Role of polymer chemistry in cell encapsulation by polyelectrolyte complexation in biomedical and biotechnological applications

Igor Lacík

Polymer Institute of the Slovak Academy of Sciences

Dept. of Special Polymers and Biopolymers

Bratislava, Slovakia

igor.lacik@savba.sk

www.polymer.sav.sk/lacik.html

OUTLINE

- **ENCAPSULATION**
  - **Why polymer chemist(ry) should enter encapsulation?**
- **POLYMERS IN CAPSULES**
  - **Polyelectrolytes as a capsule material**
  - **Case 1: *Pros and cons* of Alginate-based capsules**
  - **Case 2: Alternative capsules "PMCG"**
  - **Process, mechanism, understanding and optimization**
- **APPLICATIONS – Biomedicine & Biotechnology**
- **CONCLUSIONS**

Why polymer chemist(ry) should enter…

Premise of encapsulation:

SEMIPERMEABLE MEMBRANE
made of POLYMERS

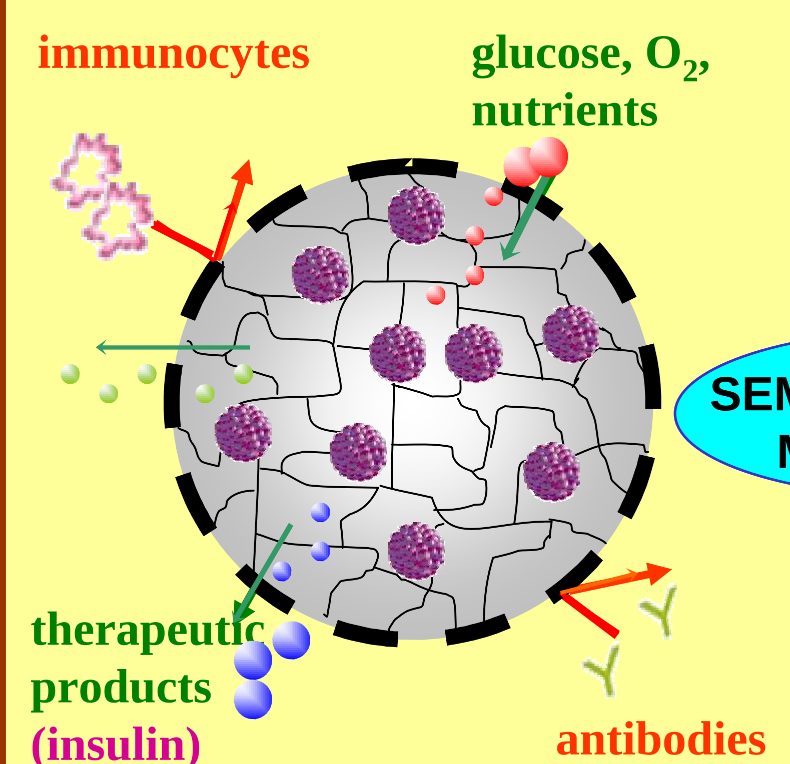
- **to concentrate encapsulated material**
- **to protect encapsulated material**
- **to release encapsulated material in a controlled way**

Why polymer chemist(ry) should enter…

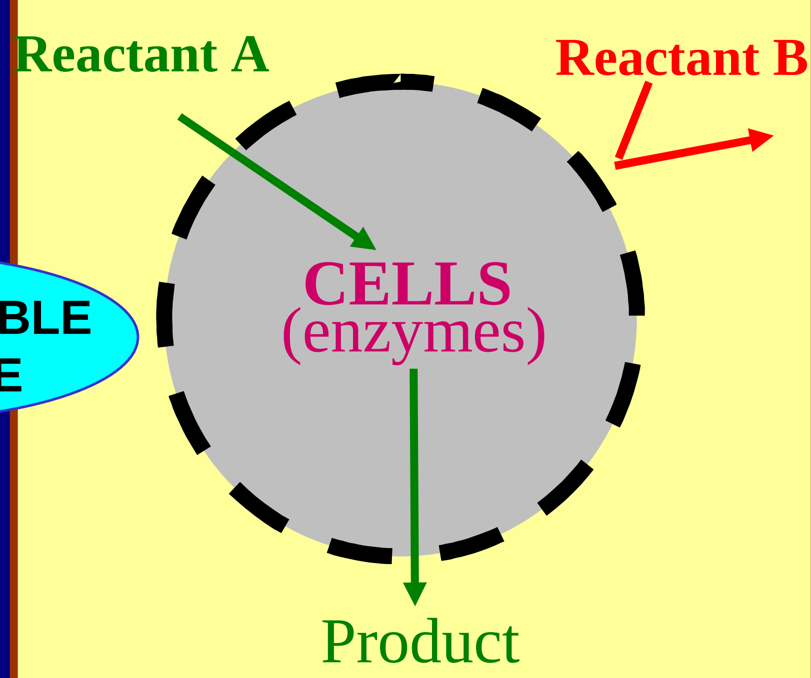
BIOMEDICINE
immunoprotection

BIOTECHNOLOGY
specifically catalyzed reactions

islets of Langerhans
diabetes treatment



chemistry
food technology
pharmaceuticals
cosmetics



SEMIPERMEABLE MEMBRANE

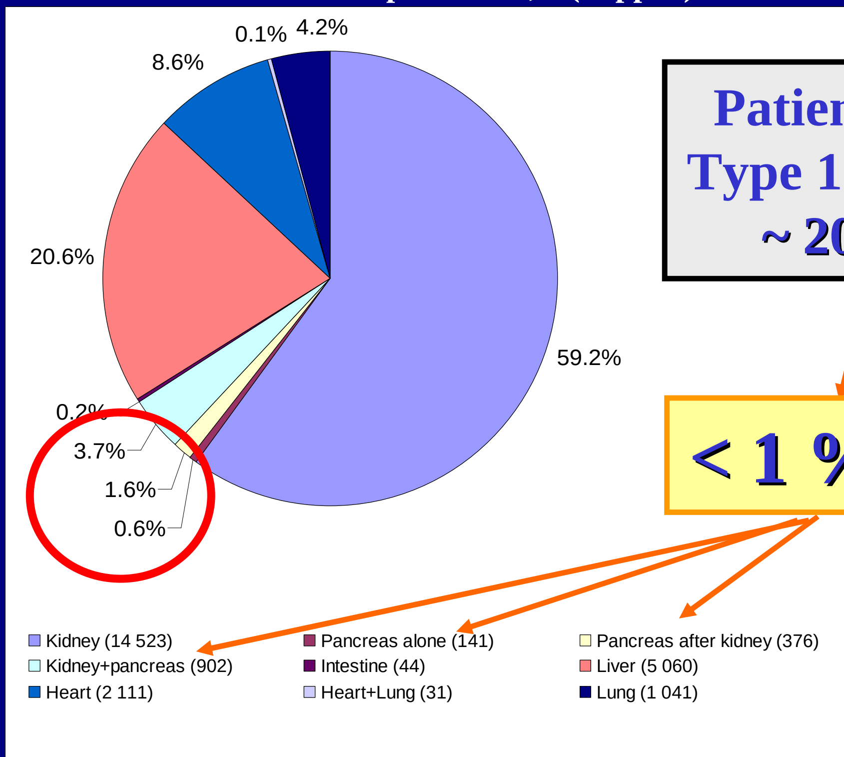
- still in development stage
- a lot of involvement (companies, universities)

- already as industrial process

Detour #1: pancreas (whole organ) transplantation

USA: Number of organ transplantations in 2002 (24 544)

Port et al. Am J Transplant 2004, 4 (Suppl.9): 7-12



Patients with Type 1 Diabetes ~ 200 000

< 1 %

Success rate of pancreas transplantation 1996-2001

C.N.Street et al, Int J Biochem Cell Biol 2004

Problems in this strategy of diabetes treatment:

- Ø donors
- Ø major operation
- Ø cost

? TRANSPLANTANTION OF VIABLE CELLS?

Detour #2: Cell therapy in diabetes treatment

Method	Results	Drawbacks

Edmonton protocol

- human islets to liver portal vein (11 000/kg)
- islet isolation protocol!!!
- selection of immunosuppressive drugs!!!

Shapiro et al. *N Engl J Med* 2000,**343**,230

- **100 % of patients: physiological blood glucose level in 7 patients within 1 year**
- follow-ups: Edmonton & tens of other groups USA/Canada/Europe

follow up: Bertuzzi et al. *Diabetologia* 2002,**45**,77

- cell source (2 donors/1 transplant)
- **immune response (allo-, auto-)**
- **?** long-term effect of immunosuppressive drugs

http://www.med.ualberta.ca/islet/

Stem cells and precursor cells

- embryonic cells
- adult pancreatic cells
- other sources

Street et al, *Int. J. Biochem Cell Biol* 2004, **36**,667

- successful experiments in mice
- **a number of open questions**
- **cell cluster producing insulin**

- **autoimmune response**
- balance between proliferation and insulin production
- **?** unknown effects (tumors…)

Cell encapsulation

- immunoisolation of donor cells behind semipermeable membrane
- **immunosuppression-free**

- first test in 1994
- “boom”in 90-ties
- allo- and **xenotransplanted** cells to different recipients

deVos et al, *Diabetologia* 2002, **45**, 159

Orive et al, *Nature Med* 2003, **9**, 104

- progress???
- **definite type and material remains to be answered**

Why polymer chemist(ry) should enter…

…to designed semipermeable membrane

biomedical applications

Biological criteria

- biocompatibility
- resistance to biodegradation
- non-cytotoxicity (host & transplanted cells)

Physical and chemical criteria

- mechanical stability
- chemical stability
- adjustment
  - size/shape
  - mechanical properties
  - permeability
  - membrane thickness

Process criteria

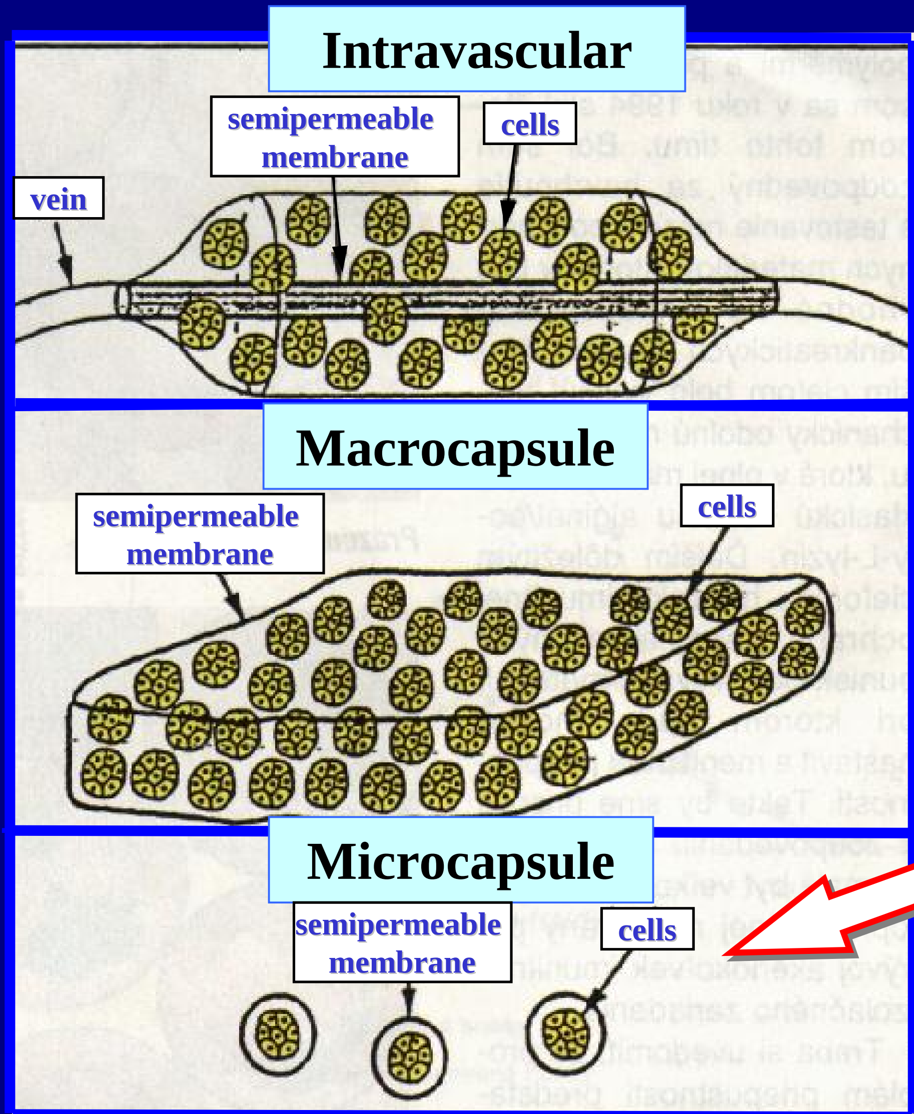
- homogeneity
- capacity
- reproducibility
- availability

biotechnological applications

igor.lacik@savba.sk

Why polymer chemist(ry) should enter…

Semipermeable membrane = polymer



polyacrylates

? major surgery / thrombosis / retrieval

- modified cellulose
- poly(acrylonitrile-co-vinylchloride) hollow fibres
- teflon planar membranes
- polyesters
- macrobeads of polysaccharides

? diffusion / islet necrosis / mechanical properties

ogels:

- gelling polysaccharides
  - polyelectrolyte complex
  - temperature induced sol-gel process
- solvent extraction/precipitation

? retrieval

OUTLINE

- ENCAPSULATION

  Why polymer chemist(ry) should enter encapsulation?

- POLYMERS IN CAPSULES

  Polyelectrolytes as a capsule material

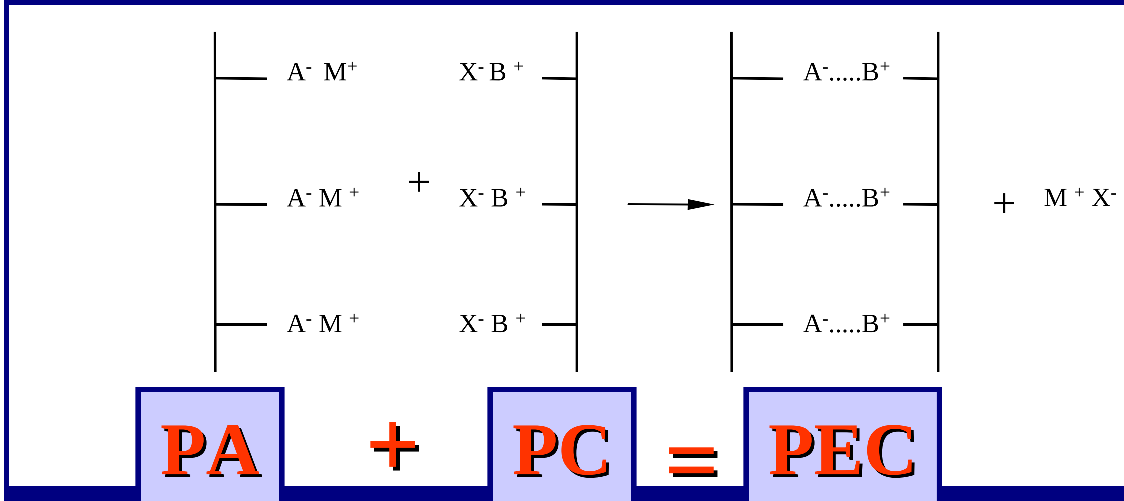
  Case 1: *Pros and cons* of Alginate-based capsules

  Case 2: Alternative capsules "PMCG"

  Process, mechanism, understanding and optimization

- APPLICATIONS – Biomedicine & Biotechnology
- CONCLUSIONS

Capsules by polyelectrolyte complexation

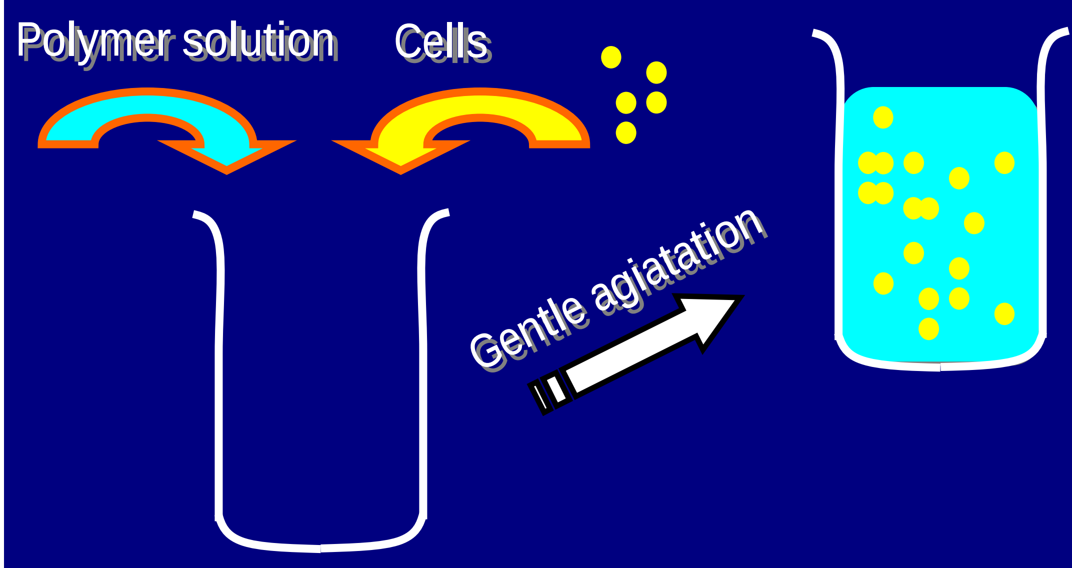


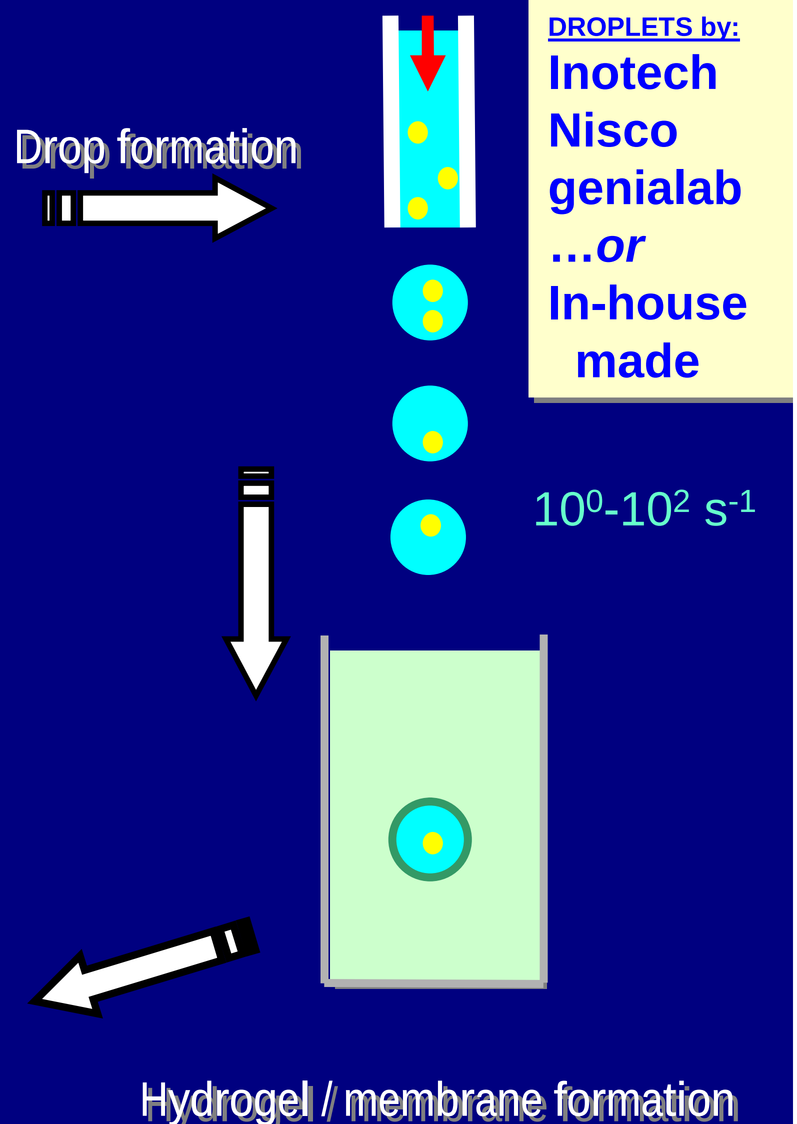
Polyanion (PA) and poly- cation (PC) via (predominantly) LONG-RANGE COULOMBIC INTERACTIONS between oppositely charged polymers will ALWAYS (!) form polyelectrolyte complex (PEC)

- **Overlapping fields**
  (i) general macromolecular chemistry
  (ii) specificity of polyelectrolyte chemistry
  (iii) hydrogels and physically crosslinked networks
  (iv) membranes.

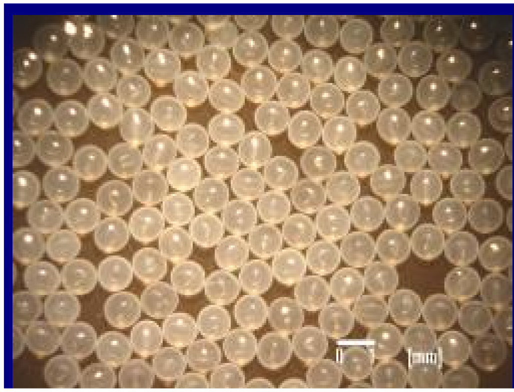
? **CAPSULE with adjustable properties**
è **serving the purpose**

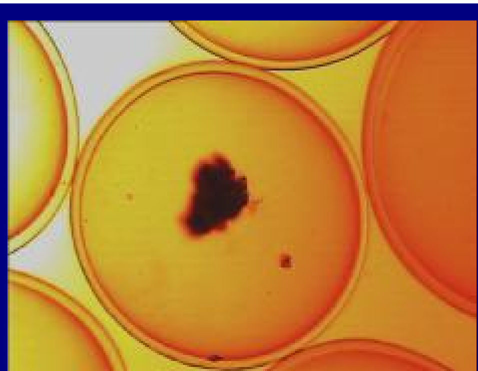
Polyelectrolyte comlexation: How the cells are encapsulated





Mild, fast, controlled procedure at physiological conditions
è POLYELECTROLYTE COMPLEXATION





Polyelectrolytes - categorization

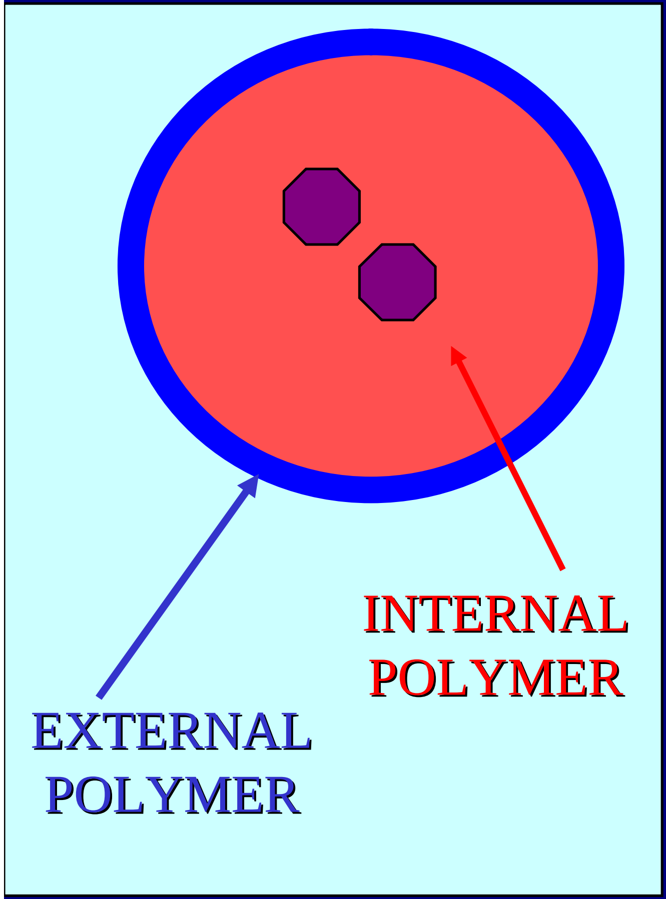
- type of charge – PA vs. PC
  charge density
  charge distributon
- acid-base characteristics
  weak vs. strong acids and bases
- molecular weight
- MWD
- chemical distribution
  (homopolym. vs. copolymers)

- hydrophobic groups
- H-bonds
- gel-formation in the presence of simple electrolytes
- origin - synthetic vs. narural vs. modified
- chain architecture – linear vs. branched
- monomer type - flexible vs rigid

Many possibilities → **ALL FACTORS NEED TO BE CONSIDERED WHEN STATING SUITABILITY OF A GIVEN POLYMER**

Polyelectrolytes - selection

Polyanion vs. Polycation



PA – "biocompatible"
– not critically toxic
– internal polymer

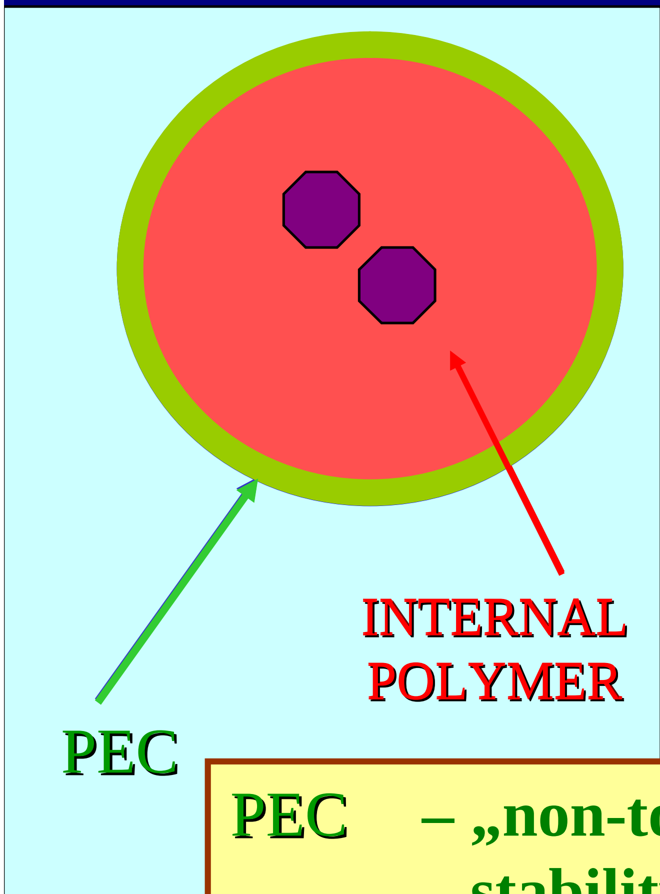
Sodium alginate
Cellulose sulfate
Carboxymethyl cellulose
Hyaluronic acid
Pectates
....
Polyacrylic acid
PolyAMPS
………..

PC – decomposition of cell walls
– positive charge has to be SATURATED
– external polymer

Chitosan
…………
Poly(L-lysine)
Poly(L-ornithine)
....
Poly(methylen-co-guanidine)
Poly(vinylamine)
Poly(ethylenimine)
Poly(DADMAC)
Poly (N-vinylpyrrolidone)
....

Polyelectrolytes - selection

Polyanion vs. Polycation



PA – biocompatible
– not critically toxic
– internal polymer

Sodium alginate
Cellulose sulfate
Carboxymethyl cellulose
Hyaluronic acid
Pectates
…………..
Polyacrylic acid
PolyAMPS
…………

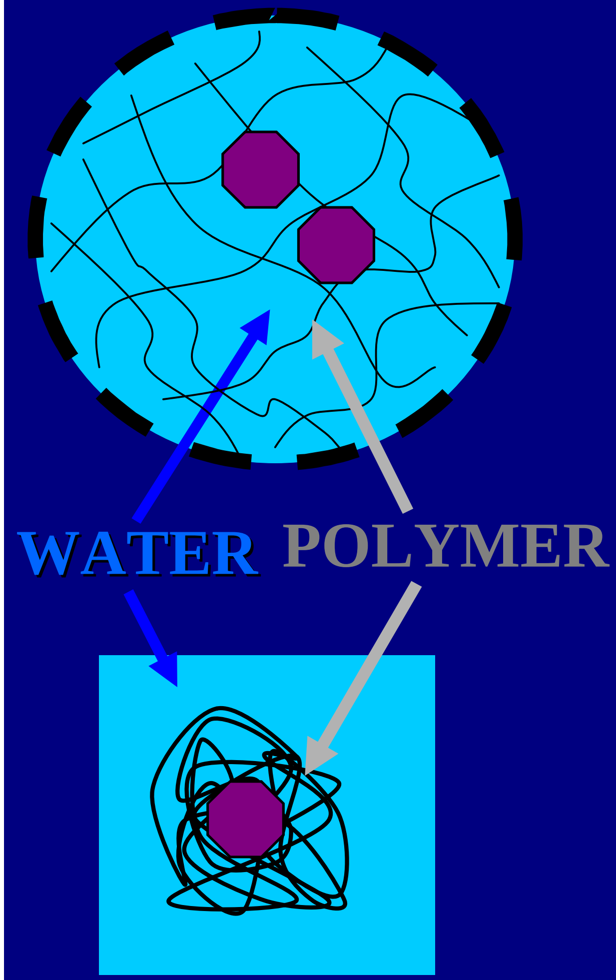
PC – decomposition of cell walls
– positive charge has to be SATURATED
– external polymer

Chitosan
…………
Poly(L-lysine)
Poly(L-ornithine)
……………
Poly(methylen-co-guanidine)
Poly(vinylamine)
Poly(ethylenimine)
Poly(DADMAC)
Poly(N-vinylpyrrolidone)
…………..

PEC – „non-toxic, biocompatible“
– stability! is critical
è multiple layers PA/PC/PA/PC/PA...

Polyelectrolytes - selection

Charge density



AIM → HYDROGEL (polym. conc. → 0 %)

- **physiological environment for cells**
- **strict selection of PE's to balance interactions between PA and PC**

STRONG INTERACTIONS → COACERVATE

polym. conc. →100 %

- **lost control over *membrane* properties**

Additional factors

è **polymer concentrations, pH, ionic strength, reaction time…**

Polyelectrolytes - selection

Molecular weight and MWD

Critical parameter for

- → solution rheology
- → drop and capsule shape and surface topology
- → mutual diffusion of PA and PC zones (pH, ionic strength)
- → osmotic pressure
- → complex stability (biocompatibility and capsule overall performance

RECOMMENDATION – internal polymer é M.h. (rigid chain, gelled ionotropically by small ions)

100 – 1000 kDa

– external polymer ê M.h. (flexible chain)

5 – 20 kDa

Consider in line with other parameters

– concentration, time of reaction, process parameters...

Polyelectrolytes - selection

Natural polymers

predominantly used as internal polymers

- advantage – ionotropically formed gels
  – diversity
  – availability and cost

- drawbacks – purity
  – standardization!!!
  – overcoming these problems ????

Synthetic polymers

- advantage – "simply" prepared, modified, purified & characterized

- drawbacks – difficult to mimic, by *e.g.* secondary structure, the advantageous properties of natural polymers

OUTLINE

- **ENCAPSULATION**

  Why polymer chemist(ry) should enter encapsulation?

- **POLYMERS IN CAPSULES**

  Polyelectrolytes as a capsule material

  Case 1: *Pros and cons* of Alginate-based capsules

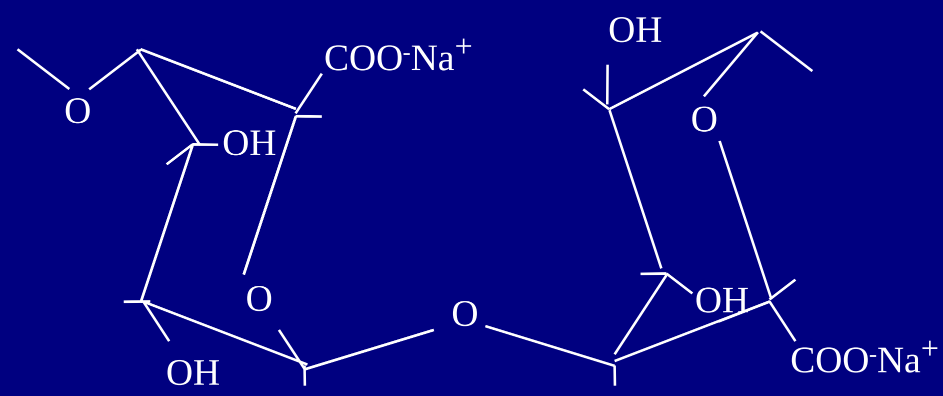
  Case 2: Alternative capsules "PMCG"

  Process, mechanism, understanding and optimization

- **APPLICATIONS – Biomedicine & Biotechnology**

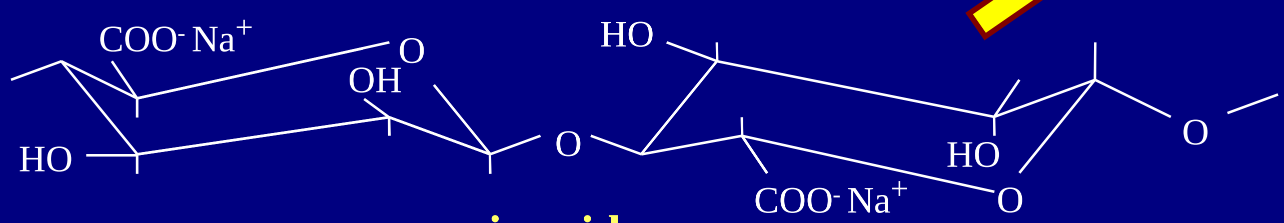
- **CONCLUSIONS**

Sodium alginate in microparticle and capsule formation

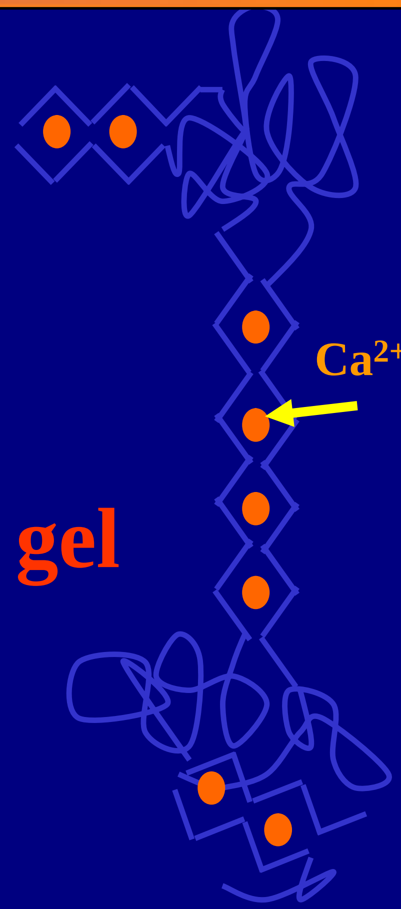


guluronic acid
(if >60% high-G alginates)

+ $CaCl_2$ = gel



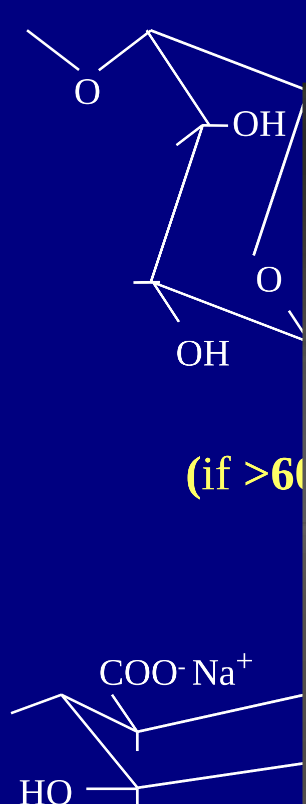
mannuronic acid
(if >60% high-M alginates)



egg-box model

EXTREMELY simple → **may cause a problem if the „polymer nature“ of alginate is underestimated**

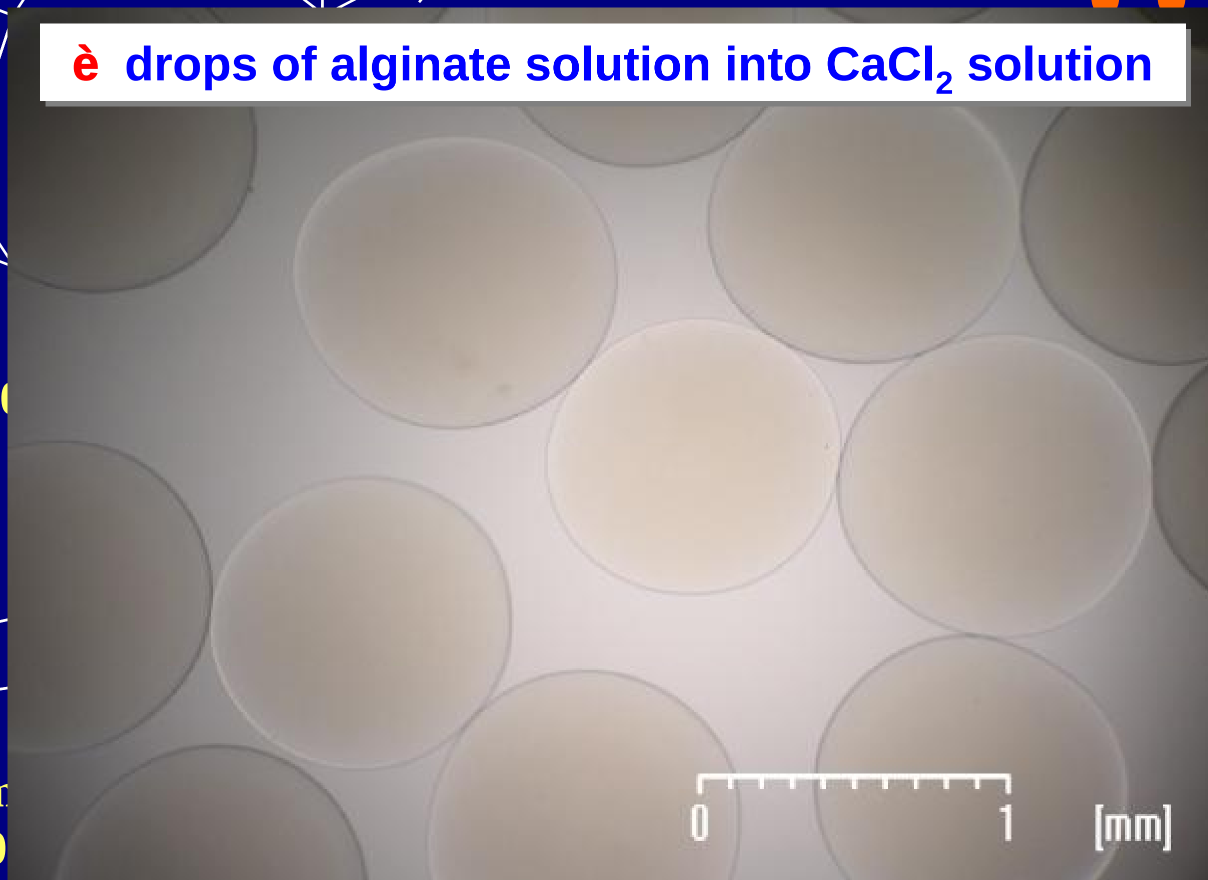
Sodium alginate in microparticle and capsule formation

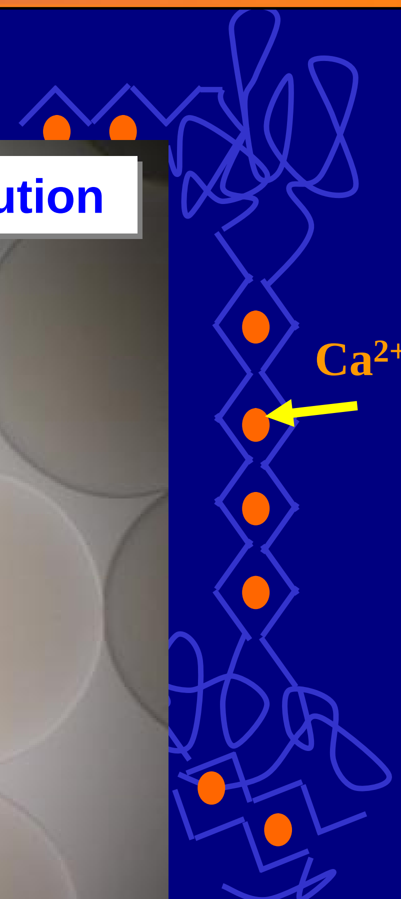


(if >60

m

(if >60





box model

EXTREMELY simple → may cause a problem if the „polymer nature“ of alginate is underestimated

Sodium alginate ionotropic gels

Bead inhomogeneity

Na-alginate into $CaCl_2$

Gell density across the capsule

? SA concentration in *core* vs *surface*

Polymer concentration è
Crucial for bead/capsule properties

SA diffusion towards gelling zone ?

low Ca^{2+}
low Na^{+}

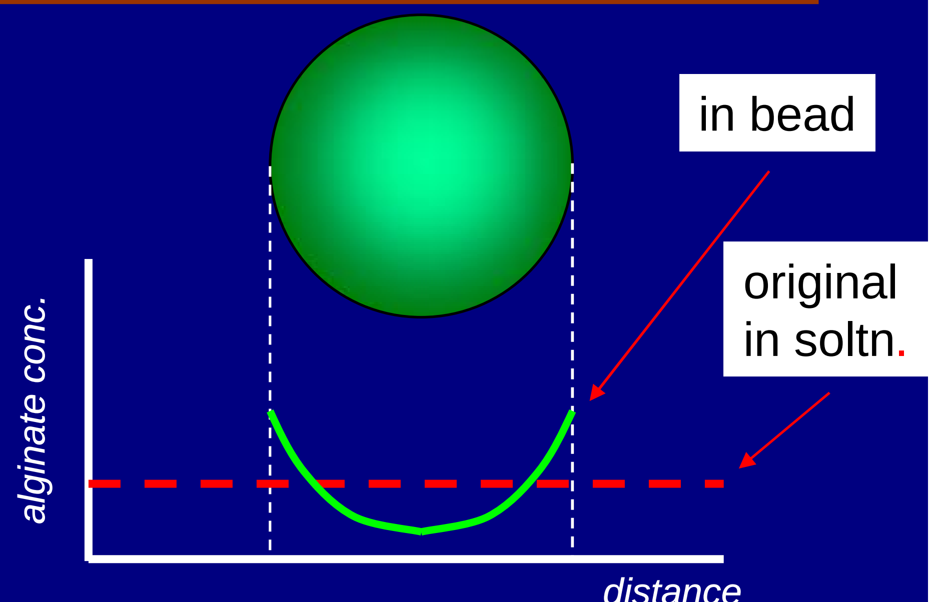
FAST

Inhomogeneous bead

high Ca^{2+}
high Na^{+}

SLOW

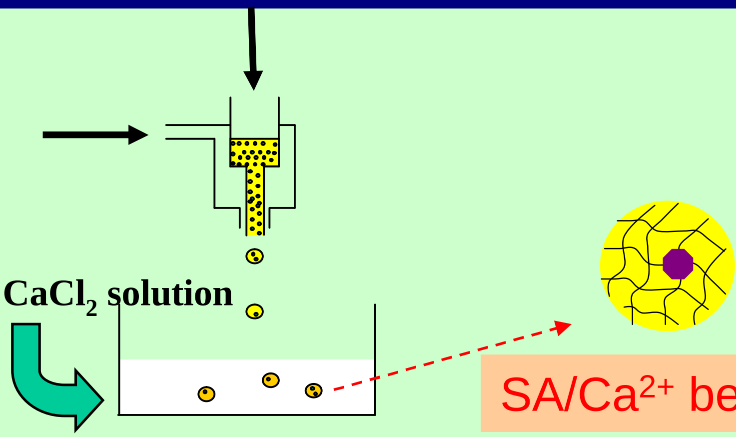
Homogeneous bead



Sodium alginate in capsules: history to presence

Lim and Sun, *Science* ***210***, 908-909,1980

1st step:
drops of SA (0.6-0.8%) containing islets of Langerhans (Wistar rats) gelled by Ca^{2+} ions



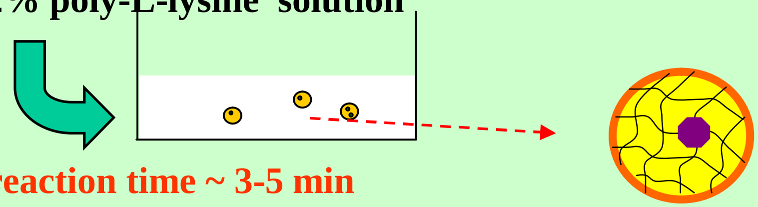
1.5% $CaCl_2$ solution

SA/Ca^{2+} bead

collection time + gelling time ~ ? + ? min

2nd step:
membrane formation

0.02% poly-L-lysine solution

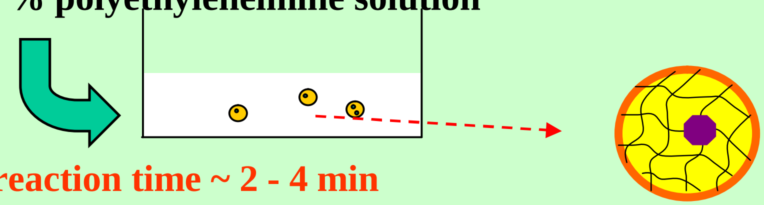


reaction time ~ 3-5 min

SA/Ca^{2+}-PLL coated bead

3rd step:
strengthening the membrane

0.2 % polyethyleneimine solution

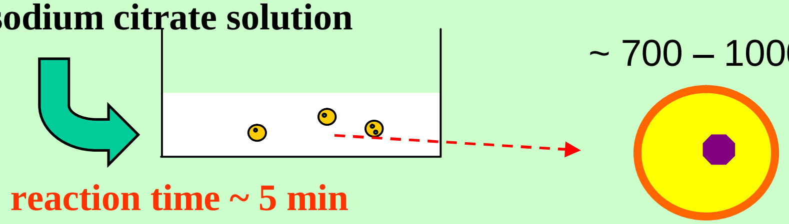


reaction time ~ 2 - 4 min

SA /Ca^{2+}- PLL/PEI coated bead

4th step:
Dissolving SA/Ca^{2+} gel

sodium citrate solution



~ 700 – 1000 μm

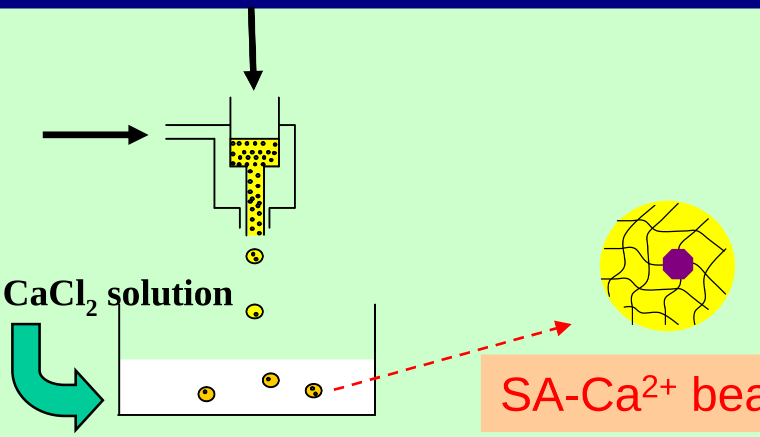
reaction time ~ 5 min

SA-PLL/PEI hollow capsule

CELL VIABILITY è four months in vitro
è 3 weeks in vivo

Sodium alginate in capsules: history to presence

1st step:
drops of SA (1-3 %) containing islets of Langerhans gelled by Ca^{2+} ions

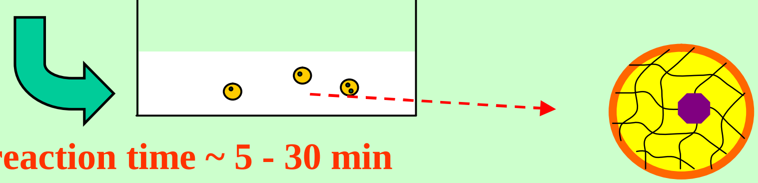


SA-Ca^{2+} bead

collection time + gelling time ~ 5 + 5 min

2nd step:
membrane formation

~ 0.02% poly-L-lysine solution



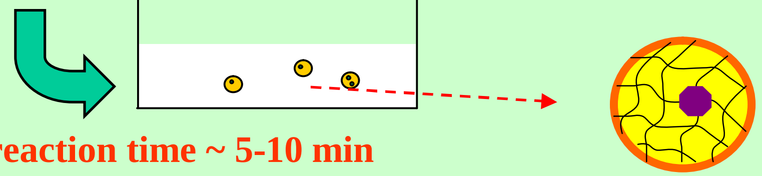
reaction time ~ 5 - 30 min

SA/Ca^{2+}-PLL coated bead

Modified protocol of Lim and Sun - till now

3rd step:
saturating residual cationic charge by SA layer

0.1 % sodium alginate solution

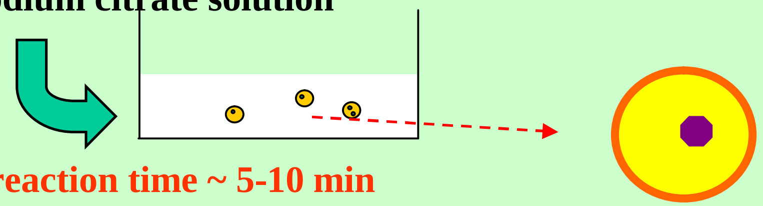


reaction time ~ 5-10 min

SA/Ca^{2+}-PLL-SA coated bead

4th step:
Dissolving SA-Ca^{2+} gel

Sodium citrate solution



reaction time ~ 5-10 min

SA-PLL-SA hollow capsule

Sodium alginate in capsules: history to presence

Patrick Soon-Shiong et al (VivoRx): a dominant influence on encapsulation community 1990-1998

purified alginate with **high G content**
(mechanical stability & biocompatibility)
ê
SA/PLL/SA hollow capsules
with
15-20 000 islets/kg (allografts)
TX site: peritoneal cavity
ê
Successful dog trials (1992)
ê
Successful clinical trial
(Lancet 1994)

Boom in the field

?

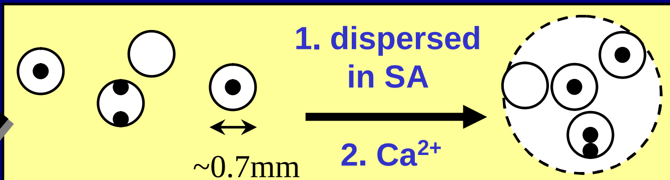
Difficult / impossible to repeat

Understanding:
(i) PEC stability
(ii) capsule formation
(Trondheim, Norway)

US Patents 1998-1999

Capsule and membrane mechanical & chemical stability

- alginate rich in G units
- alginate rich in M units for binding to PLL has to be present (multi-layer)
- no degelling of core by citrate
- stable Ba^{2+} gelation to Ca^{2+}
- anisotropic distribution of alginate in core
- absence of PLL positive charge on surface → macrocapsules

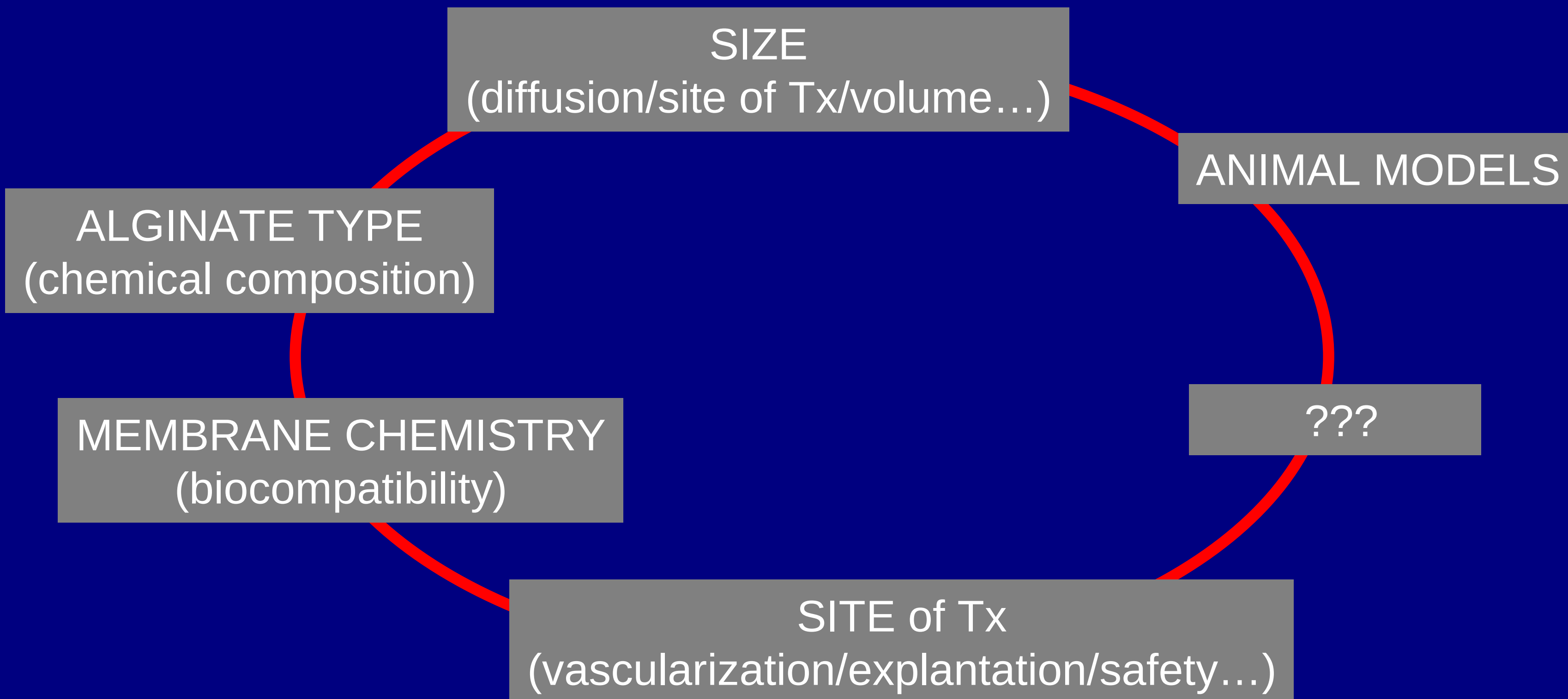


y.2005: clinical trials www.amcyte.com

igor.lacik@savba.sk

Sodium alginate in capsules: history to presence

MODIFICATIONS & **“IMPROVEMENTS”** TO SA/PLL/SA CAPSULE
→ suppress islet necrosis, improve long-term biocompatibility and durability



Sodium alginate in capsules: history to presence

MODIFICATI
→ suppress islet

ALGINATE TYPE
(chemical composition)

MEMBRANE CHEMI
(biocompatibility

- **high-M** alginate is more suitable than high-G yet biocompatibility is an issue of purity (endotoxins, mitogens, phenolic compounds)
  Klöck et al. 1997
- **high-M** alginate is more suitable than high-G for SA/PLL/SA capsules due to efficient binding to PLL
  deVos et al. (1996-2005)
- **high-G** alginate should be the main constituent in SA/PLL/SA capsules for their stability
  Soon-Shiong et al (1998)
- **high-G** alginate is more suitable than high-M for SA/PLL/SA capsules due to improved mechanical stability
  Halle et al (1999)
- **high-G** alginate is more suitable than high-M for SA-Ca^{2+} beads
  deVos et al. (1996-2005)
- **high-M** alginate is more suitable than high-G for SA-Ba^{2+} beads
  Weir et al. (2001-2005)
- **high-G vs high-M** → biocompatibility is an issue of purity
  Orive et al. (2002)
- *purity may not be a major issue* → for PEC capsules, endotoxins as polyanions are captured by cationic charge of membrane
  Wandrey (1999)

Sodium alginate in capsules: history to presence

MODIFIC
→ suppress

- **replacing PLL by poly-L-ornithine (stable complex with SA) Calafiore et al (1995-presence, clinics)**
- replacing PLL by aminopropyl silicate (stable complex, sol-gel) Sakai et al (2001 - presence)
- **SA (macro)bead containing SA-PLL-SA microcapsules (clinics: www.amcyte.com)**
- **avoiding PEC membrane – formation of stable SA/Ba^{2+} beads Weir et al (2001 - presence)**
- coating layer based on the PEG chemistry grafting PEG on PLL Hubbell (1992); **presence devoted to PEG chemistry**
- etc.

ALGINATE TYPE
(chemical composit

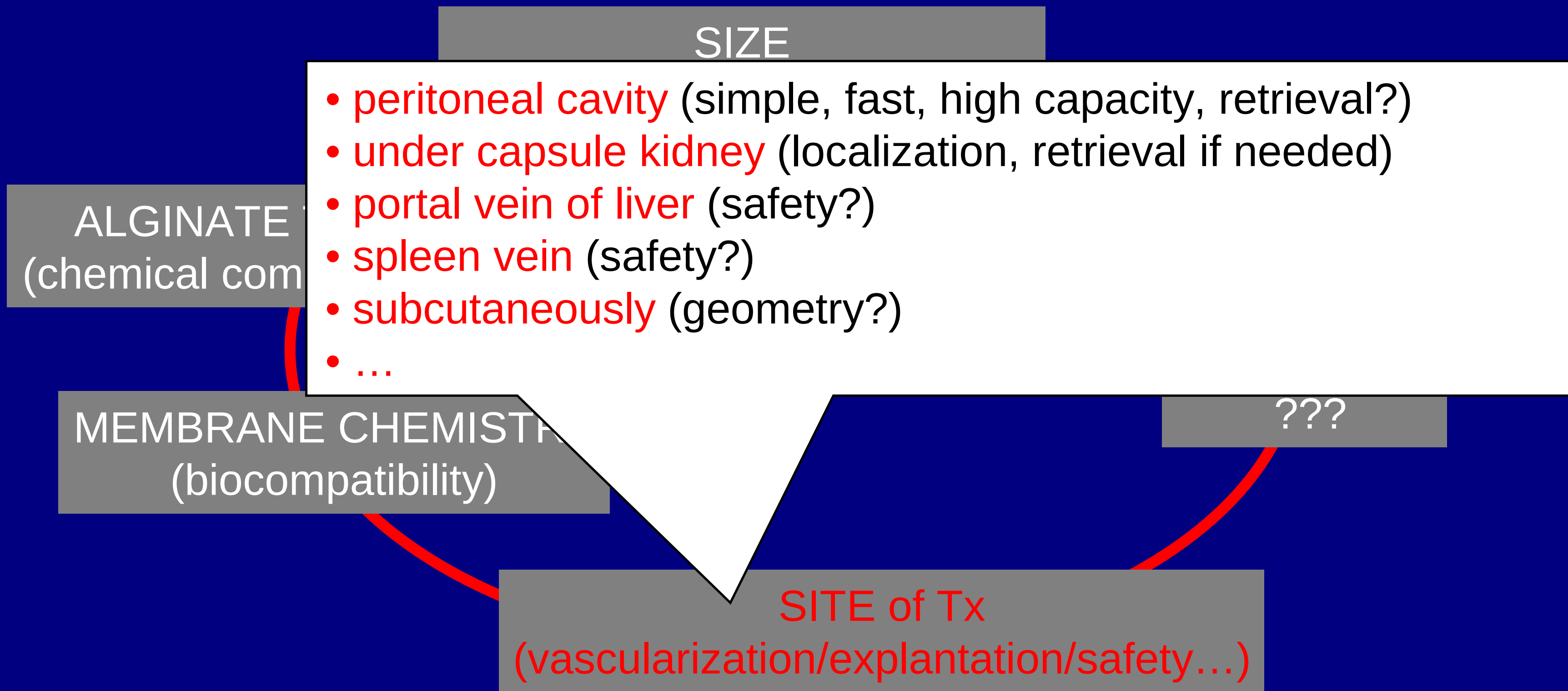
MEMBRANE CHEMIST
(biocompatibility)

???

SITE of Tx
(vascularization/explantation/safety…)

Sodium alginate in capsules: history to presence

MODIFICATIONS & **“IMPROVEMENTS”** TO SA/PLL/SA CAPSULE
→ suppress islet necrosis, improve long-term biocompatibility and durability



Sodium alginate in capsules: history to presence

MODIFICATIONS & **"IMPROVEMENTS"** TO SA/PLL/SA CAPSULE
→ suppress islet necrosis, improve long-term biocompatibility and durability

SIZE
(diffusion/site of Tx/volume…)

ANIMAL MODEL

ALGIN
(chemica

MEME
(b

Calafiore et al 1995 – 2003:
- medium and conventional size microcapsules 600 – 1000 µm
- coherent microcapsules 400 µm

smaller is better, **but** was not proven for dogs

Halle et al 1996 – 1999:
recommended size of microcapsules < 350 µm
more biocompatible than conventional size

deVos et al 1996 – 2002:
large diameter ~ 800 µm
small diameter ~ 500 µm
larger is better = a more adequate islet encapsulation

Strand et al 2002:
larger diameter ~ 500 µm
small diameter ~ 200 µm
islets in vitro = no difference between sizes of capsules at adjusted conditions for capsule formation

Sodium alginate in capsules: history to presence

MODIFICATIONS & **"IMPROVEMENTS"** TO SA/PLL/SA CAPSULE
→ suppress islet necrosis, improve long-term biocompatibility and durability

SIZE
(diffusion/site of Tx/volume…)

ANIMAL MODEL

???

SITE of Tx
(vascularization/explantation/safety…)

- typical animal donors/hosts
  - normal mice
    NOD
  - rats
  - pigs
  - dogs
- allo- vs. xenotransplantations

- **note that successful "Edmonton protocol" could avoid using animal models before the clinical trials**

Sodium alginate in capsules: history to presence

- smoothness vs. roughness, i.e. physical imperfections
  - → general opinion **smoother** is better
  - → possibly **"just rough"** is better (Prof Hilborn, Uppsala)
    - è ??? Quantification

- Ba^{2+} vs Ca^{2+}
  - → the gel/capsule chemical stability
    - è ??? Toxicity of Ba^{2+}

- homogeneous vs. heterogeneous capsule core
  - → meet both opinions
    - è ??? real evidence

- homogeneous vs. heterogeneous capsule core
  - → meet both opinions
    - è ??? real evidence

- difference in modulus of tissue and material
  - è Prof Hilborn, Uppsala

- etc

APSULE
nd durability

MAL MODEL

???

Sodium alginate in capsules: history to presence

MODIFICATIONS & **“IMPROVEMENTS”** TO SA/PLL/SA CAPSULE
→ suppress islet necrosis, improve long-term biocompatibility and durability

SIZE
(diffusion/site of Tx/volume…)

ANIMAL MODEL

???

SITE of Tx
(vascularization/explantation/safety…)

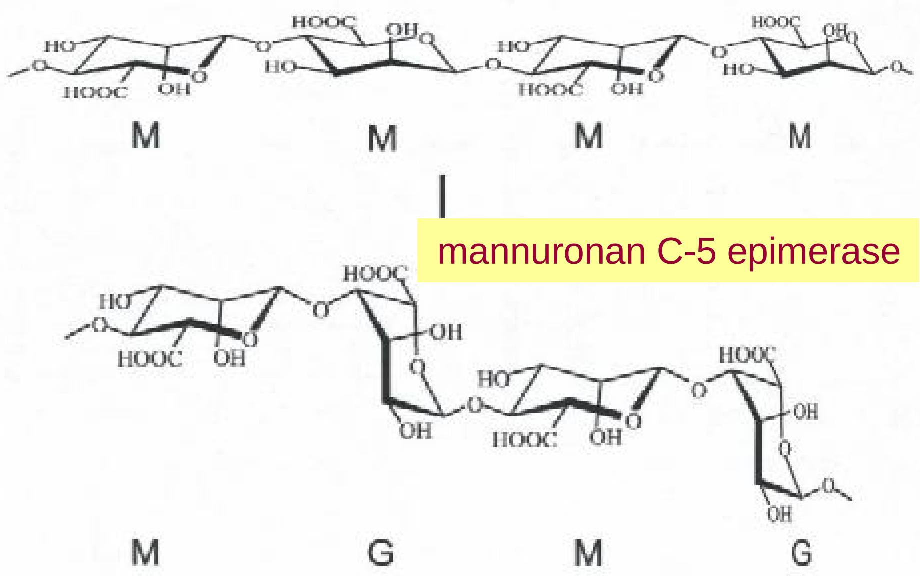
MEMBRANE CHEMISTRY
(biocompatibility)

ALGINATE TYPE
(chemical composition)

CIRCLE OUTCOME: A VERY POOR LEARNING CURVE
è struggles for one searching “some degree of exactness”

Sodium alginate in capsules: history to presence

Enzymatically tailor-made composition of sodium alginate
by group of Prof. S. Bræk, Trondheim Group



J Biomed Mater Res 64A, 540-550 (2002)

Effect on
- è chain flexibility
- è packing density
- è interactivity

Improvement
- è higher stability
- è lower permeability
- è core and coat material

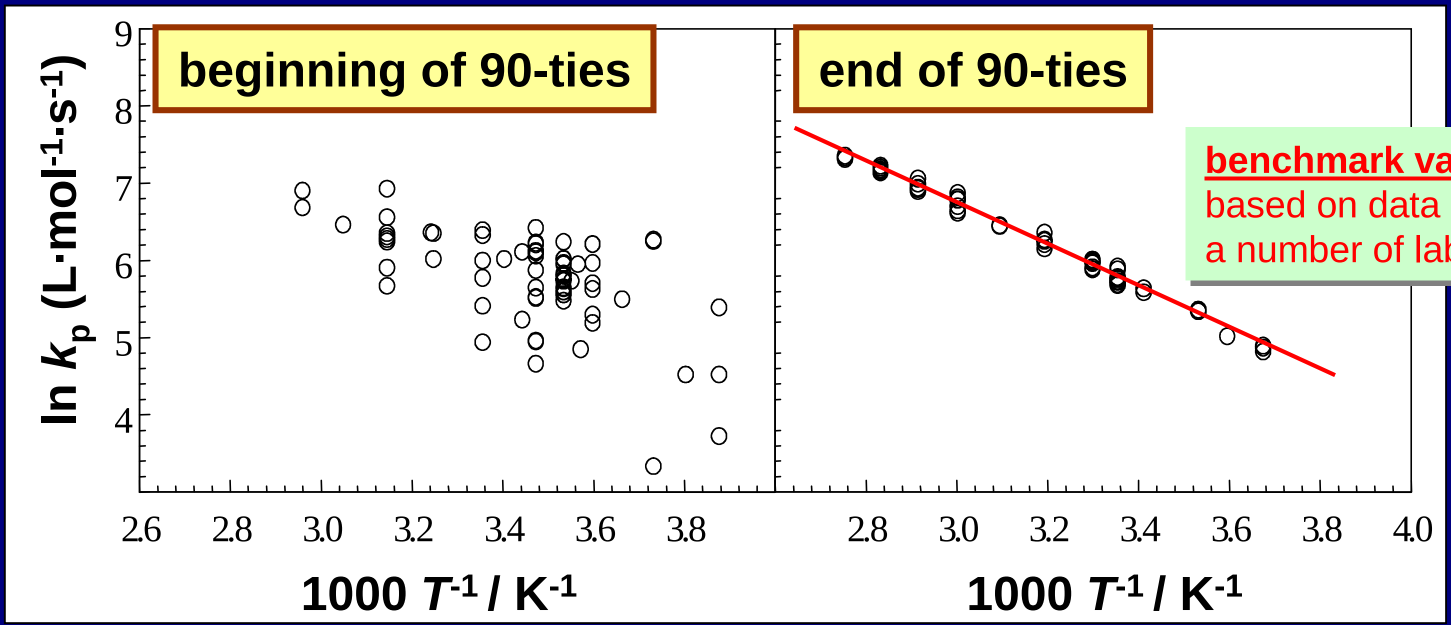
→ a new hope for alginate-based capsules

Detour #3: search for exactness (IL's another field)

IUPAC activities (www.iupac.org/projects/2004/2004-034-1-400.html)

Availability and precision of rate coefficients in free-radical polymerization

Arrhenius plot for propagation rate coefficient for ***methyl methacrylate***



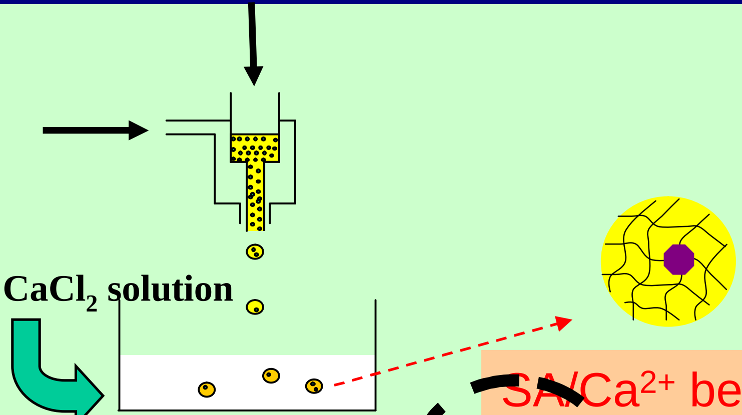
1. **achieved after recognizing drawbacks and critical discussion and setting consistency criteria**
2. **difficult topic but seems a simpler problem than capsule formation**
3. **in encapsulation field, critical discussion might be (is) missing, " blaming nature" for non-consistency**

è primary principle: consistency

Comments on process of capsule formation by PEC

(Lim & Sun, 1980)

1st step:
drops of SA (0.6-0.8%) containing islets of Langerhans (Wistar rats) gelled by Ca^{2+} ions

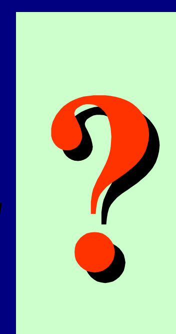


1.5% $CaCl_2$ solution

SA/Ca^{2+} bead

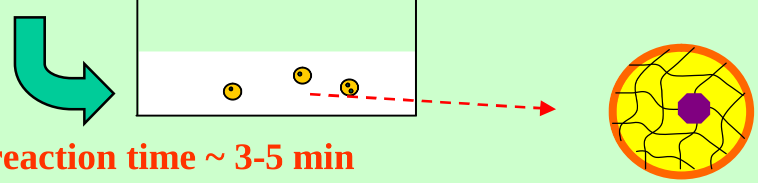
collection time + gelling time ~ ? + ? min

SIMPLE è VASTE AMOUNT OF PAPERS
è ANYBODY CAN DO IT
è ANYBODY CAN REPEAT IT



2nd step:
membrane formation

0.02% poly-L-lysine solution

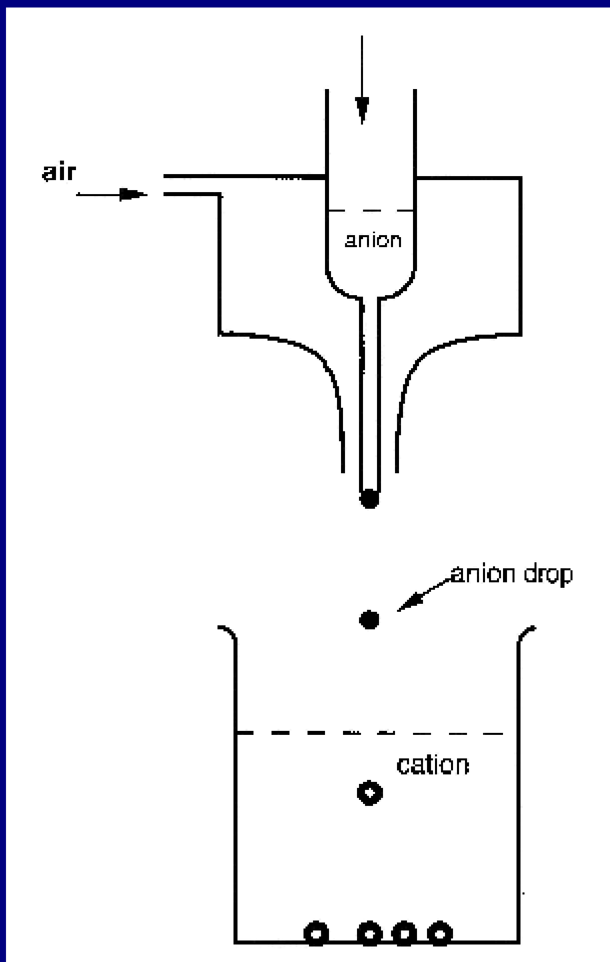


reaction time ~ 3-5 min

SA/Ca^{2+}-PLL coated bead

igor.lacik@savba.sk

Comments on process of capsule formation by PEC



Polyelectrolyte-based beads and capsules

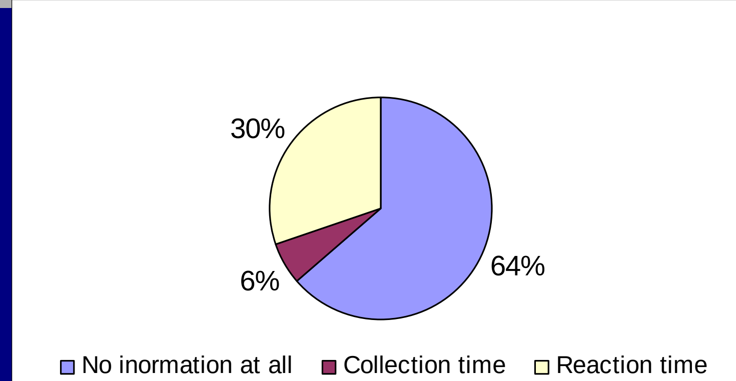
(i) time of droplet collection

(ii) time of gel/membrane formation …

i.e. discontinuous process

take the literature on PEC capsule and find this (**logically required) information**

50 randomly selected papers (>y.1999)



?

- **trivial and/or unimportant information?**
- **confidential information?**
- **hard to define?**

è comparison among the data/groups is questionable

Comments on process of capsule formation by PEC

collection & reaction times

A wrong observation: beads formed by reaction of Ca^{2+} with sodium alginate in a couple of **seconds** and a couple of **minutes** **look similar**

BUT are not identical

•Collection time 5 minutes
•Reaction time 5 minutes

ð Reaction time for individual capsules varies between 5 to 10 minutes

Polymer network

Øgel density
Øeffective molecular weight btw. crosslinks
Øgel thickness
Øswelling
Ø…..

Capsule

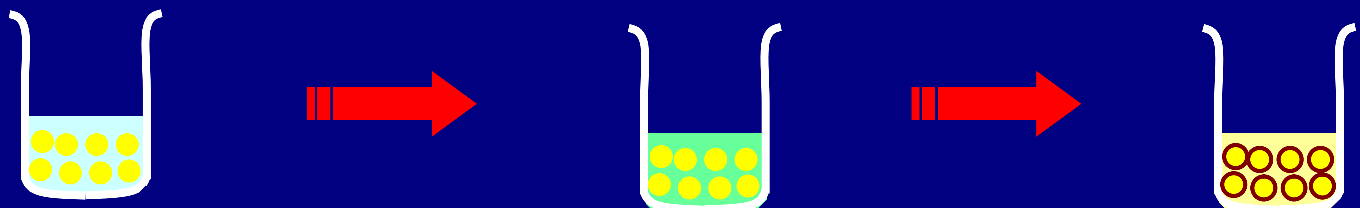
- **permeability**
- **mechanical stability**
- **chemical stability**
- **mass transfer**
- **degree of swelling (size)**
- **post-reactions (PLL, coat…)**
- **cell environment**
- **….**

Averaged properties a difficult to judge the capsule/cell performance (repeatability; discarded systems)

Comments on process of capsule formation by PEC

…limitations in SA-PLL-SA (type of) capsule:

- **capsule inter- and intrabatch homogeneity**



$CaCl_2$ solution
(GELLING REACTION)

NaCl solution
(WASH & POOL COLLECTED BEADS)

PLL solution
(MEMBRANE FORMATION)

– Ca^{2+} exchanged by Na^{+}
– time dependent

Capsule wash
ACTIVE STEP
(Strand et al 2003)

Further exploration for capsule type with:

- **"independent" adjustment of membrane properties?**
  (mechanical properties, thickness, permeability, smoothness)

OUTLINE

- **ENCAPSULATION**

  Why polymer chemist(ry) should enter encapsulation?

- **POLYMERS IN CAPSULES**

  Polyelectrolytes as a capsule material

  Case 1: *Pros and cons* of Alginate-based capsules

  Case 2: Alternative capsules “PMCG”

  Process, mechanism, understanding and optimization

- **APPLICATIONS – Biomedicine & Biotechnology**

- **CONCLUSIONS**

"PMCG" microcapsule: basic chemistry

Vanderbilt University, TN

5 components interact in one step

Polyanion solution

Polycation solution

Poly(methylene-co-guanidine) PMCG — 1-2 %

Sodium alginate (high M) — 0.6-1.2 %

$CaCl_2$ — 0.6-1.2 %

Sodium cellulose sulfate — 0.6-1.2 %

NaCl — 0.9%

Complex stability in PBS (after Ca^{2+} removal)

- - - - - - (dashed)	—— (solid)	━━ (thick)
unstable	***stable***	***highly stable***

"PMCG" microcapsule: basic chemistry

Polyanion soltn.

high viscosity (SA)

&

cellulose sulfate (CS)

ê

Polycation soltn.

poly(methylene-co-guanidine)

&

$CaCl_2$ (gelling cation)

&

NaCl (anti-gelling cation)

- **SA/CS ratio** - specifically interacting polyanions with components of PC solution

- **$CaCl_2$** - gelling with SA è spherical shape
- **PMCG** - low M.w. (Mn = 4500 g/mol) and high charge density è high mobility and reactivity, strongly interactive with CS
- **competitive reaction** between PMCG and Ca^{2+} with the anonic sites
- **anti-gelling Na^+** cations tune the gelling reaction

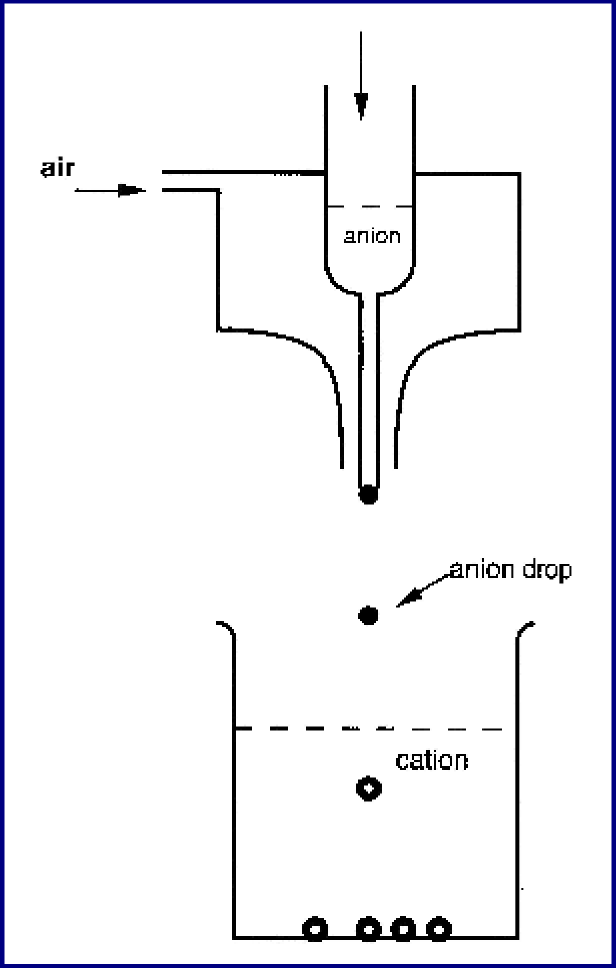
- **reaction is fast** è a few tens of seconds

- **1-step process** è no changes of membrane introduced between gel bead and membrane formation

"PMCG" microcapsule: process of preparation

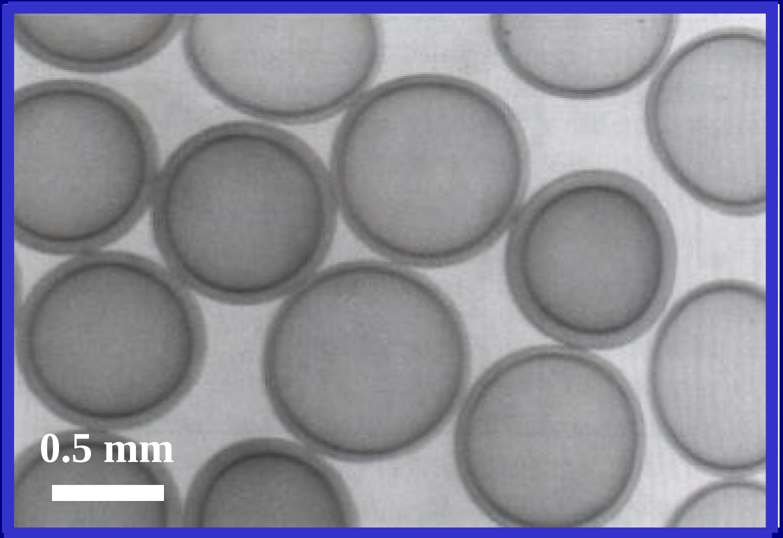
Discontinuous (beaker) collection

typically applied to the PEC-made capsules



- nominal size of droplets generated at ~ 3000/min by air-stripping. size ~ 0.8 mm
- 0.6 % SA + 0.6 % CS in PBS
- 2.2 % PMCG, 0.6 % $CaCl_2$, 1.8 % NaCl in H_2O

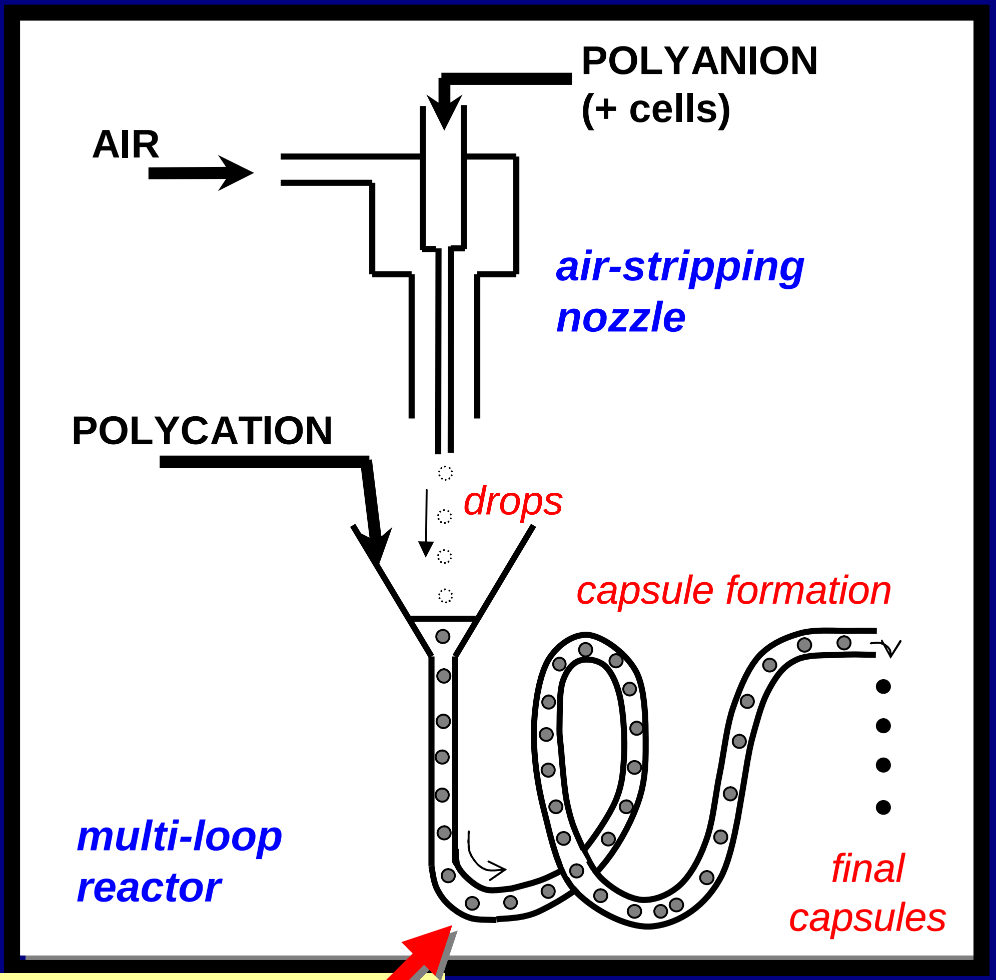
Collection time: 30 s
Reaction time: + 30 s



igor.lacik@savba.sk

"PMCG" microcapsule: process of preparation

1-step CONTINUOUS process

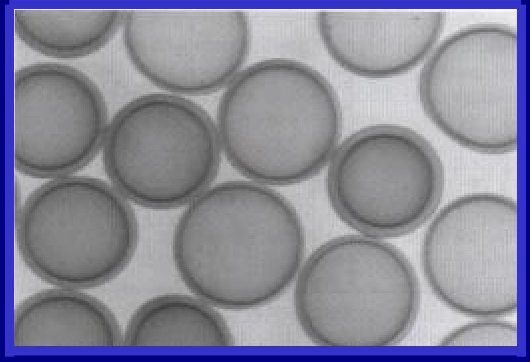


Multiloop reactor

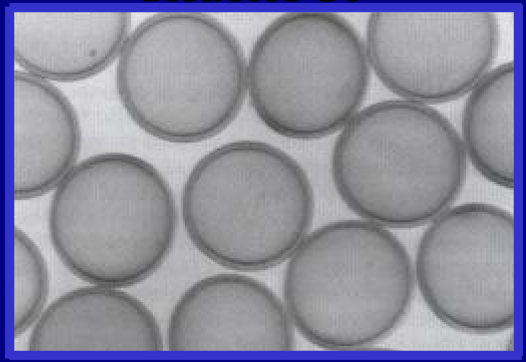
each capsule is exposed to the same reaction conditions

⇒ capsule uniformity (geometry, chemistry)

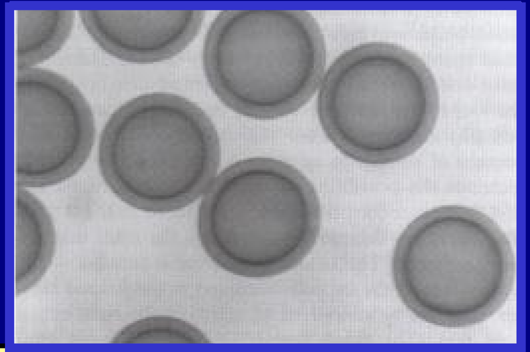
Beaker: collect 30''+ react 30''



Reactor 30''



Reactor 60''

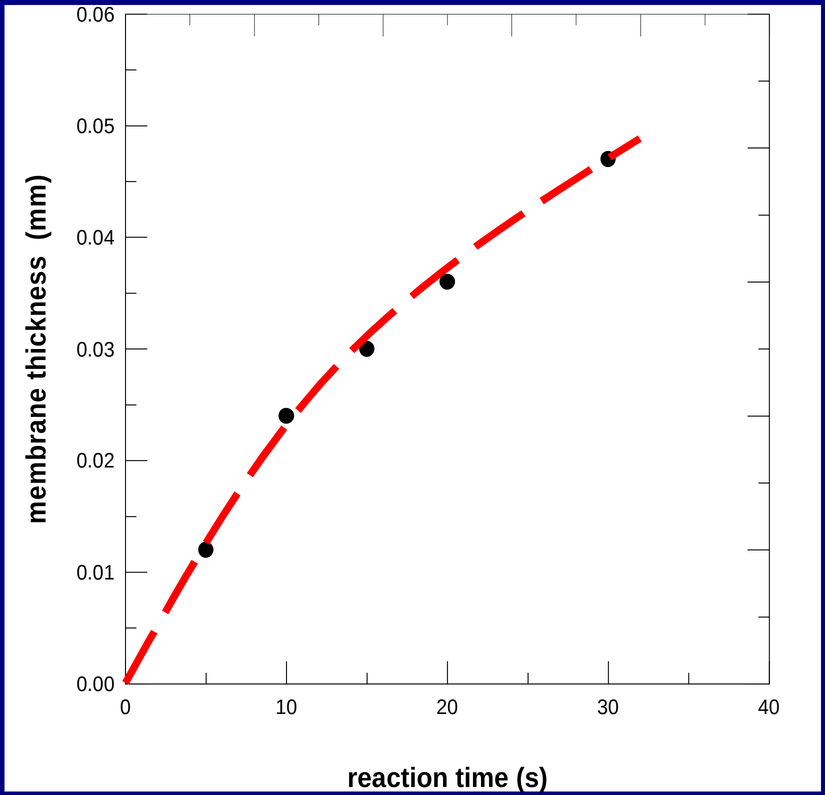


Anilkumar et al *Biotechnol. Bioeng.* **75**, 581 (2001)

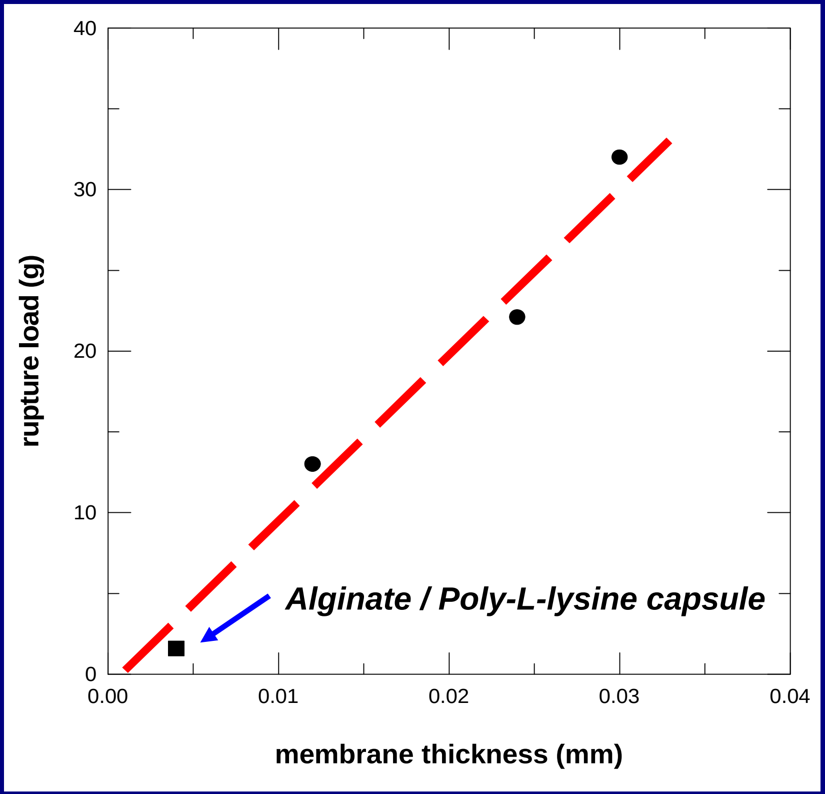
PMCG capsule: membrane formation & mechanical resistance

Conc. (wt.%): SA/CS = 0.6/0.6, PMCG 1.8 %, $CaCl_2$ 1.0 %, NaCl 0.9%

Rate of membrane formation
~ tens of second



Mechanical stability



Permeability of PMCG capsule è inverse SEC

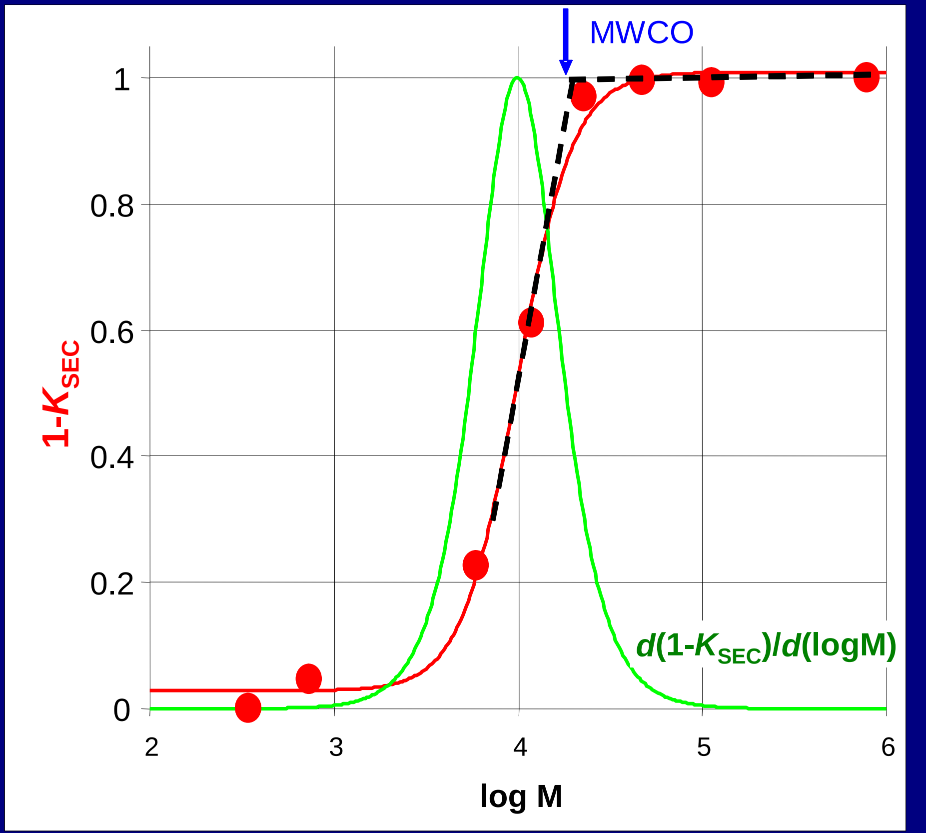
1. Capsules used as a column packing
2. Pullulan standards of different molecular weight injected on column

è molecular weight cut-off (MWCO)

è pore size distribution

column bed	~ 20 ml
eluent	0.9 % NaCl
flow rate	0.2 ml/min
pullulan conc.	1-3 mg/ml
inj. volume	100 µL

$$K_{SEC} = (V_i - V_0)/(V_t - V_0)$$

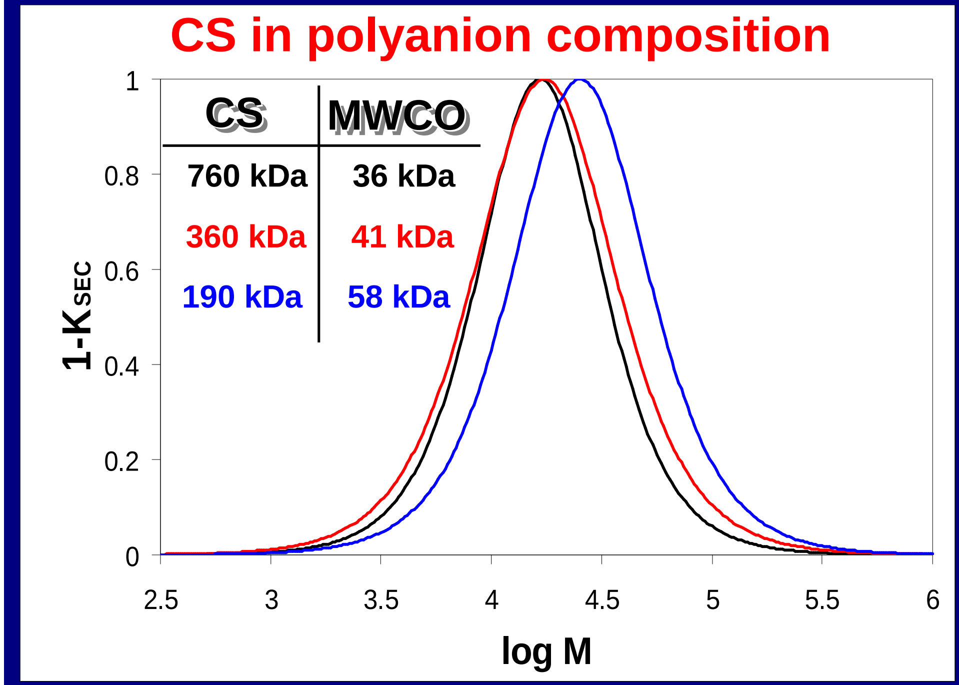


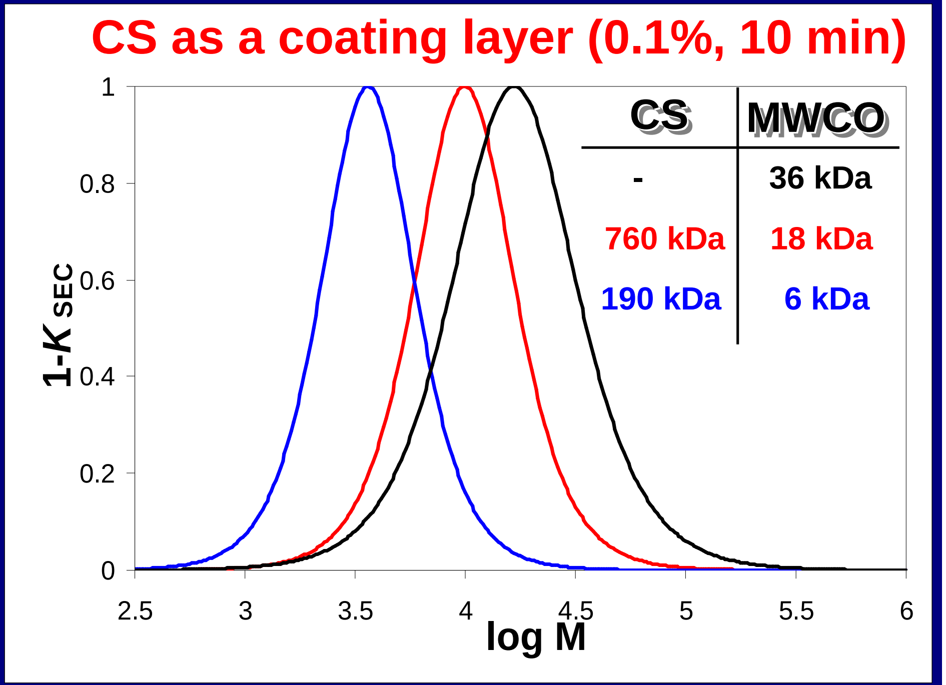
Brissova et al *Analytical Biochem.* **242**, 104-111, 1996

igor.lacik@savba.sk

Permeability of PMCG capsule è effect of MW of CS

PA: 0.9% SA, **0.9% CS** in 0.9% NaCl
PC: 1.2% PMCG, 1.0% $CaCl_2$ 0.9% NaCl, pH 7
40'' reaction time, citrate step



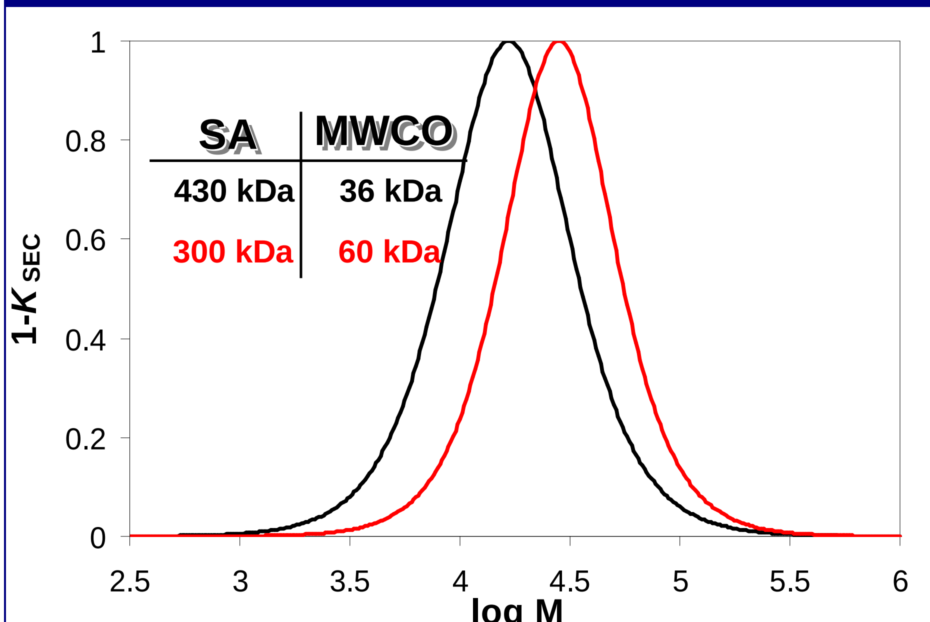


ð effect of the molecular weight of CS is important
- network density
- coating of primary membrane

Permeability of PMCG capsule è internal polymers

SA in polyanion composition

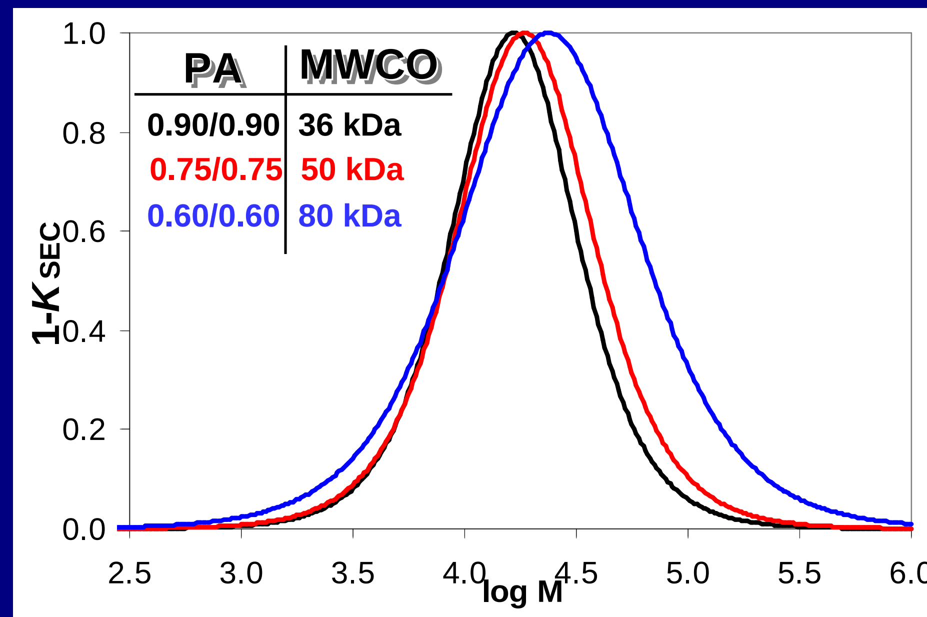
PA: **0.9% SA, 0.9% CS** in 0.9% NaCl
PC: 1.2% PMCG, 1.0% $CaCl_2$ 0.9% NaCl, pH 7
40'' reaction time, citrate step



ð refer to Wandrey 2003 – more effect of SA chemical composition than MW

Polyanion concentration

PC: 1.2% PMCG, 1.0% $CaCl_2$ 0.9% NaCl, pH 7
40'' reaction time, citrate step



ð effect on MWCO and pore size distribution

ð correlation to years at Vanderbilt?

! MW (and MWD) affects also other properties than MWCO (stability, rheology…)

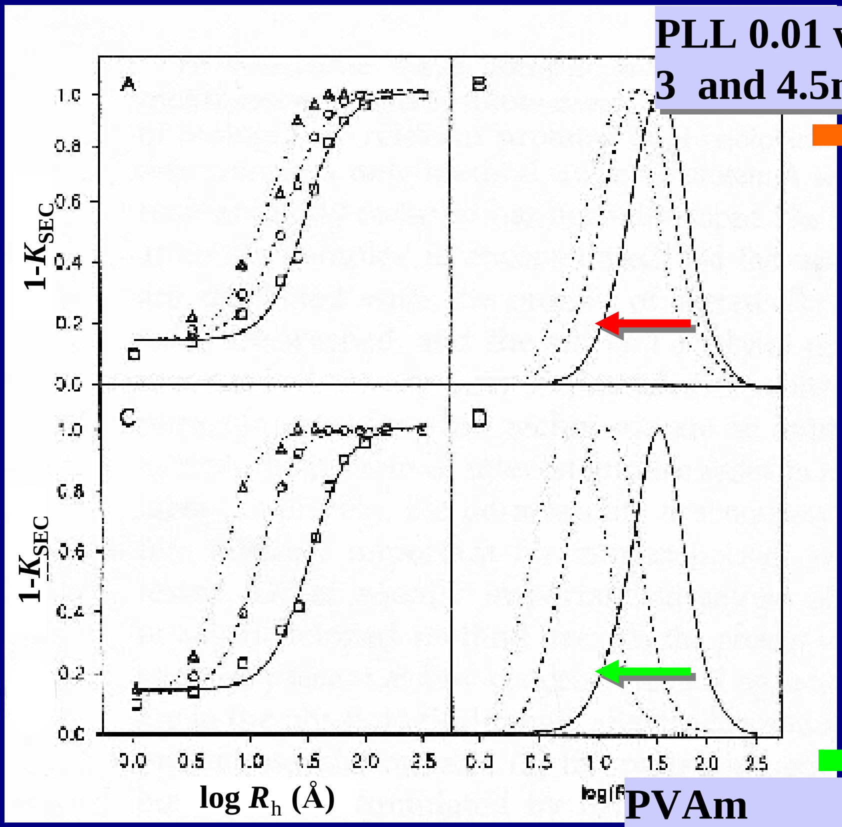
Permeability of PMCG capsule è secondary coating

Polyanion Concentration and Secondary Membrane Effects

1.8 wt% PMCG, 1.0 wt% $CaCl_2$, 0.9 wt% NaCl, 1min

SA / CS in PA solution (wt.% / wt.%)	DEXTRANS (statistical coil) [kDa]	R_h [nm]	PROTEINS (globular conformation) [kDa]
0.6 / 0.6	230	11.7	1 770
0.9 / 0.9	120	8.5	750
0.9 / 0.9	44	5.1	200
0.9 / 0.9	19	3.4	67
0.9 / 0.9	13.8	2.9	44
0.9 / 0.9	3.2	1.4	6.5

molecular weight cut-off



PLL 0.01 wt.%; 3 and 4.5min

PVAm
0.001 wt.%; 3 min
0.1 wt.%; 5 min

Mechanism of capsule formation – a kinetics view

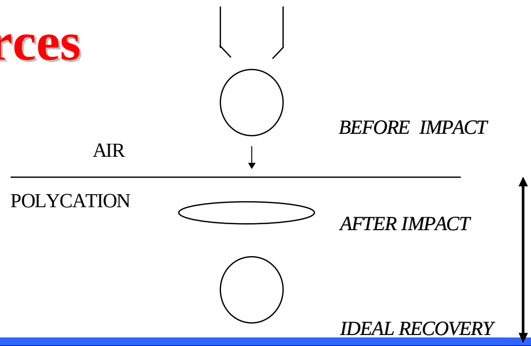
Lacik et al *J Microencapsulation* 2001

I) shape relaxation after impact by viscous forces

time ~ 10^{-3} s

a ***surface smoothness, sphericity and permeability***

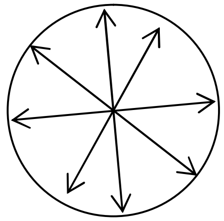
EFFECT OF GELLING CONDITIONS



II) shape relaxation by osmotic swelling

time ~ 10^{-3} - 10^{0} s

EFFECT OF MW, CONCENTRATION, REACTIVITY

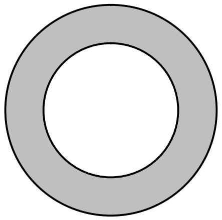


III) membrane formation

time ~ 10^{0} - 10^{2} s

a required ***thickness, mechanical and chemical stability*** and ***permeability***

OVERALL PROCESS AND CHEMISTRY CONDITIONS



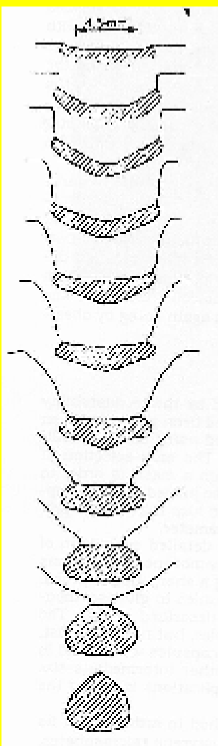
igor.lacik@savba.sk

Shape relaxation by viscous forces

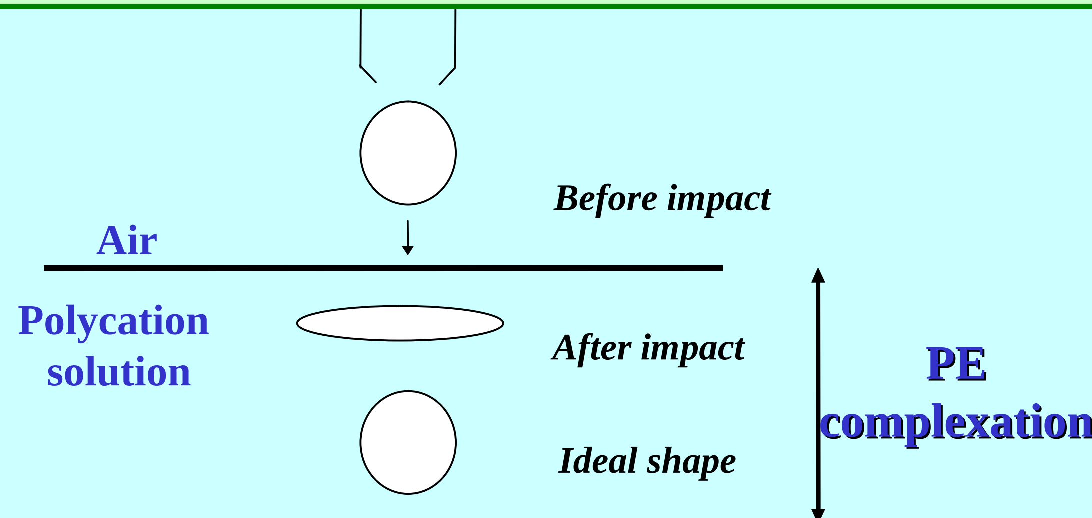
COMMON UNDERSTANDING: **NaCl provides physiological osmotic pressure**

ADDITIONAL ROLE: **Screening of ionic interactions a effect on kinetics!**

Drop penetration via air-liquid interface



Kendal et al. 1989



Time to reach the "ideal shape" is given by viscous forces

$$t \approx \frac{R^2}{5n} \qquad \left(\frac{\sim 0.05^2\,(cm^2)}{\sim 1\,(cm^2 s^{-1})} \right) \qquad \approx 10^{-3}\,s$$

ð Encapsulation process – ~~minimize time needed for shape relaxation~~:

ê drop size, é viscosity ð **OK, but limited by process slow-down the rate of gel formation !**

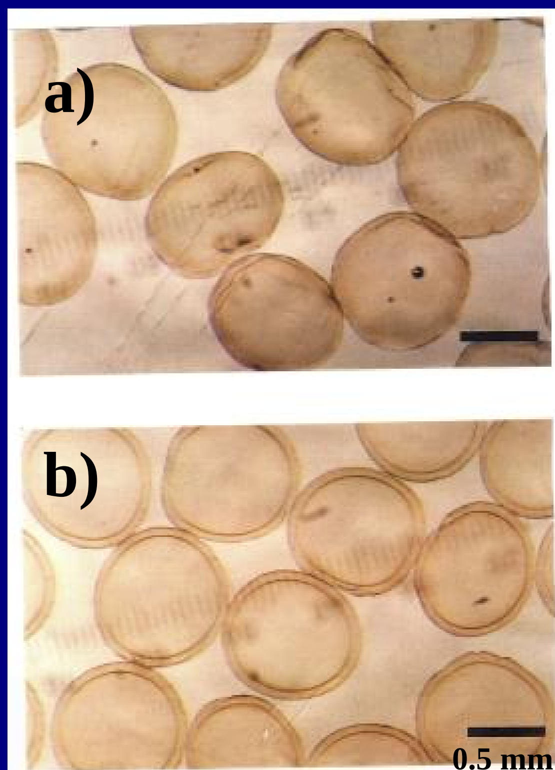
Antigelling effect of NaCl

igor.lacik@savba.sk

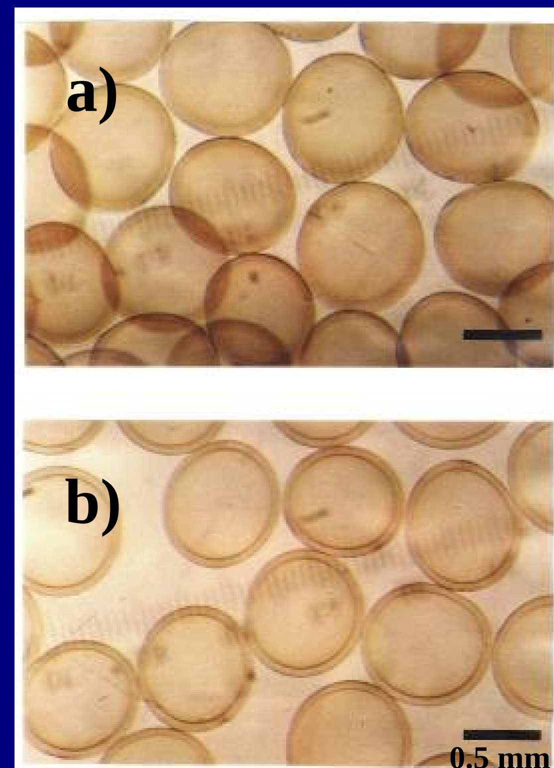
Shape relaxation è gelling conditions $[Na^+]/[Ca^{2+}]$

Optical microscope

a) Immediately after membrane formation
b) After dissolving of internal gel by citrate



without NaCl in cation solution



0.9 wt.% NaCl in cation solution

Positive effect on capsule quality

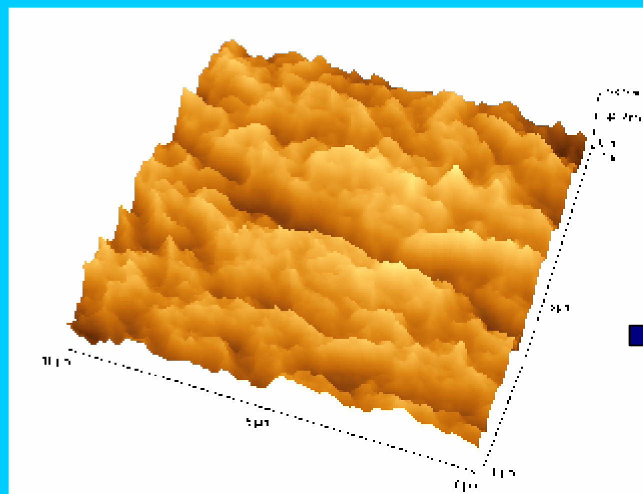
Shape relaxation è gelling conditions $[Na^+]/[Ca^{2+}]$

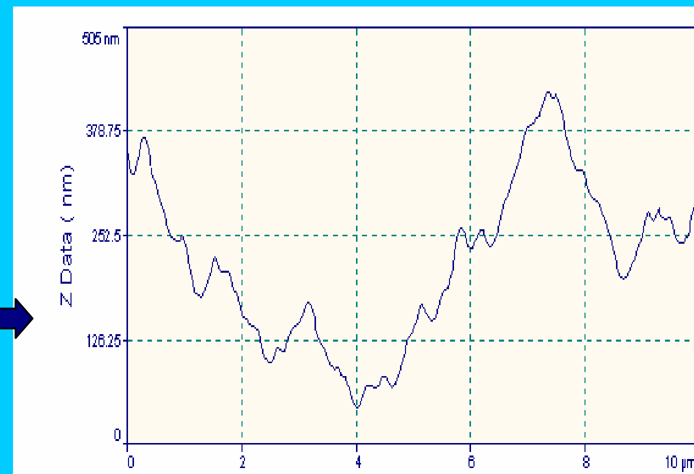
Quantification: AFM

10 x 10 mm

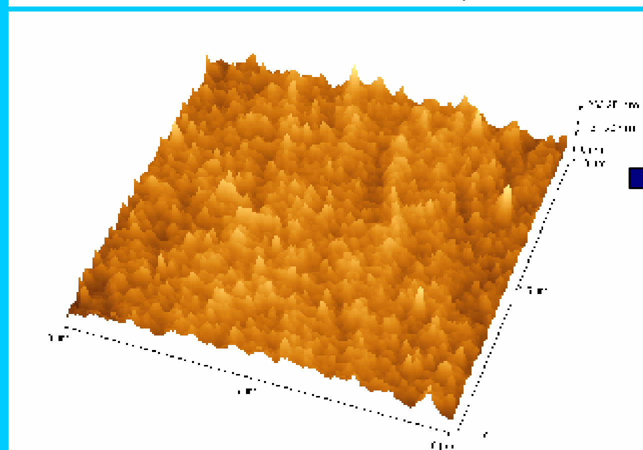
LINE PROFILES

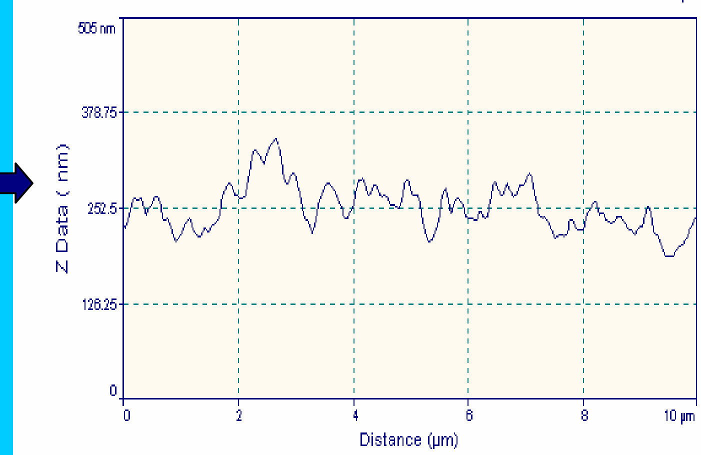
0.9 wt% of NaCl
1.0 wt.% of $CaCl_2$
1.0 wt.% of PMCG





4.5 wt% of NaCl
1.0 wt.% of $CaCl_2$
1.0 wt.% of PMCG





Shape relaxation è gelling conditions $[Na^+]/[Ca^{2+}]$

Effect on permeability

SA/CS = 0.6/0.6 (wt% / wt%), poly (methylene-co-guanidine) 1.0 wt%

$CaCl_2$ (%)	NaCl (%)	$[Ca^{2+}]/[Na^+]$	exclusion limit [kDa]
0.60	0.9	0.35	97
0.86	0.9	0.49	137
1.00	0.9	0.58	215

ì $[Ca^{2+}]/[Na^+]$ ì **MWCO**

ì $[Ca^{2+}]$/[PMCG] ì **MWCO**

Indication of the **competition processes** in the membrane formation

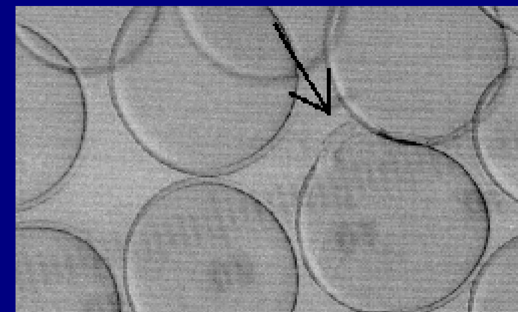
Increased NaCl concentration lowers the PA concentration at the drop surface
ð homogeneous vs. heterogeneous distribution of polyanions

Extremely simple adjustment of MWCO

Shape relaxation è gelling conditions [Na^+]/[Ca^{2+}]

Lacik et al *J Microencapsulation* 2001

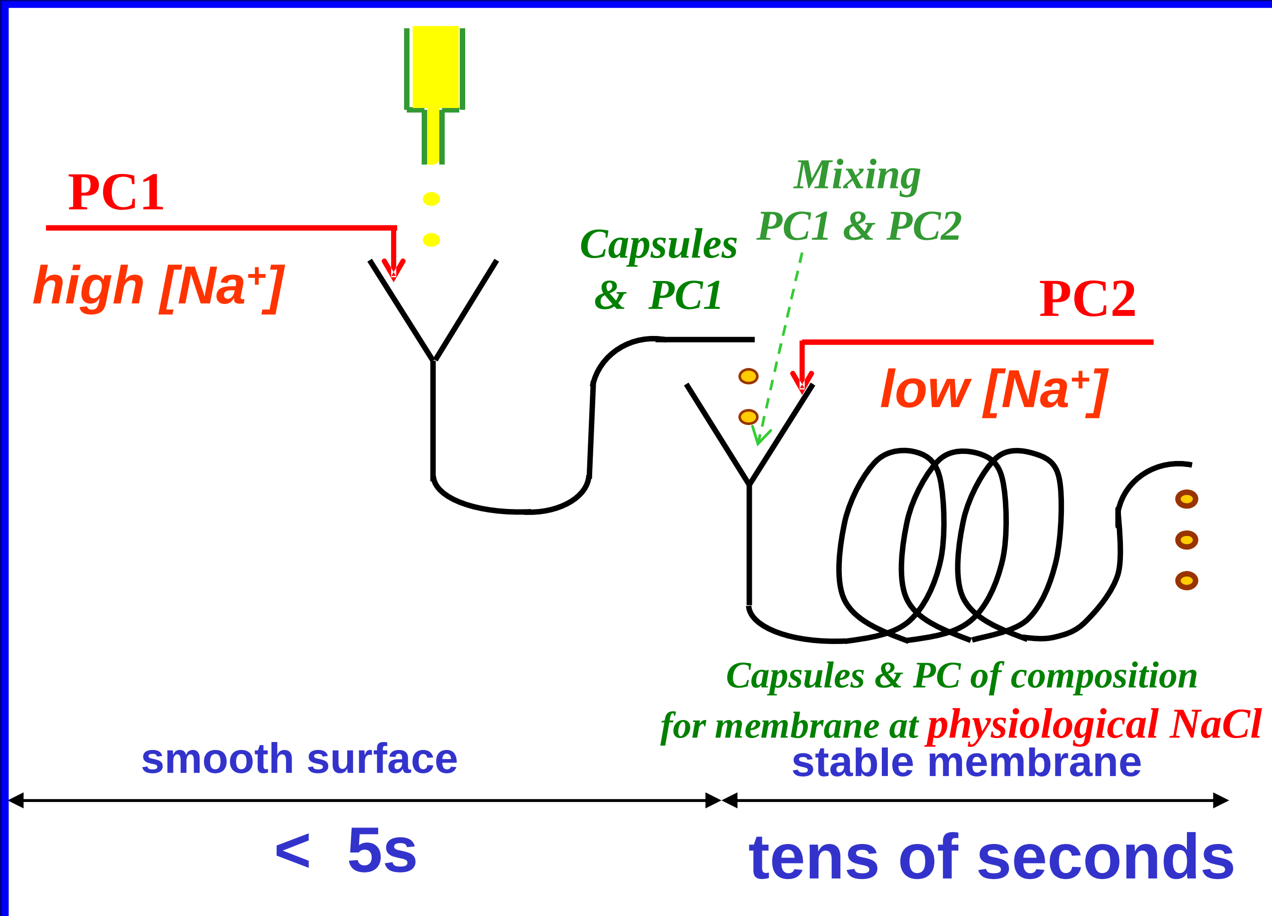
Increased ratio Na^+/Ca^{2+} leads to **smoother** surface, but **less stable** complex



Solution: capsules are formed continuously in **two steps**

$$c_{2,i} = \frac{c_i(1+k) - c_{1,i}}{k}$$

k is the flow rate ratio V_2/V_1 of PC2 and PC1



a **Capsules of different surface quality**

igor.lacik@savba.sk

Mechanism of capsule formation – a chemistry view

interactions – dilute solution

composition – real conditions

capsules are made by interactions between:

PMCG + SA

PMCG + (SA-Ca^{2+})

PMCG + CS

PMCG + SA/CS

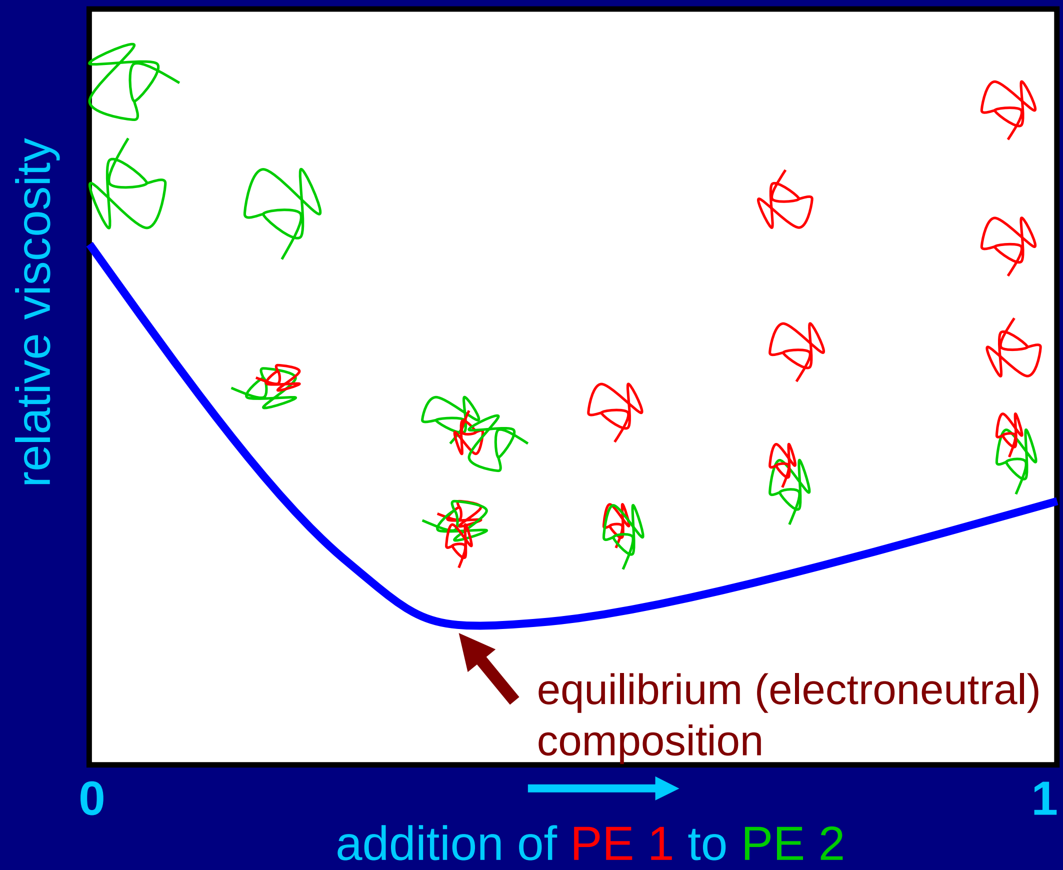
PMCG + (SA/CS-Ca^{2+})

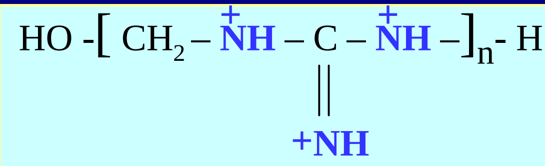
Questions:

- **what do we know about interactions?**
- **can the information on interactions help?**

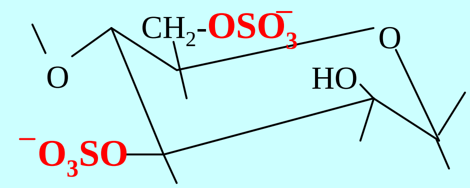
Interactions in dilute solution

viscometry (Ubelohde viscometer) at 25°C

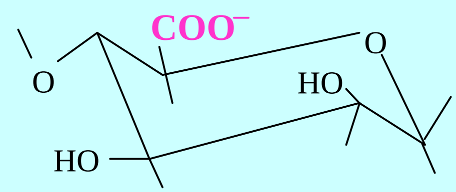




M_{monmol} = 107 g/mol
3.0 charge / unit ?



M_{monmol} = 341 g/mol
1.7 charge / unit



M_{monmol} = 198 g/mol
1.0 charge / unit

⇓ expected???

PMCG : CS ~ 1 : 2 (x_{PMCG} ~ 0.33)

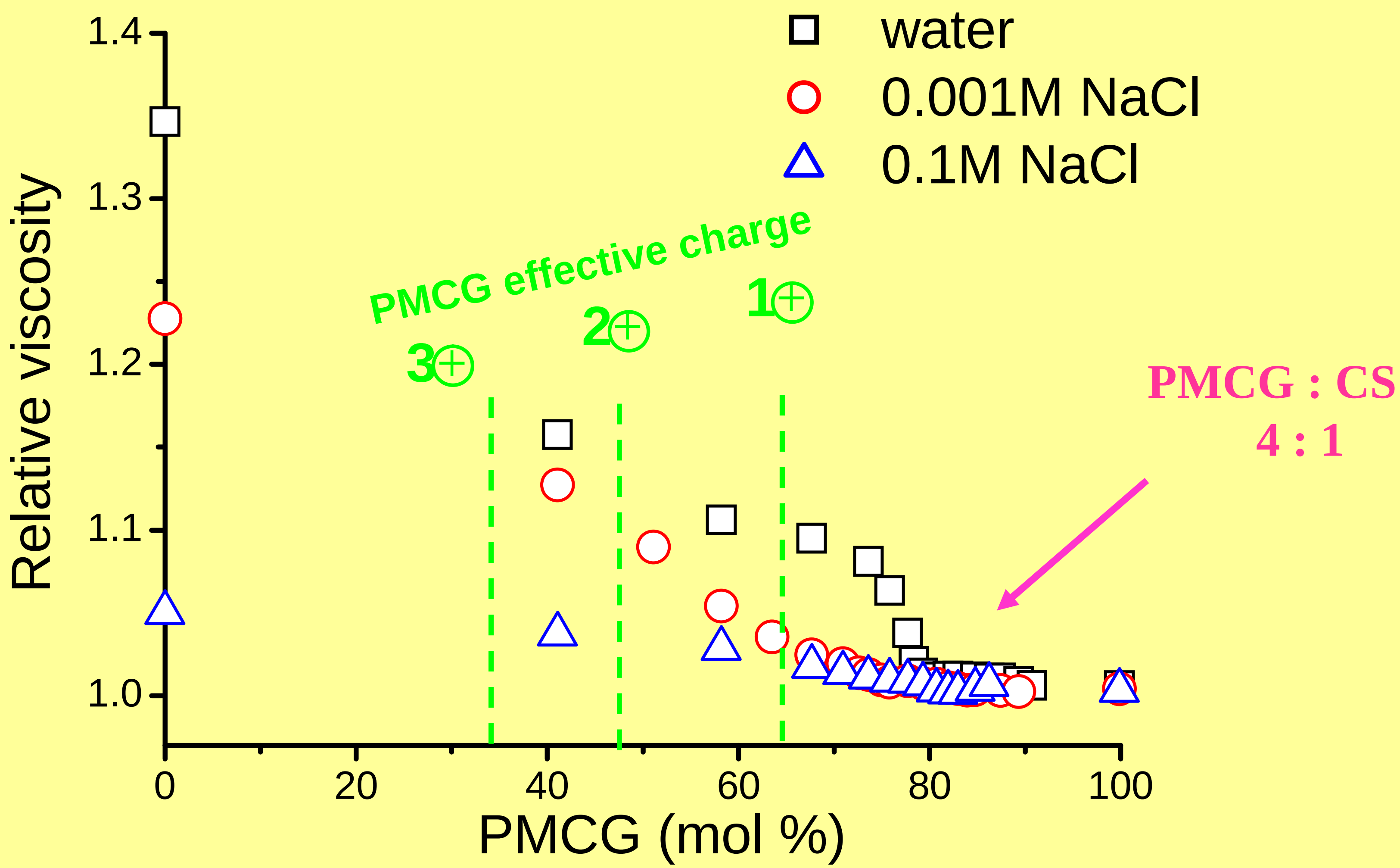
PMCG : SA ~ 1 : 3 (x_{PMCG} ~ 0.25)

- molecular weight
- concentration
- ionic strength

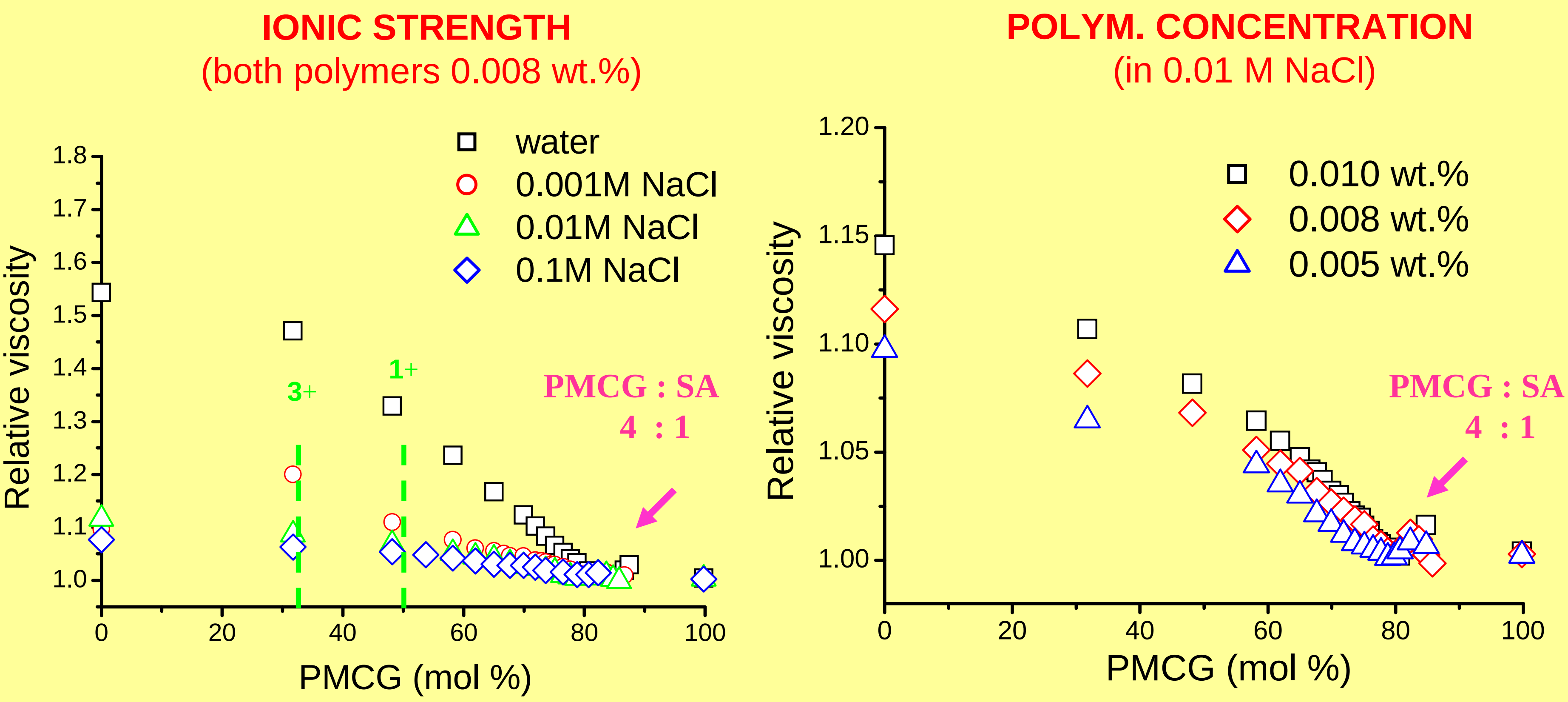
Interactions in dilute solution: PMCG - CS

IONIC STRENGTH

(both polymers 0.01wt.%)



Interactions in dilute solution: PMCG - SA



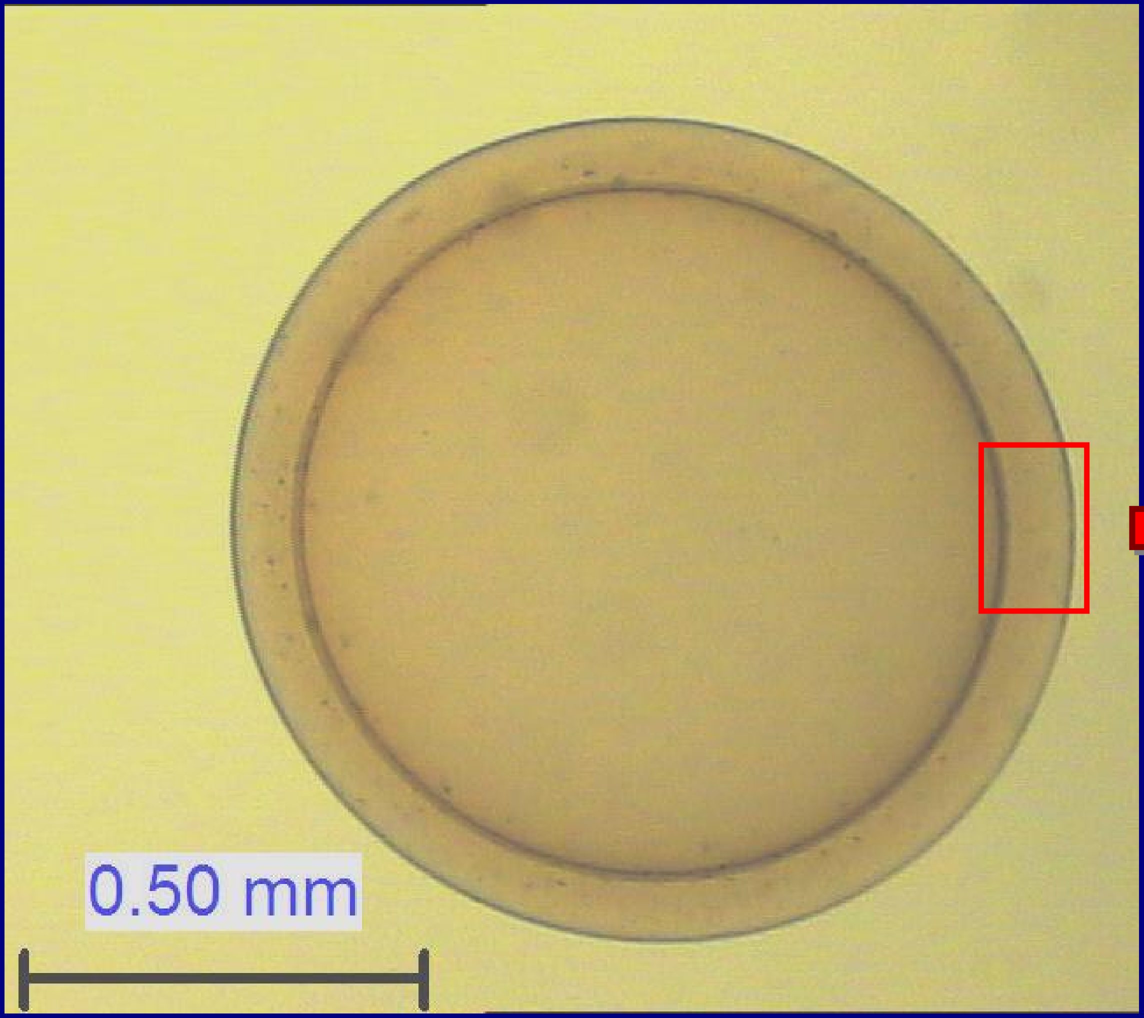
Outcomes from studies of interactions in dilute solution

- **PMCG is less (electrostaticaly) interactive than assumed (PMCG effective charge?)**
- **PMCG content in complex is higher than stechiometrical.**
- **Other than (only) ionic interactions are responsible for complex formation?**
- **similar PMCG interactivity to both SA and CS**
  SA + Ca^{2+} CS + PMCG   **SA + PMCG**

è Question: complex composition under REAL conditions?

igor.lacik@savba.sk

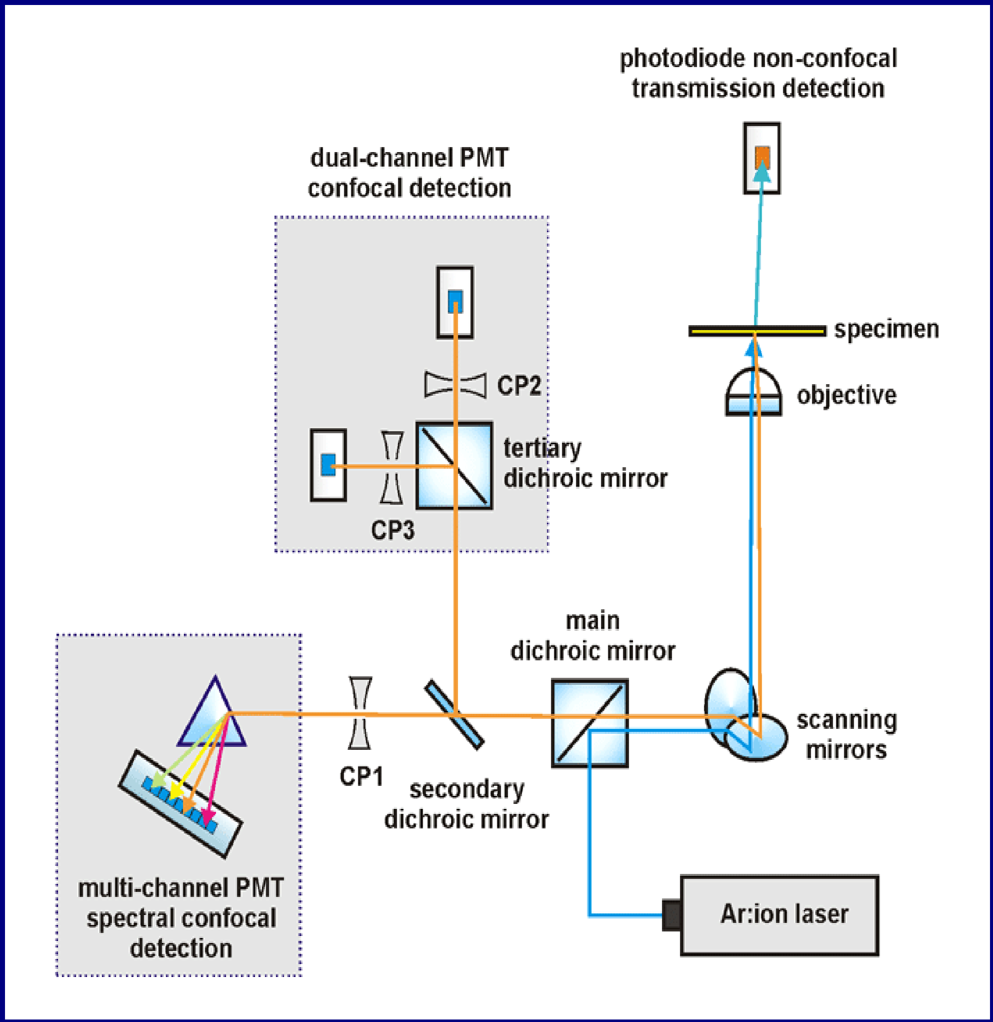
Membrane formation under real conditions



?

Confocal laser scanning microscopy in membrane characterization

Multi-spectral confocal microscope Zeiss LSM 510-META+Axiovert 200 Mot

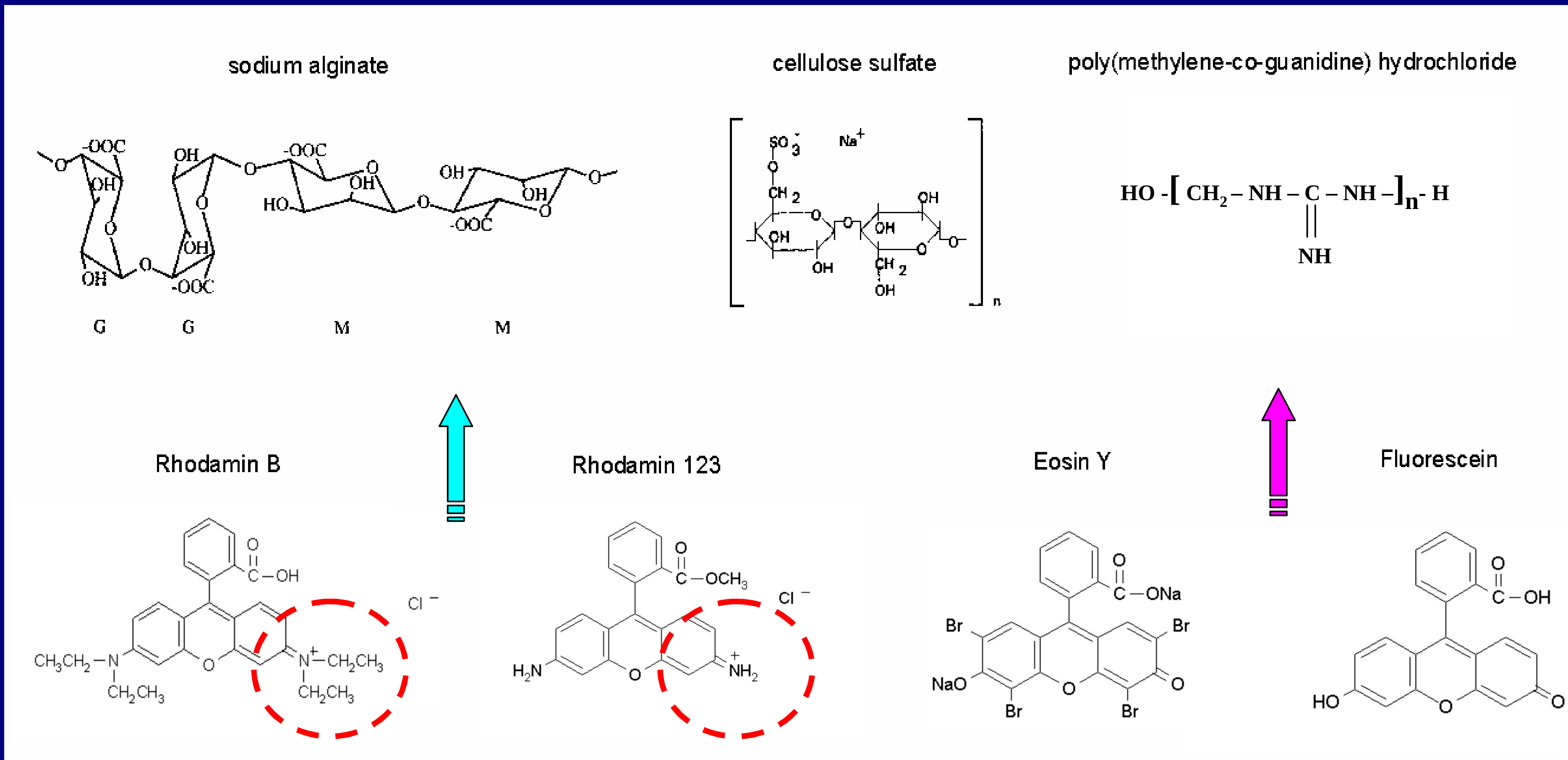


Two fluorescence/reflection channels
One transmission channel
32-channel polychromatic detector

488 nm laser line and emission bands 435-485 nm (reflection) and 535-590 (fluorescence)

Confocal laser scanning microscopy in membrane characterization

Fluorescence labels è non-covalent labeling probes after capsule formation (10^{-7} mol/L)



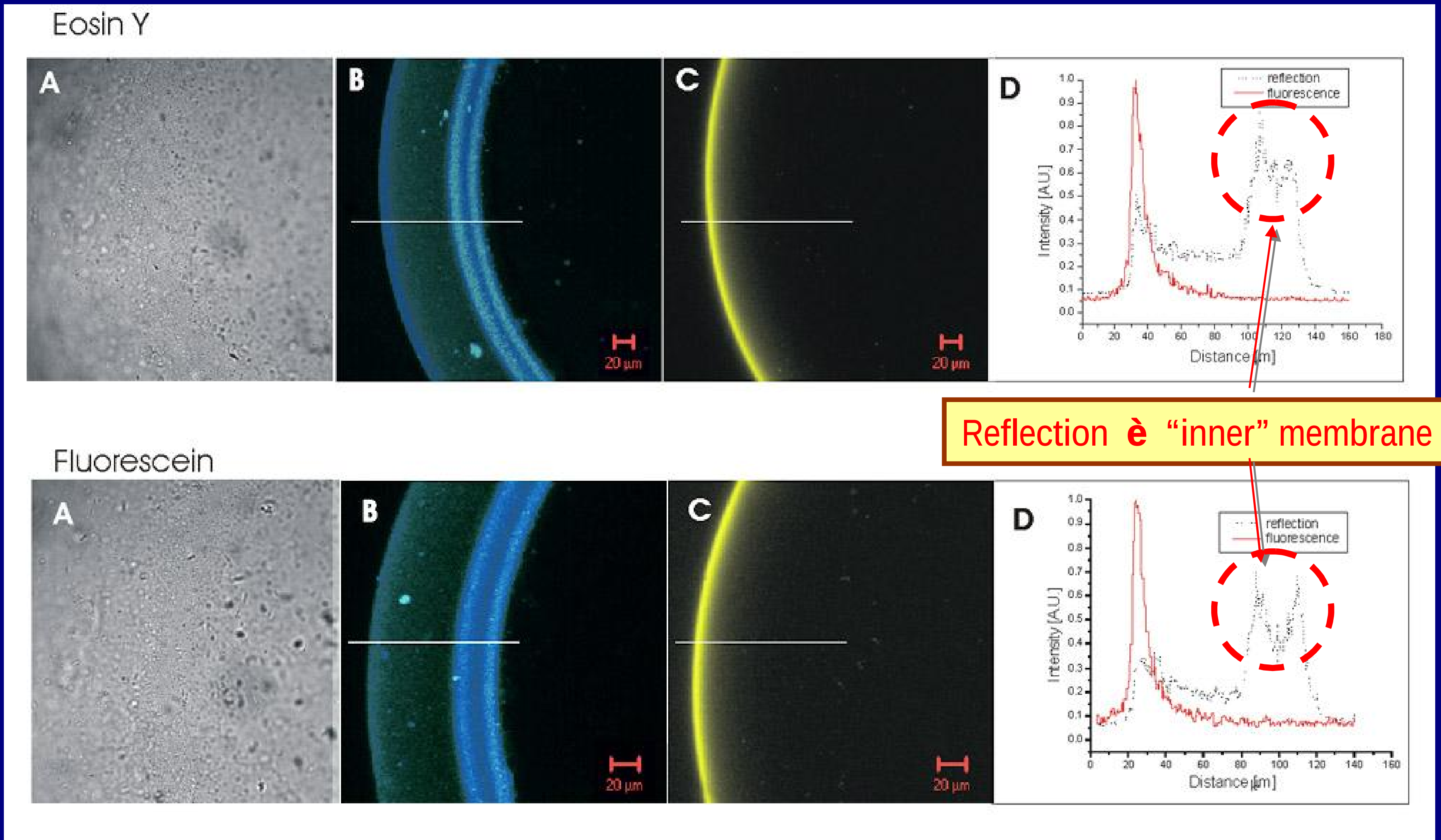
selected cation probes
(visualisation of negatively-charged polymers)

selected anion probes
(visualisation of positively-charged polymer)

Sensitive to (free, unbound) charged polymer groups

CLSM characterization of microcapsule wall – anionic probes

PA: 0.9% SA, 0.9% CS in 0.9% NaCl, PC: 1.2% PMCG, 1.0% $CaCl_2$ 0.9% NaCl, pH 7 , 40'' reaction time, citrate step



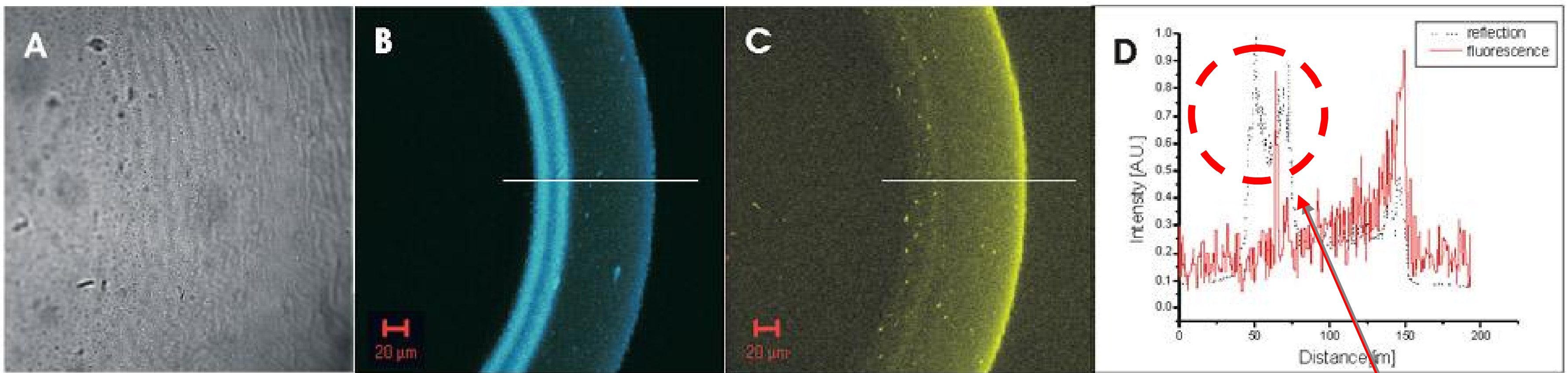
A) transmission, B) reflection, C) fluorescence, D) cross-sections (across white line in B,C)

igor.lacik@savba.sk

CLSM characterization of microcapsule wall – cationic probes

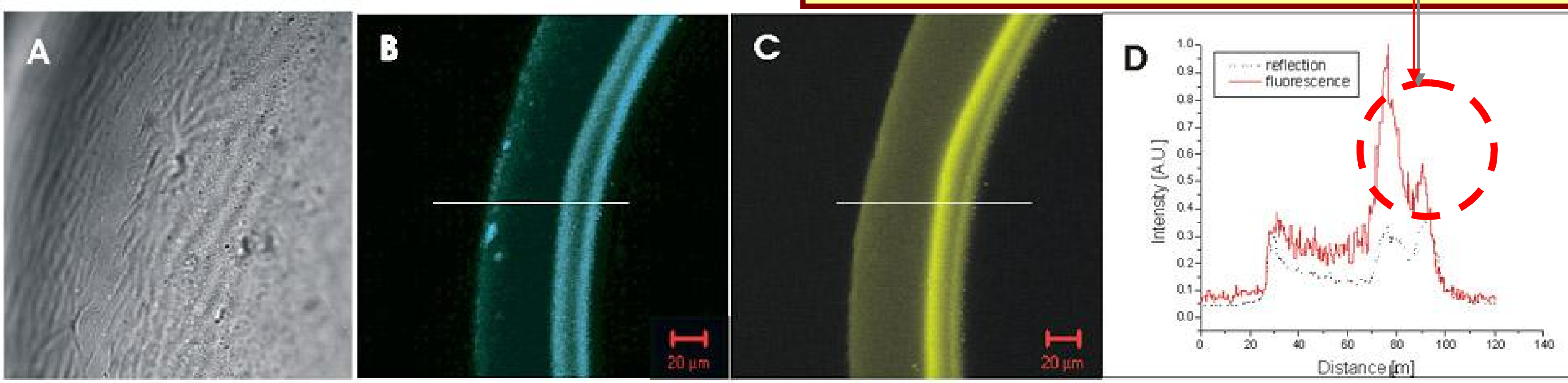
PA: 0.9% SA, 0.9% CS in 0.9% NaCl, PC: 1.2% PMCG, 1.0% $CaCl_2$ 0.9% NaCl, pH 7 , 40'' reaction time, citrate step

Rhodamin B



Reflection & fluorescence for non-bulky probes
è "inner" membrane (of anionic nature)

Rhodamin 123



A) transmission, B) reflection, C) fluorescence, D) cross-sections (**across** white line in B,C)

Dilemma between 1- and 2-step processes

1 – step

- ð capsular membrane SA/CS – PMCG is made in one step (by using loop-reactor)
- ð intra- and inter-batch homogeneity (geometry and chemistry)

2 – step

- ð 1st step: SA/CS – Ca^{2+} beads (step consisted of collection + reaction)
  2nd step: exposure to PMCG
- ð easy control of membrane thickness and shape
- ð may arise a problem with intra- and inter-batch homogeneity (an issue for large batches!) due to:

- Ø differences in collection and reaction times
- Ø diffusion of CS between 1st and 2nd steps (CS is not bound by Ca^{2+})
- Ø duration of washing steps

…what can CLSM see?

CLSM characterization of microcapsule wall: non-covalent labeling

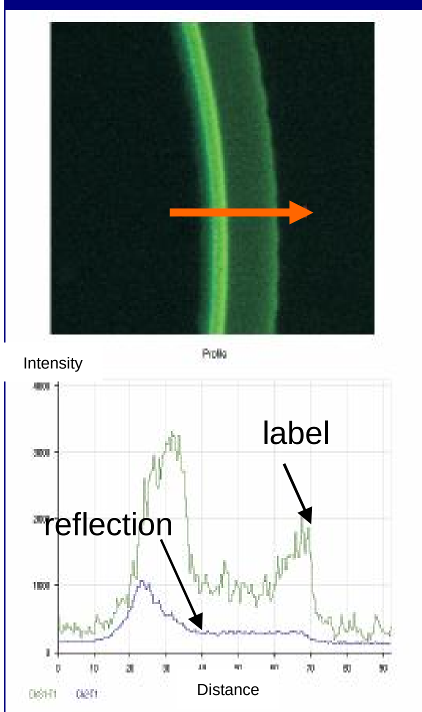
1-step process

PA: 0.9% SA, 0.9% CS in 0.9% NaCl,
PC: 1.2% PMCG, 1.0% $CaCl_2$ 0.9% NaCl, pH 7
40'' reaction time

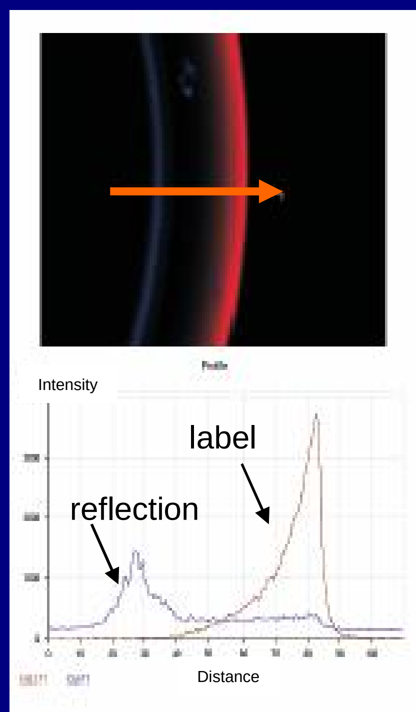
2-step process

PA: 0.9% SA, 0.9% CS in 0.9% NaCl,
Step 1: 1.0% $CaCl_2$ 0.9% NaCl, pH 7, 40'' reaction time,
Step 2: 1.2% PMCG, 0.9% NaCl, pH 7, 40'' reaction time

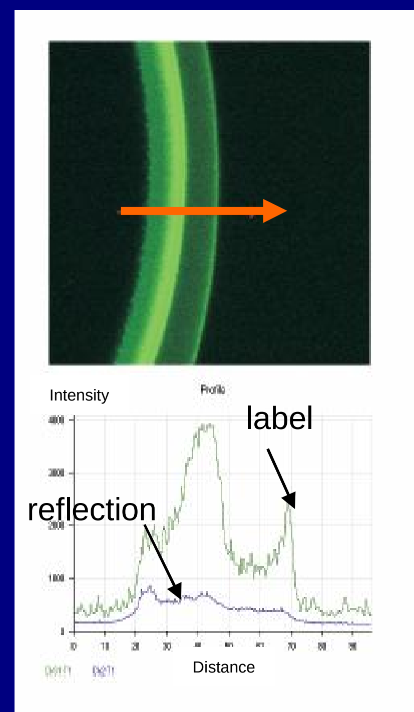
Rhodamine 123



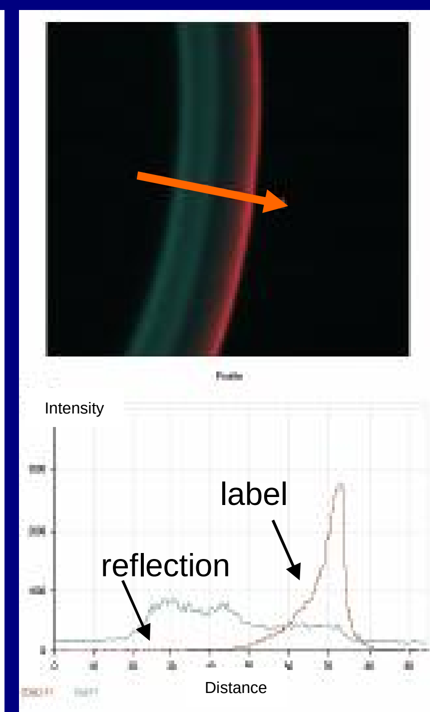
Eosin Y



Rhodamine 123



Eosin Y



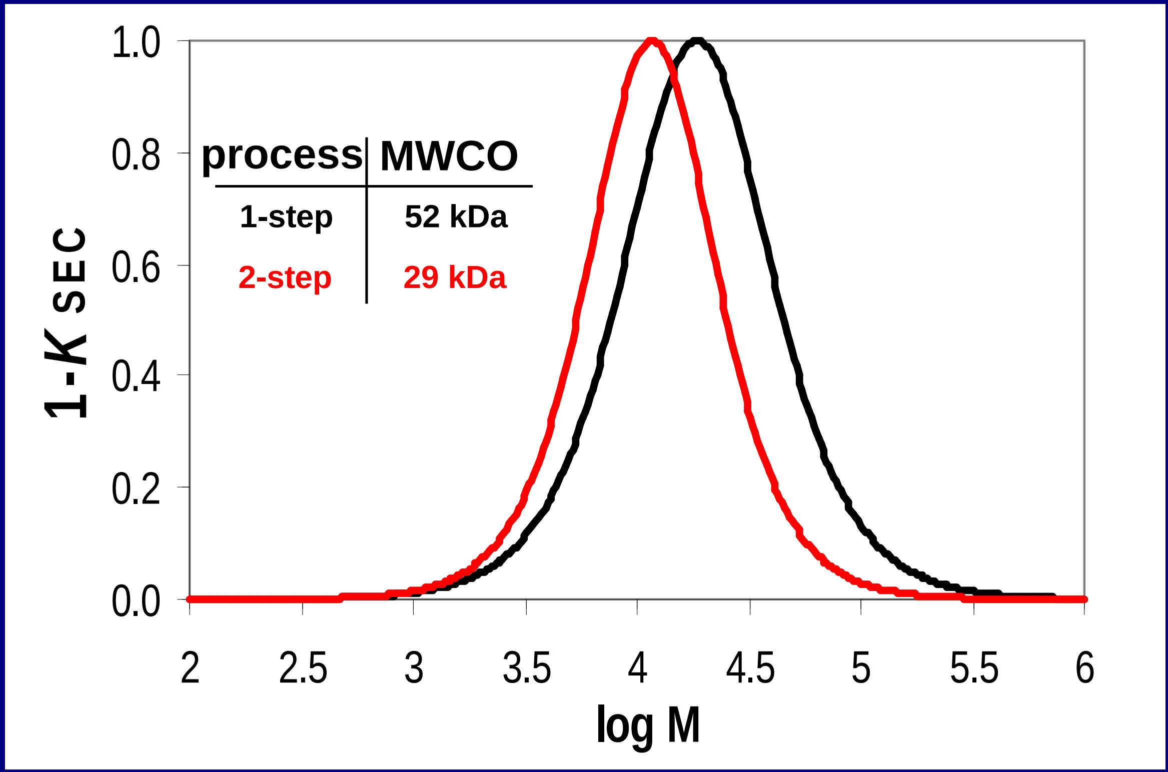
è CLSM identifies membrane composition and suggests mechanism of capsule formation

Permeability by inverse SEC è 1- vs. 2-step process

PA: 0.9% SA, 0.9% CS in 0.9% NaCl

1-step capsule: 1.2% PMCG, 2.0% $CaCl_2$ 0.9% NaCl, pH 7, 40’’ reaction time, citrate step

2-step capsule: 1[st] step: 2.0% $CaCl_2$, 0.9% NaCl 40’’
2[nd] step: 1.2% PMCG, 0.9% NaCl 40’’
citrate

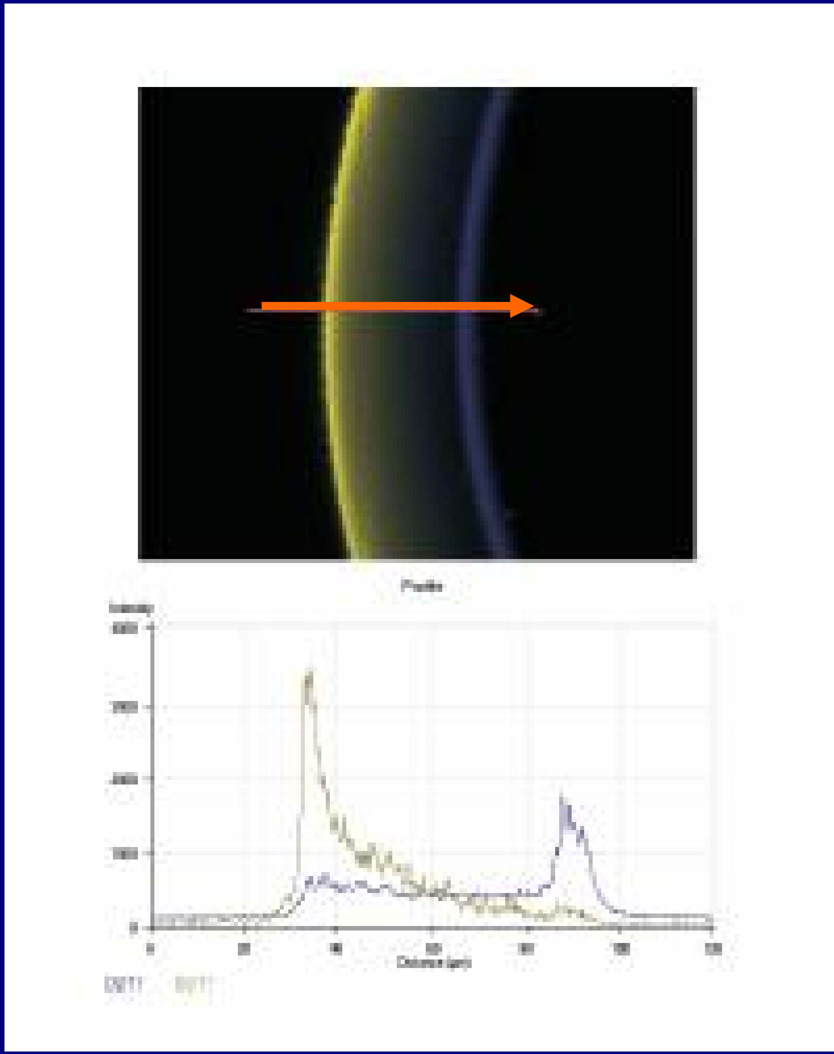


MWCO shows a denser network in 2-step process
? a higher CS concentration towards surface ?

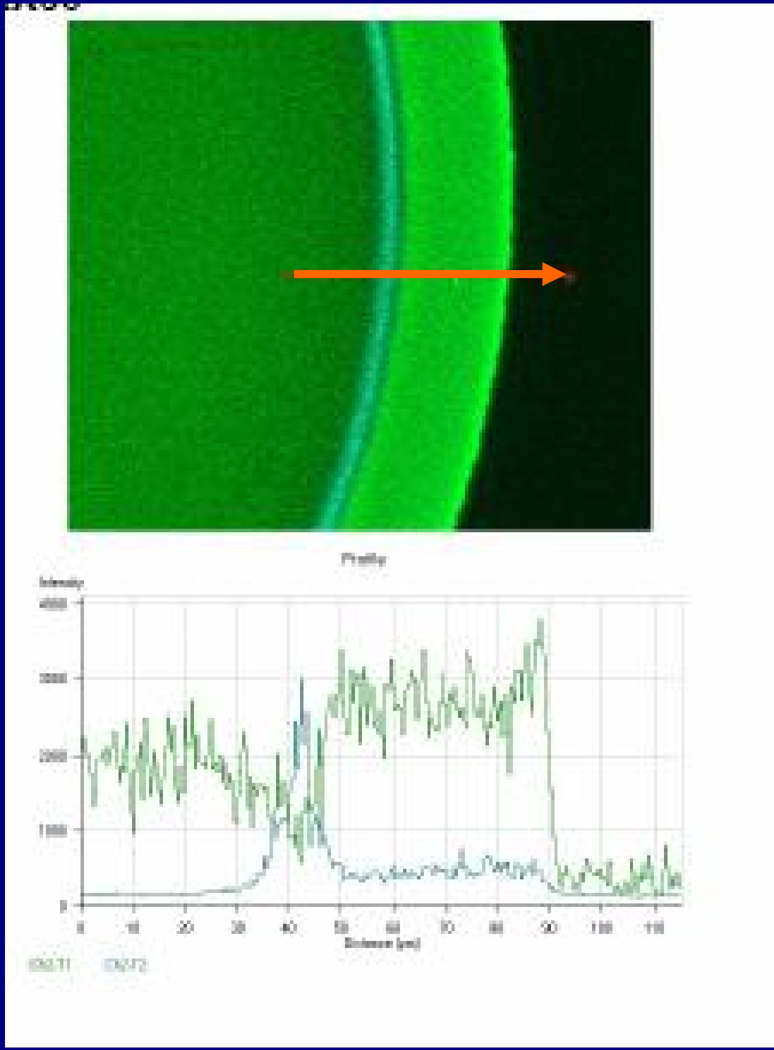
CLSM characterization of microcapsule wall – covalent labeling

PA: 0.9% SA, 0.9% CS in 0.9% NaCl, PC: 1.2% PMCG, 1.0% $CaCl_2$ 0.9% NaCl, pH 7 , 40'' reaction time

PMCG – rhodamineB-isothiocyanate



SA – fluorescein amine



igor.lacik@savba.sk

CLSM characterization of microcapsule wall

- extremely useful tool for membrane and capsule characterization
- understanding mechanism and, hence, control of capsule formation
- specifically bound labels provide sufficient primary information; covalently bound labels provide a rigorous information
- cooperation among devoted people is needed
- future: also for viability studies of encapsulated cells

OUTLINE

- **ENCAPSULATION**

  Why polymer chemist(ry) should enter encapsulation?

- **POLYMERS IN CAPSULES**

  Polyelectrolytes as a capsule material

  Case 1: *Pros and cons* of Alginate-based capsules

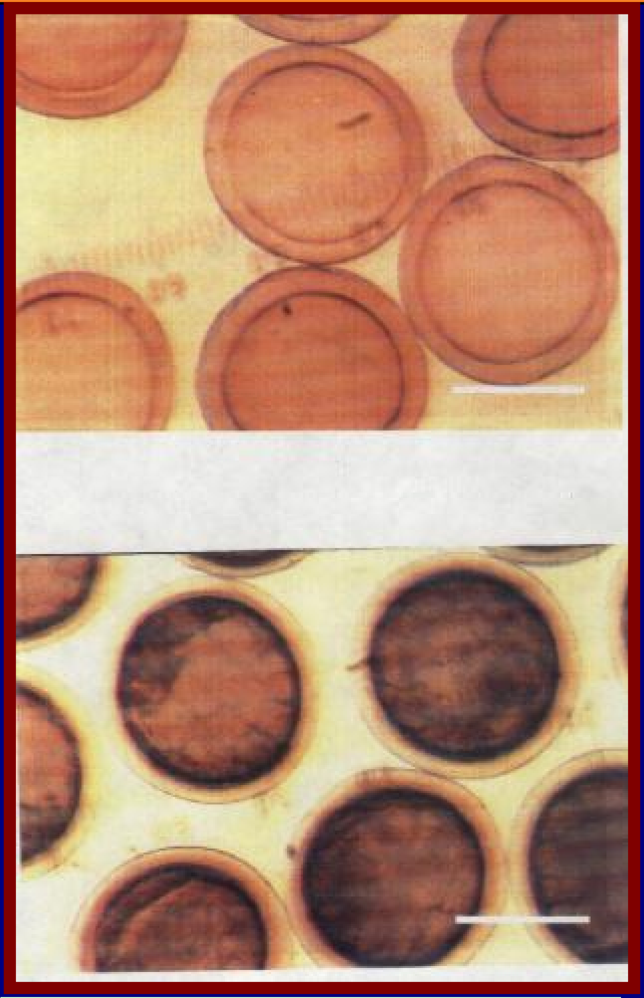
  Case 2: Alternative capsules "PMCG"

  Process, mechanism, understanding and optimization

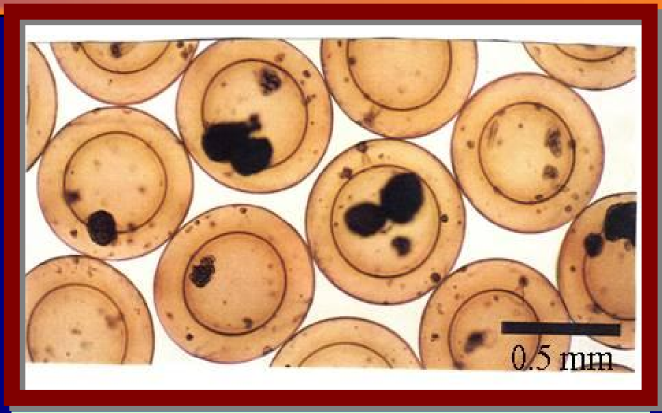
- **APPLICATIONS – Biomedicine & Biotechnology**

- **CONCLUSIONS**

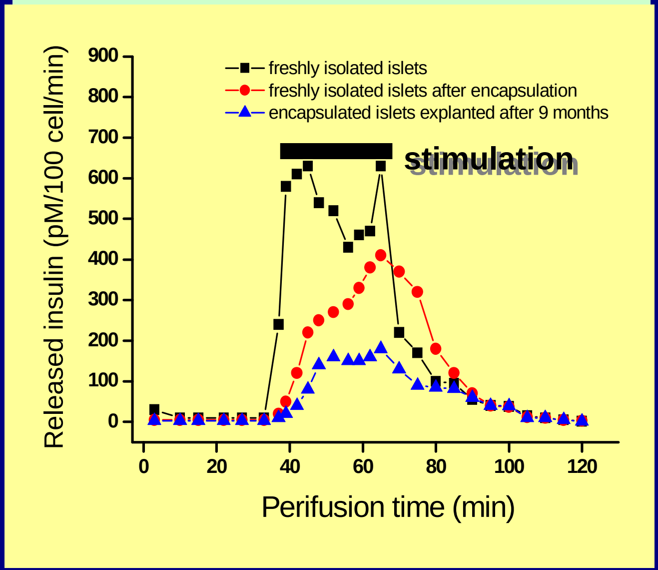
PMCG capsule for encapsulation of islets of Langerhans



Capsules before Tx to C57 mice (A) and explanted after ~ 9 months (B)



Capsules with rat islets



Perifusion in glucose medium

capsules seem to be biocompatible and cells remain viable

igor.lacik@savba.sk

PMCG capsule for encapsulation of islets of Langerhans

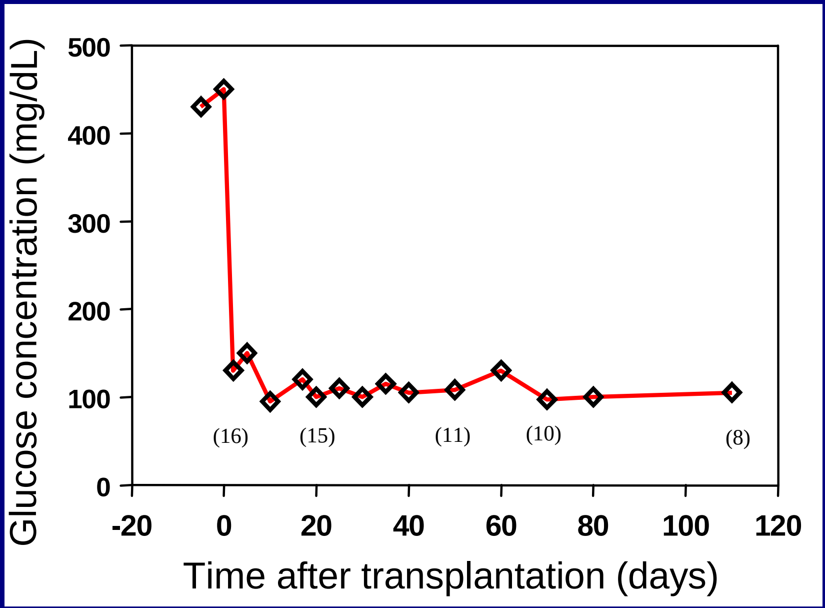
~ 1000 islets of Langerhans / mouse model intraperitoneally
capsule: 800 mm, membrane ~ 70 mm, MWCO towards dextrans

Sprague-Dawley rat islets

C57 mice

200 mg STZ/kg

MWCO 100-230 kDa

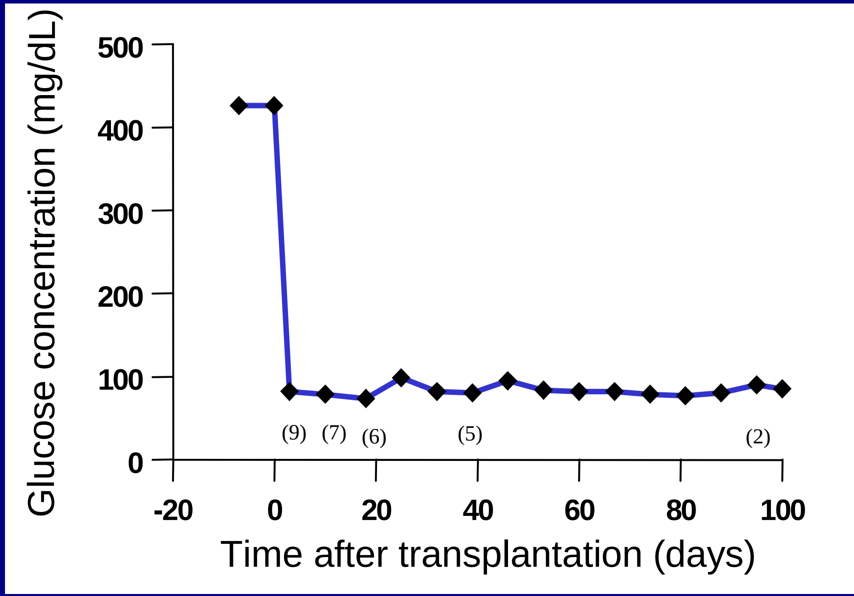


The same for NOD mice with rat islets

Neonatal pig islets

NOD mice

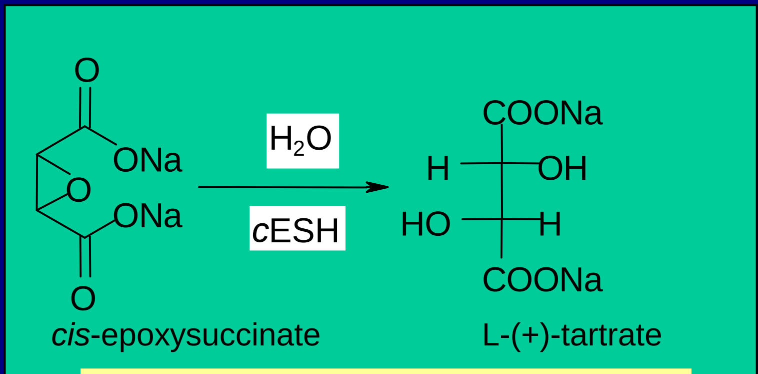
MWCO 100 kDa



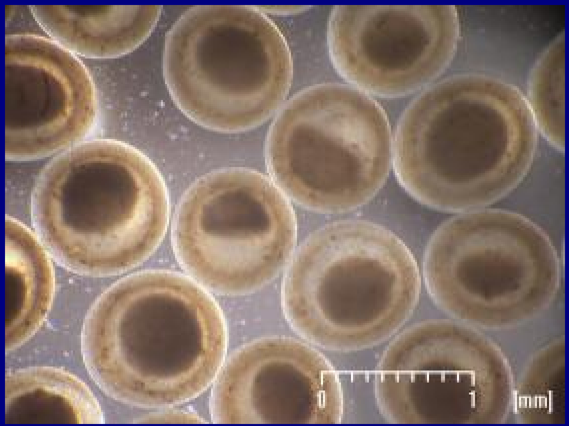
PMCG capsule used in biotechnology

Immobilization of enzymes and whole cells as catalysts for biotransformation

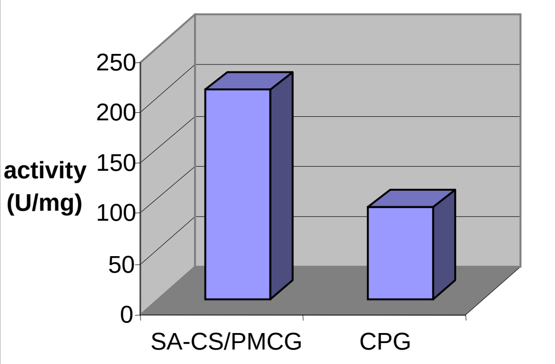
Nocardia tartaricans whole cells with *cis*-epoxysuccinate hydrolase acitvity



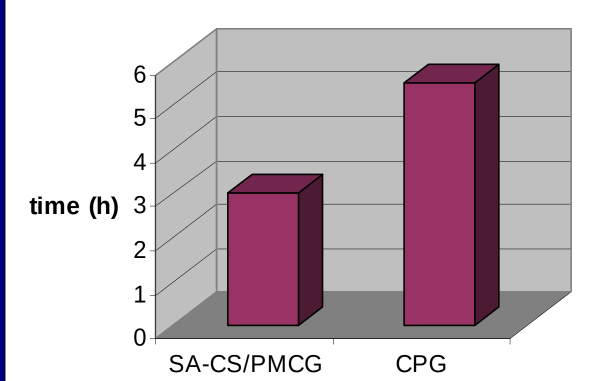
Bucko et al EMT, 2005, 36/1, 118, 2005



SA-CS/PMCG capsule vs. typical calcium pectate beads)

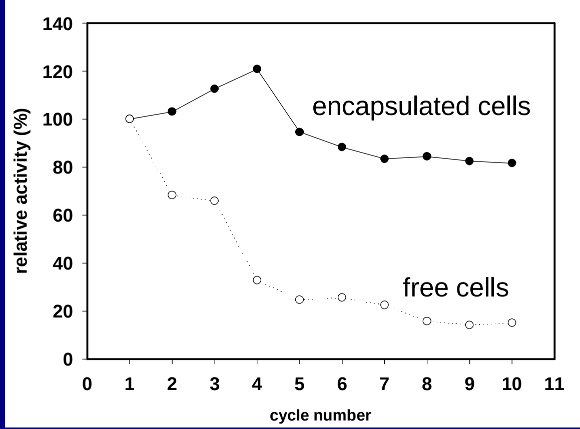


Storage stability after 35 days



Time for complete bioconversion

Operational stability



Conclusion remarks to polymers used in encapsulation of biological substances

BIOTECHNOLOGY

- industrially utilized process using mainly "simple" encapsulation systems (alginates, pectates, PVA)
- **?** effect of tight-controlled parameters on efficiency vs. cost
- a "simple" problem compared to biomedicine

Conclusion remarks to polymers used in encapsulation of biological substances

BIOMEDICINE

long-term biocompatibility – capsules tested and tailored *in vitro*, the *in vivo* performance = black-box

- inter-laboratory repeatability is poor
- polymers – lacking characteristics, purity, and standardization (and fate *in vivo* after losing performance)
- locus of transplantation?
- final composition of polymeric material? (microsphere = gelled SA?; microcapsule = ???)
- “academic” approaches – change them to the application-driven activities (+ 5 years time…?)

Concluding message è whether success or failure, always

(for *understanding the* topic of immobilization of biological substances)

1. THINK OF POLYMERS AND PROCESS, and

2. SEARCH FOR MESSAGES FROM POLYMER CHEMISTRY

Acknowledgement

Polymer Institute SAS, Bratislava (polymers)
G. Kolláriková, M. Danko, E. Hipka, S. Hanzelova, M.Stach, A. Durisova

International laser center, Bratislava (AFM, CLSM, SIMS)
D.Chorvát, Jr., J. Podskočová

Institute of Chemistry SAS, Bratislava (enzyme engineering)
P. Gemeiner, M. Bučko, A. Vikartovská, J. Nahalka

Institute of experimental endocrinology, Bratislava (islets)
V. Štrbák, J. Benický, M. Najvirtová, Z. Bačová

Slovak grant agency for support of science and technology
APVT, BASF Ludwigshafen, Slovak Grant Agency VEGA, COST 840

Acknowledgement

Nashville Cell Encapsulation Team, Vanderbilt Uni (NASA, NIH, NSF)

T. G. Wang	PI
A. V. Anilkumar	reactor design
M. Brissova	capsule design, permeability
D. Hunkeler	capsule design
A. Prokop	capsule design
A. Powers	medical center
K. Xu	AFM