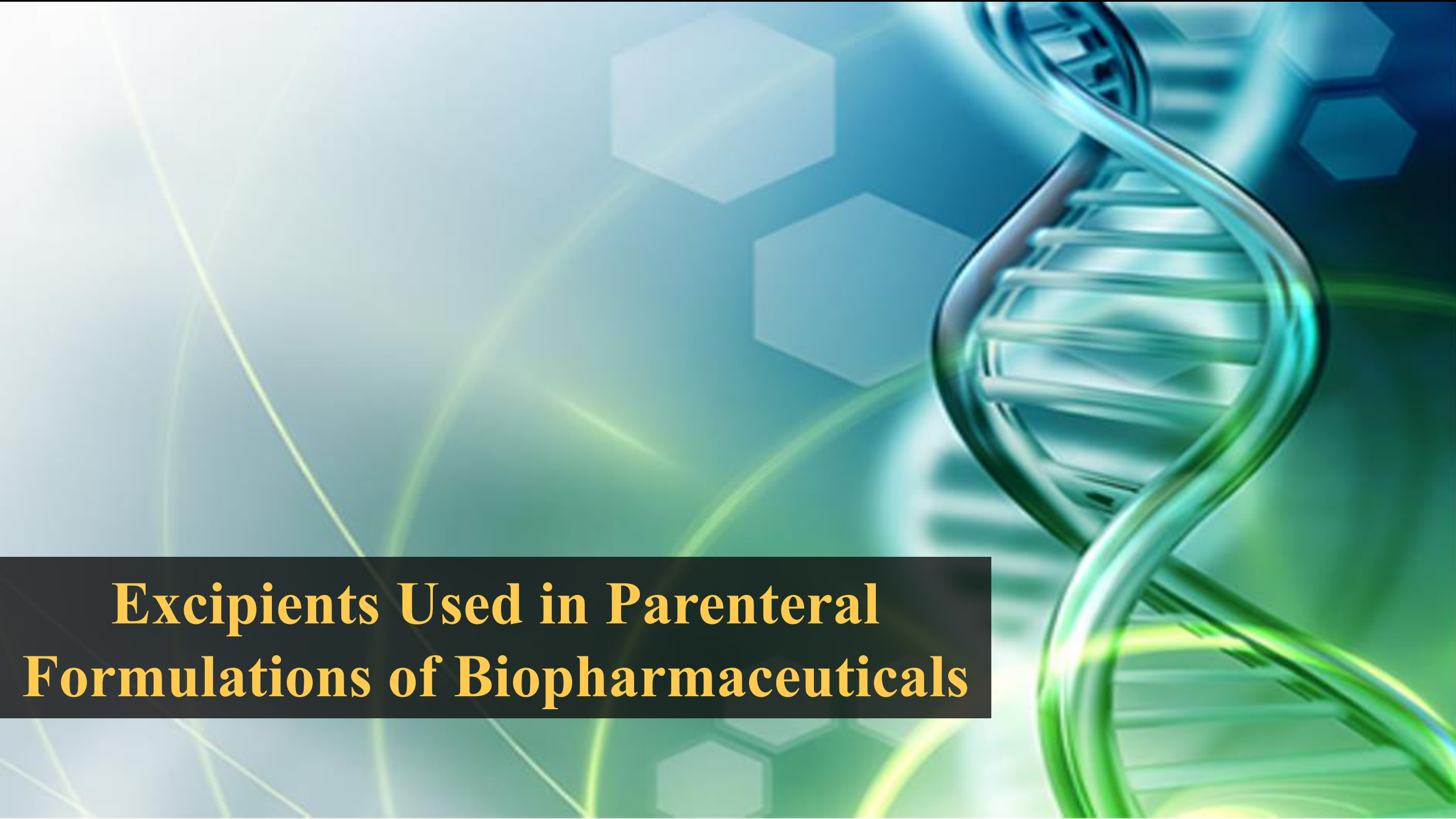
Excipients Used in Parenteral
Formulations of Biopharmaceuticals

Excipients in Biopharmaceuticals

- As other dosage form, biopharmaceuticals also contains number of **excipients** that are selected to serve different purposes.
- Our concern is that the biopharmaceuticals are a complex dosage form that required special consideration in formulation as follow:

1. Most of these product are **designed for parenteral administration** which is a quiet challenging dosage form.
2. The nature of protein which can be considered as **unstable** product due to multiple ways of instability that turns the protein inactive.
3. The special processing in included in formulation of biotech product such as **freeze drying and aseptic preparations**.
4. In addition, if the dosage form is designed for **multiple injection system,** this will add additional complexity to the dosage form.

- Both the choice of the excipient and its concentration are important. For instance, low concentrations of polysorbates may stabilize the protein (see below),while higher concentrations may cause denaturation.

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Protein Instability

Proteins are unstable

Chemical instability ⟷ **Physical instability**

- Deamidation
- Oxidation
- Proteolysis (hydrolysis)
- Disulfide shuffling
- Racemization
- Beta elimination
- ...

- Conformational
  - Unfolding
  - Misfolding
- Colloidal
  - Aggregation
  - Precipitation
- Adsorption

Each degradation reaction can induce another one
Multiple degradation processes occur at different rates, yielding numerous degradation products

Example of Excipients in Marketed Biopharmaceuticals

Excipient class	Function	Examples
Buffers	pH control, tonicity	Histidine, phosphate, acetate, citrate, succinate
Salts	Tonicity, stabilization, viscosity reduction	Sodium chloride
Sugars[a], polyols	Tonicity, stabilization, cryoprotection, lyoprotection[b], bulking agent[b], reconstitution improvement[b]	Sucrose, trehalose, mannitol, sorbitol
Surfactants	Adsorption prevention, solubilization, stabilization, reconstitution improvement[b]	Polysorbate 20, polysorbate 80, poloxamer 188
Amino acids	Stabilization, viscosity reduction, tonicity, pH control, bulking agent[b]	Arginine, glycine, histidine, lysine, proline
Anti-oxidants	Oxidation prevention	Methionine, sodium edetate
Preservatives[c]	Bacterial growth prevention	m-cresol, benzyl alcohol, phenol

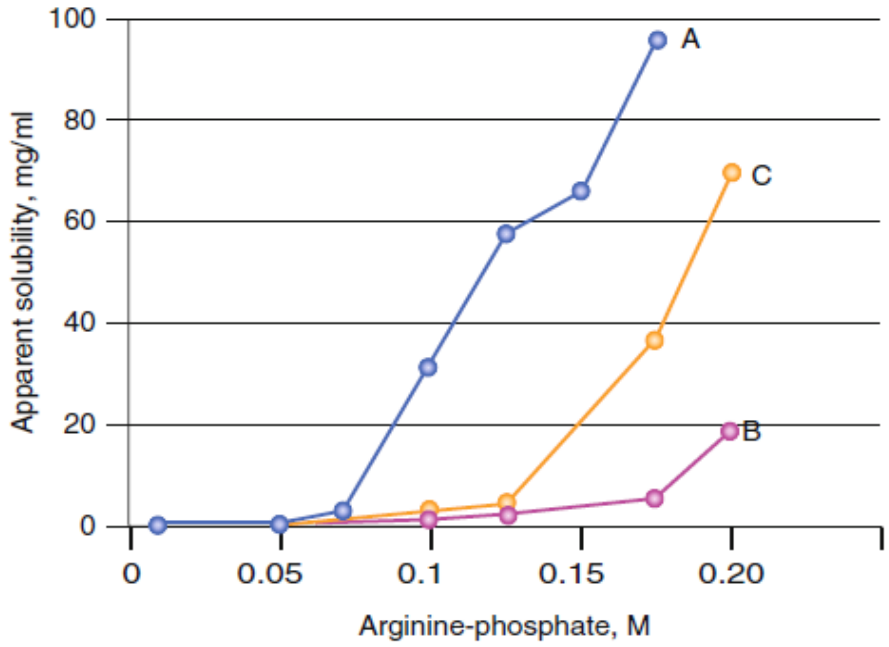
Adapted from Weinbuch et al. (2018)
[a]Only non-reducing sugars
[b]For freeze-dried products
[c]Multi-dose containers

Table 5.6 ■ Common excipients in protein drug products

Solubility Enhancement

- 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:

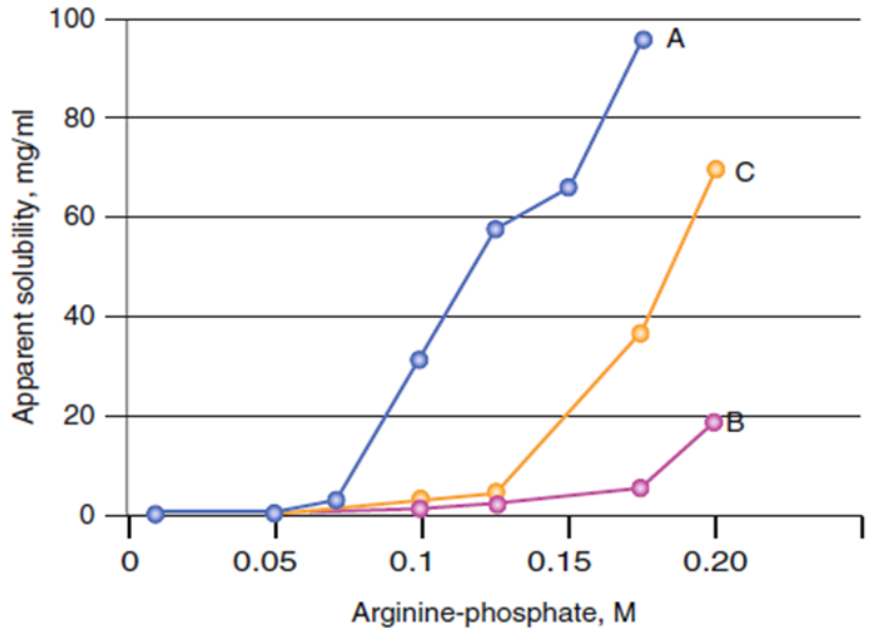
1. **Selection of the proper pH** and ionic strength conditions.
2. **Addition of amino acids** such as lysine or arginine (used to solubilize tissue plasminogen activator, t-PA).
3. **Surfactants** such as sodium dodecyl sulfate to solubilize non-glycosylated IL-2 can also help to increase the solubility.



Effect of arginine on type I and type II alteplase at pH 7.2 and 25 °C. A type I alteplase, B type II alteplase, C 50:50 mixture of type I and type II alteplase.

Solubility Enhancement

- The mechanism of action of these solubility enhancers **depends on the type of enhancer** and the **protein involved** and is not always fully understood.
- This figure clearly indicates the dramatic effect of arginine concentration on the apparent solubility of t-PA.
- It is believed that arginine will **increase the hydrogen bonding** ability of the protein.
- In the above examples, aggregation is **physical in natur**e, i.e., based on hydrophobic and/or electrostatic interactions between molecules.



Solubility Enhancement

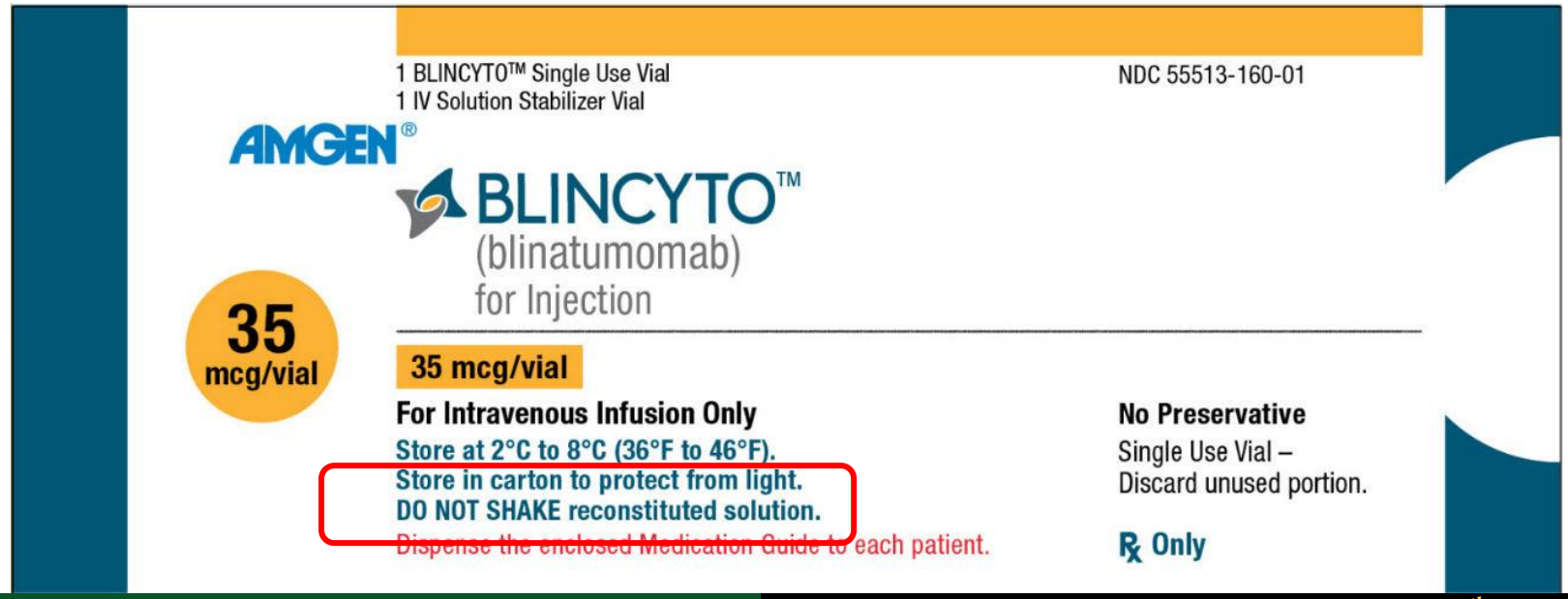
- However, aggregation based on the formation of **covalent bridges between** molecules through disulfide bonds and ester or amide linkages has been described as well.
- In those cases, proper conditions should be found to avoid these chemical reactions such as controlling pH of the solution and adding anti-adsorption agents.

Anti-adsorption and Anti-aggregation Agents:

- Most proteins are **prone to adsorb** to interfaces.
- **Anti-adsorption** agents are added to **reduce adsorption** of the active protein to interfaces.
- Some proteins tend to **expose hydrophobic sites**, normally present in the core of the native protein structure 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).
- **For solid surfaces** (such as protein- container wall):
- Adsorbed, partially unfolded protein molecules not only present a **loss of API** but also may **form aggregates**, leave the surface, return to the aqueous phase, and form **larger aggregates**, and precipitate.

Anti-adsorption and Anti-aggregation Agents

- A similar situation may occur at **gas-liquid** interfaces.
- For some proteins the reconstitution protocol for the freeze-dried cake stipulates to **swirl** the vial **instead of shaking** it to avoid protein exposure to large liquid-air interfaces.



Anti-adsorption and Anti-aggregation Agents

- Techniques used to decrease (or prevent) adsorption and suppress aggregation.

1) For interface-induced aggregation:

a) Use of surfactant: surfactants will adsorb at the interfaces → make the interface more hydrophilic → Protein accumulation at the interface is suppressed and thereby aggregate formation.

- The most commonly used surfactants for parenteral use are poly-sorbate 20, 80. and Poloxamer 188.

b) Use of human serum albumin: also prevent adsorption.

- Albumin is **commonly avoided** nowadays due to probability of **transferring infections** and the **interference with the analysis** of the original drug.

Anti-adsorption and Anti-aggregation Agents

2) **For the aggregates that occur in the bulk:** these are formed because of colloidal and/or conformational instability.

a) **Adding sugars:** Glucose may perfectly act as a conformational stabilizer

- However this may cause additional instability through **Millard reaction (***Primary amino groups of the proteins react with the reducing sugar, resulting in brownish/yellow solutions*)

b) **Selecting a proper pH and buffer medium for maximum stability**.

- Variation in pH of the medium can cause protein instability and aggregation.

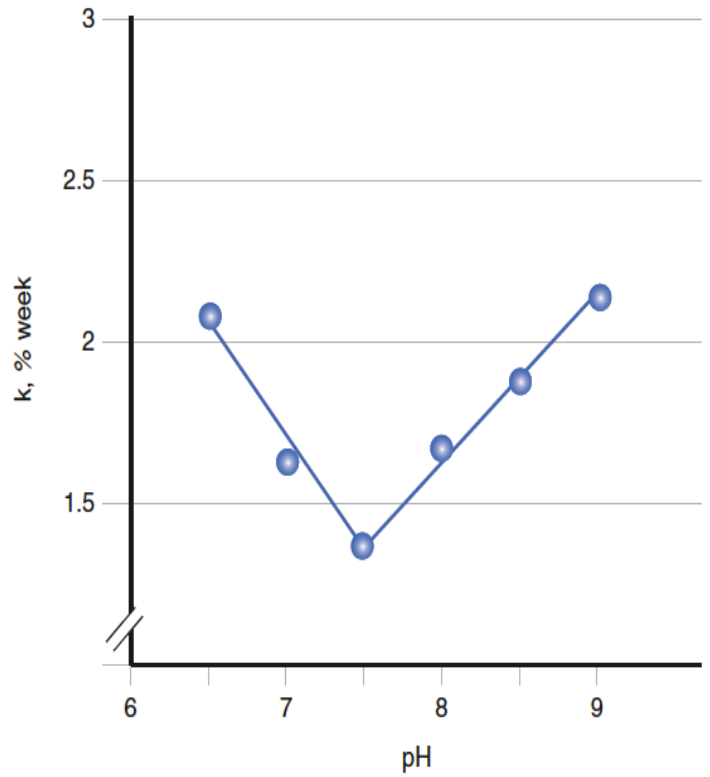
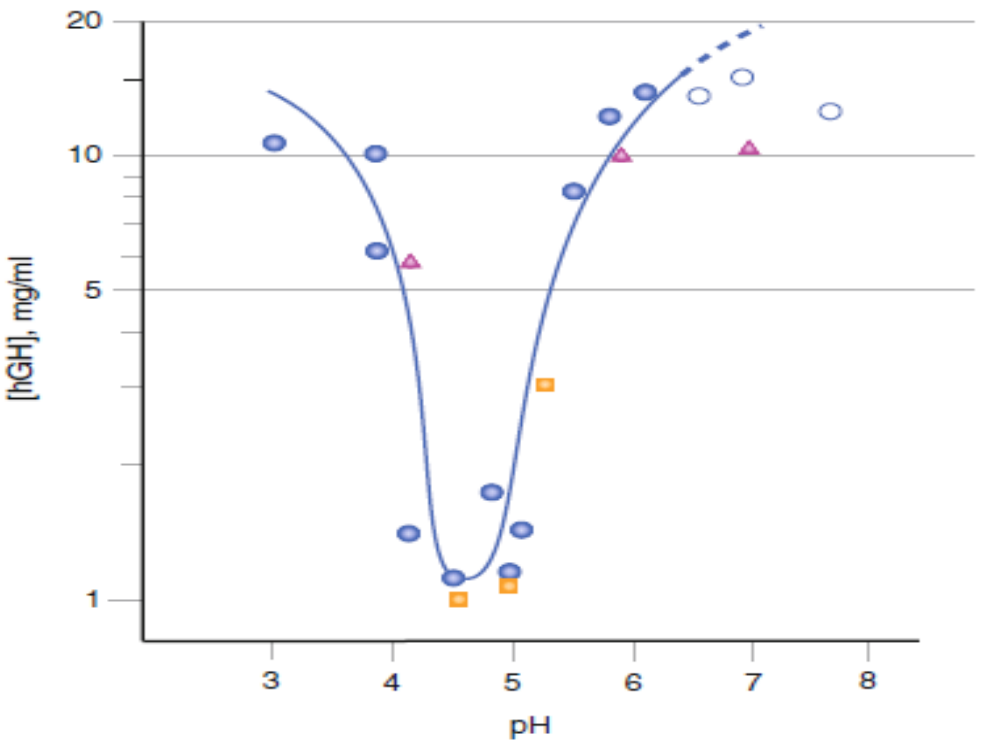


Figure 5.6 ■ pH stability profile (at 25 °C) of monomeric recombinant α1-antitrypsin (rAAT) by size exclusion-HPLC assay,

Buffer Components

- Buffer selection is an important part of the formulation process, **because** of the pH dependence of protein **solubility** and **physical and chemical stability**.
- Buffer systems regularly encountered in biotech formulations are **phosphate, citrate, and acetate**.
- **A good example on pH effect on solubility**: the importance of the isoelectric point (pI) (which is the pH at which protein carries NO charge (neutral)→ **lowest solubility**) is the solubility profile of human growth hormone (hGH, pI around 5)



*A plot of the solubility of various forms of hGH (**human growth hormone**)as a function of pH.*
Open circles means completely soluble.
pI here =~ 5

Buffer Components

- **Other Example on pH effect of Protein Solubility and Stability:**
- The protein **aggregation** due to the short (temporary) changes of pH that may occur during freeze drying process.
- This change in pH **happened** when one of the buffer components is crystallizing and the other is not.
- In a phosphate buffer,Na_2HPO_4 crystallizes faster than NaH_2PO_4. This causes a **pronounced drop in pH** during the freezing step.

- **Note**: this can be decrease by using cryoprotectant such as sugar trehalose

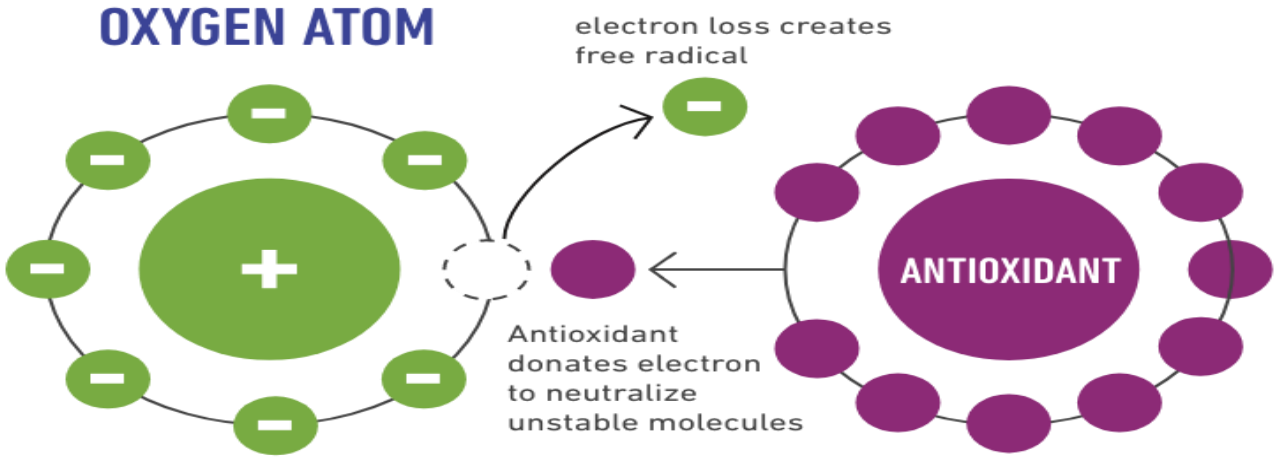
Preservatives and Antioxidants

- **Protection against oxidation:**
- Methionine, cysteine, tryptophan, tyrosine, and histidine are amino acids that are readily oxidized.
- Proteins **rich in these amino acids** are liable to oxidative degradation.
- These changes can be decreased (or prevented) by:

1. **Replacement of oxygen** by inert gases (e.g. argon) in the vials helps to reduce oxidative stress.
2. **Decrease the headspace in** the vial (such as in pre-filled syringes) will decrease the amount of oxygen available and decrease the oxidative stress.
3. **Addition of antioxidants** such as methionine that competes with the methionine residues for oxidation.

Preservatives and Antioxidants

- **Note**: some antioxidant can act as an **oxidant** in certain condition that need to be consider.
- Ascorbic acid, for example, can act as an oxidant in the presence of a number of heavy metals.
- So, if ascorbic acid had to be used for any reason we need to add chelating agents such as **EDTA** to reduce the effect of heavy metal.



Preservatives and Antioxidants

- **Preservation:**
- Certain proteins are formulated in containers designed for **multiple injection schemes**.
- After administering the first dose, **contamination with microorganisms may occur**.
- **Preservatives** must be added to **minimize growth**.
- Common antimicrobial agents include phenol, meta-cresol, benzyl alcohol, and chlorobutanol.
- Usually, these preservatives are present in **concentrations that are bacteriostatic** rather than bactericidal in nature. →
- **They can interact with the protein**, which may compromise both the activity of the protein and the effectivity of the preservative.

Cryoprotectant

- **Due to heat instability** of biopharmaceuticals → **freeze drying has become** the gold standard drying process for dosages that need to be in dry state for maximum stability.
- Freeze drying includes **removing of water by sublimation** (from solid phase into vapor phase without passing in liquid phase).
- These include freezing of the product first, then decreasing the pressure above the product so the temperature and pressure will be below that of **triple point**.
- The condition could be at -52˚C and 0.08 mbar.
- Then increasing the temperature slightly so water can sublimate.

Cryoprotectant

- During freezing stage, **ice crystal may form** and grow causing structural changes and protein instability

- **Cryoprotectants are excipients** that protect a protein during freezing or in the frozen state (mainly sugars: sucrose, trehalose and sugar alcohols: mannitol, sorbitol).
- These work by **increasing the solute concentration** and **lower the melting point** (keep water as liquid as possible).
- This will **prevent rapid ice formation** which is the cause of **structural changes** in which it takes out **structural water** out of the material or cells and cause deformation.
- So cryoprotectants keep the structure integrity during freezing stage.