Pharmaceutical Formulations of Biotech Products

Lecture-3

by
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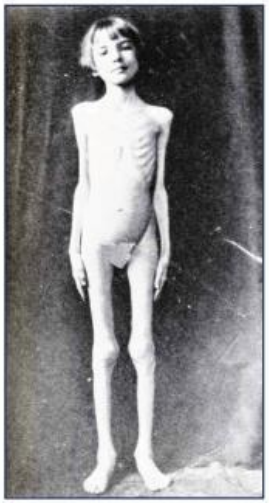
- The discovery of insulin in 1922 marked a major breakthrough in medicine and therapy in patients with diabetes.
- the insulin story began on October 31, 1920, when Dr. Fredrik Banting noted an **idea for an experiment to isolate an internal secretion from the pancreas from dogs**.

- Many hurdles remain in the prevention and treatment of diabetes because **of high prices** and **poor availability of** insulin extracted from animals
- Biotechnology offers insulin production

Insulin

The Journal of Clinical Investigation REVIEW SERIES: 100TH ANNIVERSARY OF INSULIN'S DISCOVERY

A



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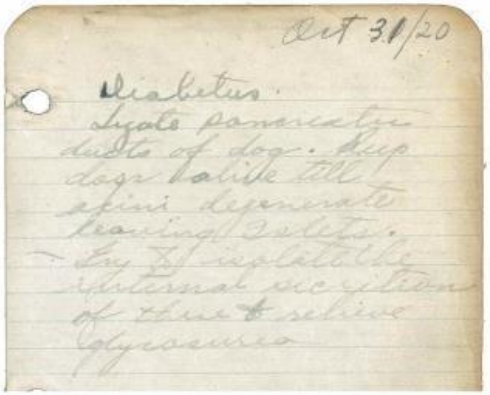

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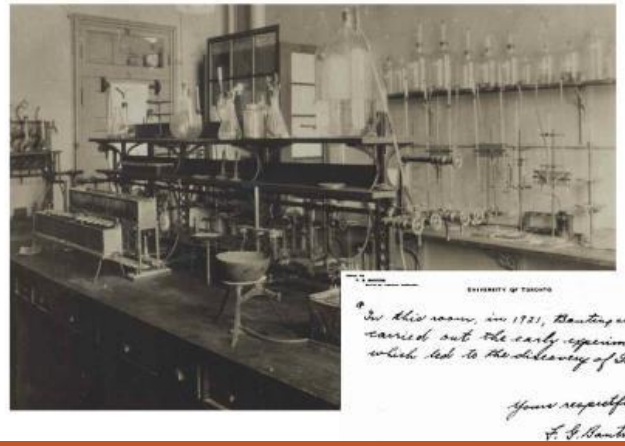

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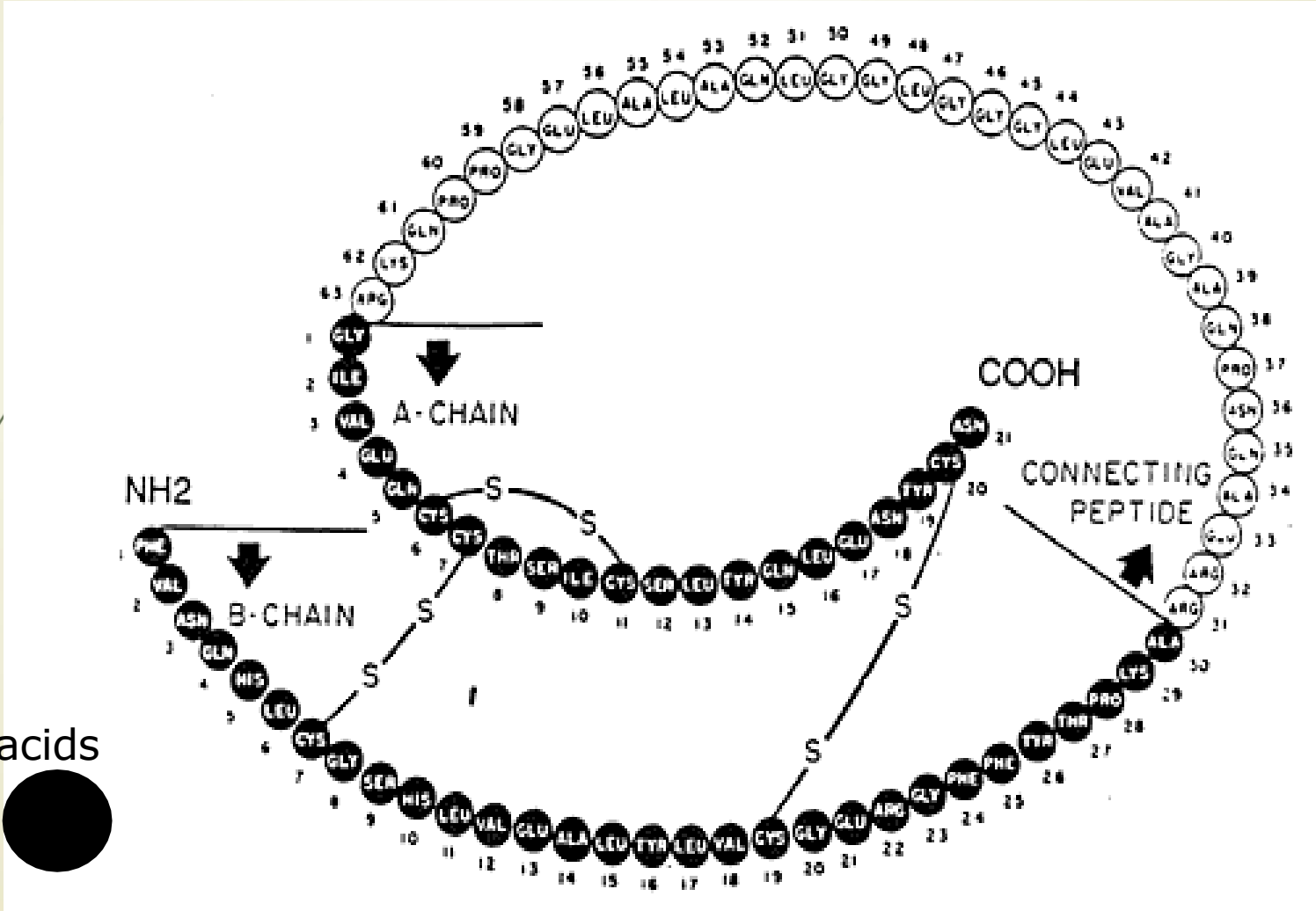
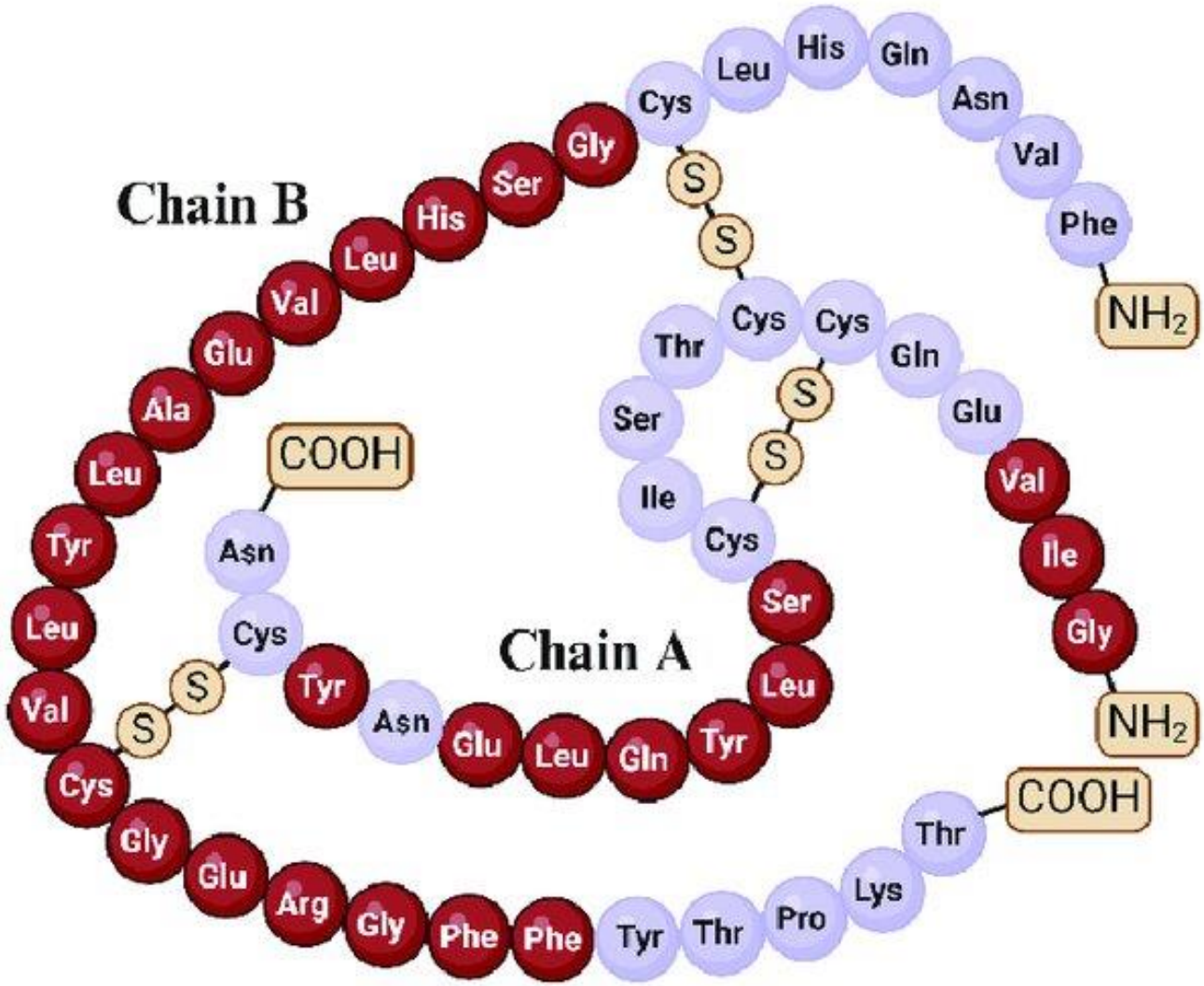
F

Insulin structure



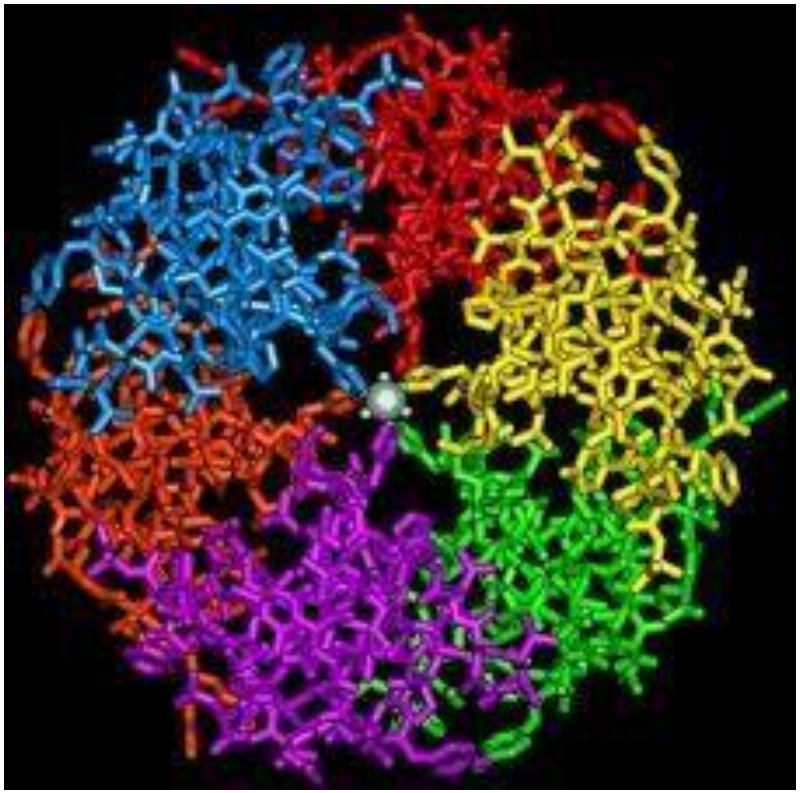
Amino acids


Chain B
Chain A
COOH
NH2
NH2
COOH
Gly
Ser
His
Leu
Val
Glu
Ala
Leu
Tyr
Leu
Val
Cys
Gly
Glu
Arg
Gly
Phe
Phe
Tyr
Thr
Pro
Lys
Thr
Asn
Cys
Tyr
Asn
Glu
Leu
Gln
Tyr
Leu
Ser
Cys
Cys
Leu
His
Gln
Asn
Val
Phe
Thr
Ser
Ile
Cys
Gln
Glu
Val
Ile
Gly
S

- Understanding insulin structure and amino acid sequence led to recombinant insulin production via Ecoli

- Due to its nature , as procaryotic creature, E. Coli could not produce the insulin in its proper 3D structure , instead, the insulin produced by E. Coli **lose** the proper structure and **fold** into hard insoluble structures called **inclusion bodies**
- Inclusion bodies required chemical modifications to harvest the pure and effective insulin

Schematic representation of insulin association in presence of zinc and phenolic antimicrobial preservatives



New method ?

- Using yeasts instead of bacteria have the advantages of producing insulin in the desired form.
- However, Yeast can not conjugate the A and B chains of insulin together which requires production of A chain , and B chain then joining them chemically

- Refer to slide 4

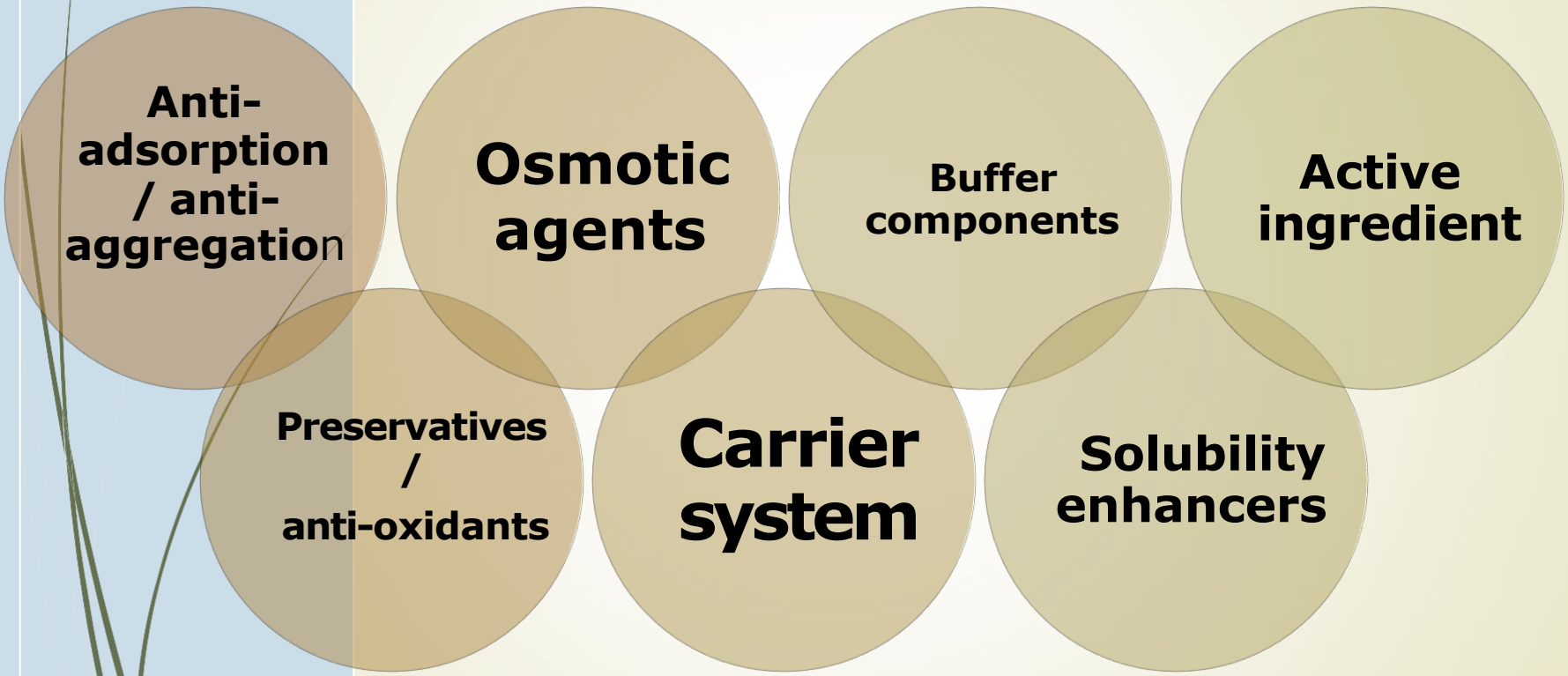
Why cannot biotech. Produce chemicals??

- **In a protein formulation** (active substance), a number of **excipients selected** to serve different purposes.

- This formulation design should be carried out with great care) to ensure : **Therapeutic effectiveness and safe products.**

- **The nature of the protein** (e.g. **lability-rapid change** or **destroyed)** and its **therapeutic use** (e.g. multiple injection systems) can **make these formulations quite complex in term of excipients profile and technology** (**freeze-drying, aseptic preparation**).

Components found in parenteral formulations of biotech products



Note: All of the above are not necessarily present in one particular protein formulation

Proteins, in particular those that are non-glycosylated, may **have a tendency to aggregate and precipitate**.

Approaches that can be used **to enhance solubility** include:

1. **Selection of the proper pH and ionic strength** conditions

2. **Addition of amino acids**, such as **lysine or arginine** (used to **solubilize tissue plasminogen activator**, t-PA)

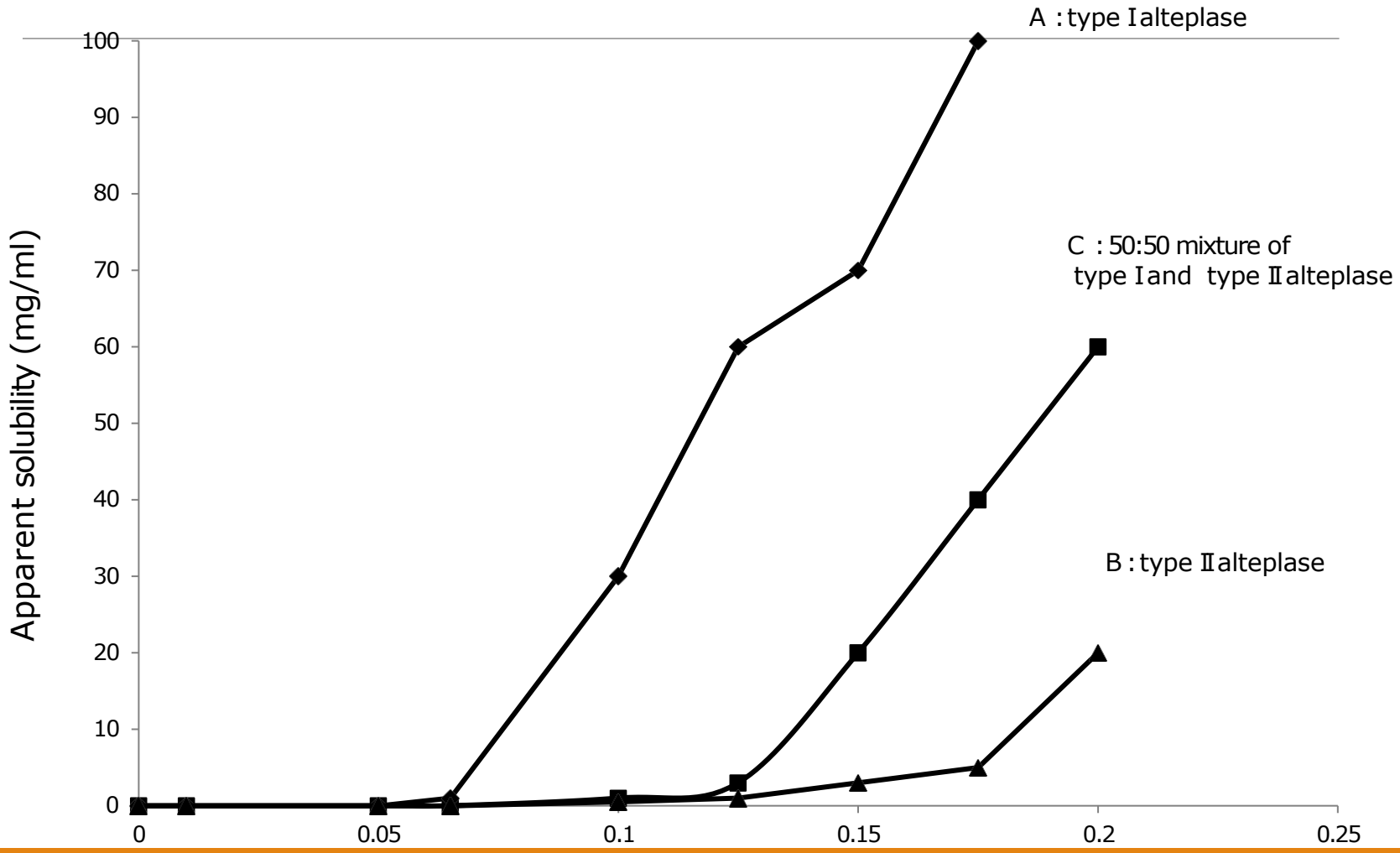
3. **Addition of surfactants** such as **sodium dodecylsulfate**, to **solubilize non-glycosylate IL-2 (interleukin-2)** can also help to increase the solubility.

The mechanism of action of these solubility enhancers

depends on

Type of enhancer and **protein involved** and is not always fully understood.

Figure 1: Shows the effect of arginine concentration on the solubility of t-PA (alteplase) at **pH 7.2** and **25°C.**



aggregation is physical in nature, i.e. **based on hydrophobic and/ or electrostatic interactions between molecules.**

By

Formation of covalent bridges between molecules through disulfide bonds, and ester or amide linkages.

avoid

In these cases proper conditions should be found to avoid these chemical reactions (**the figure above clearly indicates the dramatic effect of this basic amino acid on the apparent solubility of t-PA**).

3. Anti-adsorption and anti-aggregation agents

- **Anti-adsorption agents** (**added to reduce adorption of the active protein to interfaces**).

  - Some **proteins** normally have **hydrophobic sites in the core** structure.

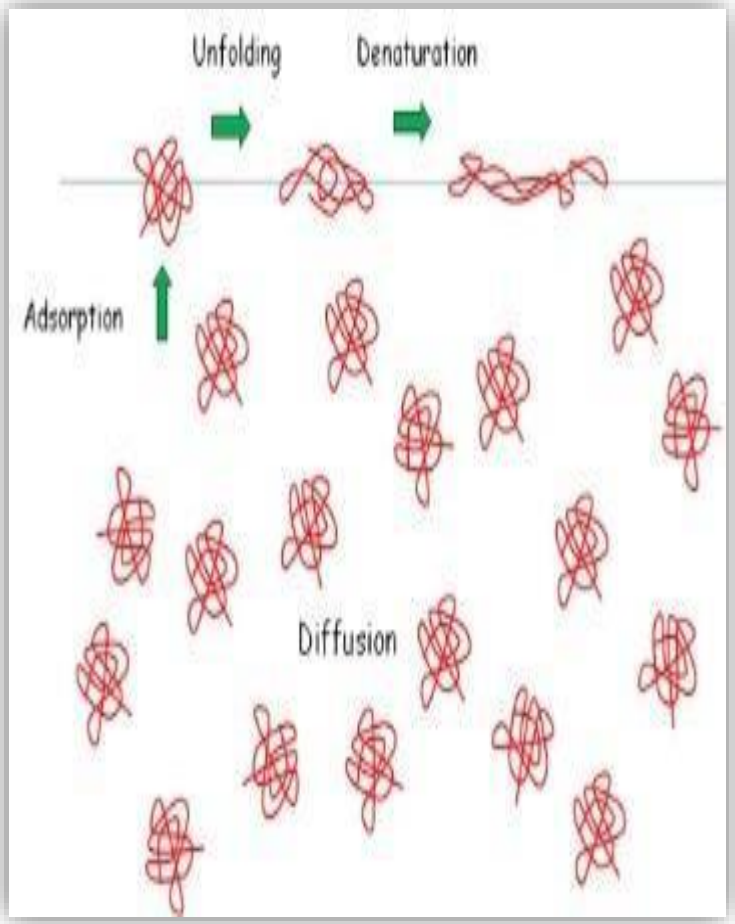
They tend to **expose hydrophobic sites** when an interface is present.

- These **interfaces** can be **water/air, water/container wall** or **interfaces formed between the aqueous phase and utensils** used to administer the drug (e.g. **catheter, needle).**

- These **adsorbed**, **p a r t i a l l y unfolded protein molecules form aggregates**, leave the surface, return to the aqueous phase, **form larger aggregates and precipitate**.

Example:

The proposed mechanism for aggregation of insulin in aqueous media through contact with a hydrophobic surface (or water-air interface) is presented in Figure 2.



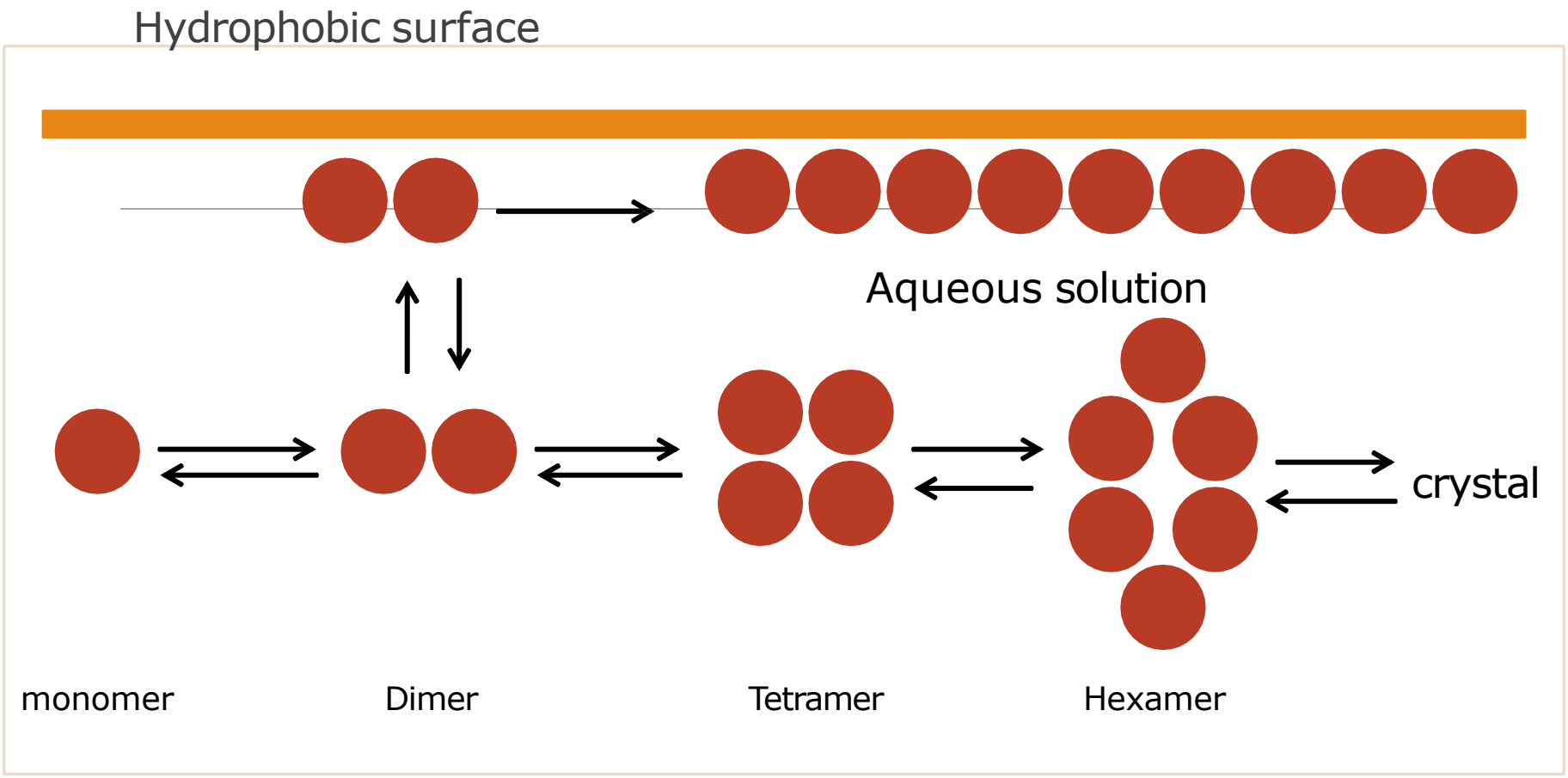


Figure 2 Reversible self-association of insulin, its adsorption to the hydrophobic interface and irreversible aggregation in the adsorbed protein film

Native insulin in solution is in an **equilibrium state** between monomeric, dimeric, tetrameric and hexameric form.

- The relative **abundance** of the different aggregation states depend **on the pH, insulin concentration, ionic strength and specific excipients (Zn^{2+} and phenol)**.

- **Suggestion:** dimeric form of insulin adsorbs to hydrophobic interfaces and subsequently forms larger aggregates at the interface.

This adsorption explains why anti-adhesion agents can also act as anti-aggregation agents.

Albumin

has strong tendency to adsorb to surface and is therefore added relatively high concentration (1 %) as ani adhesion agent to protein formulations

MECHANISM:

albumin competes with the therapeutic protein for binding sites

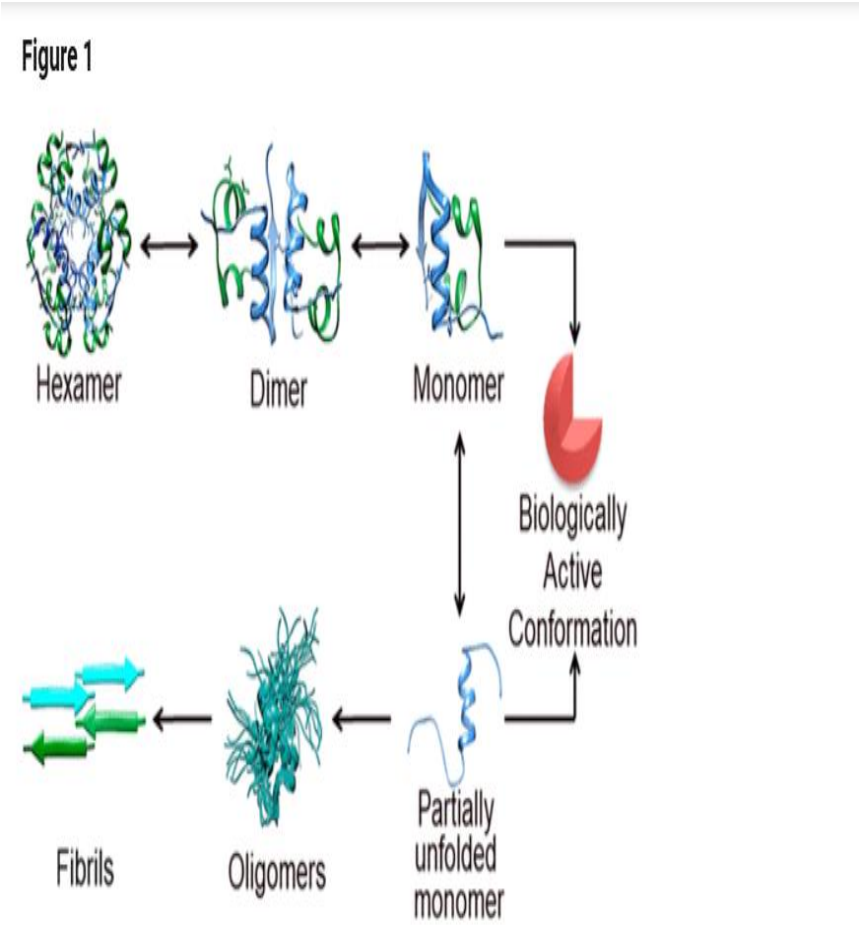
prevents adhesion of the therapeutically active agent

by

combination of its binding tendency and abundant presence.

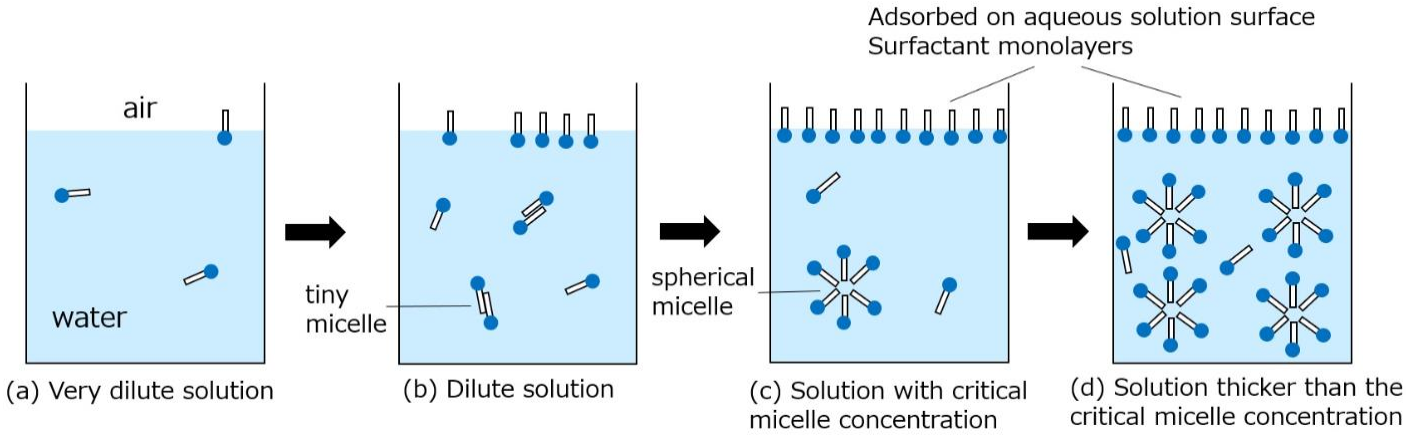
- **Insulin** is one of the many proteins that can form fibrillar **precipitates** (long rod-shaped structures with diameters in the 0.1 µm range). Which can be prevented by:

1. **Low concentrations of phospholipids and surfactants** (as a fibrillation-inhibitory effect).
2. The **selection of the proper pH** to prevent this unwanted phenomenon.



- **Apart from albumin, surfactants can also prevent adhesion to interfaces and precipitation.**

Readily adsorb to hydrophobic interfaces with their own hydrophobic groups and render this interface hydrophilic by exposing their hydrophilic groups phase.



Important notes:

Insulin has as **isoelectric point (PI) of 5.3 in the denatured state**; thus, the insulin molecule is **negatively charged at neutral pH**

charge-state of insulin used in formulation development.

2. Insulin ability to readily associate into diamer and higher order state (**The deriving force for dimerization** appears to be the formation of favorable **hydrophobic interactions** at the **C-terminus of the B-chain**).

Excipients added to insulin:

1. **Insulin can associate into discrete hexameric complexes in the presence of various divalent metal ions, such as zinc** at **0.33 g-** atom/ monomer, where each zinc ion (a total of two) is coordinated by His^{B10} residue from three monomers.

- The ability to form discrete hexamers in the presence of zinc has been used to develop therapeutically useful formulation of insulin.

- **Commercial insulin** preparations also **contain phenolic excipients** (e.g., **phenol, m-cresol, or methyl-paraben**).

Benefits:

A. **Act as anti microbial agents**.

B. **Bind to specific sites on insulin hexamers, causing a conformationl change that increases the chemical stability of insulin in commercial preparations.**

(This reduce high-molecular-weight polymer formation)

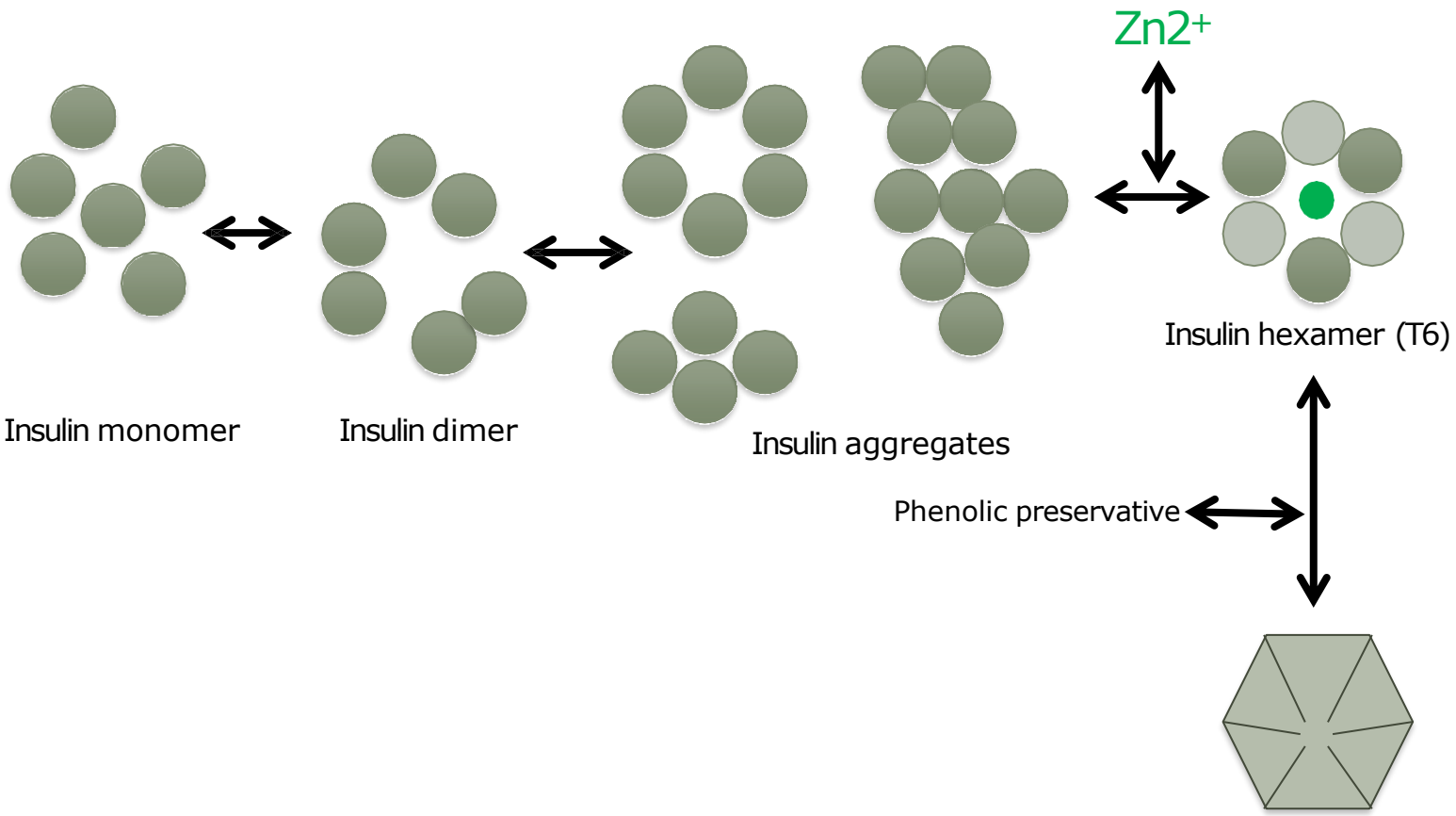
C. Modern insulin formulation may contain an **isotonicty agent (glycerol or NaCl)**

minimize the subcutaneous tissue damage and pain on injection.

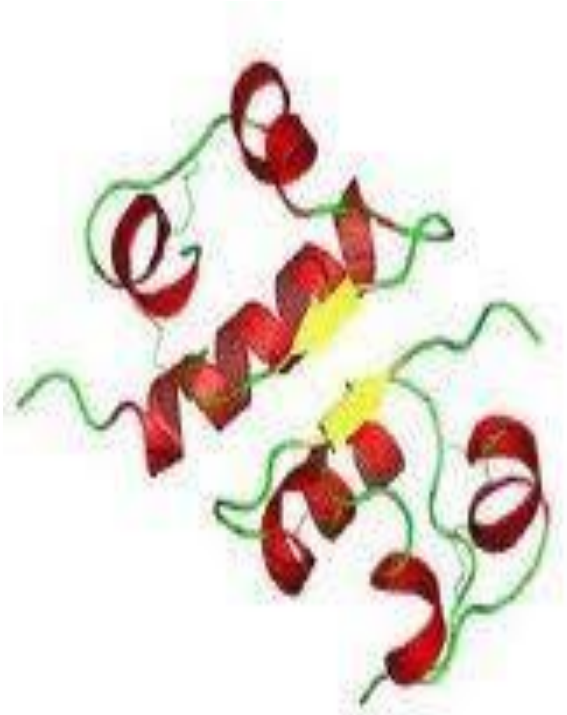
D. physiologic buffer (sodium phosphate)

minimize pH drift in some pH-sensitive formulations.

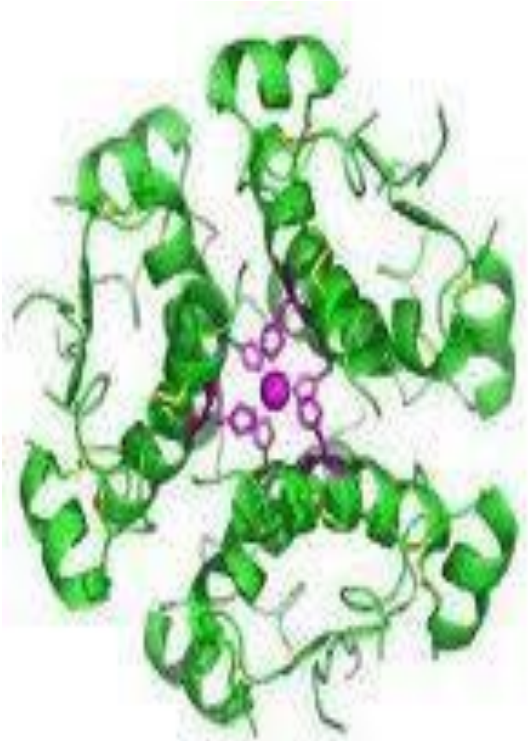
Schematic representation of insulin association in presence of **zinc** and **phenolic antimicrobial preservatives**



T-state dimer and hexamer



Insulin Dimer



Insulin Hexamer


Thank You