# Pharmaceutical Formulations of Biotech Products

## Lecture-4

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. Frederick 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

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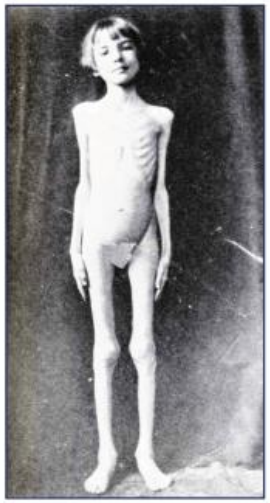

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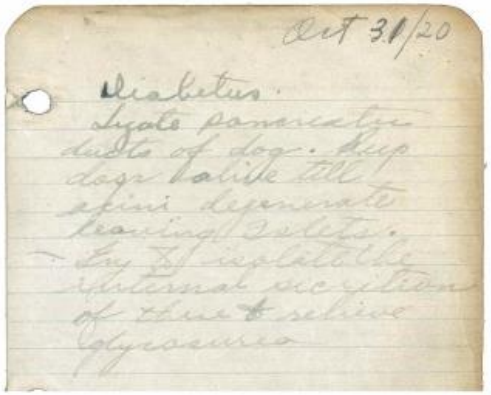


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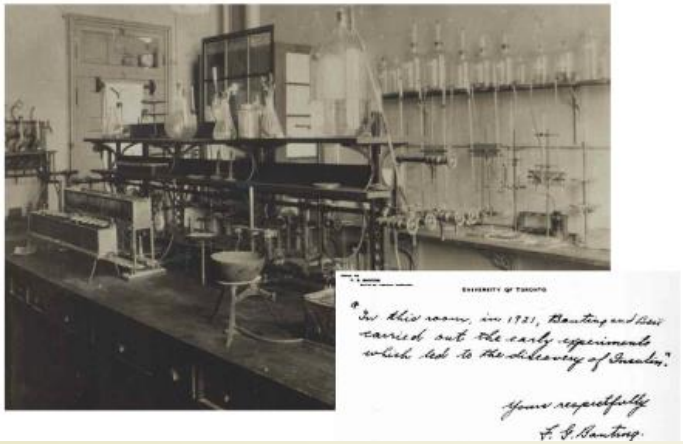


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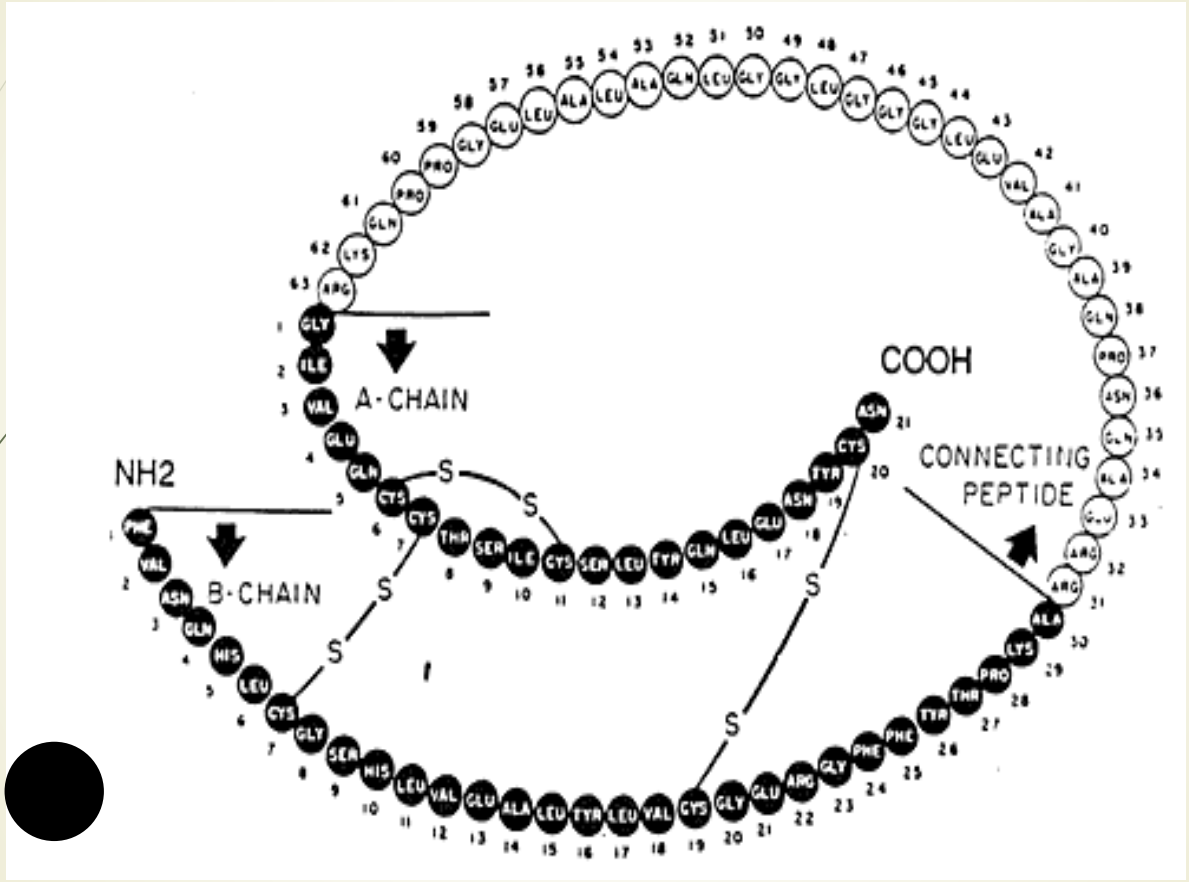
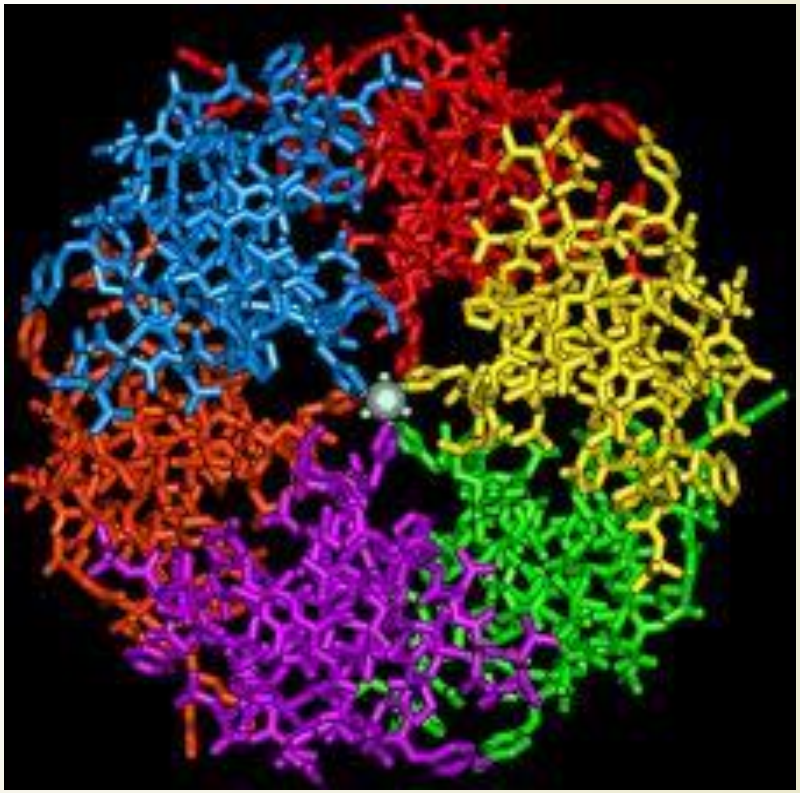
# Insulin structure




Amino acids

- 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 structed 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 cant biotech. Produce chemicals??

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

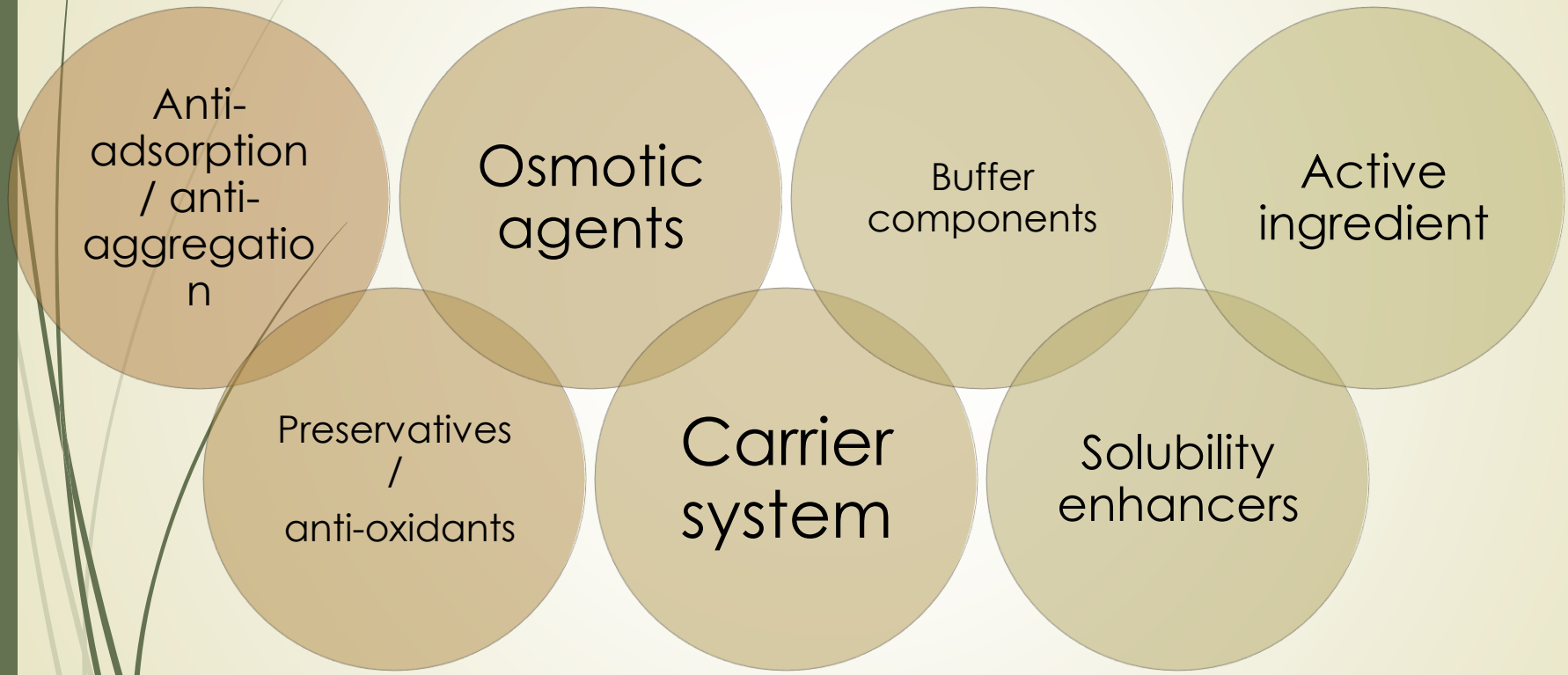
(This formulation design should be carried out with great care)

**Therapeutic effectiveness and safe products.**

to ensure

- **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

# 2. Solubility Enhancers

- **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)

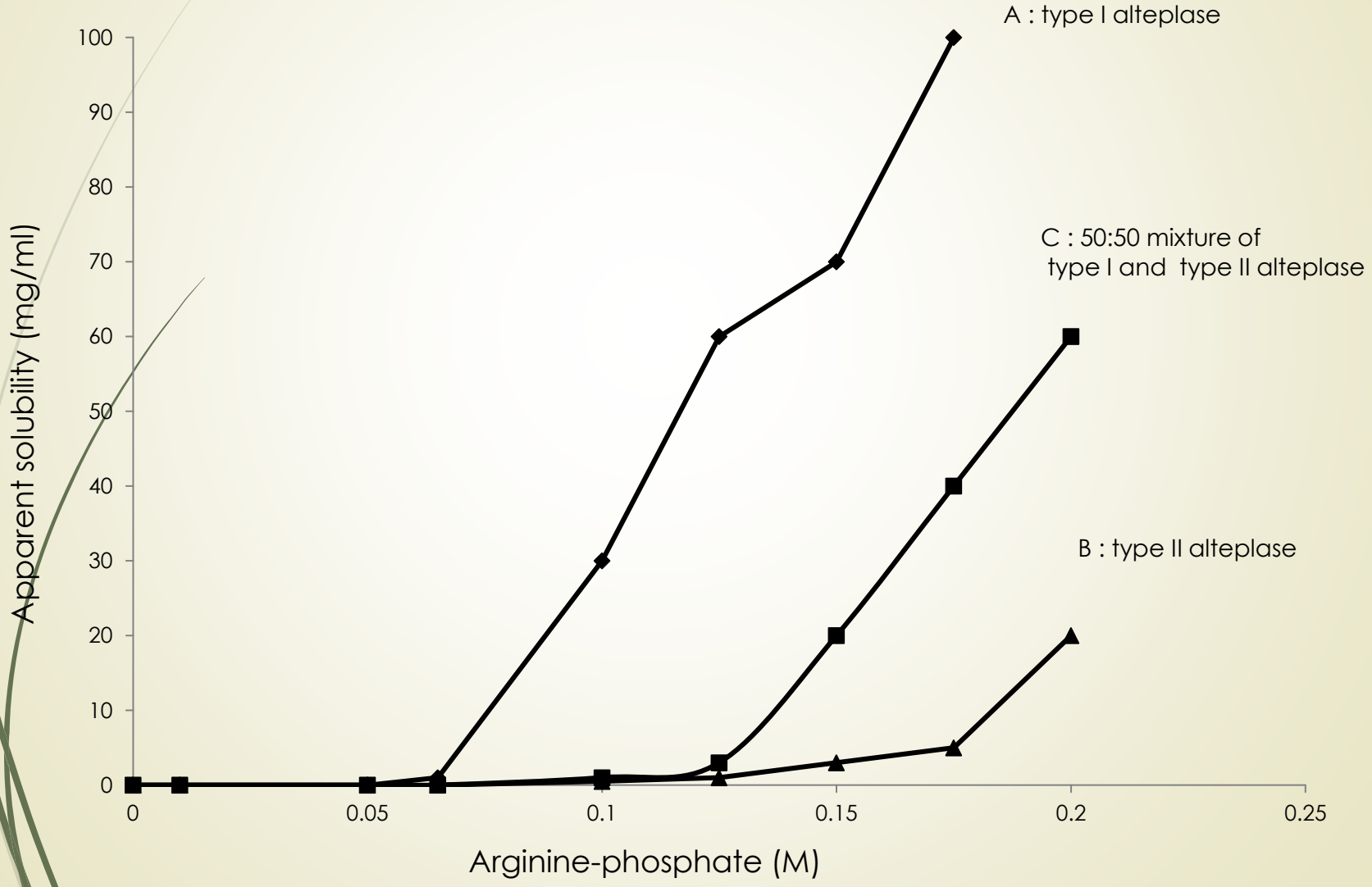
3. **Addition of surfactants** such as **sodium dodecylsulfate**, to **solubilize non-glycosylate IL-2 (interleukin-2)** can also help to increase the solubility.

**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 adsorption of the active protein to interfaces**).

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

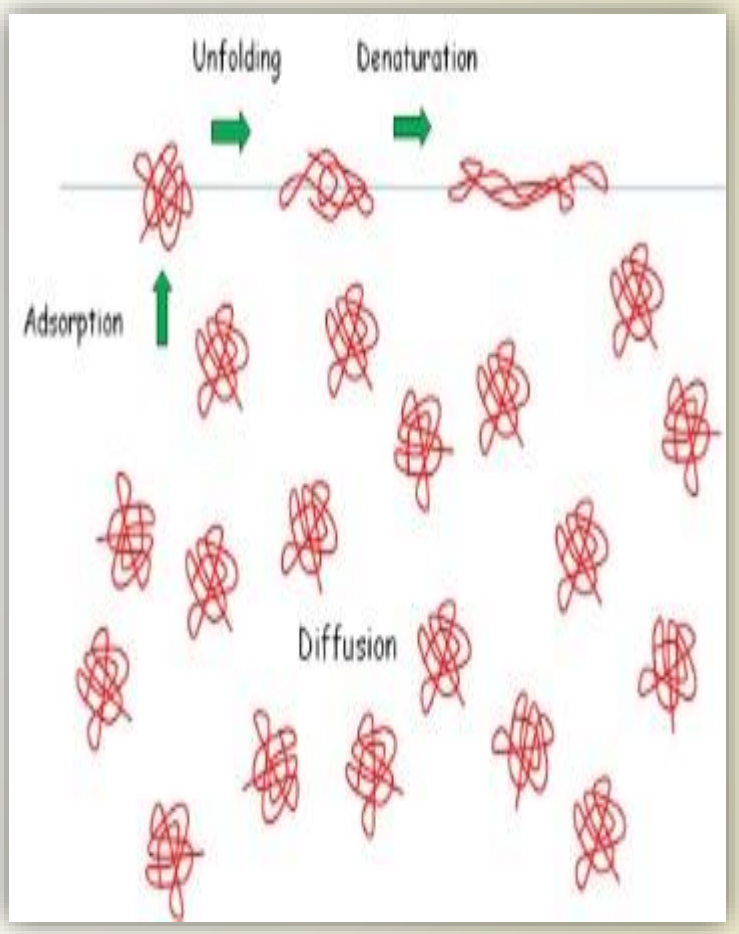
They tend to **expose hydrophobic sites** when an interface is present.

- 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**, **partially 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.



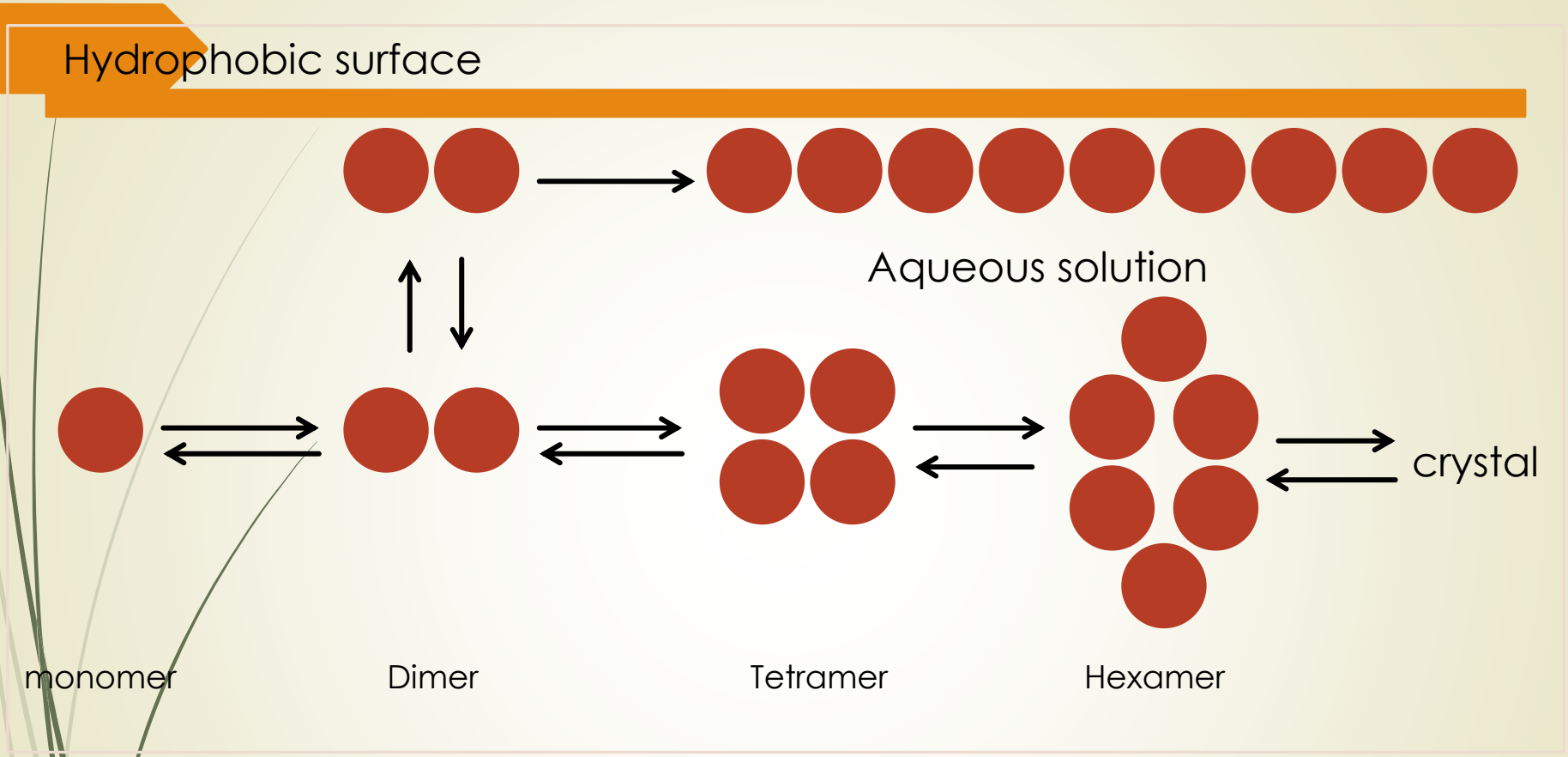
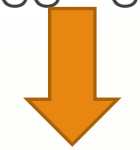



Figure 2 Reversible self-association of insulin, its adsorption to the hydrophobic interface and irreversible aggregation in the adsorbed protein film

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

- The relative **abundance** of the different aggregation states **depends 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.**

**Ex: Albumin (strong tendency to adsorb to surfaces)** and is therefore added in relatively high concentration (e.g. 1%) **as an anti-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).

This 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)

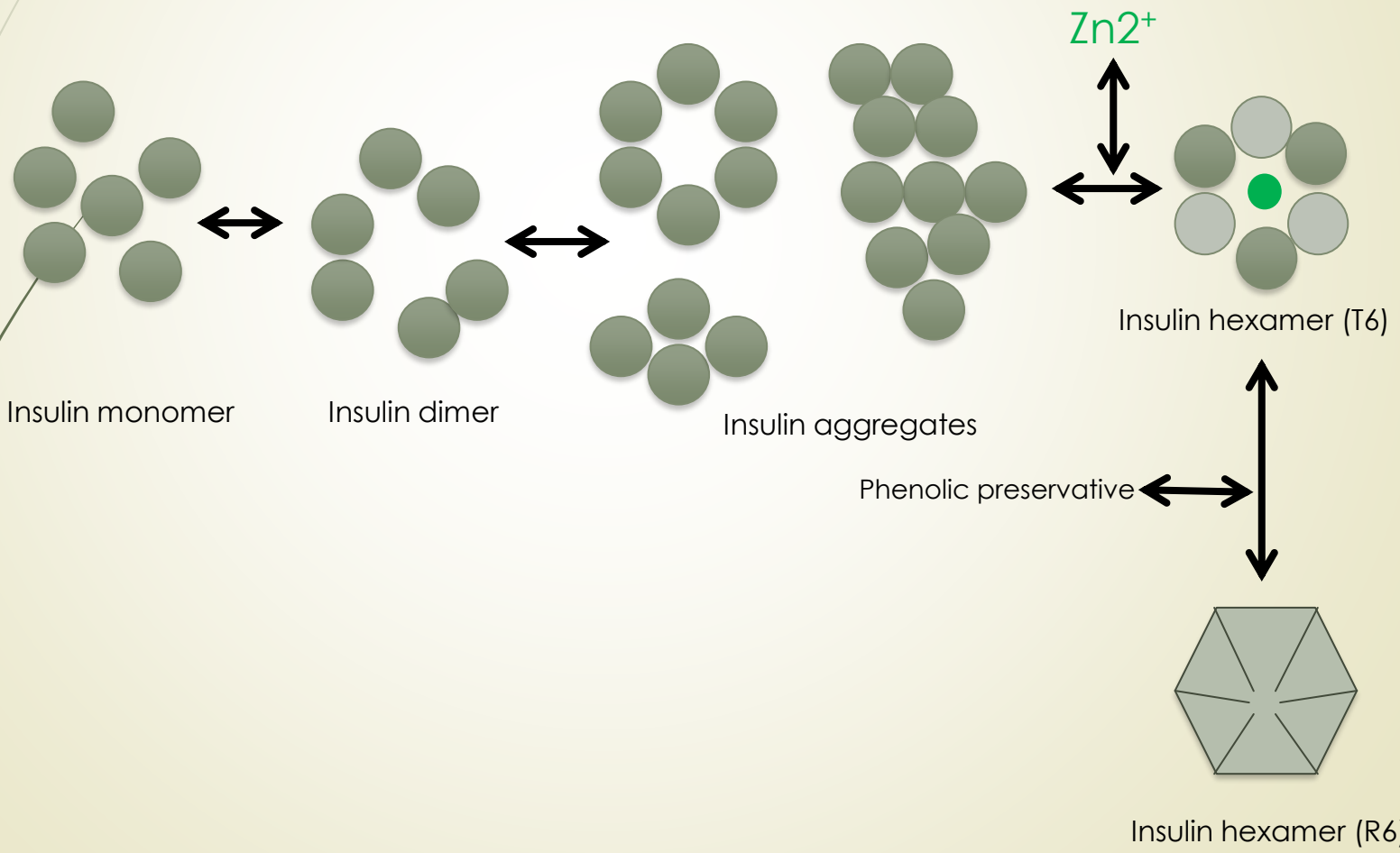
C. Modern insulin formulation may contain an **isotonicty agent (glycerol or NaCl)**

minimize the subcutaneous tissue damage and pine on injection.

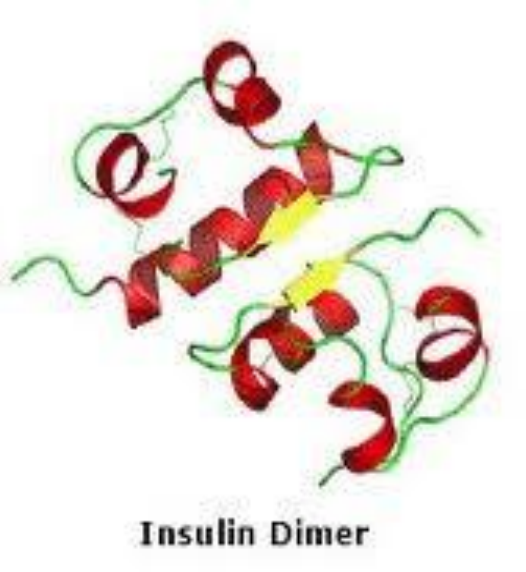
**D. physiologic buffer (sodium phosphate)**

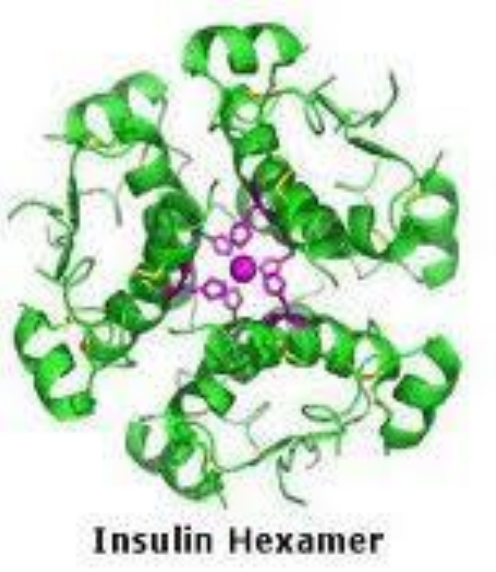
minimize pH drift in some pH-sensitive formulations.

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



# T-state dimer and hexamer




Thank You.