Formulation of biotech products

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Acknowlegedments

Statement of conflict

- None

Learning objectives and outcomes

1) Knowledge of measures to remove microbial contaminants and their limitations
2) Knowledge of conventional measures to inhibit drug degradation and their limitations
3) Knowledge of newer approaches for protein stabilization and improving protein delivery

Challenges of biotech drugs

Biopharmaceuticals have lengthy, expensive and complicated formulation processes in comparison to those of low molecular weight drugs

Chemical stability

Sterilization

Physical stability

Delivery kinetics

Administration routes

Targeting

Sustained action

Presystemic metabolism

Half-life

Clearance by MPS

Bioavailability

Antigenicity

Formulation strategies and tools

Stabilization

Crystallization

PEGylation

Aseptic processing

Bioconjugation

Protease inhibitors

Localized Delivery

Absorption enhancers

Nasal delivery

Bioadhesion

Pulmonary delivery

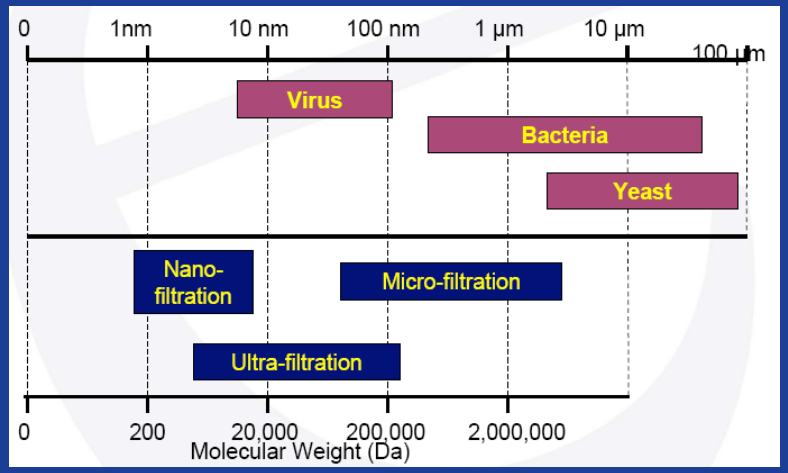
Microneedles

Sterility/purity of a biotech product – the challenges

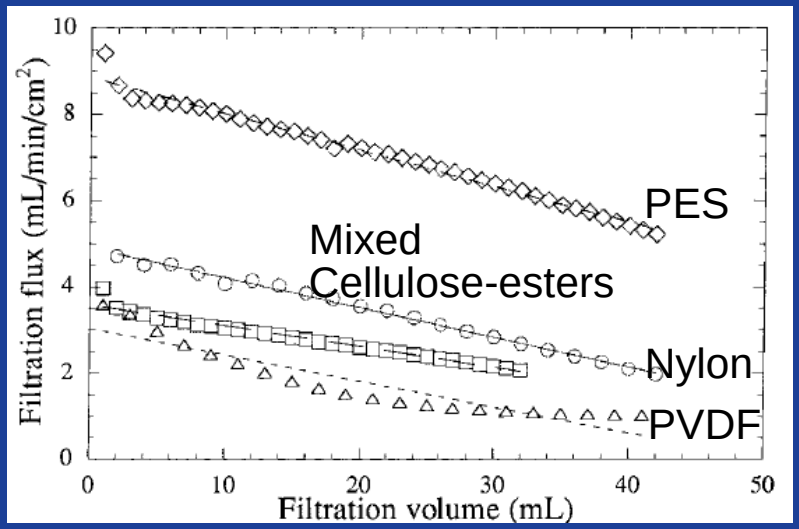
- Chemical and physical instability of drug
- Bacterial contaminants
- Viral contaminants (from production cell line or process-related)
- Pyrogens (from production or process-related)

Elimination and killing of bacteria (yeast)

- Sterilization?
- Aseptic processing – sterile filtration (0.22 μm)
- Low protein binding filters, eg, hydrophilic PVDF, PES



http://www.edenbiodesign.com/documents/info_eden_biodesign1.pdf



rhHG filtration through 0.22 μm filters

Maa and Hsu. *J PharmSci* 1998;87:808–812

Elimination and killing of viruses

- Sources: cell banks, human/animal materials (plasma); viral seeds; culture media; affinity matrices
- Aim: load of 10^{-6}; 1 - 2 validated clearance steps

- Inactivation (preferred): heat, chemical
- Clearance:
  - precipitation
  - chromatography
  - ultracentrifugation
  - filtration

Table 3. Validated Virus Clearance Steps in the Talecris Albumin Products (20% and 25%) Production Process[6]

Process Steps	Reduction Factor (log_{10}) for Enveloped Viral			Reduction Factor (log_{10}) for Non-Enveloped Viruses		
	HIV	BVDV	PRV	Reo	HAV	PPV
Fraction II + III separation	3.4	3.6	3.9	2.1	1.4	1.0
Depth filtration	3.4	<1.0	≥3.4	4.9	2.0	4.2
Precipitation/acetone suspension	≥5.1	7.5	≥4.2	2.3	ND	ND
Pasteurization	≥5.9	≥5.2	≥4.8	5.6	4.4	1.6
Total reduction factor (log_{10})	**≥17.8**	**≥16.3**	**≥16.3**	**14.9**	**7.8**	**6.8**

HIV: Human immunodeficiency virus
BVDV: Bovine viral diarrhea virus, a model for HCV
PRV: Pseudorabies virus, a model for HBV and herpes viruses
HAV: Hepatitis A virus
Reo 3: Reovirus type 3, a model for viruses resistant to physicochemical agents
PPV: Porcine parvovirus, a model for human parvovirus B19
ND: Not done

http://www.plasbumin.com/images/table_3.gif

Virus clearance by filtration

- Size exclusion and/or adsorptive retention
- Direct flow or tangential flow

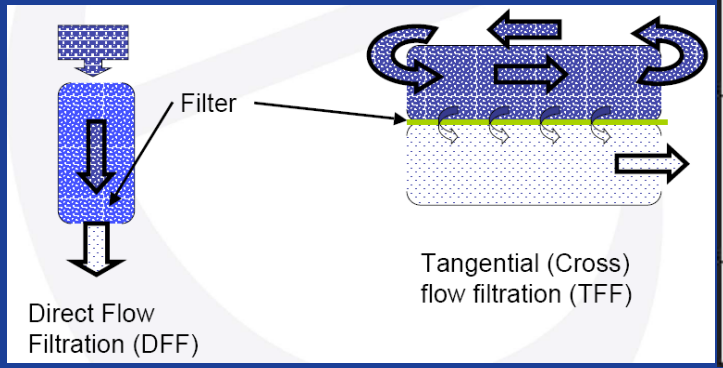


Table 1. Commercially available virus filtration products

Company	Product	Virus Retention Claim	Virus Size
Asahi-Kasei	Planova 15N	>6.2 log Parvovirus >6.7 log Poliovirus	18–26 nm 28–30 nm
	Planova 20N	>4.3 log Parvovirus >5.4 log Encephalomyocarditis	18–26 nm 28–30 nm
	Planova 35N	>5.9 log Bovine Viral Diarrhea virus >7.3 log HIV	40–70 nm 80–130 nm
Millipore	Viresolve NFP	>4 log ϕX-174 bacteriophage	28 nm
	Viresolve NFR	>6 log Retrovirus	80–130 nm
Pall	ULtipor DV20	>3 log PP7 bacteriophage >6 log PR772 bacteriophage	26 nm 76–88 nm
	ULtipor DV50	>6 log PR772 bacteriophage	76–88 nm
Sartorius	Virosart CPV	>4 log PP7 bacteriophage >6 log Retrovirus	26 nm 80–130 nm

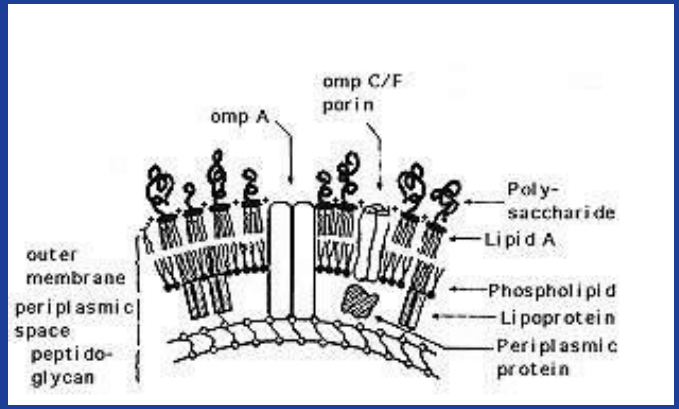
http://www.edenbiodesign.com/documents/info_eden_biodesign1.pdf

Kern G and Krishnan M. *Biopharm Int* Oct 2006

Pyrogen removal

- Maximum endotoxin levels:
  5 EU/kg product/h

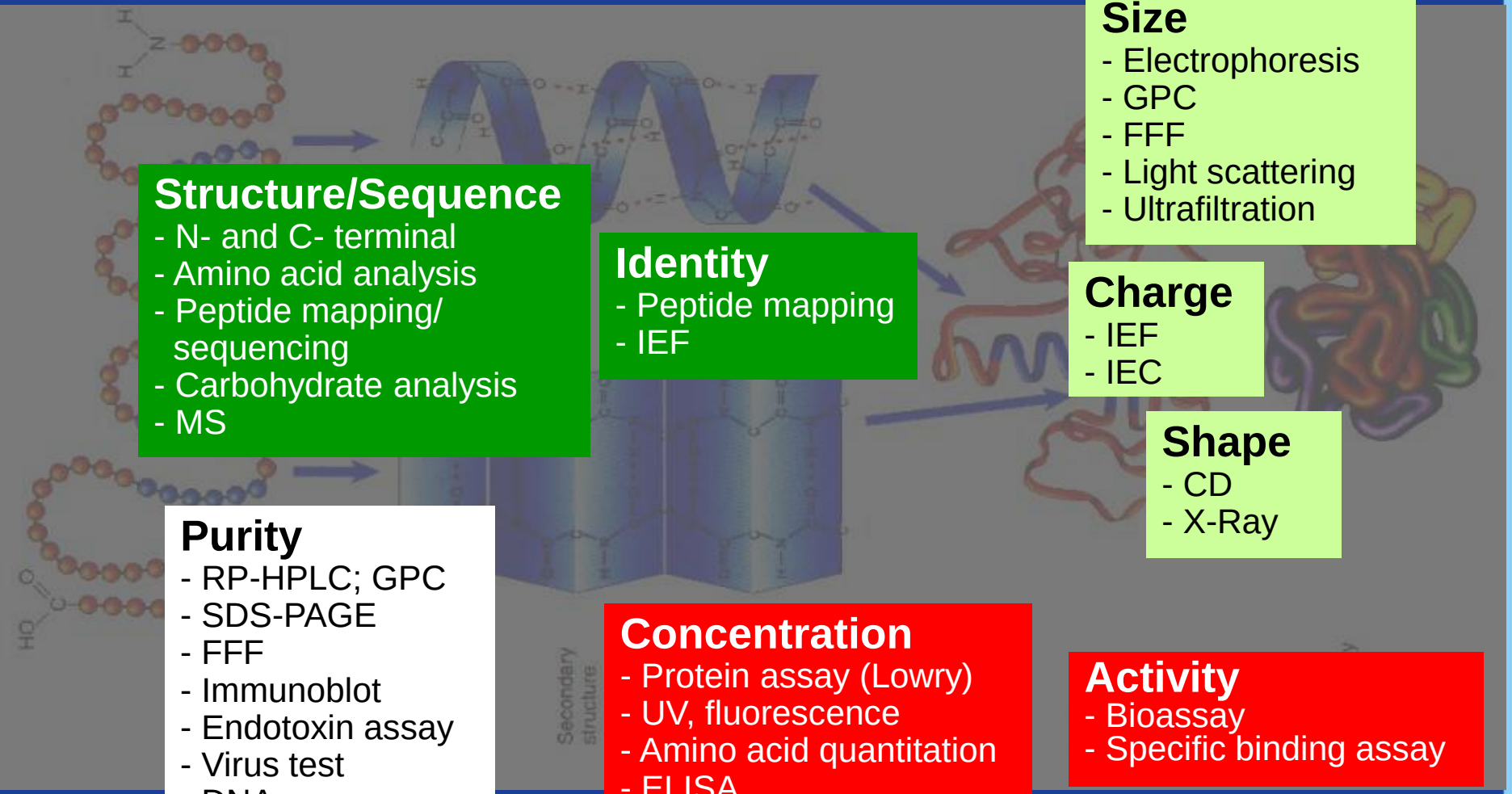
- Detection:
  - rabbit
  - LAL
  - monocyte activation/cytokine assay



www.bact.wisc.edu/
themicrobialworld/endo1.jpg

- Removal:
  - ion exchange chromtaography
  - ultrafiltration
  - inactivation (heat, oxidation, hydrolysis)

Instability of protein requires extensive protein analytics



Size
- Electrophoresis
- GPC
- FFF
- Light scattering
- Ultrafiltration

Structure/Sequence
- N- and C- terminal
- Amino acid analysis
- Peptide mapping/ sequencing
- Carbohydrate analysis
- MS

Identity
- Peptide mapping
- IEF

Charge
- IEF
- IEC

Shape
- CD
- X-Ray

Purity
- RP-HPLC; GPC
- SDS-PAGE
- FFF
- Immunoblot
- Endotoxin assay
- Virus test
- DNA assay

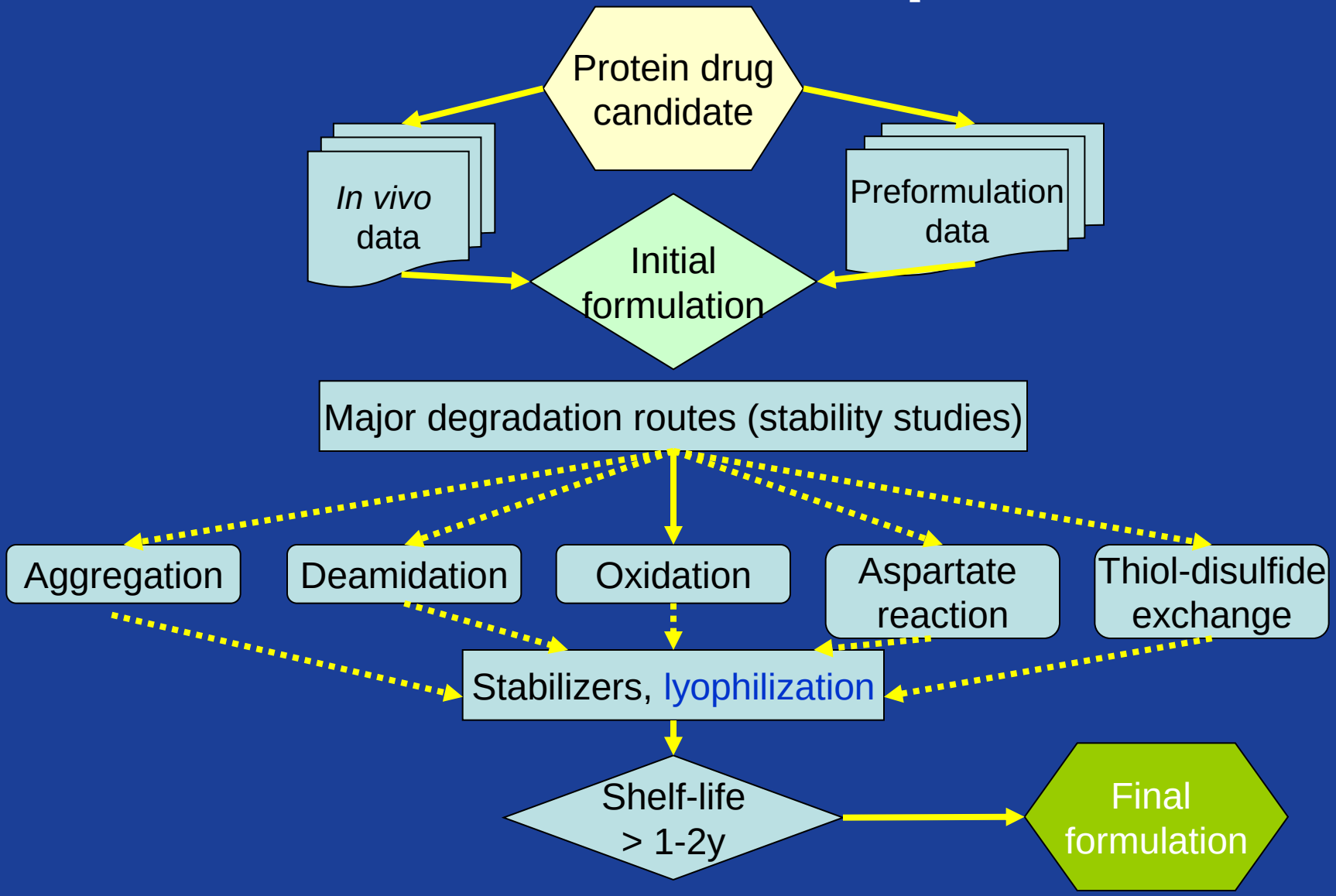
Concentration
- Protein assay (Lowry)
- UV, fluorescence
- Amino acid quantitation
- ELISA

Activity
- Bioassay
- Specific binding assay

Additives needed to produce water-soluble injectable solutions

• pH	4-6
• Buffers	Citrate, acetate, glycine, histidine, succinate, phosphate, tris
• Antioxidants	Ascorbic acid, citric acid, Met, Cys, EDTA (O_2 removal/protection; protection against light)
• Preservatives	Phenol, benzyl alcohol, benzoic acid, parabens
• Surfactants	Polysorbate, poloxamer, albumin
• H-bonding promoters	Sugars, glycols
• Steric shields	PVP, dextran, PEG, albumin, poloxamer
• Complexation agents	Zn, Mg
• Cryoprotectants	Sugars, sugar alcohols, glass forming agents
• (Chemical stabilization)	(Pegylation, replacement of amino acids)

Formulation development



Chemical and physical stability – stress factors and effects

- pH
- Ionic strength
- Temp, O_2

- Agitation, shear forces
  Stirring, pumping, filtration, filling
- Interfaces
- Freezing
- Freeze-drying
- Moisture

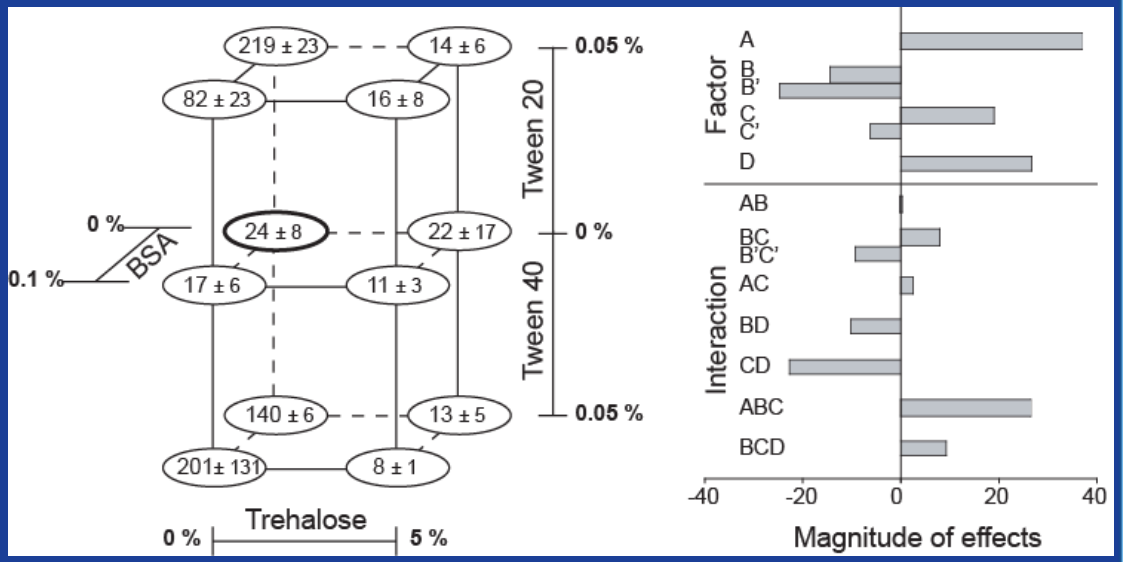
- Deamidation (Asn, Gln)
- Cleavage (Asp-X)
- Oxidation (Met, Cys, His, Trp, Tyr)
- Thiol disulfide exchange (Cys)

- Conformational changes
- Aggregation
- Adsorption

Formulation?

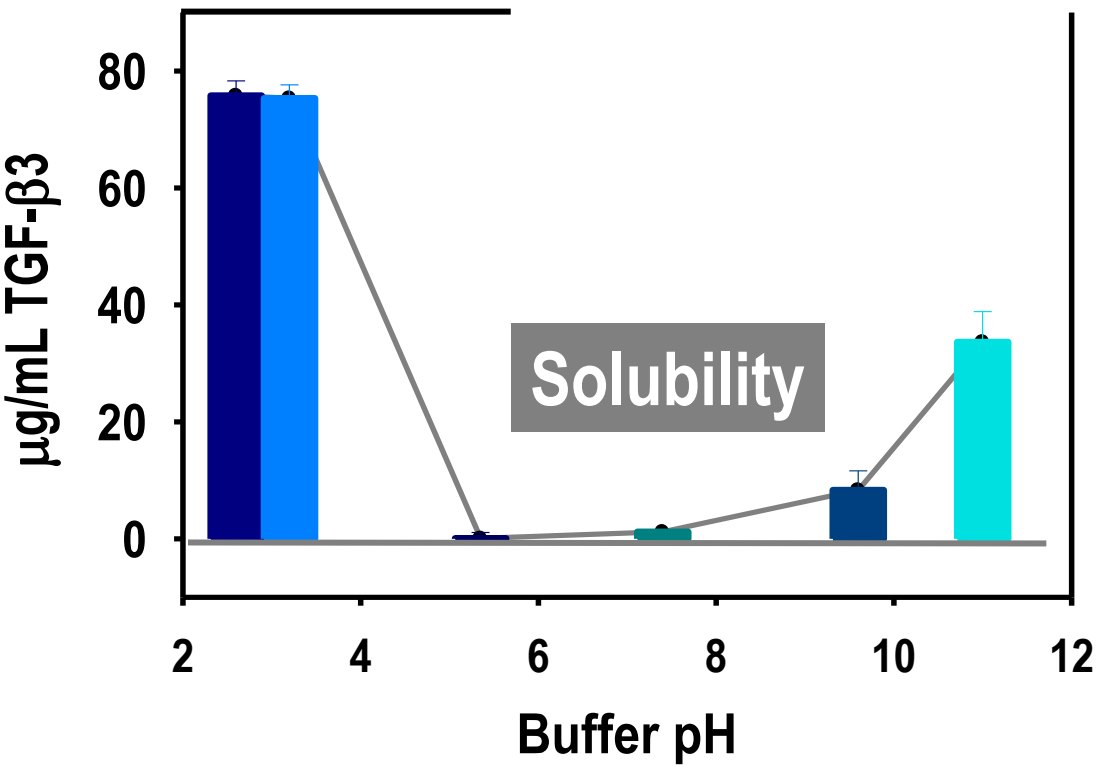
Intuition ? or Experimental factorial design?

Illustrated by Leigh Rondano,
Boehringer Ingelheim Pharmaceuticals



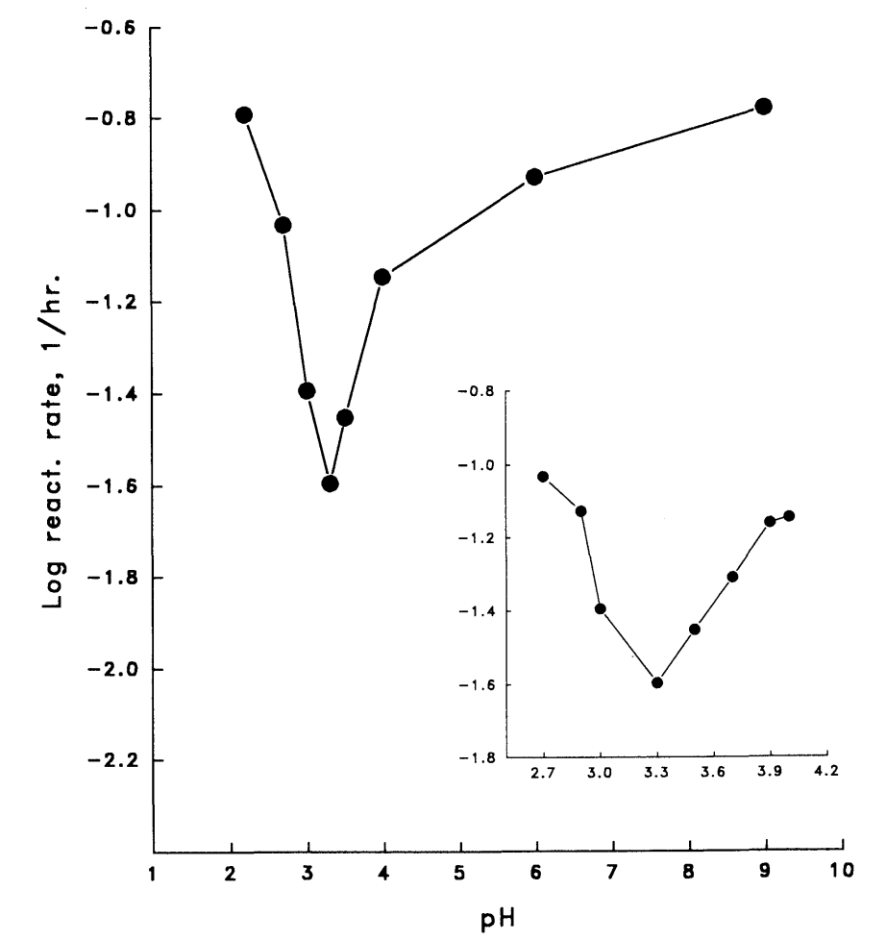
Pfister L. PhD-thesis, ETH Zurich, 2007

Effect of pH on solubility of TGF-β3



Jen AH et al. *J Control Rel* 2001;78:25–34

Effect of pH on sCT degradation



Degradation of salmon calcitonin in 0.01 M citric acid / 0.02 M phosphate and in 0.01 M HCl / 0.02 M borate buffer (pH 9) solution at 70 ° C

Lee KC et al. *Pharm Res* 1992;9:1521–1522

Effect of shaking on stability and antigenicity of various insulins

Table 3
Long-term physical stability of various insulins evaluated by shake testing[a]

Sample[b]	$T_{50\%}$ (days)[c]	Antigenicity (Relative IgG-titres in mice; %)
Zn^{2+}-insulin	0.5±0.3	100.0
Zn-free insulin	0.4±0.2	
F750	18.4±2.8	1.18
F2000	20.7±4.1	0
K750	4.3±1.1	0.96
K2000	8.6±1.7	

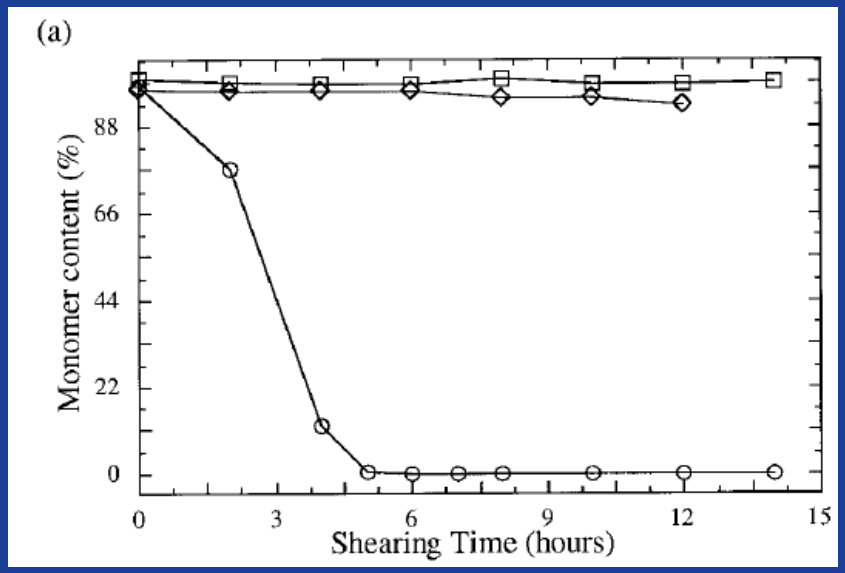
[a] Accelerated shake-test done at 100 strokes/min and 37 °C.
[b] Protein solutions prepared in PBS (38.0 mM, pH 7.4) containing 0.01% Na-azide.
[c] Elapsed time to 50% of the initial protein concentration remaining (mean±standard deviation).

Hinds and Kim. *ADDR* 2002;54:505–530

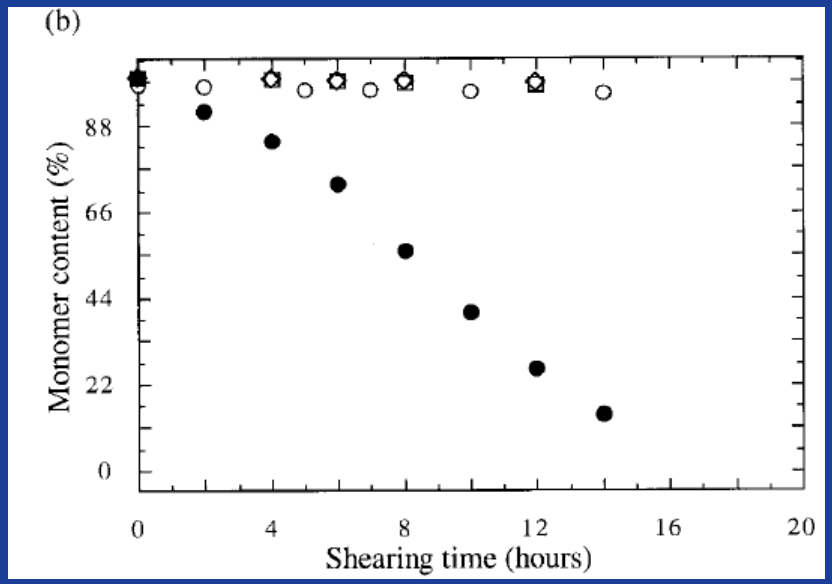
Effect of shearing on aggregation of proteins

Protein monomer content under shearing (concentric cylinder device; 1500 rpm)

Maa and Hsu. *J Pharm Sci* 87:808–812 (1998)



in presence of air-liquid interface;
Key: ○ rhGH; □ rhDNAse; ◊ rt-PA



in presence of 1% PS beads (4-150 μm)
Key: (●) rhGH; (○) rhGH + 0.05% polysorbate 20; (□) rhDNAse; (◊) rt-PA

Shelf-life of marketed sCT products

Product	Adminis-tration	Storage	Excipients
Miacalcin® Novartis	sc, im	2-8 ° C, 2 w at RT	Acetate buffer, phenol
Forcaltonin® Unigene	sc, im	2-8 ° C	Acetate buffer
Miacalcin® Novartis	Nasal spray	15-25 ° C for 4 w	HCl, benzalkonium chloride
Fortical® Unigen	Nasal spray	20-25 ° C for 30 d	Citrate buffer, phenyl ethyl alcohol, benzyl alcohol, polysorbate 80

Freeze-drying may increase protein stability – critical steps

- **Freezing**
  - ➔ structural perturbations (‘cold denaturation’)
  - ➔ pH-shifts
  - ➔ increasing concentration of protein and additives
  - ➔ protein adsorption to ice-liquid interface
  - ➔ mechanical stress

- **Dehydration**
  - ➔ structural perturbations (loss of H-bonds; unfolding)
  - ➔ structural damage (aggregation)

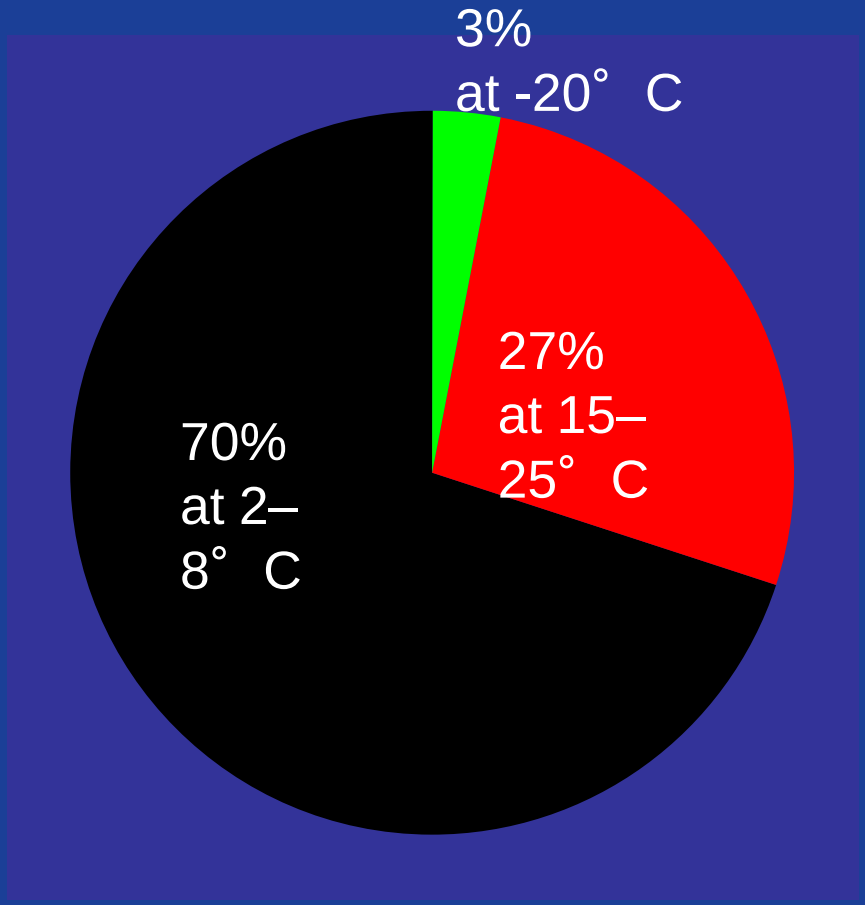
Freeze-drying – excipients

- Buffering agents
- Antioxidants
- Surfactants
- Complexation agents
- Chelators
- Preservatives
- Tonicifiers
- Cryo-/lyoprotectants: PEG, sugars, mannitol, lactose, trehalose, albumin
- Bulking agents: mannitol, sorbitol, glycine, arginine
- Collapse temp. modifiers: dextran, albumin, gelatine

Cryo-/lyoprotectants, bulking agents, collapse temperature modifiers

- Cryo-/lyoprotectants:
  - replace water for hydrogen bonding
  - increase T_g and collapse T of cake (*cave* sorbitol!)
  - adsorb moisture from stoppers
  - prevent overdrying during secondary drying step
  - 350–500 moles of sugar/mole protein
- Bulking agents: elegance of cake
- Collapse temp. modifiers: increase collapse temperature

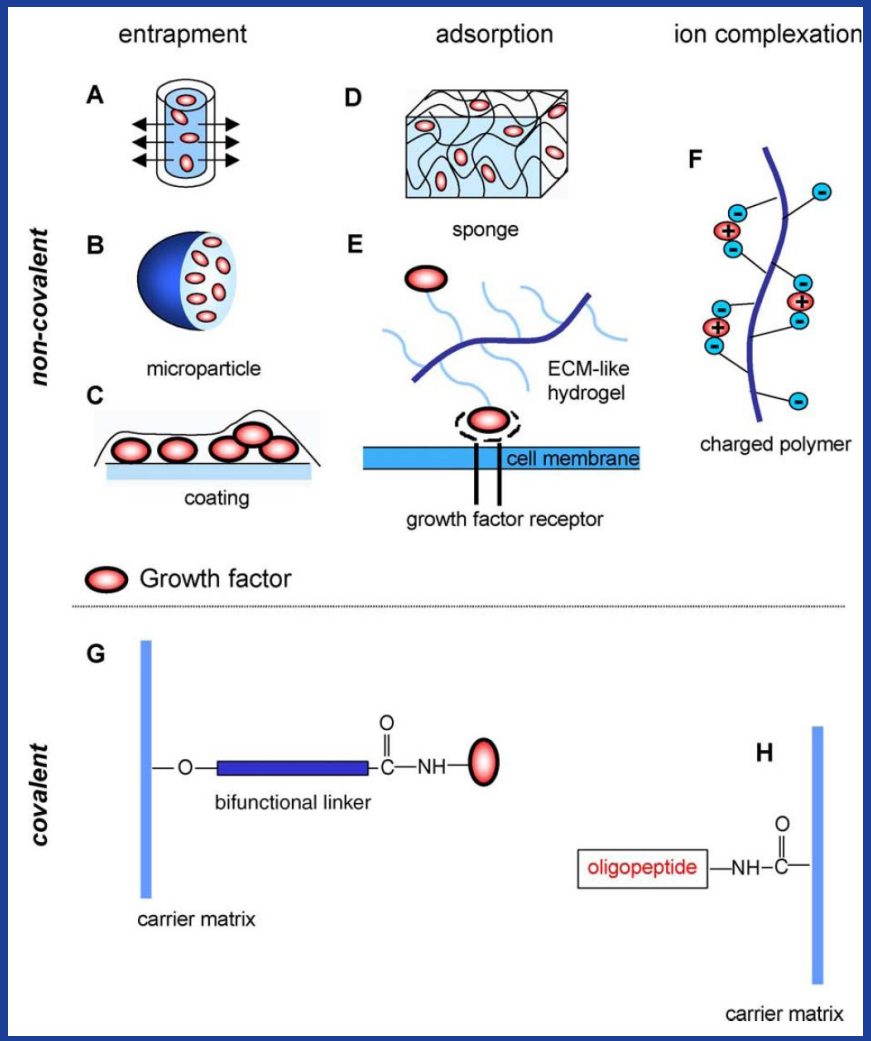
Storage conditions for lyophilized FDA-approved proteins



Example: trastuzumab (Herceptin®)

- Recombinant humanized monoclonal antibody against HER2/neu receptor of tumor cells
- **Lyophilisate**
- Components:
  histidin HCl/histidin, trehalose, polysorbate 20
- Storage: 2–8 ° C
- Incompatibilities: glucose, other drugs ➔ aggregation
- pH after reconstitution: 6
- Reconstitution: avoid shaking and foam formation
- Injection: empty syringe slowly to avoid aggregation

New approaches for protein stabilization (and controlled release)



Luginbuehl V et al. *Eur J Pharm Biopharm* 2004;58:197–208

Adsorption on nanocarriers

Activity of soy bean peroxidase exposed to 95° C.

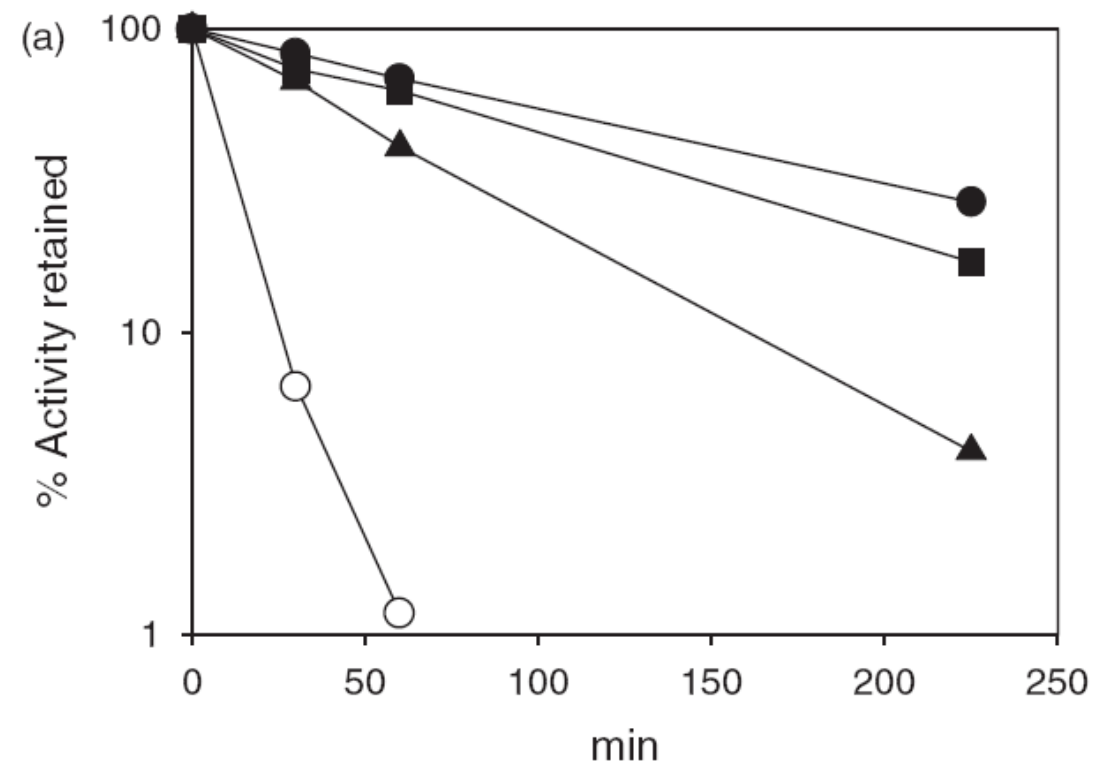
(○) enzyme in solution

enzyme adsorbed on nanocarriers:

(●) fullerenes;

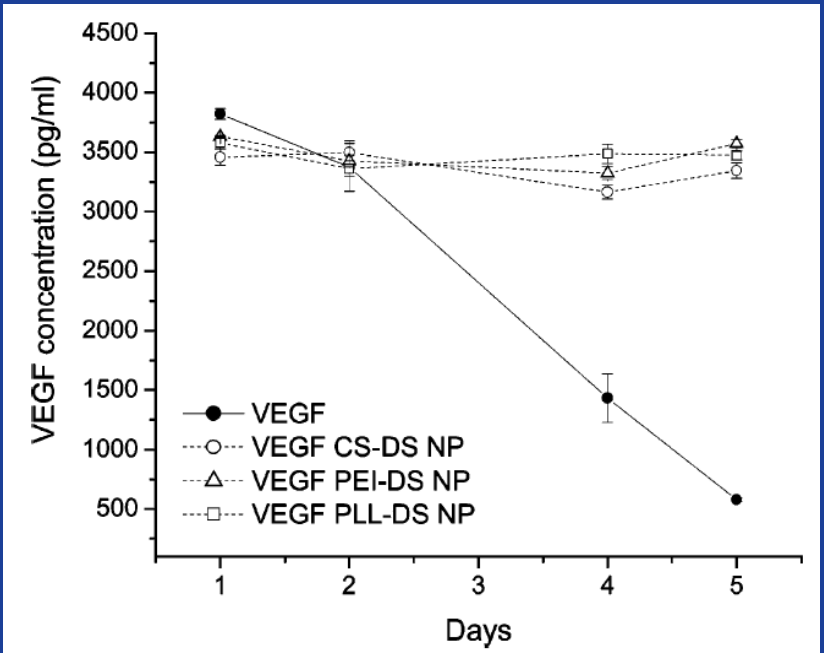
(■) carbon nanotubes;

(▲) graphite flakes;



Asuri P et al. *J Nanosci Nanotechnol* 2007;7:1675–1678

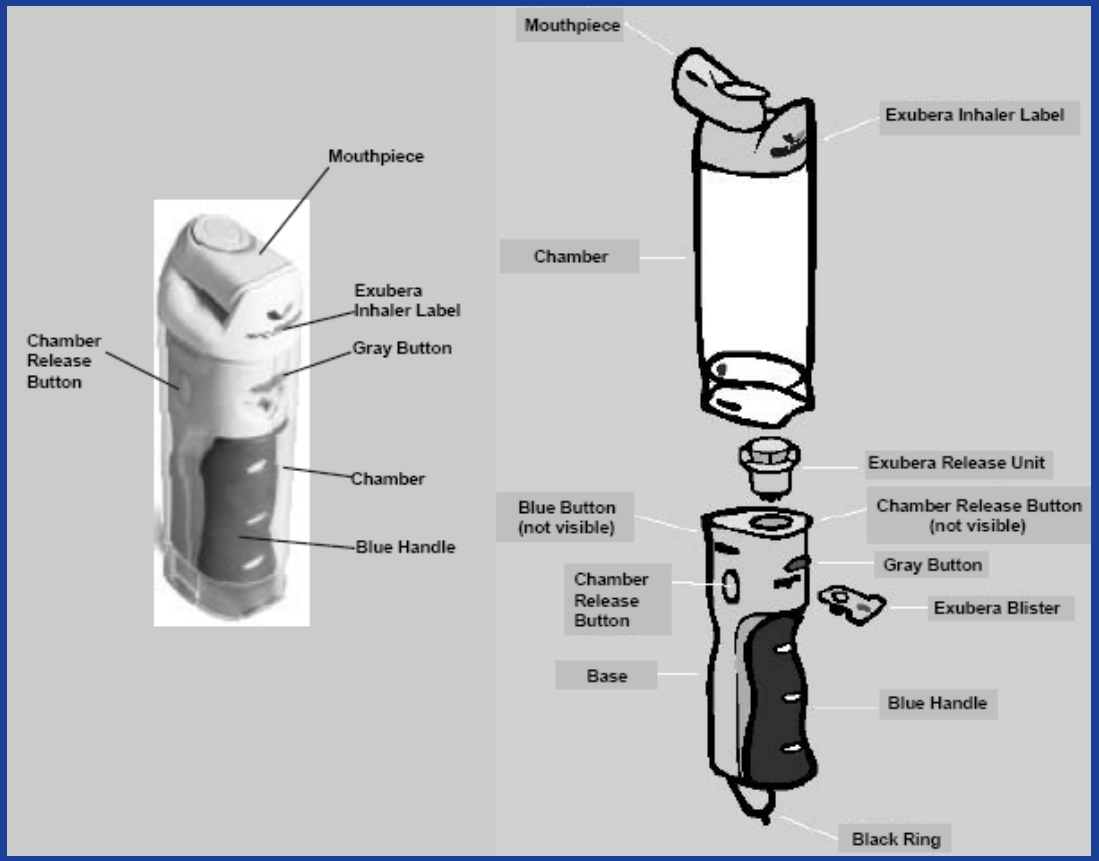
VEGF-polyelectrolyte nanoparticles



Bioactive VEGF in medium from human umbilical vascular endothelial cells (HUVEC) after incubation of
(●)VEGF solution,
or VEGF-polyelectrolyte nanoparticles:
(○) dextran sulfate-chitosan;
(△) dextran sulfate -polyethyleneimine;
(□) dextran polyethyleneimine

Huang M et al. *Biomacromolecules* 2007;8:1607–1614

Nasal spray of insulin (Exubera®)

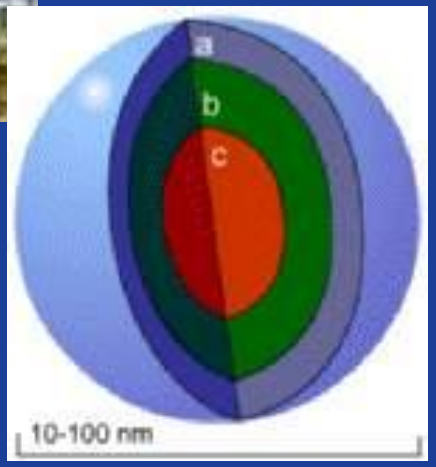


<u>Components</u>
Insulin, 1 mg
Mannitol
Glycin
Na-citrat
NaOH

Pulmonal inhalation pulmozyme (rhDNAse; dornase-α)

- Inhalation solution (2.5 mg protein +$CaCl_2$ +NaCl; not buffered!; 2.5 ml WFI)
- Incompatibilities: other drugs or excipients
- Storage: 2–8˚ C; at 30˚ C for max. 24 h)

Have we met the learning objectives?



ETH Zurich –
a world full of proteins and more!