Permeability of Microcapsules by Inverse Size Exclusion Chromatography

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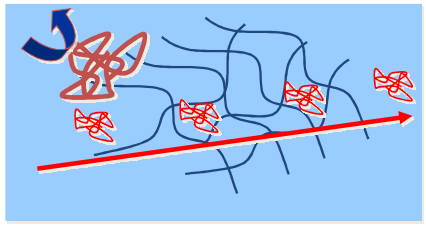
Introduction: permeability of solutes via hydrogel matrix

$P = D \times K$



P permeability

D diffusion coefficient → driving force to move molecules
- obstruction from matrix
- hydrodynamic drag
- heterogeneity of matrix
- interactions

K partition coefficient → equilibrium distribution
- pore size and pore size distribution

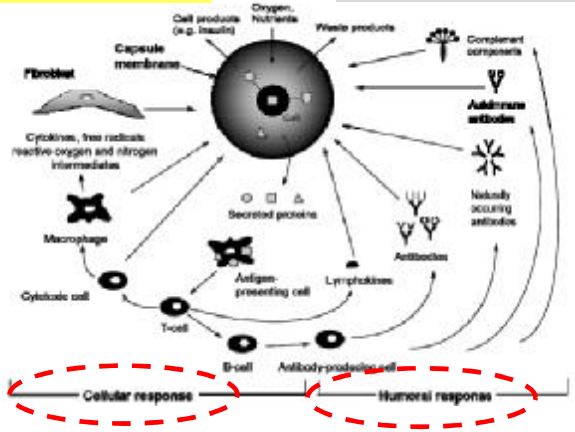
Outline

- INTRODUCTION
  - Permeability of microcapsules
  - Experimental techniques
- INVERSE SIZE EXCLUSION CHROMATOGRAPHY
  - Principle
  - Evaluation
  - Representative results
    - Case 1: PMCG microcapsules
    - Case 2: "COST865" microcapsules
- OPTIONAL TECHNIQUES
- CONCLUSIONS

Introduction: microcapsule for islet transplantation

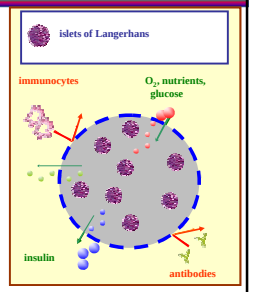
Immunoisolating device

Colton, C. K. (1995) Implantable biohybrid artificial organs. Cell Transplant. 4, 415-436.



Introduction: microcapsule for islet transplantation

Membrane role:
(1) provide immuneprotection
(2) ensure cell viability



Ø **exclude immune cells**
è ***mm*-range**

Ø **exclude soluble components** able to start immune reaction
è ***nm*-range**

Ø **allow for permeation** of nutrients, O_2, insulin
è ***nm*-range**

?

Introduction: target in microcapsule characterization

Molecular weight cut-off (MWCO)

q **the lowest size (nm) and/or**
the lowest molecular weight (Da)
of a solute which can permeate through the membrane

….in addition, the functional semipermeable membrane has to exhibit proper **diffusion properties** (a "YES" is often "automatically" assumed)

Introduction: microcapsule for islet transplantation

Nanometer range: molecular weight vs size of some proteins

Sample	MW	R_g (nm)
Thyroglobulin	670.000	8.60
β-Galactosidase	518.000	6.86
Apoferritin	443.000	6.06
Catalase	232.000	5.23
Glucose oxidase	186.000	5.20
γ-Globulin	158.000	5.23
Alcohol dehydrogenase	150.000	4.55
Albumin (dimer)	132.000	4.76
Alkaline phosphatase	86.000	3.30
Transferrin	77.000	3.92
Albumin	66.000	3.62
Ovalalbumin	44.000	2.83
β-Lactoglobuline	35.000	2.70
Hemoglobin (dimer)	32.000	2.40
Carbonic anhydrase	29.000	2.01
Chymotrypsinogen	25.700	2.50
Ovomucoid	25.000	2.75
Myoglobin	17.000	1.91
α-Lactalbumin	15.500	2.02
Lysozyme	14.000	1.85
Ribonuclease	13.700	1.75
Cytochrome c	11.700	1.63
Aprotinin	6.700	1.50
Insulin	5.700	1.34

M. Briššová, M. Petro, I.Lacík, A.C. Powers, T.G. Wang, Analytical Biochem. 242, 104-111, 1996

TNF 51,000

IL-1β 17,500

Q1: Can the hydrogel be designed to have such "nm" control over the pore size?

Q2: What exactly is the proper "nm range"? **è** transplantation results should tell.

Introduction: methods for permeability characterization

U. Schuldt, D. Hunkeler, *Minerva Biotecnologica* 2000, *12*, 249..

Category	Specification
Solute type	Proteins – unlabeled, radiolabeled Dextrans Pullulans
Analytical method	Protein assay kit UV-VIS spectrometry Radioactivity SEC or inverse SEC Fluorescence microscopy
Static/dynamic methods	Inverse SEC Incubation
Direction of diffusion	Into the capsule (ingress) Out the capsule (egress)
Parameter	Molecular weight cut-off Release or binding protein Pore size distribution

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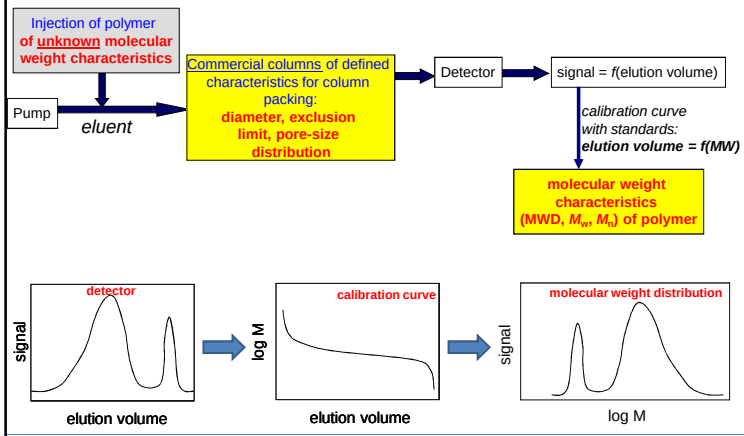
Inverse size-exclusion chromatography: principle

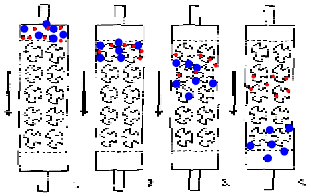
Size-exclusion chromatography (SEC)



Inverse size-exclusion chromatography: principle

Size-exclusion chromatography (SEC)

Column separation technique based on **enthalpy-free partitioning** of analyzed polymer chains of different length (size) between mobile and stationary phases



$$V_e = V_0 + K_{SEC}(V_t - V_0)$$

V_e - elution volume for given size

V_0 - free (interstitial) volume between particles of column packing

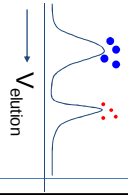
V_t - total (interstital plus pore) volume

K_{SEC} - partition coefficient $0 \le K_{SEC} \le 1$

solute exluded

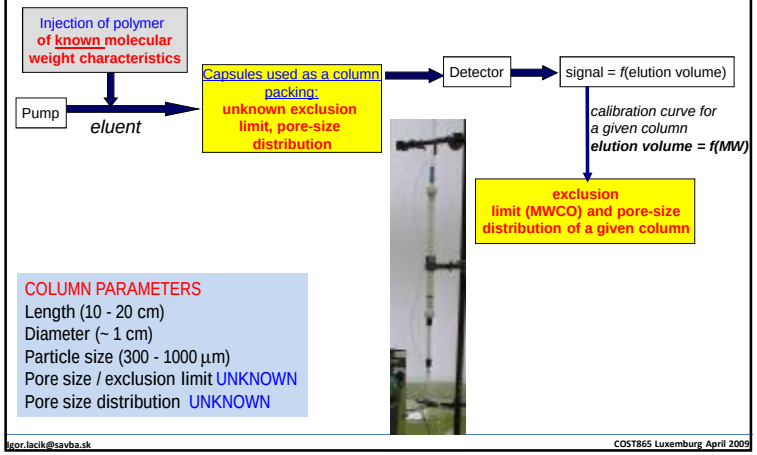
solute permeated

COLUMN PARAMETERS
Length (30 – 60 cm)
Diameter (~ 1 cm)
Particle size (5-20 μm)
Pore size / exclusion limit (100 – 10 000 Å)
Pore size distributions (narrow)



Inverse size-exclusion chromatography: principle

Inverse size-exclusion chromatography



COLUMN PARAMETERS
Length (10 - 20 cm)
Diameter (~ 1 cm)
Particle size (300 - 1000 μm)
Pore size / exclusion limit UNKNOWN
Pore size distribution UNKNOWN

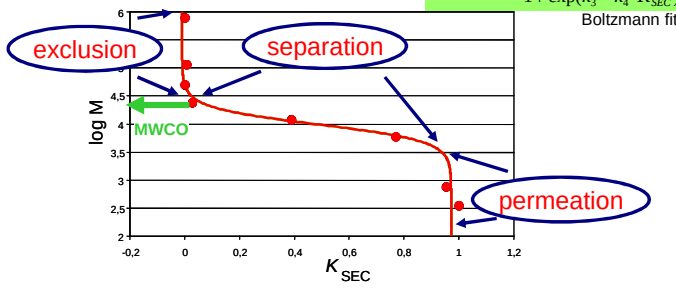
Inverse size-exclusion chromatography: principle

Inverse size-exclusion chromatography: evaluation

Step 2: Evaluation of elution curves

$$\log M = k_1 + \frac{k_2}{1+\exp(k_3 - k_4 \cdot K_{SEC})}$$

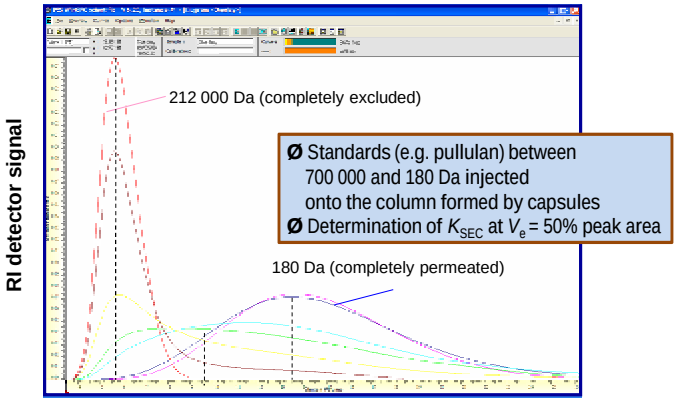
Boltzmann fit



è **calibration curve** for the column (made of capsules)

Inverse size-exclusion chromatography: evaluation

Step 1: Measurement of elution curves



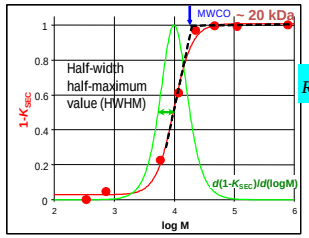
Ø Standards (e.g. pullulan) between 700 000 and 180 Da injected onto the column formed by capsules

Ø Determination of K_{SEC} at V_e = 50% peak area

Inverse size-exclusion chromatography: evaluation

Step 3: Further processing of calibration curve

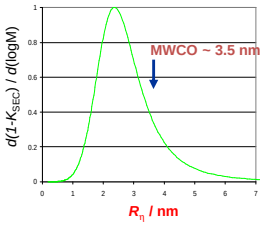
MWCO and PSD based on MW of pullulans



$$R_\eta = 0.015 * (\overline{M}_w^{pull})^{0.55}$$

radius of pores in *nm*

MWCO and PSD based on SIZE of pullulans



MWCO based on SIZE of proteins

Pullulan / kDa MEASURED	R_η / nm	Protein / kDa ESTIMATED
70	6.7	400
35	4.6	150
20	3.5	70
15	2.2	20
4	2.4	6

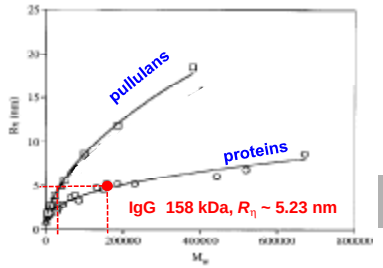
$$M_w^{protein} = \left(\frac{\overline{R}_h}{0.051}\right)^{2.645}$$

MWCO to proteins
Brissova, Petro, Lacik, Powers, Wang. *Analytical Biochem.* **242**, 104-111, 1996

Molecular weight cut-off: to remember…

1. **MWCO is a size-related parameter**

2. **MWCO when expressed by "molecular weight", it is a solute-type related parameter (polysaccharide ≠ protein)**



Brissova, Petro, Lacik, Powers, Wang. *Analytical Biochem.* **242**, 104-111, 1996

Inverse size-exclusion chromatography: Case study #1

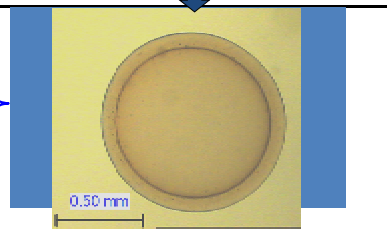
"PMCG" microcapsule

Lacik, Brissova, Anilkumar, Powers, Wang. *J Biomed Mater Res* 39, 52-60, 1998

sodium alginate
+
cellulose sulfate

droplet of polyanion solution

poly(methylene-co-guanidine)
PMCG
+
$CaCl_2$
+
NaCl



Inverse size-exclusion chromatography: expt. conditions

COLUMN PARAMETERS

* resolution

Omnifit glass column with adjustable plungers

Length	10 - 30 cm
Diameter	1 cm
Microcapsule volume	10 - 20 ml*
Microcapsule size	tested up to ~1.5 mm*

ELUENT

Saline solution or any culture media (with NaN_3)
Flow rate 0.1 – 0.2 ml/min*

TESTING SOLUTE TYPE

Pullulan narrow distributed standards, PDI ~ 1.1 (note: dextrans ~ 1.5)
Proteins (may interact ð enthalpic separation?)

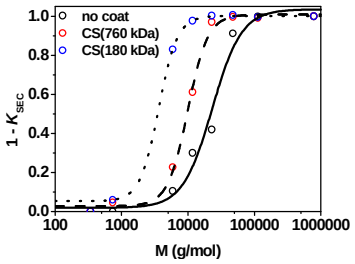
HARDWARE (~ 30 k€)

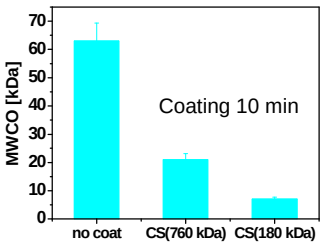
Degasser - HPLC Pump – Injector – RI Detector – (Software)

TIME OF ANALYSIS AND EVALUATION ~ 3 days

Inverse size-exclusion chromatography: Case study #1

"PMCG" microcapsule
Coating by cellulose sulfate (MW dependence)

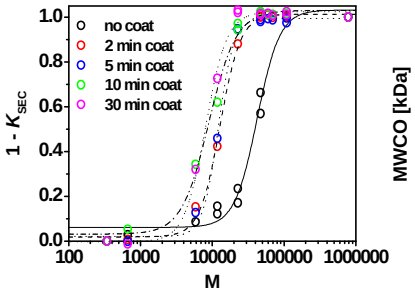


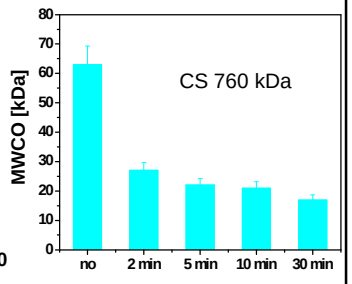


è tuning the MWCO values

Inverse size-exclusion chromatography: Case study #1

"PMCG" microcapsule
Coating by cellulose sulfate (time dependence)

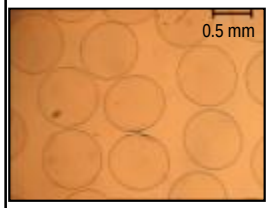


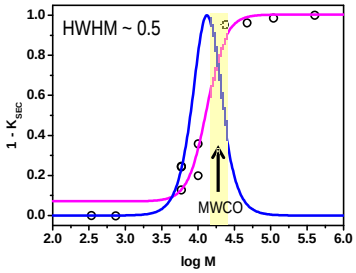


è tuning the MWCO values

Inverse size-exclusion chromatography: Case study #2

COST865: Alginate / PLL microcapsule (Paul)

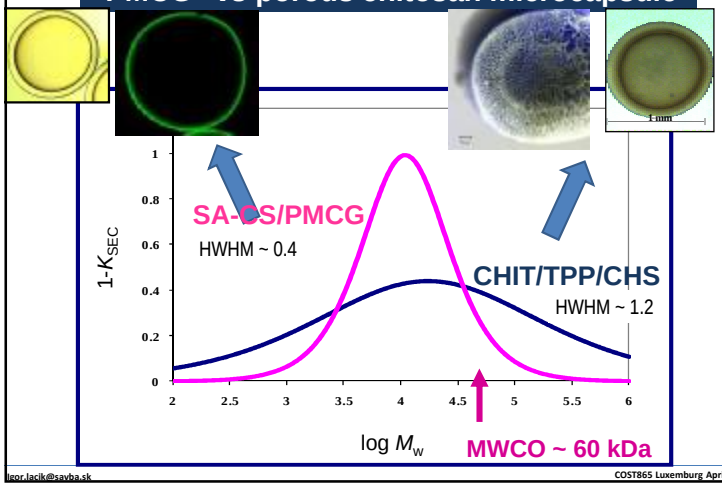




MWCO (pullulans) ~ 15 – 25 kDa ~ 6 – 8 nm ~ **MWCO (protein) 40–100 kDa**

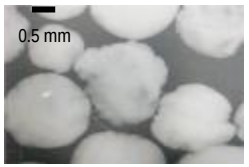
Inverse size-exclusion chromatography: Case study #1

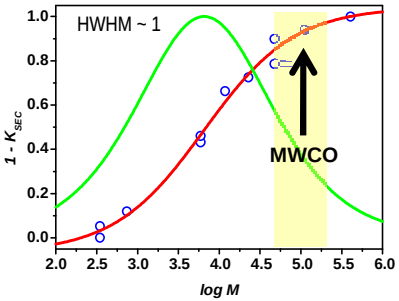
"PMCG" vs porous chitosan microcapsule



Inverse size-exclusion chromatography: Case study #2

COST865: PVOH microcapsule (Marion)



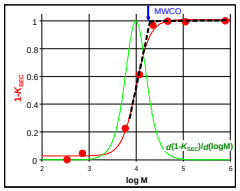


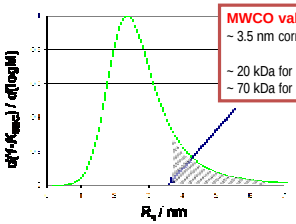
q broad pore size distribution, similar to chitosan microcapsules

q MWCO ~ 100 kDa (pullulans) ~ 16 nm ~ 800 kDa (proteins)

Inverse size exclusion chromatography: final remarks

molecular weight cut-off (MWCO) and effective pore size distribution





MWCO value
~ 3.5 nm corresponds to:

~ 20 kDa for pullulans
~ 70 kDa for a protein

è extremely valuable tool for (1) comparison among the batches and (2) optimization process, (3) indirectly, stability studies (eluent can be saline solution as well as media / artificial body fluids)

è in Bratislava, we are convinced this is true; to my knowledge, currently no other groups use it

- è it should not be the "cost" issue…(30 k€)
- è it may be the "fear" from chromatography
- è it can be the "limited amount of capsules" typically made (?), note: I-SEC requires > 10 ml

è may underestimate the MWCO compared to the direct (long-term) ingress measurements

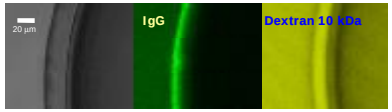
- è usually MWCO is determined as K_{SEC} ~ 0.9 – 1.0

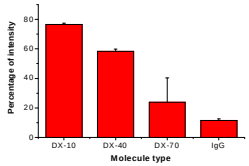
Optional techniques

è a number of techniques have been available and are in use

è **Polymer Institute in Bratislava** in cooperation with International laser centre

è **CLSM:** ingress of fluorescently labeled dextrans (?) and proteins (IgG)





I. Lacík, D. Chorvát, Jr. Visualisation techniques in the characterization of polymer microcapsules: CLSM and AFM. In ***The bioartificial pancreas and other biohybrid therapies***, Halle, J. P., de Vos, P. and Rosenberg, L., Eds., Research Signpost 2009, pp. 137-175

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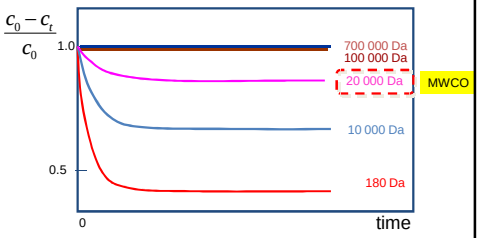
Optional techniques

è **Static incubation:** ingress of dextrans or pullulans from supernatant
(note: dextrans are polydisperse è pullulans prefered)

M. Briššová, M. Petro, I.Lacík, A.C. Powers, T.G. Wang - "Evaluation of Microcapsule Permeability via Inverse Size Exclusion Chromatography", *Analytical Biochem.* **242**, 104-111, 1996

MWCO: search for the first solute which concentration starts to decrease (injector + RI detector)
Partition coefficient:
Can be correlated to I-SEC



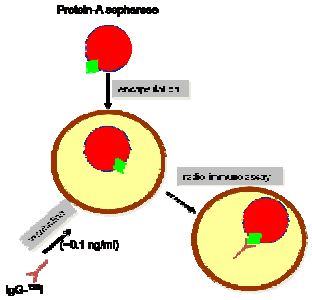
EPFL: incubation in a cocktail of standards, quantity analyzed on SEC columns

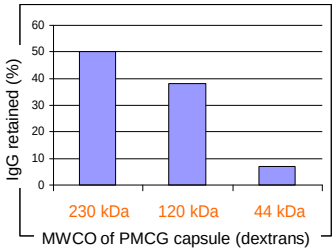
Bartkowiak, A and Hunkeler, D (1999) Alginate-oligochitosan microcapsules: A mechanistic study relating membrane and capsule properties to reaction conditions Chem Mater 11, 2486-2492

Optional techniques

Briššová, M; Lacík, I; Anilkumar, A V; Powers, A C and Wang, T G (1998) Control and Measurement of Permeability for Design of Microcapsule Cell Delivery System J Biomed Mater Res 39, 61-70,

1. immobilization of Protein-A Sepharose particles, which bind IgG
2. measurement of bound radiolabeled IgG in the capsule





Mørch, Y. A., Donati, I., Strand, B. L. and Skjåk-Bræk. G. (2006), Effect of Ca^{2+}, Ba^{2+}, and Sr^{2+} on Alginate Microbeads Biomacromolecules 7, 1471.

- CLSM and RIA of alginate beads
- Permeable to IgG

Acknowledgement

Dept of Special Polymers and Biopolymers
Polymer Institute SAS Bratislava
www.polymer.sav.sk/lacik.html

Conclusions

1. Permeability properties represent an important material characteristics and, therefore, have to accompany any microcapsule development

2. Different experimental approaches and/or the same experimental approaches performed at different laboratories may lead to discrepancies
   - ð COST865 tries to find a solution ð precise description
   - ð to compare various microcapsules, the analysis in one laboratory is recommended as the first “practical” step

3. The permeability (and other) properties of microcapsules after application, i.e. after explantation, are almost completely missing
   - ð here, the inverse SEC is not suitable because of a limited amount of capsules
   - ð the egress/ingress methods needed only a few capsules are recommended and should be regularly used

Acknowledgement

Slovak Agency for Science and Development, Projects # 51-033205, 51-016002

The Chicago Diabetes Project: Global collaboration for a functional cure coordinated by the University of Illinois at Chicago and the Christopher Foundation

COST 865