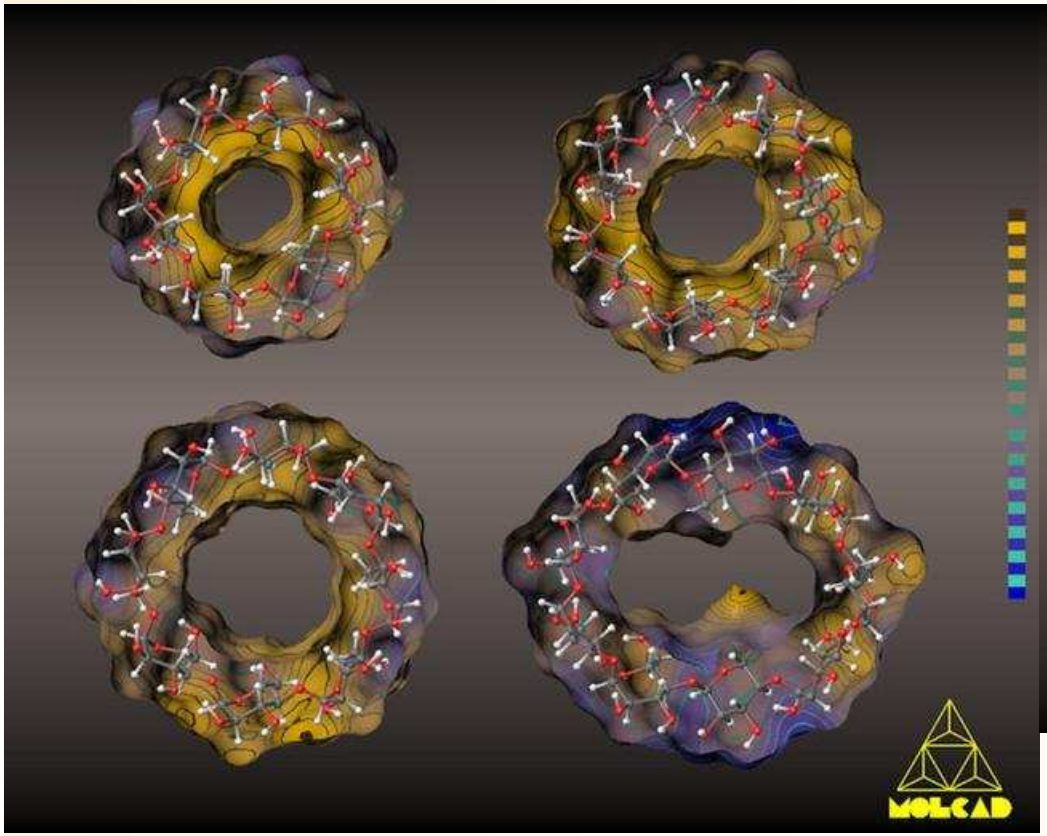
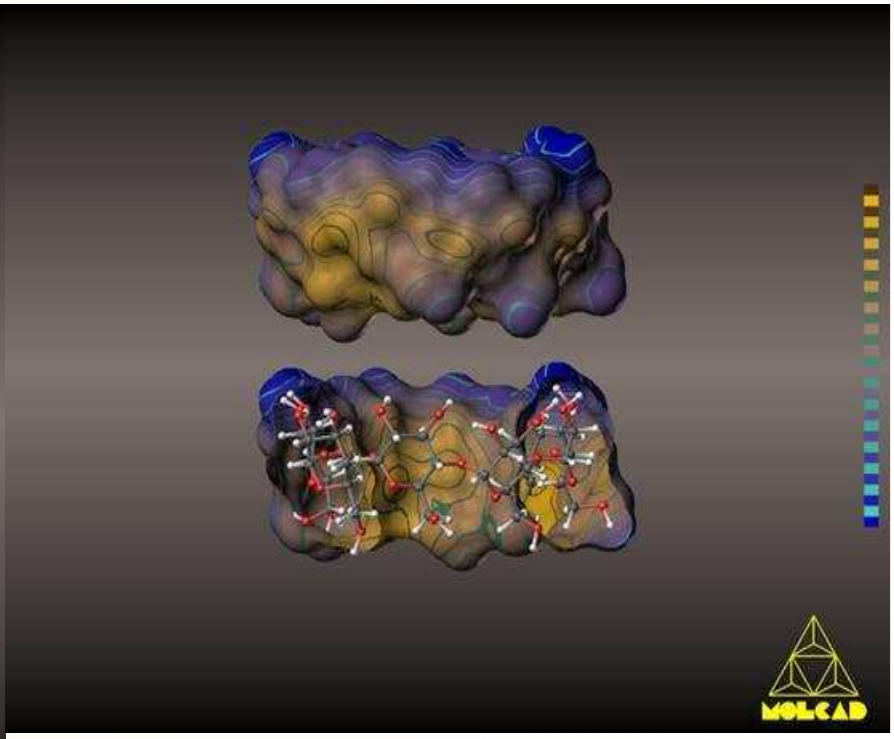
Cyclodextrin – protein interactions

Practical aspects

Cyclodextrins are molecular containers

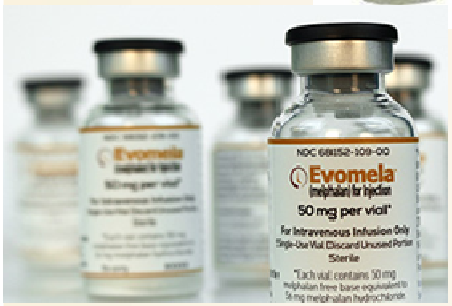
CYCLOLAB
The Cyclodextrin Company



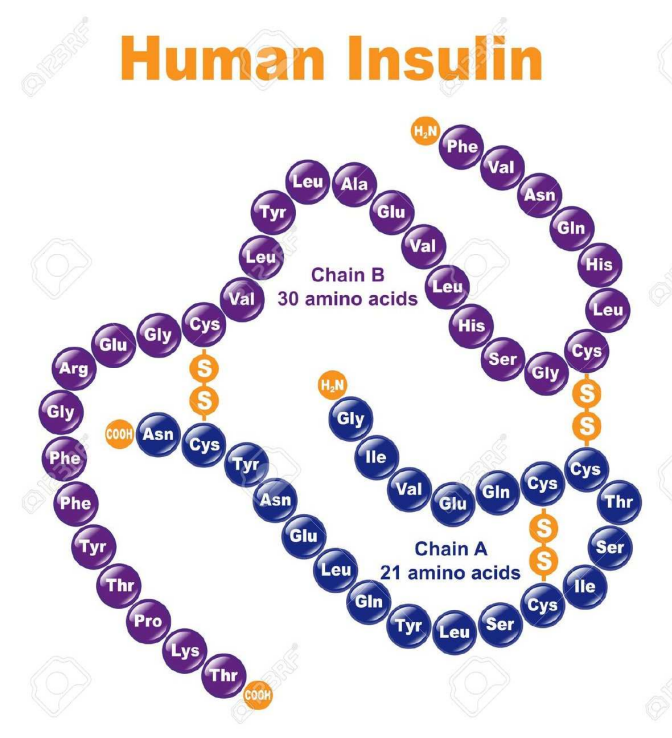


Small molecules formulated with CDs (61 products in 2017)

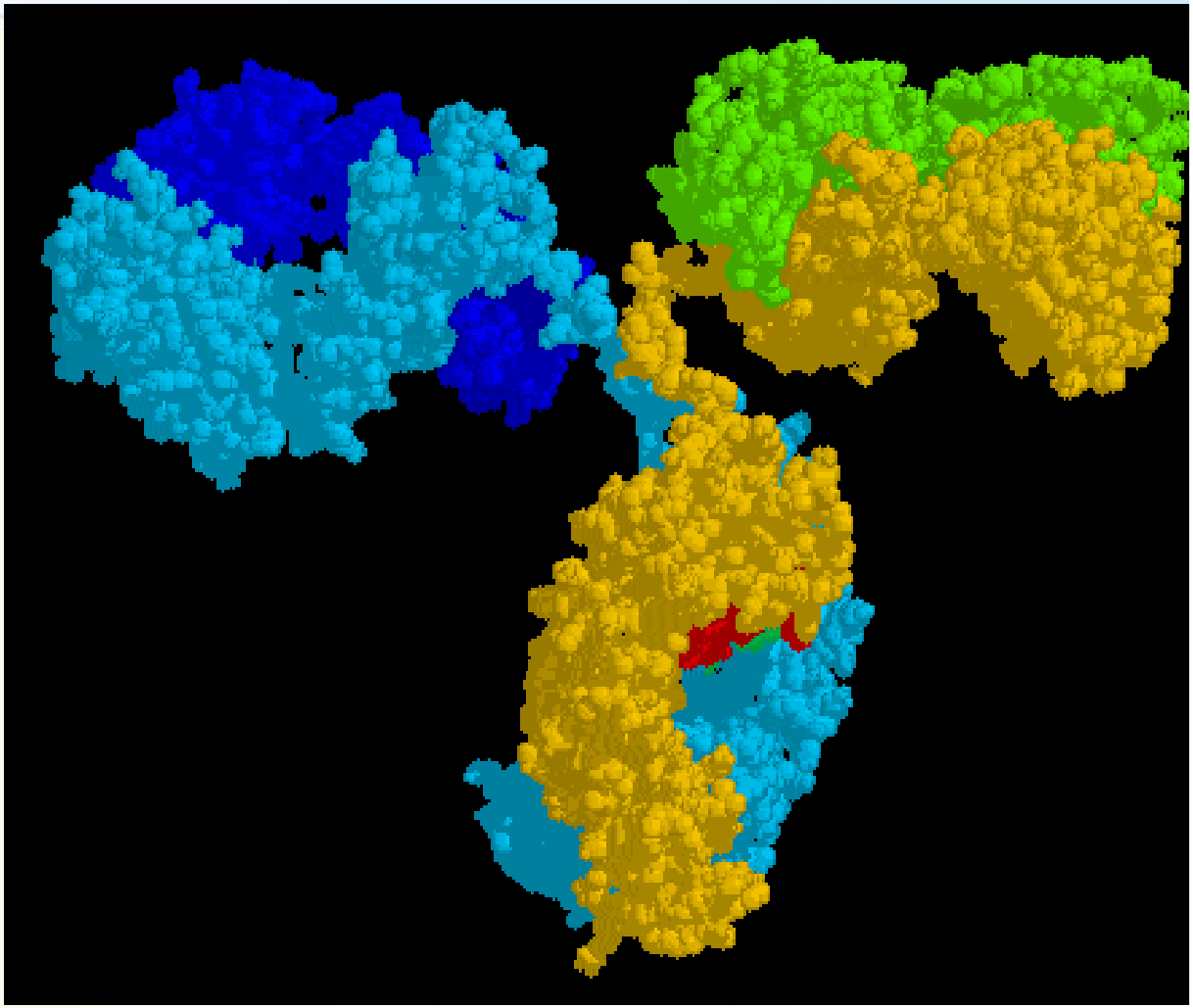
CYCLOLAB The Cyclodextrin Company



Biological active substances, peptides, proteins:

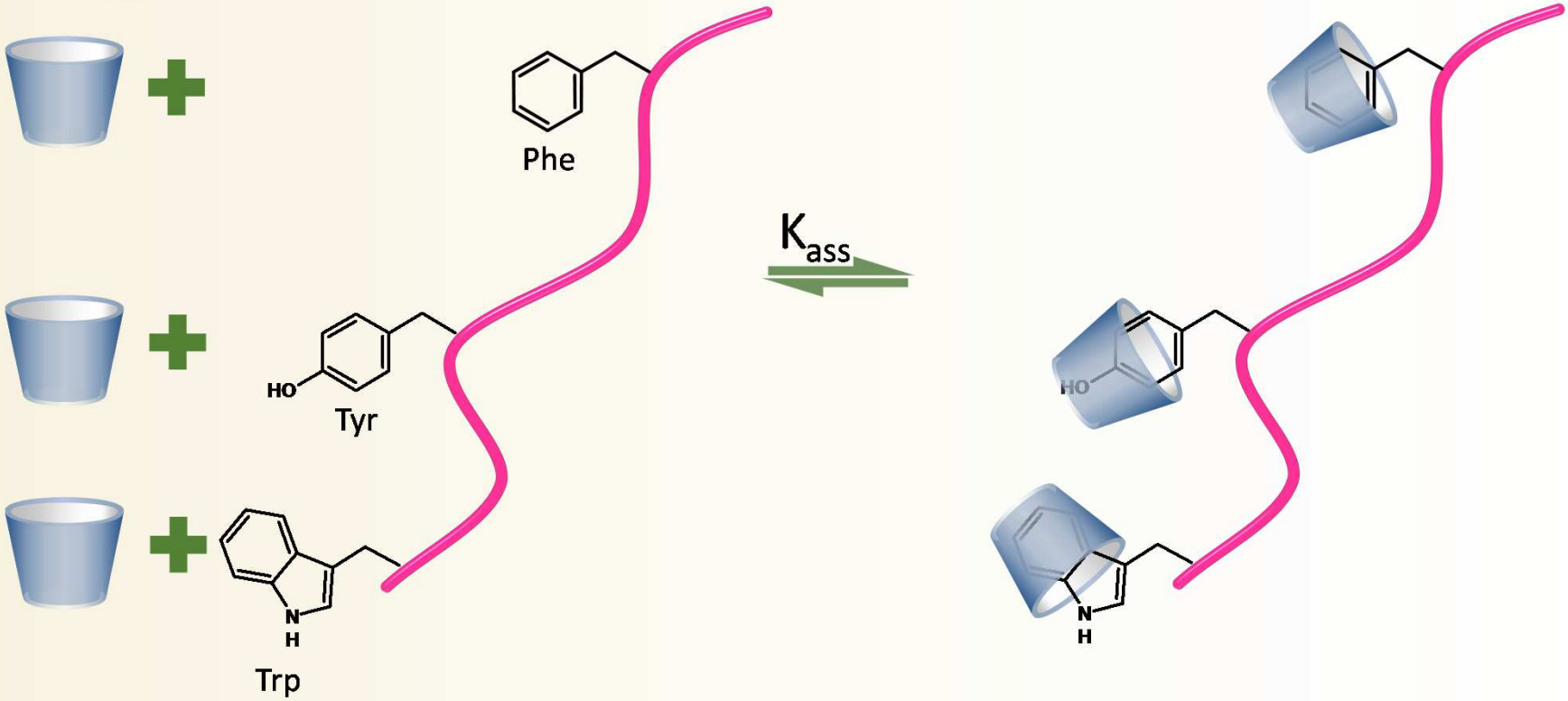


Peptide hormones (Inzulin)
5808 Da



Monoclonal antibodies
~1300 amino acids, 150 000 Da

Protein – CD interactions



Association dominantly on aromatic amoni acids

Trp > Phe > Tyr order of affinity

(Szente-Szejtli,1980)

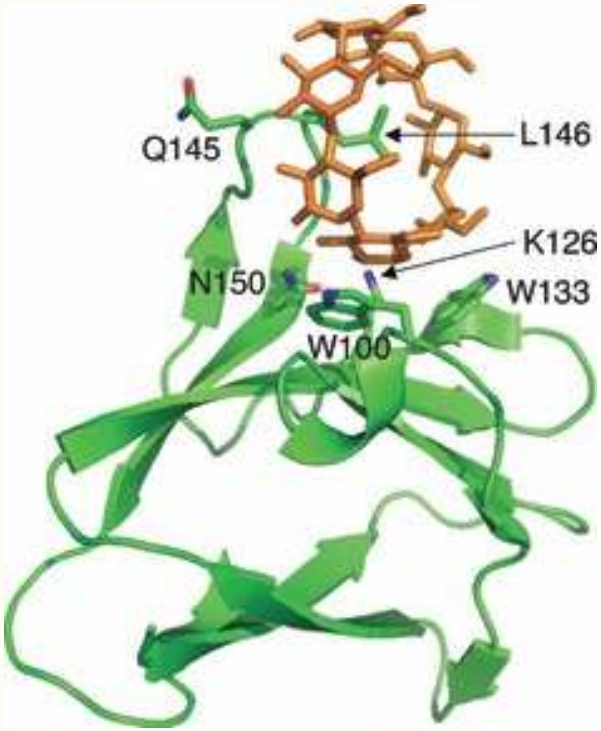
Characteristics of protein-CD interaction

- CDs attract to hydrophobic regions of the proteins
- The interactions are host-guest and electrostatic type
- CDs act like artificial chaperons
- Certain CDs act like caotropic agents and delay protein-protein interaction and thereby folding in solution

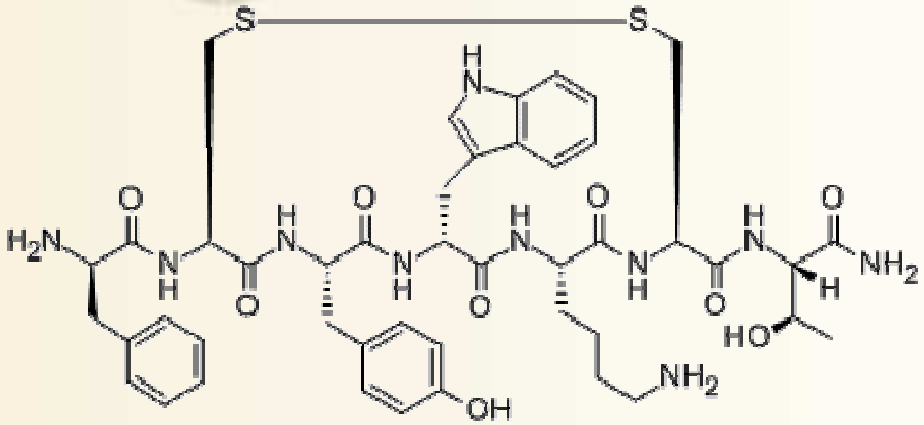
Protein stabilizing agents
(Han, 2007)

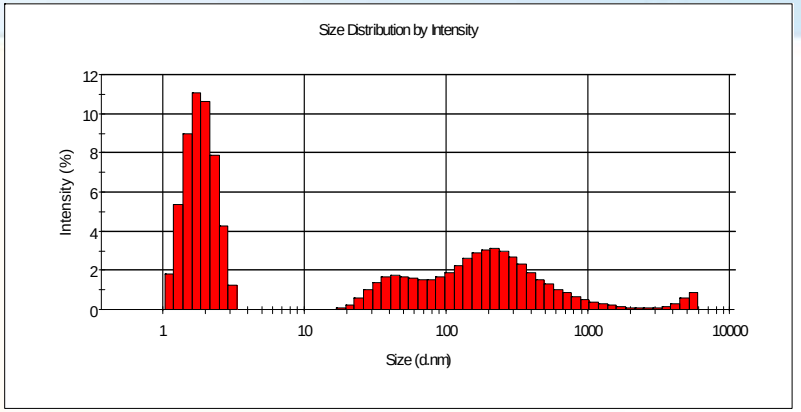
- **Agents affecting osmosis (trehalose)**
- **Surface active agents (Tween)**
- **Polymers(PEG)**
- **Poliols: glycerin, polysaccharides**
- **Cyclodextrins**

Cyclodextrin's effect on protein aggregation

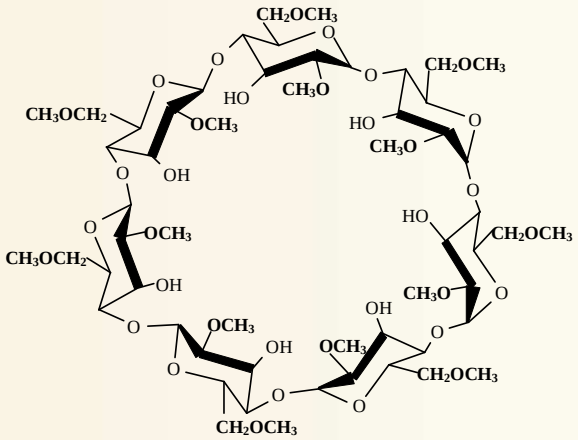


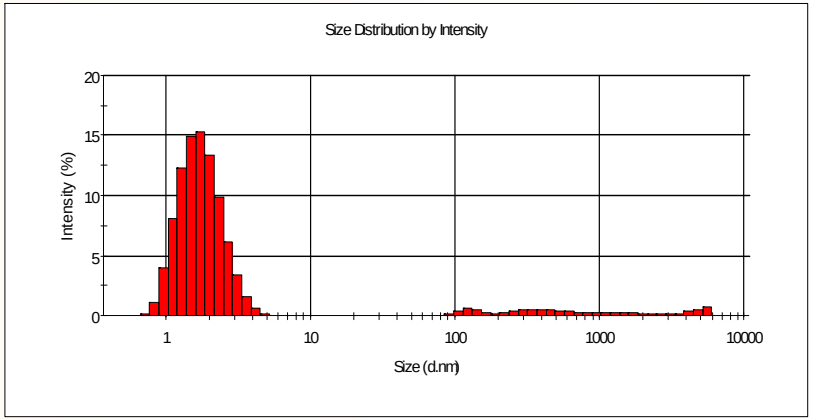
A stomatostatin analogue heptapeptide's aggragation in buffer





Without additive



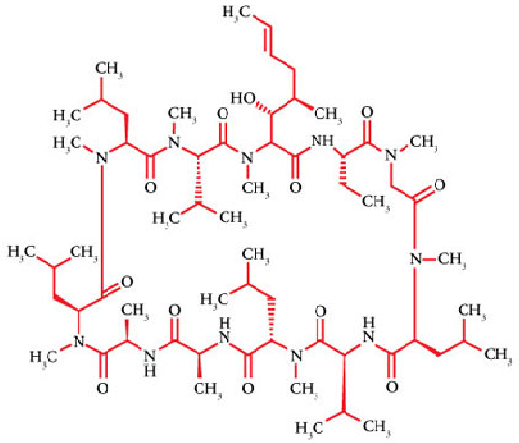


With DIMEB-CD

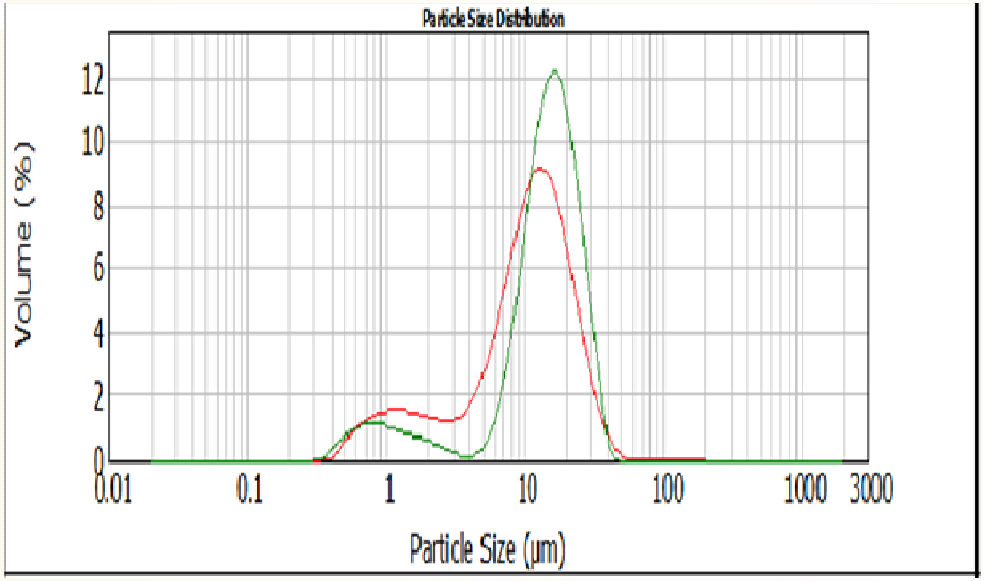
TT-232, heptapeptide: D-Phe-Cys-Tyr-D-Trp-Lys-Cys-Thr-NH2

Kéri, Gy et al. ANTICANCER RESEARCH 27: 4015-4020 (2007)

Preventing aggregation of CyclosporinA

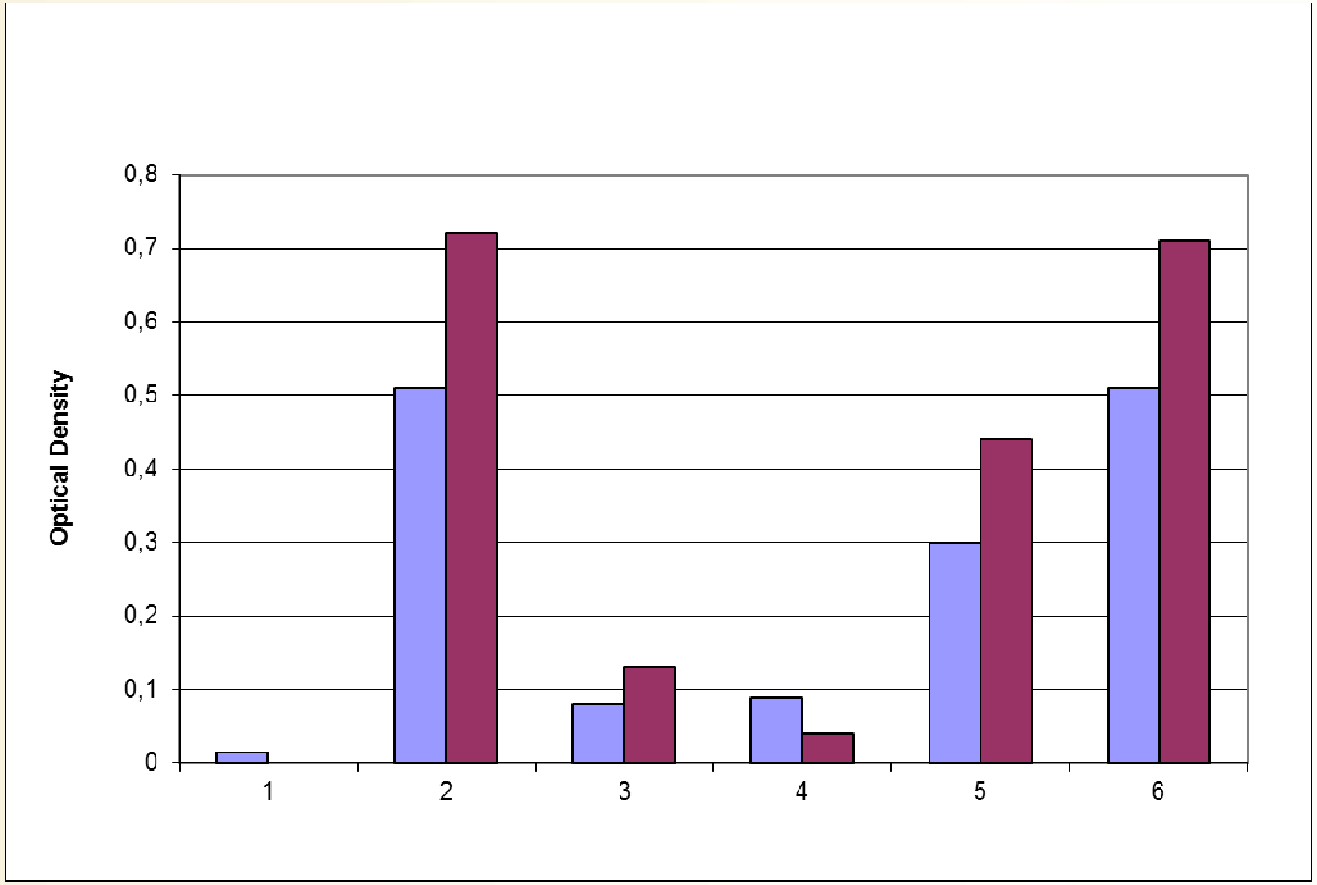


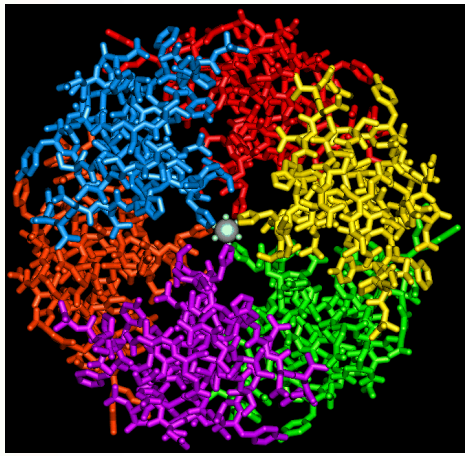
Cyclosporin A



Particle Size distribution in commercial formulation és a CD enabled formulation (Boukhris et al 2012)

CDs effect on insulin aggregation *(„Shaken, not stirred!”)*

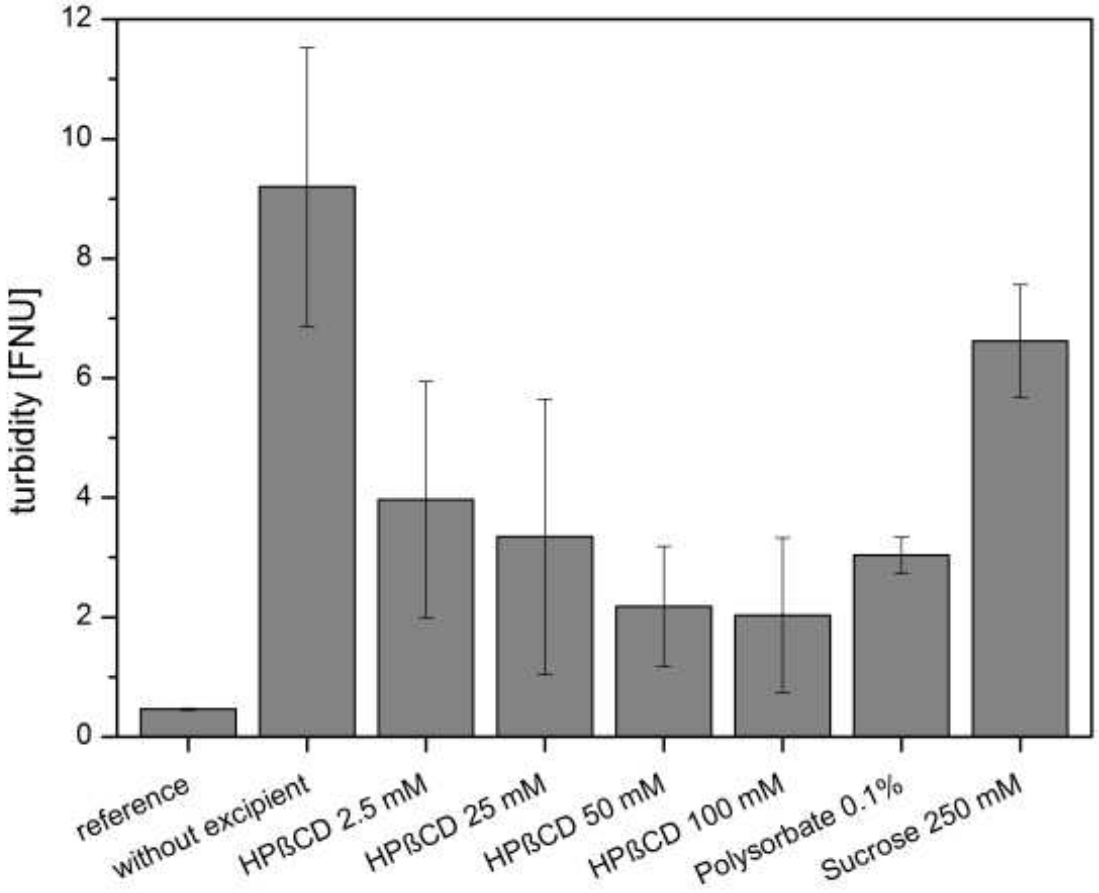


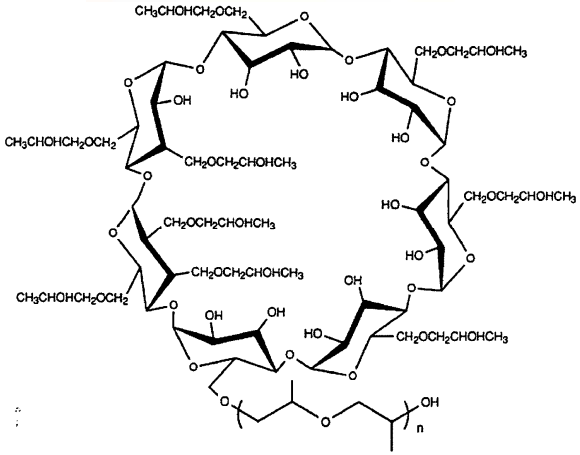


insulin hexamer

1. Control, 2. Stirred without CD, 3. HPBCD, 4. alfa-CD, 5.beta-CD, 6. gamma-CD (Banga J., 1993.)

Aggregation of Immunglobulin B by strirring
(Hartl et al, J. Pharm. Sci. 2013)



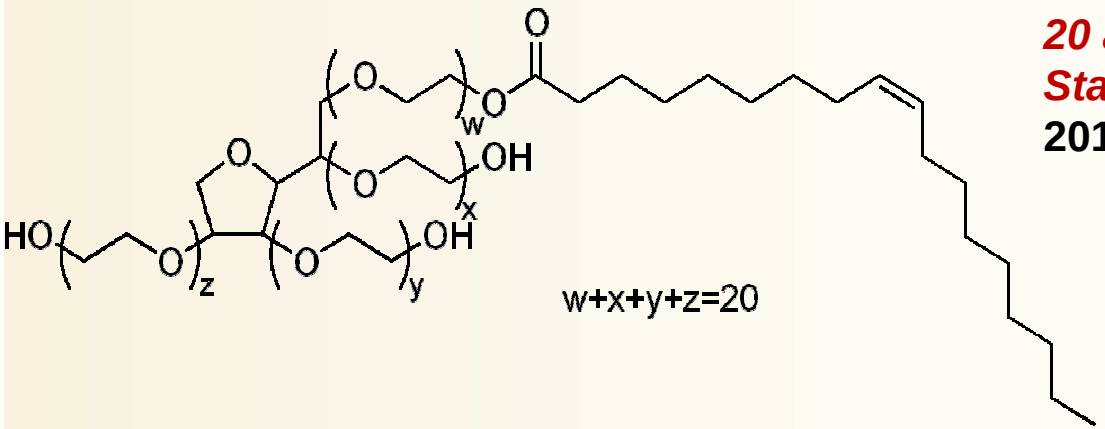


HPBCD

Turbidity of 1.8 mg/mL IgGB aqueous solution after 1 h stirring

Cyclodextrins or Tween?

- **Tween-like detergents efficiently prevent protin aggregation, prevent immunogenicity**
- **Polisorbate 80 is the most common, they are widely used**
- **However, their degradataion lead to aldehydes, epoxy-acids and peroxides damaging proteins**



Polyoxyethylene (20) sorbitan mono-oleate

Kishore. R: ***Degradation of Polysorbates 20 and 80 and its Potential Impact on the Stability of Biotherapeutics.*** **Pharm. Res. 2011. 28(5): p. 1194-1210**

Some protein based product, their shelf-lives and additives

Product	Active	Shelf Life	Ingredients
Remicade	infliximab	3 years at 2 °C – 8 °C.	dibasic sodium phosphate dihydrate, monobasic sodium phosphate monohydrate, **polysorbate 80**, and sucrose. No preservatives are present.
Humira	Adalimumab	2 years at 2 °C – 8 °C.	sodium chloride, monobasic sodium phosphate dihydrate, dibasic sodium phosphate dihydrate, sodium citrate, citric acid monohydrate, mannitol, **polysorbate 80** and water for injections
Herceptin	trastuzumab	4 years at 2 °C – 8 °C.	histidine hydrochloride, histidine, trehalose dihydrate, **polysorbate 20**
Vetsulin	porcine insulin zinc suspension	**42 days**	zinc chloride, methylparaben, sodium chloride, sodium acetate, water
NovoLog	insulin aspart	**28 days**	glycerin, phenol, metacresol, zinc, disodium hydrogen phosphate dihydrate, sodium chloride and water for injection
ORTHOCLONE	muromonab-CD3	**9 months**	sodium phosphate, monobasic sodium phosphate, dibasic sodium chloride **polysorbate 80** water
Oncaspar	pegaspargase	**8 months (2-5°C)**	dibasic sodium phosphate dihydrate, dibasic sodium phosphate heptahydrate, sodium chloride, water for injection

What is the technological advantage of using CDs?

- **Increased stability**
- **No peroxide formation, no corresponding immunogenicity**
- **Prevention of aggregation**
- **IP protection „*Compositions of matter*” life cycle management**

Effect of charged CDs on the aggregation of monoclonal antibodies (mABs)

mAb: polyionic protein with hydrophobic surfaces

- The hydrophobic surface induces aggregation
- The net surface charge is minimal at pH 5 - 7.5 → no electrostatic repulsion

Consequence: mAb-mAb aggregation increases

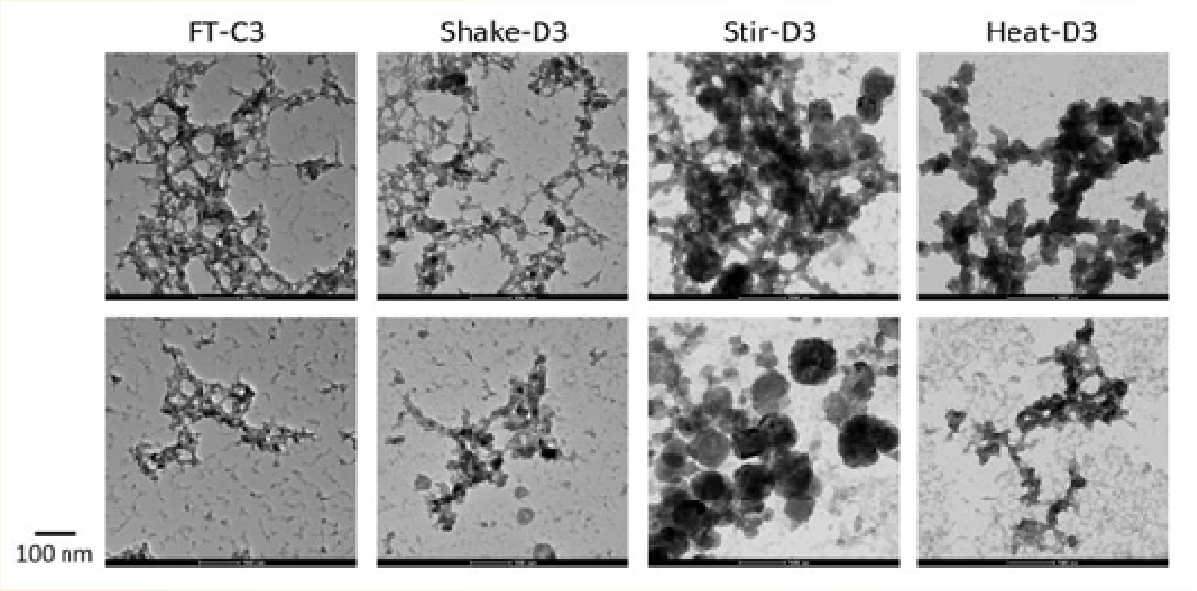
- Masking of the hydrophobic surface reduces van der Waals interactions
- Increase in the surface charge increases electrostatic repulsion

Result: mAb-mAb aggregation decreases

Results of protein aggregation on prouct

- Decreased effect
- Altered pharmacokinetics
- Immunogencity, irritation, anaphylaxis
- Short shelf-life, poor stability

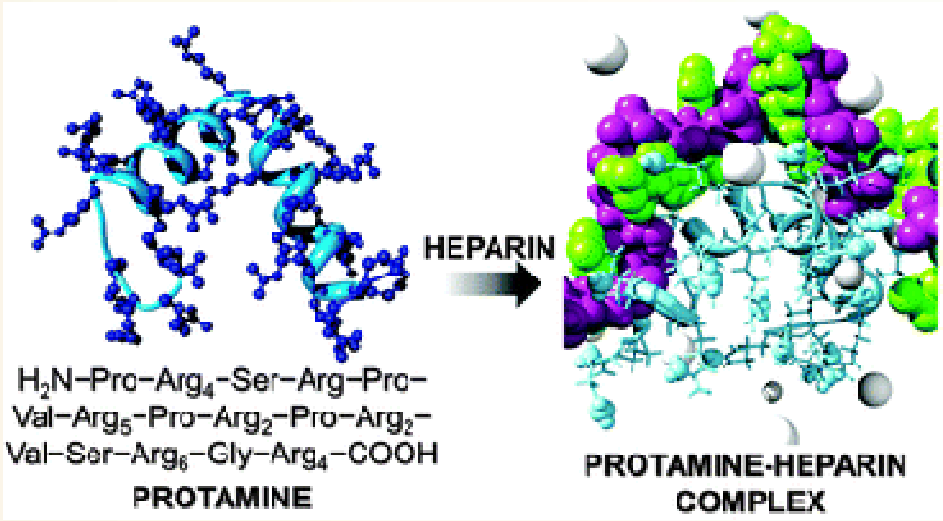
mABs are particularly prone to aggregation



IgG mAb aggregation (Hartl, 2013)

How do polysaccharides work?

Stabilization by *„physical glycosilation”*: Anionic oligo- és polysaccharides act as „chaperone”



These polysaccharides may be:

- **Carboxyalkyl dextranes**
- **Ionic cyclodextrins (pl. carboxyalkyl, sulfoalkyl, etc)**

CD's effect on proteins surface adsorbtion

The Cyclodextrin Company

Practical example: albumin, globulin és lysozyme adsorb on contact lenses

US005364637A

United States Patent [19]

De et al.

[11] **Patent Number: 5,364,637**

[45] **Date of Patent: Nov. 15, 1994**

[54] **METHOD AND COMPOSITION FOR CLEANING CONTACT LENSES WITH CYCLODEXTRINS**

[75] Inventors: **Nimai C. De**, Rochester; **David J. Heiler**, Avon; **David A. Marsh**; **Suzanne F. Groemminger**, both of Rochester, all of N.Y.

[73] Assignee: **Bausch & Lomb Incorporated**, Rochester, N.Y.

[21] Appl. No.: **852,427**

[22] Filed: **Mar. 16, 1992**

Related U.S. Application Data

[63] Continuation of Ser. No. 602,447, Oct. 22, 1990, abandoned.

[51] **Int. Cl.**[5] **A61K 9/20**; A61K 31/715

[52] **U.S. Cl.** **424/464**; 424/429; 514/839; 514/840

[58] **Field of Search** 424/464, 427, 429; 514/58, 839, 840

[56] **References Cited**

U.S. PATENT DOCUMENTS

3,882,036 5/1975 Krezanoski et al. 514/643

Primary Examiner—Thurman K. Page
Assistant Examiner—James M. Spear
Attorney, Agent, or Firm—Craig E. Larson

[57] **ABSTRACT**

Contact lenses are cleaned by contacting the lenses with a composition containing an effective amount of one or more cyclodextrins. The compositions can also be employed at elevated temperatures or may contain suitable antimicrobial agents in order to simultaneously clean and disinfect the lenses.

10 Claims, No Drawings

The product contains

HPBCD

Thank you!

For more details, please contact us!