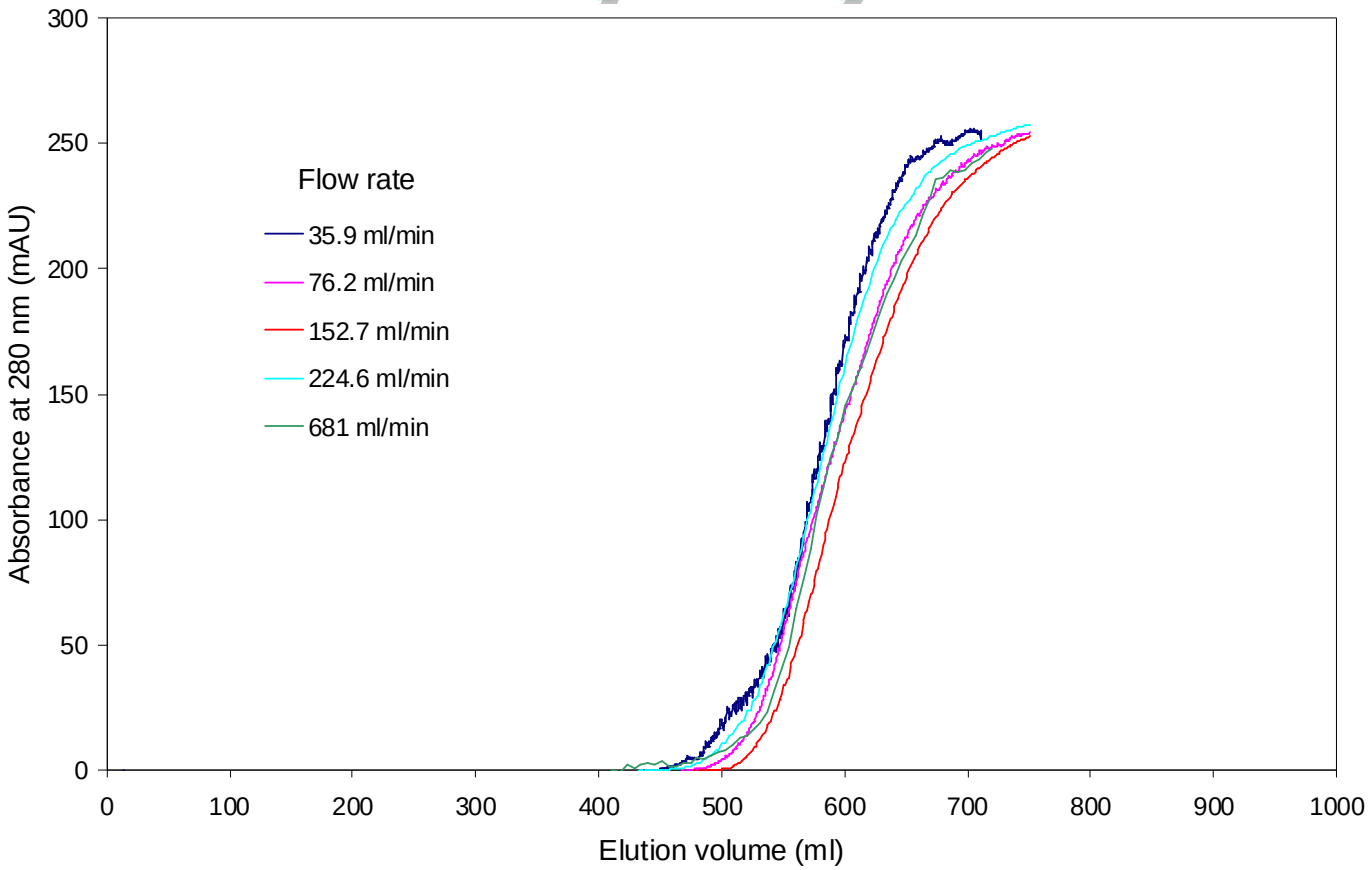
$$HETP = A + \frac{B}{u} + C * u$$

Van Deemter's equation

$$HETP = A + \frac{B}{u} + C * u * f(\lambda)$$

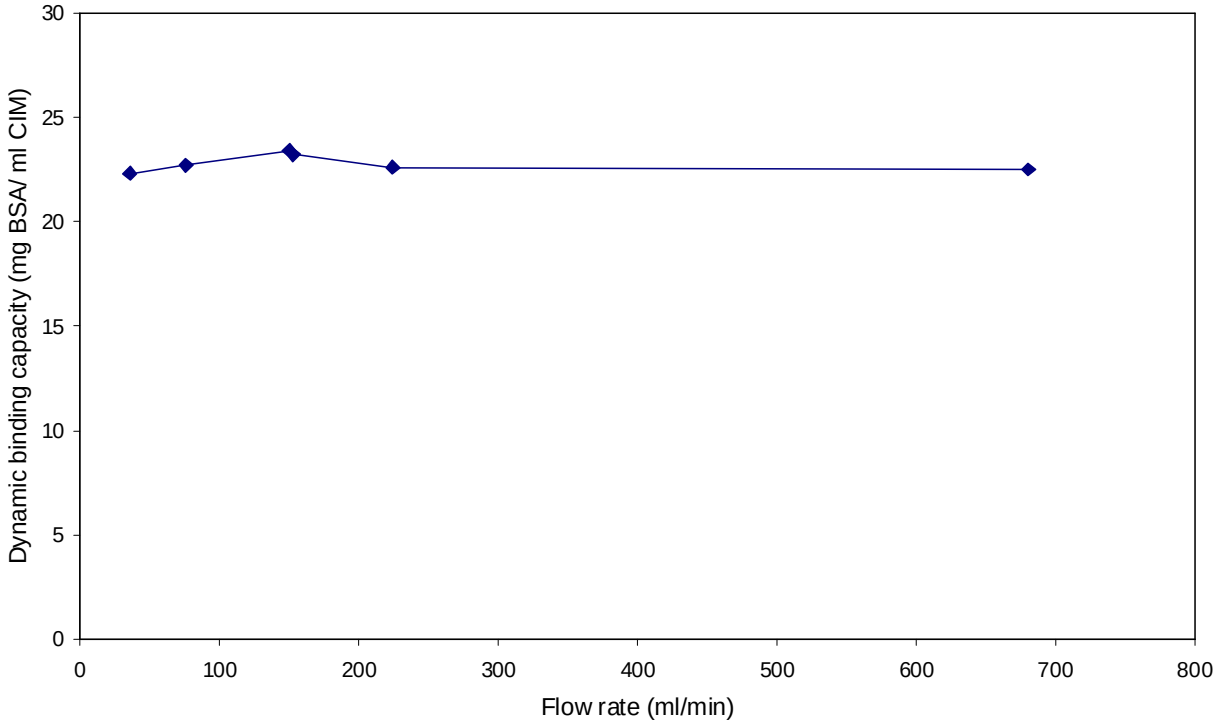
Rodrigues' equation

Flow independent dynamic binding capacity



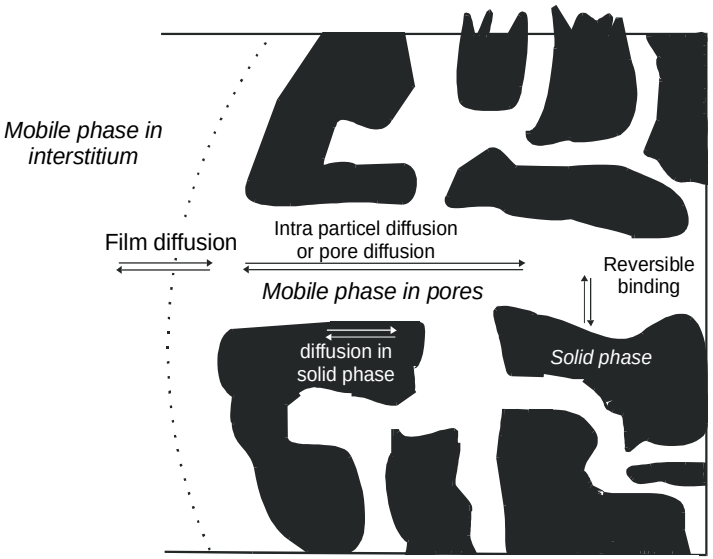
BSA breakthrough curves obtained at different flow rates on a CIM® 80 ml monolithic column

Effect of the flow rate on the maximal dynamic binding capacity



Maximal binding capacity obtained at different flow rates on a CIM® 80 ml monolithic column

Effect of the structure on ligand accesibility

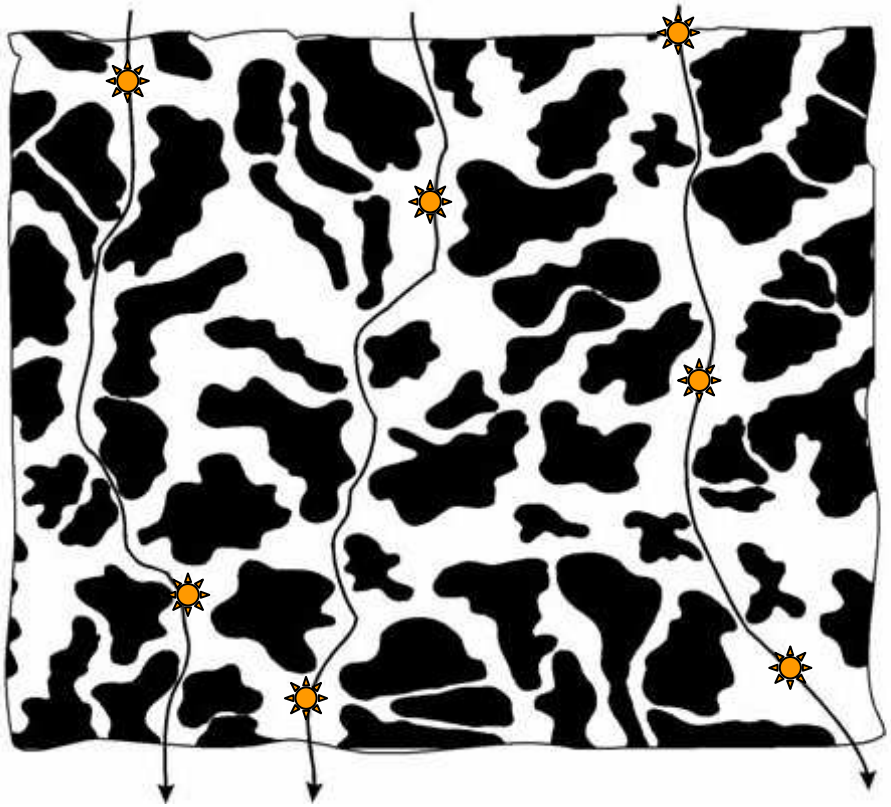


Accesibility might be restricted for large molecules

Monolith

Pores are interconnected channels – all surface is accesible for large molecules

High pDNA & virus binding capacity



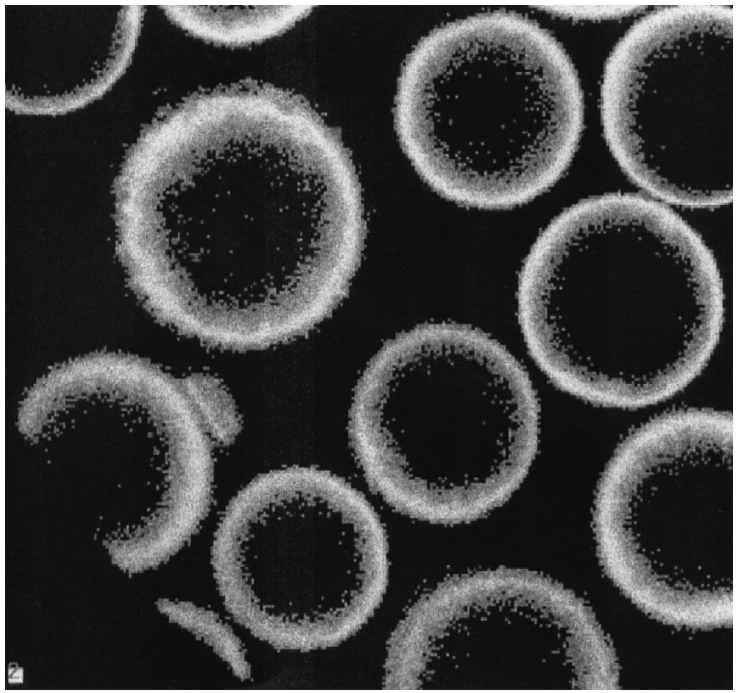
Pores too small for pDNA & viruses!

- Binding mostly on outer surface
- Too small surface area
- Small binding capacities

Large flow through pores

- Internal surface accessible
- High binding capacities

Effect of molecule size on surface accesibility



Confocal images of colored DNA on the chromatographic particles – no DNA penetration into the particles.

Ljunglöf et al., J. Chromatogr. A, 844 (1999) 129.

Conclusions

❖Monolithic GMA/EDMA polymers represent a new and innovative type of stationary phases for rapid chromatographic analysis of large biomolecules

❖In contrast to conventional stationary phases monoliths are formed from single piece of highly porous polymeric material, giving them higher permeability and consequently lower back pressure than conventional sorbents.

❖Because of mass transfer governed by convection these chromatographic materials maintain high separation efficiency, even at high flow-rates. From same reason dynamic binding capacity is independent of linear velocity.

❖Due to large pore size monolithic chromatographic materials enable good surface accessibility even for extremely large biomolecules like pDNA and viruses.

Thank you