

**A**  
**REPORT**  
**ON**  
**STABILITY OF POLYPEPTIDES AND PROTEINS**

SUBMITTED BY:

SR. NO.	NAME	ID NO.
1.	GUNJA CHATURVEDI	2008H146101

SUBMITTED FOR THE PARTIAL FULFILLMENT OF THE REQUIREMENTS OF THE COURSE  
**ADVANCED PHYSICAL PHARMACEUTICS (PHA G542)**



**BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE**  
**PILANI (RAJASTHAN)**  
**AUGUST, 2009**

NPS EX. 2039

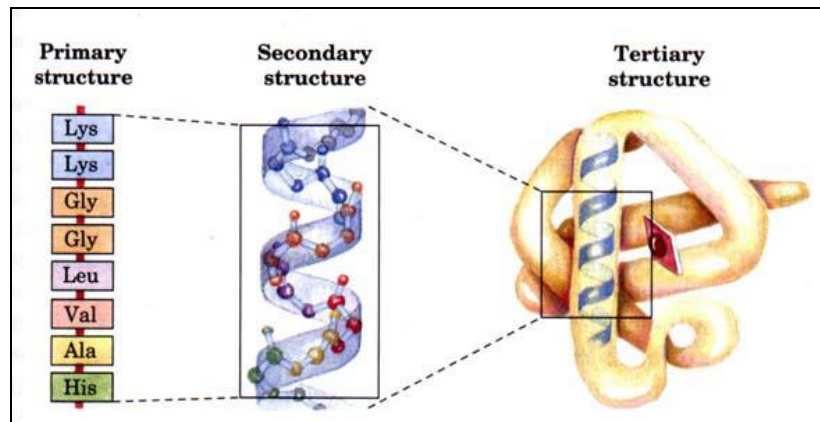
1

## STABILITY OF POLYPEPTIDES AND PROTEINS

Background:

Proteins comprise an extremely heterogeneous class of biological macromolecules. They are often unstable when not in their native environments, which can vary considerably among cell compartments and extracellular fluids. Their properties make them particularly difficult to formulate but, with right approach, they can be developed into effective therapies. Proteins and polypeptides are fast becoming an important segment of the pharmaceutical industry. Although there have been tremendous advances in production of the active pharmaceutical ingredient (API), production of the peptide-based drug products is still a significant challenge.

Peptides are defined as polypeptides of less than 50 residues or so and lacking any organized tertiary or globular structure. Some do adopt secondary structure, although this tends to be limited, for example a single turn of an  $\alpha$ -helix. While their smaller size makes them easier to deliver across biological barriers than larger proteins, their formulation can be problematic.



Mainly because of their chemical instability or degradation like by hydrolysis and racemization and physical degradation depending upon their molecular weight, they undergo denaturation, aggregation and precipitation; they are very challenging to be formulated in desired dosage form.

Proteins and peptides exhibit the following challenges to the formulation scientists:

- They exhibit maximal chemical instability.

- They tend to self associate.
- They adopt multiple conformers.
- They can exhibit complex physical instabilities, such as gel formation.

Chemical and physical properties of peptides and proteins have been studied extensively and the thermodynamics of protein structure have also been studied in detail and reported. But because of the complicated degradation mechanisms, it is generally more difficult to predict the stability of peptide and protein pharmaceuticals.

Proteins and peptides undergo degradation by two mechanisms:

- a) Physical mechanisms
- b) Chemical mechanisms

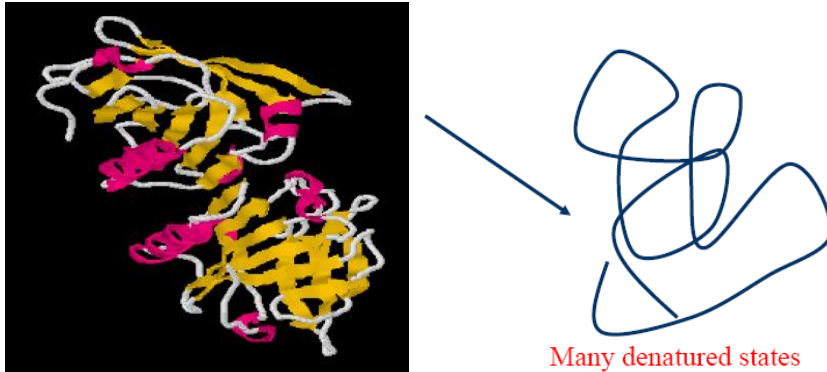
#### **PHYSICAL INSTABILITY:**

Physical instability or noncovalent changes are generally observed in case of larger peptides and proteins. Physical degradation includes ***denaturation, self association, aggregation, adsorption, and gelation.***

***Denaturation:*** protein Denaturation is mainly associated with any modification in conformation not accompanied by rupture of peptide bonds and ultimate step might correspond to a totally unfolded polypeptide structure which can be reversible or irreversible. It can also results in loss of bioactivity mainly because of the alteration the tertiary structure of the proteins. Furthermore, exposure of hydrophobic groups upon Denaturation often leads to adsorption on the surfaces, aggregation, and precipitation. Denaturation sometimes also triggers the chemical degradation pathways often not seen with the native or natural tertiary (and/or quaternary) structure. Other effects of Denaturation are:

- Decreased solubility
- Altered water binding capacity
- Destruction of toxins

- Improved digestibility
- Increased intrinsic viscosity
- Inability to crystallize



Denatured proteins

#### Causes of protein Denaturation:

##### 1. Temperature fluctuation

- Effect of increased temperature:
  - Affect interactions of tertiary structure
  - Increased flexibility → reversible
  - H-bonds begin to break → water interaction
  - Increased water binding
  - Increased viscosity of solution
  - Structures different from native protein
- Effect of decreased temperature:
  - Can result in Denaturation(for e.g.Gliadins, egg and milk proteins)
  - Remain active( for e.g.Some lipases and oxidases and Release from sub-cellular compartments)
  - Proteins with high hydrophobic/polar amino
  - residues and structures dependent on hydrophobic interactions

2. Water content affects heat Denaturation
3. Mechanical treatments
4. Hydrostatic Pressure
5. Irradiation
6. Heavy metal salts act to denature proteins in much the same manner as acids and bases. **Heavy metal salts** usually contain  $\text{Hg}^{+2}$ ,  $\text{Pb}^{+2}$ ,  $\text{Ag}^{+1}$ ,  $\text{Tl}^{+1}$ ,  $\text{Cd}^{+2}$  and other metals with high atomic weights. Since salts are ionic they disrupt salt bridges in proteins. The reaction of a heavy metal salt with a protein usually leads to an insoluble metal protein salt.
7. Heavy metals may also disrupt disulfide bonds because of their high affinity and attraction for sulfur and will also lead to the denaturation of proteins

**Self association:** The propensity of peptides to *self-associate* is connected with their physical instability. While self-association of peptides for e.g. melittin and corticotrophin – releasing factor (CRF), the relationship between these metastable oligomeric species and larger aggregates has been investigated, but still unclear. Noncovalent aggregation has been suggested for many other proteins, but not always confirmed. For e.g. a conjugate formed between a vinca alkaloid and a monoclonal antibody exhibited aggregation in solution, the mechanism of which (covalent or noncovalent) was not clear. Aggregates formed upon agitation of insulin solutions in the presence of hydrophobic surfaces (Teflon) were dissociated with urea, suggesting noncovalent aggregation.

**Aggregation** can lead to either amorphous or ordered forms. Ordered aggregates usually take the form of fibrils; these fibrillar structures are the basis for the most common type of the aggregation seen for peptides, namely gelation. Gelation is the last step in a pathway that starts with the formation of peptides protofibrils that exhibit  $\beta$ -sheet structure. The protofibrils then associate to form mature fibrils, which propagate and intertwine, resulting in gelation.

**Detection of aggregates:** Insoluble aggregates can be detected by FTIR, Raman, and electron spin resonance spectroscopy, or light scattering techniques (UV absorption). Soluble aggregates can be detected by HP-SEC (High Performance Size Exclusion Chromatography), found in many proteins like hGH, insulin, interferon-2 (IL-2), anti trypsin-a1, IFN-g, basic fibroblast growth factor and IFN-b.

# Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

## Real-Time Litigation Alerts



Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

## Advanced Docket Research



With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

## Analytics At Your Fingertips



Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

## API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

## LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

## FINANCIAL INSTITUTIONS

Litigation and bankruptcy checks for companies and debtors.

## E-DISCOVERY AND LEGAL VENDORS

Sync your system to PACER to automate legal marketing.