INTRODUCTION
# CHAPTER-1

## CHAPTER -1. Introduction

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1. INTRODUCTION

The progress of suitable vehicles has become a significant challenge for pharmaceutical researchers to advocate unstable macromolecule compounds like proteins based biopharmaceuticals including gene therapy. As their usefulness is extremely restricted by their capability to penetrate the biological environment, its prospect as therapeutic agents evidently depends on the sketch of an appropriate medium for their release to the body.

1.1 DELIVERY CONSIDERATIONS OF BIOTECHNOLOGICAL PRODUCTS

Proteinous substances are measured as therapeutic modalities to fight human diseases all the time as the trade opening of peptide hormone insulin, hormone thyroid, and antihemophilic factor in near the beginning of 20th century. Generally formed from innate sources, early request of bio molecules been hampered by complex and valuable developmental events. Amid progresses in rDNA skills and solid-phase synthesis, public concern in protein and peptide remedies has significantly augmented over the duration. Accordingly, approximately more than two hundred proteins as well as peptides have got US-FDA sanctions for serving a variety of individual disorders. Even as contemporary genomic and proteomic expertise enable quick selection of novel proteins as probable drug entrant, aim of release systems for these biologics relics complicated specially to realize site-specific biological activities \(^1\). Biopharmaceuticals especially proteins and peptides with established activity on the molecular, cellular intensity frequently fail to
generate sufficient efficiency when practically observed *in vivo*, principally due to insufficient pharmacokinetic profile. These comprise:

1. Poor oral bioavailability,
2. Poor stability and shelf time,
3. Immunological complications,
4. Minute plasma half-life
5. Reduced penetration transversely via biological membranes².

Requires thorough knowledge on the below mentioned points

Peptide and Protein structure

Stability profile

Physical stability

Chemical stability

Obstacles to biomolecules Delivery

Enzymatic barrier

Intestinal epithelial barriers

Capillary endothelial barriers

Blood barriers to peptide and protein delivery

Brain barrier (BBB)

Lymphatic Transportation of Proteins

Colorectal transport

Pulmonary transport

Site-specific Protein Modification (protein engg.)

Enzyme – PEG conjugates

Protein glycosylation

Modification of Proteases into Peptide Ligases

Production of site specific nuclease.

Production of artificial semisynthetic Oxidoreductases (flavor-enzymes)

Toxicity Profile Characterization

a) Classes of toxicity of proteins and peptides 3.

Modules of Remedial proteins as well as peptides:

Whether logically occurring, genetically architecture, or Partially synthetic, there are broad variety of protein as well as peptide drugs, with (1) Bacterial or plant toxins, (2) Antibody-based drugs., (3) Hormones and growth factors, (4) Drug-activating enzymes and (5) Clotting factors and anticoagulants.
For bio molecule associates of big magnitude, site-special release might be attained via “passive targeting” i.e., the superior withholding of large molecules in cellular networks by way of weakly formed blood vessels (eg., angiogenic tumors and inflamed joints). This event is recognized as the EPR (Enhanced Permeability and Retention) result

1.2 BIOLOGICAL / BIOCHEMICAL NECESSITIES OF BIOMOLECULAR DRUGS

Prior to a protein medicine can be examined as a candidate for targeted delivery, there are general considerations in regard to its biological and biochemical characters. Distinctly small unprocessed medicine substances, biomolecular proteins frequently need complex higher commands of organization (e.g., derived, tertiary, quaternary) in a bid to use their biological actions. Several physicochemical alterations of the resident structure can have important impact on a protein’s biological actions in vivo. Comparatively poor physical stability of bio molecules, modifications of higher order configuration may arise during preparation and storage. If one time partially or completely opened, proteins can go through further modifications by aggregation with other protein particles or form macroscopic assemblies in a precipitation process. The chemical volatility may be the consequence of additional bond structure (e.g., formation of disulfide bond) along with/otherwise separation. However being ventured to the place of action, protein drugs are contented to insensitive physiological factors like shear pressure in the flow, lysis of proteins in the plasma, short pH in endosomes, beside with digestive enzymes in
lysosomes, all of which might lead to adverse alterations in protein configuration. The immune recognition of a protein medicine is an extra key experimental worry since it stimulates disagreeable host resistant responses (i.e., synthesis of antibodies) upon the drug substances. Obligation of own antibodies to therapeutic proteins proliferates their approval from the blood, consequences in loss of efficiency, and averts repeated supervision of the medicines. The agents that donate to such resistant confictions include

(1) Biomolecules of other than human source (e.g., microbial poisons, enzymes),

(2) Existence of impurity or lumps

(3) The resistant class or inherited backdrop of being patients 7.

The biological actions of peptides is principally directed by means of their mass, surface chemistry (e.g., potential, glycosyl reaction), and active centre defending (e.g., PEGylation).

Modalities are essential to sell abroad protein based drugs prior towards their destruction or reprocess rear to the group surface. For this rationale, Biomolecule-drug couple can be complete by means of sticking to permit the release of drugs in definite biological milieus. On the other hand, for drug substance to achieve their intended place of accomplishment, activities must be completed to make sure their way out from endocytosis or other hydrolytic enzymes compartment 8.
The vast prospective of protein drugs has rejuvenated the technical society in investigation of superior ways to realize precise causes of infections\textsuperscript{9}.

### 1.3 POLYMERIC NANOPARTICULATE SYSTEMS

There are some apprehensions about the safeguard of nanoparticles insinuated in the individual body and delivery into the surroundings. There is some advancement in investigations to deal with these problems\textsuperscript{10}. Since there are no USFDA instructions to control nanotechnology but as goods are organized to enter marketplace, these are approved to be in position. A growing use of nanobiotechnology in the pharmaceutical and biotechnology manufacturing will increase in near future. Nanotechnology will be realistic at all stages of drug progresses from formulations for most favourable delivery to investigative applications in medical trials\textsuperscript{11}.

Progress in biotechnology has endorsed the cost-effective and large-scale manufacture of remedially important peptide and protein medicines to be used to contest poorly forbidden diseases. The speedy progress in cell biology, conversely, has not been correspondingly matched with the development in the formulation of release systems for such next inventive medicines\textsuperscript{12}.

Several endeavours have contended these troubles by chemical modification or by co administration of adjuvant to eliminate undesirable properties of protein based drugs such as chemical and enzymatic instability, poor assimilation through biological membranes, rapid plasma clearance and immunogenicity\textsuperscript{13}.
Colloidal carriers like nanoparticles have been accustomed as material avenues in close proximity to change and get better pharmacokinetic and pharmacodynamic property of different kinds of medicines. They are mainly used *in vivo* to guard the medicine unit in the complete distribution, confine right to use of the drug to the preferred sites and to convey the drug at a controlled and sustained rate to the site of action. A variety of polymers are widely habituated in the preparation of nanoparticles for medicine releases investigations to increase beneficial advantages, while diminishing adverse effects\textsuperscript{14, 15}.

The goals for nanoparticle enmeshing of drugs are improved release and uptake by cells or the decrease in poison effect of the free medicine to non target organs. The physical and chemical property of nanoparticles and their behaviour on disclosure to normal functioning media are significantly conquered by their compound shapes and surface features.

Colloidal polymer particles involve nanoparticles, nano capsules, microspheres and microcapsules. Nanoparticles and microspheres are huge strategies, through a grade-controlling polymer medium, all the way through which medicament is dissolved or spread\textsuperscript{16}. 
Figure.1.1: Technique of medicine release from colloidal systems.


Colloidal systems are defined as particulate dispersions or solid particles with size range of 10-1000 nm. The medicine is dissolved, entrapped, immobilized or fixed to a nanoparticle matrix. Depending in advance technique of research, nanoparticles, nanospheres or nanocapsules can be acquired\textsuperscript{17}. Nanoshells are structures in which the medicine is limited to a hollow enclosed by a distinctive polymer film, while nanospheres are matrix systems in which the drug is physically and constantly diffused.

In modern years, eco-friendly polymeric nanoparticles, mainly individuals covered by hydrophilic polymer such as poly ethylene glycol (PEG) known as long-circulating particles, have been used as prospective drug release devices due to their capacity to travel for a long-lasting period of time to duck a
exact organ, as carrier of DNA in genetic material treatment, and their capability to distribute proteins, protein amides and Nucleic acids. The key goals in scheming nanoparticles as a release system are to organize particle size, surface properties and release of pharmacological dynamic agents in bid to realize the site-specific utilization of the medicine at the therapeutically best possible rate and dosage schedule\textsuperscript{18}.

Although liposomes are used as potential carriers with unique advantages including protecting drugs from deterioration, targeting to location of action in addition decreased poisonous or adverse effects; their usages are inadequate due to intrinsic trouble such as low encapsulation efficiency, quick leakage of water-soluble medicine in the presence of blood mechanism and poor storage stability\textsuperscript{19}. On the other side polymeric nano particles present a few precise benefits above liposomes. For example, they assist to augment the strength of medicine/biomolecules and acquire useful controlled release patterns. The rewards by means of nanoparticles as a drug release methods comprise the following:

1. Particle size with surface characteristics of nanoparticles can be simply manipulated to attain together passive and active drug target after systemic administration.

2. They control and sustain release of the medicine through the transportation and at the site of localization, altering organ distribution of
the drug and successive clearance of the drug so as to get improve in drug therapeutic efficiency plus reduction in adverse effects.

3. Controlled release and particle degradation features can be readily modulated by the choice of matrix constituents. Medicine load is moderately tall and drugs can be built-in or captivated on the systems without any chemical reaction, this is a significant aspect for preserving the drug activity.

4. point-specific target can be attained by attaching to group icons outside of particles or using captivating control.

5. The group can be used for a range of routes of regime\textsuperscript{20}.

\textbf{Figure 1.2} : Some of the advantages of controlled drug release technology
1.4 CARRIERS

The selection of carrier depends on a number of factors; which include the character of the protein to be delivered, the device for delivery, the site of action, the disease condition, and the nature and safety of the carrier. When a medicinal agent is condensed within or inclined to a polymer or lipids, medicine safety and efficiency can be enhanced. Further, this has provided momentum for active learning in the design of appropriate carriers, quick delivery systems and paths for delivery during different gates in the body.

CS is capable to release epithelial firm junctures to allow for and strengthen in paracellular transportation of macrobiomolecular medicines. A plenty of cationic or anionic CS secondary products have been produced and tested by mutual addition of enzyme inhibiting particles to the CS backbone, enzyme degradation of the medicine to be delivered, and capacity can be disallowed. CS and CS derivatives simply form micro- and nano particles, which are being researched as delivery vehicles for vaccines in mucosal vaccination studies, and extra chromosomal DNA in non-viral gene treatment together with radioactive substances. TMC at different grades of quaternization raises the penetration and incorporation of neutral and cationic peptide analogues over gut epithelia.

CS after protonation (pH<6.5), is capable to raise the paracellular penetration of peptide medicines over the gut epithelia.
The idea of embodying a medicine into a polymeric or biomolecular particulate transporter was launched by the medical scientist as a way to transform the physical, chemical and biological qualities of entrapped medicines\textsuperscript{24}.

In order to overcome the clearance/opsonization process, it is appropriate to coat the particles with polymers which render them undetected by the RES and thereby raising the retention time of the micro particles carrier in the general circulation.

In general, to impart \textit{in vivo} longevity to drug carriers, certain synthetic and natural polymers are being studied \textsuperscript{25}. Albumin nanoparticles are currently the topic of clinical studies for anticancer medicine release purposes. Majority of the ‘nanoparticle formulations’ mentioned are not stringently or exclusively nanoparticulate in the common sense to their size as many of them are below 100 nm and some medicine delivery carriers include particles up to quite a few hundreds of nm. In many instances, the skill to produce very tiny particles did not exist in the beginning stages of growth, but at present there is a growing modification in their dimension and it is appropriate in this view to converse this group of goods collectively. The way of management may be oral, systemic (SC, IM, IA, IV) and by dermal.

Surface remodeling of nanoparticles tender promise for medical usages such as drug target in tenure of cellular bonding, incursion and transcellular conveying. Carbohydrate binding ligands on the outside of biodegradable poly
lactide nanospheres were formed to combine at superior rates by cell membranes\textsuperscript{26}. Such improved loyalty may lead to a better activity of the medicines offered as such or included in nanoparticles\textsuperscript{27}.

**Table 1.1:** Micro and nanoparticles characteristics

<table>
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<tr>
<th>S.No.</th>
<th>character</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>mass</td>
<td>thickness, consistency, circulation</td>
</tr>
<tr>
<td>2.</td>
<td>number</td>
<td>solidity, R. index, Lyphophilicity/ hydrophilicity, Non specific binding, Autoflourescence</td>
</tr>
<tr>
<td>3.</td>
<td>Surface chemistry</td>
<td>immediate groups, point of functionalization, Count</td>
</tr>
<tr>
<td>4.</td>
<td>Special properties</td>
<td>detectable dyes/ flourescence, immense-paramagnetic</td>
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**1.5 CHITOSAN AND MUCOADHESION**

Mucoadhesion can be seen anywhere in which two superficial, one is a mucous membrane, adhere to each other. This has been of significance in the pharmaceutical discipline in order to improve controlled drug release, or to transport complicated molecules (proteins and oligonucleotides) into the
general circulation. Mucoadherent resources are water loving macromolecules which include numerous hydrogen bond forming groups, the carbomers and CS being two familiar examples. The method by which mucosticky takes position has been assumed to have two platforms, to make contact with (wet) stage and compact step (the organization of the bonding agent exchanges). The comparative significance of every step will hang on the unit principle. For paradigm, adsorption is a responding stage if the specified quantity form is not functional directly to the mucosa of concern, while centre is critical if the expression is viewing substantial dislodging pressure. Adhesive joint malfunction will predictably occur as a outcome of more hydration of a dosage form, or as a effect of epithelia or mucus return. Novel mucoadhesive resources by best adhesive properties are currently being improved, and these must augment the prospective usage of this skill.

Mucous layers are the wet surface lining the fortifications of a variety of body cavities resembling as the git and pulmonary tracts. They are accorded with a connective tissue coat (the lamella proper) over which is an epithelial stratum, the surface of which is finished wet frequently by the existence of a mucus cover. The epithelia can be both single layered (e.g. the stomach, small and large intestine and bronchi) or multifaceted/stratified (e.g. in the oesophagus, vagina and cornea). The previous include goblet cells which produce mucus openly against the epithelial surfaces, the second hold, or are adjoining to tissues holdings, and specific glands such as salivary glands that produce mucus on the epithelia externally \textsuperscript{29}. For sticking together to occur,
molecules have to bond crossways the boundary. The chemical bond can approach up in the subsequent way:

   Ionic bonds- are formed which contain two counters charged ions draw each other via electrostatic exchanges to shape a tough union (e.g. in a saline crystal).

   Covalent bonds are formed wherever electrons are mutual, in couples, connecting the bonded atom in bid to fill the orbital in both. These are also strong bonds.

   Hydrogen bonds-here a hydrogen atom, while covalently bonded having tendency to attract atoms similarly as oxygen, fluorine or nitrogen, carries a small positive charge and as a effect is paying concentration to erstwhile electronegative atoms. The hydrogen can consequently be reflection of, as being common, and the bond produced is usually weaker than ionic or covalent bonds.

   Van-der-Waals bonds—these are a few of the slight forms of interface that happened to be from bipole–bipole and bipole-induced bipole attractions in water loving molecules, and scattering services with absence of polar substances.

   Hydrophobic bonds—more precisely described as the hydro hating effect, these are indirect bonds (such lots only emerge to be drawn in to all other) that take place when absent of polar groups are nearby in a watery solution. H$_2$O molecules neighbouring to absent polar groups outline hydrogen bonded
structure, which lower the system entropy. At present there is consequently an increase in the tendency of non-polar groups to associate with each other to minimize the effect 30.

In modern years, eco-friendly polymeric systems encompassed importance for plan of surgical equipments, synthetic organs, and medicine release systems with diverse routes of management, vehicles for entrapped enzymes and cells, biosensors, eye inserts, and equipments for skeletal deformity usages. These polymers are categorized as moreover artificial (PE, PA, PAD) and normal (PAA, PS,). PS-based polymers symbolize a main class of biomaterials, which contain alginate, dextran, chitosan, carageenan, and agarose 31. CS, B (1, 4)2-amino-2-D-glucose, is a cationic biopolymer formed by alkaline N-deacetylation of chitin, which is the major constituent of the shells of krill, shrimp, and crab 32. CS has produced many biomedical usages, together with tissue manufacturing, due to its, eco-friendly, less poison effect, and degradation in the body by biocatalysts like lysozyme and chitosanase, which have opened positive routes for fabricating drug release in vivo in the cure of a variety of diseases. These CS-based release systems vary from microparticles to nanoparticles, gels and films 33.

In recent times, it was narrated that the mutual affection of SH2 blocks to polymers significantly increases their mucoadherent and penetration plats devoid of affecting biodegradability. CS has immunostimulatory, anti-inflammatory activity, and is capable to increase transcellular and paracellular transportation crosswise to the mucosal epithelial membrane.
Unchanged nanoparticles of CS have a elevated zeta potential than TMC and enhanced constructive charge. This gives explanation for the better early adsorption of mucin through the unchanged particles at 1hr point in time. The condensed mucin adsorption on thiolated CS is accredited to the diminishing +ve charge of TCN and later decreased electrostatic interface with -ve charged mucin. Whereas unchanged nanoparticles of CS reach adsorption equilibrium at 1 hour of incubation with mucin, Thiolated chitosans exhibit a stable raise in mucin adsorption up to 12 hours. This improved mucoadherent property may be accredited to the development of SH₂-SH₂ bonds linking thiol groups on CS and cysteine-rich sub domains of mucus glycoprotein’s 34.

Thiolated polymers or selected thiomers are mucoadherent based polymers, which exhibit thiol attitude side chains. Based on thiol, disulfide replaced reactions or a easy oxidation procedure disulfide bonds are produced by involving such polymers and cysteine-rich subdomains of mucus glycoproteins structure over the mucus thick membrane 35. Thiomers enhance, so the natural mechanism of secreted mucus glycoproteins, which are also covalently anchored in the mucus membrane by the formation of disulfide bonds—the bridging organizations are most commonly encountered in biological systems. So far the cationic thiomers CS–thioglycolic acid, CS–thiobutylamidine, CS–cysteine, as well as and the anionic thiomers poly (acrylic acid)–cysteamine, carboxy-methylcellulose–cysteine, poly (acrylic acid) –cysteine, alginate–cysteine, chitosan-cycloextrins and have been produced 36.
**Table 1.2:** Chitosan based drug delivery systems prepared by different methods for various kinds of drugs.

<table>
<thead>
<tr>
<th>Formulation type</th>
<th>Principle involved</th>
<th>Medicine</th>
</tr>
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<tbody>
<tr>
<td>Pills</td>
<td>Coating, Matrix</td>
<td>PropranolHcl, 1,3-dimethyl-7H-purine-2,6-dione (T), Di-clo, 2-hydroxy benzoic acid(SA) , 3,7-dimethyl-1-(5-oxohexyl)-3,7-dihydro-1H-purine-2,6-dione(P).</td>
</tr>
<tr>
<td>Capsules</td>
<td>shell Capsule</td>
<td>Insulin, 5-amino salicylic acid</td>
</tr>
<tr>
<td>Microparticles and Microspheres</td>
<td>Ionic gelation, Spray-drying, Emulsion cross-linking, Sieving method, Coacervation/precipitation</td>
<td>Prednisolone$^{37}$, interleukin2, propranolol-Hcl,Glipizide$^{38}$, Felodipine$^{39}$,clozapine,bovine serum albumin,ketoprofen Oxytetracycline, Famotidine$^{40}$.</td>
</tr>
<tr>
<td>Nanoparticles</td>
<td>coalescence, reverse micellar method,Emulsion-droplet coacervation, precipitation, ionic gelation</td>
<td>cyclosporine A, Casein bovine serum albumin Gadopenteticacid, DNA, $^{41}$ insulin, doxorubicin, ricin.</td>
</tr>
<tr>
<td>Beads</td>
<td>Precipitation, Coacervation</td>
<td>lidocaine-HCL Adriamycin, salbutamol sulfate, nifedipine, riboflavin, bovine serum albumin$^{42}$,</td>
</tr>
<tr>
<td>Films</td>
<td>Solution casting</td>
<td>granulocyte-macrophage colony-stimulating factor, testosterone,</td>
</tr>
<tr>
<td></td>
<td>Gel</td>
<td>mixed-linking</td>
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</table>

In overall, the formation of nanoparticles mainly depends on the concentrations of polymer ratios, and process parameters. Each and every part of preparation needs to be optimized to get good results i.e. drug loading efficiency, drug release, shape of particles and size etc.<sup>45</sup>

### 1.6 INFLAMMATION

Inflammation is a condition of changes occurring in living tissues when invaded by germs or characterised by heat, pain, redness and swelling. Tissue damage or aggravation initiated by entry of bacteria or of distinct irritant leading to inflammation. It is significant non-specific mechanism of protection. Initially constriction and then dilatation of blood vessels of affected site is followed by escape of polymorphonuclear cells into tissue. Microorganisms are phagocytosed and destroyed. Out pouring of plasma helps to dilute the poisonous substances. A fibrin barricade is laid, serving to wall off the site of infection. Inflammation is the response of the active tissues to damage; it
comprises of systemic reaction (having neuronal and hormonal adjustments and escalation of the lymphoreticulo system) and local reaction (pain, redness, warmth and swelling).

Figure 1.3: Release of Inflammatory Mediators from Mast Cells

The three significant phases of inflammation that deliver themselves voluntarily to extent are erythematic (local vasodilatation), edema (increased capillary permeability) and formation of granular tissue.

Compounds claimed to possess antiinflammatory activity can be estimated moreover by their capability to decrease one or supplementary of these peculiar in experimentally invoked inflammation or by evaluating their antinflammatory action in a investigational arthritis created in animals.  

The generally engaged methods are:-
Erythematic assays.

Edema assays.

Granuloma assays.

Experimental arthritis assays.

Miscellaneous:

Localised inflammation produced by intrapleural injection Terpentine or by intraperitonial injection of Formaldehyde;

Arthus reaction (sensitised reaction to antigens);

The inflamed paw technique and adjuvant arthritis model (by intra-plantar inj.of Napthoylheparine or Carrageenin).

1.7 **ENZYME THERAPY**

Enzymes are proteins that serve as biologic catalysts. The enzymes are highly specific for their substrates, without eliciting any side effects\(^{47}\). They are water soluble, therefore formulation of dosage form of enzyme is relatively easy. The most important property being their effectiveness in biological environment, enzymes are therapeutically vulnerable.

The use of enzymes in therapeutics is as old as the knowledge of their existence. Enzyme preparations have been used as digestive aids, digestive enzymes and topically for the treatment of burns. They are used as selective chemotherapeutic agents for the treatment of neoplastic diseases, thrombolic
diseases as antiinflammatory agents, in enzyme replacement remedy, and in the curing of inherited storage sickness.

**Table 1.3:** Therapeutic applications of enzymes⁴⁸.

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteases: Pepsin, Trypsin, Chymotrypsin, Papain, Ficin, Bromelein, Amylase, Cellulase and Lipases.</td>
<td>Digestive aids</td>
</tr>
<tr>
<td>Urokinase, Streptokinase, Tissue Plasminogen activator (tPA) and Chymotrypsin.</td>
<td>Thrombolytic enzymes</td>
</tr>
<tr>
<td>L-Asparginase, L-Arginase, and Carboxylase.</td>
<td>Anti-neoplastic</td>
</tr>
<tr>
<td>Serratiopeptidase, Chymotrypsin, Trypsin, Lysozyme.</td>
<td>Antiinflammatory</td>
</tr>
<tr>
<td>Dnase and Superoxide dismutase thromboplastin and Thrombin.</td>
<td>Clotting factors</td>
</tr>
</tbody>
</table>

**Problems associated with Enzyme Therapy:**

Therapeutic enzymes are extremely effective because of their substrate explicitness i.e. the capability to strike only element or only alone metabolic course and extra ordinary catalytic power. Despite having the ideal properties of a drug molecule, the uses of therapeutic enzymes remain truly rare. There are several reasons controlling the everyday scientific use of biocatalysts.
**Economical reasons:**

Enzymes are heterogeneous proteins obtained from animals or vegetable organs or microrganisms. The availability of pure and homologous enzymes is relatively low and expensive\(^49\). The development of concurrent biotechnological approaches linked with cloning of expressing the required enzyme into the culture medium can answer case.

**Nature of enzyme:**

The typical problems in enzyme therapy arise from the complexity and the efficacy of the natural bio-defence mechanism. Biocatalysts are complicated biological macromolecules, which are frequently heterogeneous for the cured patient. These foreign proteins are quickly adsorbed on or entrapped in lymphoid tissues, mostly of the lungs and spleen, and then recognized by immuno-competant cells. This can direct to prominent immune response, associated with the systemic management of remedial enzymes.

**Molecular size:**

An enzyme molecule cannot easily penetrate the plasma membrane because of its larger molecular size. If the enzyme enters into the cell either by pinocytosis or phagocytosis, the risk of proteolysis by lysosomal enzymes is acute. In the blood stream, the enzyme may be inactivated due to adsorption of plasma components or by leukocyte phagocytosis, or by partial proteolysis. Enzyme monomer smaller than 60,000 daltons, can pass through the glomerular filter and are discarded in the urine.
**Stability factors:**

The storage stability of therapeutic enzymes is very poor, which requires their storage at low temperature and away from light. The instability in enzyme arrangements requires fast consumption of therapeutic enzyme solutions and leads to regular research of new entirety during long-lasting therapy.

**Other factors:**

Erstwhile factors comprise of recurrent non-specific poison of enzymes, their rapid degradation and inactivation in the body via endogenous protease or depletory agents. Moderately rapid elimination of the enzymes from the circulation and low shelf life of bacterial biocatalysts at bodily normal pH and temperature resulting in the configuration “unfolding“ of the dynamic structure. The unfolding or changes in native structure reduces the circulation half-life and activity of the enzyme in-vivo. Many enzymes, when given orally, are quickly destroyed by proteolysis following denaturation by gastric hydrochloric acid. Thrombolytic enzymes, when administered intravenously cause severe haemorrhage.

**1.8 SIGNIFICANCE OF STUDY**

The extension of a dosage form to make likely improvement in the assimilation of peptide and protein medicines by means of the gut is one of the main challenges in present scenario for the medical field. Oral management is the largely suitable route for release of remedial agents compared to various other routes and offer several advantages over other routes for example:
(i). Ease of ingestion.

(ii). Pain avoidance

(iii). Versatility (to accommodate various types of drug candidates).

(iv). Greater flexibility in dosage form design.

(v). Good patient compliance.

(vi). No need of sterilisation and also economical.

Since there are some hurdles associated with oral delivery of Peptide and Proteins, like;

Chemical and Enzymatic instability.

Reduced absorption and penetration throughout biological membranes.

Limited gastro intestinal residence time.

The above mentioned problems can be rectified by using carrier mediated delivery systems such as liposomes, microspheres, microparticles and nanoparticulate carriers. Nanoparticulate carriers have great potential for delivery of peptides and proteins in comparison to other carriers like liposomes, microspheres, etc\textsuperscript{51}. They offer several advantages such as,

(a). Delivery of bioactive agents by nanoparticles can help in preventing its degradation.

(b). Nanoparticles can be targeted by modifying their surface.

(c). Dose of the therapeutic agent can be reduced.
(d). Controlled and sustained release of the drug.

(e). Promise of the stability of drugs in the gastrointestinal tract and their bioavailability improvement.

Chitosan based nanoparticulate systems have following advantages for oral delivery of peptides and proteins:

1. Mucoadhesive, Biocompatibility, Biodegradable.

2. Enhanced uptake through gastrointestinal tract.(by increasing trans-cellular plus para-cellular transfer crosswise the mucosal epithelium).

3. Good swelling and solubilising property of chitosan (pH-dependent).

4. To reduce frequency and dose of therapeutic agent.

In a process to enlarge the stability of chitosan nanoparticles as well as to stop an immediate desorption of enzyme due to burst release in gastrointestinal fluids particularly in stomach, a coating process with sodium alginate was proposed in this work\textsuperscript{52}.

The enzyme selected for the study purpose is Serratiopeptidase which has good antiinflammatory activity. Currently it is available in enteric coated tablet form, but this dosage form still lacks some of delivery aspects e.g.:

1. Peculiar dose response after administration.

2. No guarantee of stability in gastrointestinal tract.

3. Loss of enzyme activity due to harsh compressional force used during tablet compression.
4. Poor patient compliance due to more frequency of dosage administration.

5. Short biological half-life.\textsuperscript{53}

Thus, Serratiopeptidase delivery via chitosan based nanoparticulate carrier system may enhance GI stability and absorption of enzyme. In addition, controlling the release of enzyme for prolonged effect will lead to increased patient compliance. Apart from this, chitosan itself is antinflammatory and immunostimulatory agent which will synergise the serratiopeptidase activity.