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Novel Method of Drug Delivery in Peptide / Protein Therapeutics

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Abstract:

The most prevalent substances in biological cells are proteins and peptides. Proteins are made up of more than 50 amino acids. peptides are little chains of amino acids that are more easily broken down. the novel drug delivery technology uses proteins and Peptides to deliver drugs.It might not cause discomfort or illness comparable to old needle stick wounds, allowing the patient to be cured. these microneedles, which are currently used for biosensors, fluid samplers, and micro-analysis, have the capacity to transport proteins and peptides in addition to vaccines, insulin, growth hormones, etc. In the very near future, many currently used organic-based medicines are anticipated to be replaced by protein- and peptide-based medications, which are quickly emerging as a highly important class of therapeutic agents. Biotechnology technologies will be used to make peptide and protein therapeutics on a big scale, and they will then be made commercially available for therapeutic use. The pharmaceutical industry is now faced with the pressing task of creating effective delivery methods for the effective distribution of these complex therapeutics in biologically active form. The research into their practical & efficient distribution through non-invasive method has increased due to their requirement in the clinical & therapeutic fields.

Keywords **—Protein and peptides, novel approaches, different routes of administration**

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I. INTRODUCTION

The novel drug delivery technology uses proteins and peptides. The most prevalent substances in biological cells are proteins and peptides.

PROTEIN: - Proteins are a mixed polymer with a high molecular weight that is made up of alpha amino acids and is connected by peptide linkages. By combining the same 20 amino acids in various combinations and sequences, cells may create proteins with remarkably varied characteristics and functions. All molecules made up of more than 50 amino acids are referred to as proteins.

PEPTIDES: -Peptides are little chains of amino acids that generally contain 2 to 50 amino acids. Proteins are composed of amino acids as well. Because they are smaller and more easily broken down than proteins, peptides may be easier for the body to absorb.

STRUCTURE OF PROTEIN

There are 4 types of structure of protein: -

- Primary structure
- Secondary structure
- Tertiary structure
- Quaternary structure

PRIMARY STRUCTURE: -The amount, type, and sequence of amino acids that make up a protein's primary structure are also referred to as its polypeptide chain. In this structure, the N terminal of amino acids is always visible at the left end of the polypeptide chain, while the C terminal is visible at the right side. An insulin molecule serves as an example of primary structure.

SECONDARY STRUCTURE: - A protein's secondary structure is made up of long polypeptide chains that are folded or collided in various geometric configurations. Alpha helical structure and beta pleated sheet are the two different protein secondary structure layouts.

TERTIARY STRUCTUTRE: - The three-dimensional coiling and folding of the chain that occurs in the tertiary structure of proteins is stabilised by interactions between amino acid sequences. These interactions, which primarily form (H-) bonded interactions, cause the (R-) group to form on the side chains of the amino acids as a result of this folding. Any other irregular form, such as an elapsed globe, can be the ultimate shape of a protein's tertiary structure.

QUATENARY STRUCTURE: - Haemoglobin possesses a quaternary structure, which is created when two or more polypeptide chains are held together by noncovalent bonds, an example of a protein's quaternary structure.

II. TYPES OF PROTEIN AND PEPTIDE: - Depending on the number of amino acids they are classified as follows:

PEPETIDES:-

- Polypeptides
- Oligo peptides

PROTEINS:-

- Fibrous proteins
- Globular proteins
- Oligomeric proteins

ROLE OF PROTEIN

STRUCTURAL FUNCTIONS:

Collagen and elastin, which are present in bone matrix, the vascular system, and other organs, are accountable for the body's strength.

DYNAMIC FUNCTIONS:

in addition to their role in genetic regulation and muscular contraction, act as membrane receptors, hormones, blood clotting agents, immunoglobulins, and enzymes.

III. NEED OF PROTEIN AND PEPTIDE DRUG DELIVERY SYSTEM

1. Organic molecules and biological cells both heavily rely on proteins and peptides.

2. Diseases like diabetes mellitus are brought on by an absence of proteins and peptides. (Due to a deficiency of a protein known as INSULIN)

3. These days, protein and peptide-based medications also utilise hybridoma and R-DNA technologies.

ADVANTAGES

1. RBC are mostly produced via erythropoietin.

2. Heart attacks and strokes are treated with the protein tissue plasminogen activator.

3. The treatment of labour pain involves the administration of oxytocin.

4. Bradykinin stimulates the circulation in the extremities.

5. Somatostatin reduces gastric ulcer haemorrhage.

6. Ovulation is induced by gonadotropin.

7. Insulin keeps blood sugar levels stable.

IV. FUNCTION OF PROTEIN AND PEPTIDE DRUG DELIVER SYSTEM

1. Small molecule and biological molecule transportation and storage.

2. Muscle contraction-based coordinated motion.

3. Fibrous protein support for mechanical stability.

4. Nerve impulse production and transmission.

5. Biochemical processes involving enzymatic catalysis.

6. Antibodies that safeguard the immune system.

7. The use of hormones to control growth and differentiation.

V. CHALLENGES IN PROTEIN AND PEPTIDE DRUG DELIVERY SYSTEM

Large molecular size results in low permeability, which is further accompanied by enzyme sensitivity, a short plasma half-life, immunogenicity, aggregate formation, denaturation, and protein binding.

PHYSIOLOGICAL BARRIER IN DELIVERY OF PROTEIN AND PEPTIDE

- Enzymatic barriers
- Intestinal Epithelial Barrier
- Capillary Endothelial Barrier
- Blood Brain Barrier(BBB)
- Chemical Barrier

ENZYMATIC BARRIER

The architecture Intact polypeptides and proteins are effectively blocked from absorption by the GI tract. Knowing the different types and distributions of proteases, their preferred substrates, and the likelihood of coming into contact with peptides or proteins can help one understand the function of the enzymatic barrier in peptide and protein absorption. Accordingly, the proteases may be divided into five families: - Aspartic Proteases, Cysteine Proteases, Metallo Protease, Serinyl Proteases, Threonine Proteases.

INTESTINAL EPITHELIAL BARRIER

serves as a barrier to prevent the passage of protein medications through the intestinal epithelium. Several processes are involved in the movement of peptide protein drugs across the intestinal epithelium, including-

- Carrier mediate transport
- Endocytosis and transcytosis
- Paracellular movement

CARRIER MEDIATE TRANSPORT

Facilitated diffusion or passive transport: No energy is used in this phase, and movement solely occurs from a location of greater concentration to one of lower concentration. However, compared to what would be predicted from simple physicochemical considerations, substances can move through the concentration gradient that exists across the membrane more quickly since the carrier is there. Active transport or Pump-like active transport systems, in this kind of transport, a specialised carrier is necessary in addition to the expenditure of energy. Additionally, due to cellular metabolic activity, materials can be carried "uphill," or against a concentration gradient.

ENDOCYTOSIS

• Endocytosis is a process by which proteins and peptides that are too big to be taken up via carrier-mediated transport are taken up by cells.

• The various endocytosis mechanisms include phagocytosis (eating of cells) and pinocytosis (cell drinking).

PARACELLULAR MOVEMENT: The movement of medicines across the GI epithelial cells' junction.

• There are two mechanisms for medication absorption:

1. Epithelial cell permeation across tight junctions (insulin)

2.Persorption

The epithelial mucosa of the small intestine acts as a barrier to the permeability of macromolecules.

CAPILLARY ENDOTHELIAL BARRIER

Peptides and proteins must either travel across the endothelial cells themselves or travel in between the endothelial cells in order to traverse the capillary endothelium. The endothelial passage therefore presents a metabolic or enzymatic barrier to the solution passage because cytoplasmic enzymes may modify or metabolise soluble substances that cross the endothelial cell membrane.

BLOOD BRAIN BARRIER(BBB)

A significant barrier to the transport of proteins to the brain compartment is the blood-brain barrier (BBB). There are various barriers in it, but the blood-cerebrospinal fluid (blood-CSF) barrier and the vascular BBB are the two that can best be defined. The BBB is made up of a monolayer of cells at both locations, held together by tight junctions and equipped with additional mechanisms that prevent or slow the leaking of plasma into the CNS. Small, lipophilic, uncharged molecules and gases can flow across the BBB. Proteins and other large molecules have difficulty crossing the BBB.

CHEMICAL BARRIER

pH: - Solution For purposes of stability, pH is crucial. The pH of least degradation for simple peptides should be determined. The pH of peptide formulations is often somewhat acidic (3-5). To prevent aggregation, the pH of proteins is adjusted away from the isoelectric ph. At pH 5.4, insulin is more stable. However, the pH range for insulin injection is between 2.5 and 3.5 or between 7 and 8.

VI. PHARMACEUTICAL APPROCHES OF PROTEIN AND PEPTIDE DDS

The protein and peptides are having different approaches they have follows

1. Chemical modification

2. Enzyme inhibitors

3. Penetration enhancers

4. Formulation vehicle

5. Mucoadhesive polymeric system

CHEMICAL MODIFICATION

To increase enzymatic stability and membrane permeations, it is crucial to chemically modify protein and peptide drug delivery systems. It is appropriate for lowering Immunogenicity. The following two types of modifications include the chemical modification:

1. Amino acid modification: This strategy is one of the most crucial ones for changing the physiological characteristics of proteins and peptide drug delivery systems. It involves replacing D and L amino acids. De-amino vasopressin and desmopressin are two examples.

Application: Increasing membrane permeability and maintaining enzymatic stability need the alteration of amino acids.

2. Hydrophobization: Lipophilic molecules are used to modify surfaces. Using NOBEX INSULIN as an illustration,

ENZYME INHIBITOR

The enzymatic method of the protein and peptide drug delivery systems is the enzyme (protease) inhibitors. With the aid of a range of proteolytic enzymes, the GIT and liver play a significant role in the metabolism of proteins and peptides into smaller fragments of two to ten amino acids. In order to change the environment for the stability of the enzymes and decrease the proteolytic activity, these protease inhibitors are CO-administered with proteins and peptides. Aspartic proteases (Pepsin, Rennin), Cysteinyl proteases (Papain, Endopeptidase), Serinyl proteases (Thrombin, Trypsin), and Metallo proteases are the four

different categories of enzyme proteases inhibitors (Carboxypeptidase).

PENETRATION ENHANCER

One of the most crucial ingredients in the formulation of proteins and peptides, penetration enhancers cause disruption of mucosal barriers and may be used to increase the membrane permeations of macromolecules like proteins and peptides. Surfactants (Polysorbate, SLS, Pluronic F-68), chelating agents (EDTA), Fatty acids (Sodium Carprate), Mucoadhesive Polymeric Systems (Thiomers, Cellulose Derivatives), and Phospholipids are among the several groups of substances that are primarily employed as permeation enhancers (PC). The fundamental mechanism of penetration enhancers is the disruption of the lipid bilayer of the lipid membrane, which results in increased permeability. Detergent and surfactant molecules promote the transcellular transport of the medicinal material.

FORMULATION VEHICLE

It is possible to successfully distribute thermoplastic proteins or peptides orally by employing a variety of carrier systems, including

1. To prevent the instability of long-term storage of numerous emulsions, dry emulsions are formed.

2. Microspheres: Microspheres are the homogeneous drug distribution used in protein peptide drugs for oral medication administration. prevention of proteolytic degradations in the stomach.

3.Liposomes are tiny microscopic vesicles that totally block the breakdown of insulin molecules in intestinal fluid by enclosing an aqueous volume with a membrane made of lipid molecules.

4. Nanoparticles: Particles in the nanometric size range are absorbed intact by the intestinal epithelium and are less likely to be broken down by enzymes.

MUCOADHESIVE POLYMER SYSTEM

The mucoadhesive polymeric system is crucial to maintaining the therapeutic effectiveness of the drug and preventing the issue associated with first pass or presystemic metabolism. the length of time that this drug delivery mechanism remains at the site of action and if the drug clearance rate is rising or decreasing. Thiomers, polyacrylic acid derivatives, and derivatives of cellulose are a few examples. Thiomers are thought to have higher mucoadhesive qualities because of covalent connections that form between their thiol groups and the cysteine-rich regions of mucus glycoproteins. Stronger mucoadhesive qualities result from a higher concentration of thiol groups.

VII. ROUTES OF DRUG DELIVERY IN PROTEIN AND PEPTIDE DDS

ORAL ROUTE OF ADMINISTRATION

From the patient's perspective, oral administration is the most common method. Convenience, acceptance, and excellent patient compliance are the main benefits of this method. The fundamental obstacles to effective protein and peptide oral administration are comparable to those facing classical medication candidates, although they are more evident in the case of peptide/protein moieties. Poor peptide/protein intrinsic membrane permeability is the fundamental obstacle to successful oral administration, vulnerability to intestinal proteases and peptidases' enzymatic assault, rapid evacuation upon absorption, aggregation and adsorption are examples of physical instability.

Various approaches for delivery of protein and peptide to overcome the problem by oral route

A chemical synthesis that modifies prodrugs and their analogues worked on to Improve Membrane Permeations and Enzymatic Stability. It can be used to lessen immunogenicity.

- Modifications to amino acids: -The substitution of the D- and L-amino acids is crucial in the modification of amino acids, one of the key strategies for changing the

physiological properties of proteins and peptides.

- Hydrophobization:-Using the NODEX TECHNOLOGY process of hydrophobization, the required amphiphilic oligomers are created by joining polyethylene glycol (PEG) with alkyl groups or fatty acid radicals. To create desirable amphiphilic products that may pass through the aqueous and lipid layer of the mucosa and withstand degradation, these oligomers are coupled to proteins or peptides.
- Enzyme inhibitors • To block proteolytic activity and stop protein breakdown, protease inhibitors are co-administered with proteins and peptides. For instance: aprotinin (inhibitor of trypsin and chymotrypsin), amastatin, bestatin, boroleucine, and puromycin (aminopeptidase inhibitors)
- Bio adhesive systems: • By virtue of their mucoadhesive qualities, they increase the amount of contact between the dosage form and the intestinal mucosa and help the medicine work at concentrated levels locally. A steep concentration is maintained and an increase in medication absorption and local delivery is observed as a result of greater contact with the absorbing mucosa. The in vivo absorption of vasopressin hydroxy propyl methacrylate nanoparticles via the rat gut, for instance, is enhanced.
- Particulate Carriers: They come in both replicative and non-replicative varieties. Attenuated or genetically modified strains of bacteria and viruses, such as the Vaccine virus and attenuated Salmonella strains, make up the replicating systems. Polymeric and lipid-containing particles are the non-replicating particulate systems. They enclose the medications within the Articles, providing a protective covering.
- Carrier systems: This approach is particularly useful when a specific tissue or organ is to be influenced by poorly absorbed peptides or proteins that are unstable in the GI lumen.
- Lipid carriers and emulsions: Drugs that are soluble in lipids as well as in water can be contained in lipidosomes, which are composed of bilayers with an aqueous core. Additionally, fat emulsions and solid lipid nanospheres can be employed.
- Emulsomes: -Colloidal medication delivery units called emulsomes, a lipoidal drug delivery system that was created employing a reasonably high lecithin content (5–10%). At room temperature, the interior phase is still solid or almost solid. Internal phase: macromolecule (Insulin) for oral delivery and external phase: widow palmitic acid in octyl decyl triglyceride.

ADVANCEMENT IN ORAL PROTEIN AND PEPTIDE DDS

A technology called CAPIC, created by Biosante Pharma, uses calcium phosphate to deliver an oral version of insulin. The calcium phosphate-PEG-insulin-casein oral delivery system is made using nanoparticulate technology, which involves synthesising calcium phosphate particles with insulin while also including PEG-3350.In diabetic mice, oral insulin treatment resulted in a decrease and maintenance of normal BSL. Cyclosporin is marketed by Novartis and Roche Pharmaceuticals and has a 30% bioavailability.

PULMONARY ROUTE OF ADMINISTRATION

By passing via the alveoli instead of the harsh circumstances of the stomach and first pass metabolism, particles can be absorbed into the systemic circulation. It is possible to tailor particle properties like aerodynamic diameter to transport particles to certain lung regions.

Stokes' Law provides the formula for the aerodynamic diameter, da, which is

da=(p/0)0.5dg.

Where dg is the geometric dimension of the particle and p is the particle density (1 g/cm3), respectively. Particles are deposited according to their aerodynamic diameter, which ranges from 2 to 30 micrometres for the trachea and bronchi to 10 to 30 micrometres for the oropharynx. Alveolar region: less than 2 micrometres.

Advantages:

- Reduced dosage required.
- Rapid absorption because to the alveolar epithelial membrane's thinness

Disadvantages:

- Lung inflammation may be seen.
- Lung hydrolytic enzymes caused a lower degree of bioavailability.
- The majority of the cells that make up the central airway epithelium are ciliated columnar cells, and these cells exhibit tight intercellular connections that severely restrict the paracellular transport of proteins and peptides.

TRANSDERMAL ROUTE OFADMINISTRATION

The medication is designed as a patch, which is placed on the skin and allows for drug absorption via the skin.

Advantages:

Better and increased patient adherence and it is feasible to administer medication under controlled conditions, preventing hazardous consequences. Additionally, medications having a shorter half-life can be given. It is feasible to administer medications with a low therapeutic index.

Disadvantages:

Due to their enormous molecular weight, the stratum corneum's hydrophilicity, and lipophilicity, most protein medicines have a poor rate of penetration, high interpatient and intrapatient variability with this method.

APPROACHES FOR TRANSDERMAL PROTEIN AND PEPTIDE DELIVERY

IONTOPHORESIS: - Ion or charged molecule migration is induced by the process of iontophoresis, which involves allowing an electric current to pass through an electrolyte medium. Protein and peptide molecules must contain charge in order to conduct iontophoresis. The pH and ionic strength of the solution are regulated to achieve this. Under the influence of an electric current, protein and peptide (charged molecules) permeate through the skin because they are attracted to the same charge on an electrode. The stratum corneum is covered with two electrodes, one of which has a drug reservoir put into it. When current is given, the permeability of the skin is enhanced, allowing drug molecules to pass through the epidermis, dermis, papillary layer, subdermal tissue, and blood vessels. For instance, the effective delivery of insulin, TRH, vasopressin, and leuprolide done by this technique.

PHONOPHORESIS: - Using a coupling contact agent, ultrasound is administered to the skin in this technique. The heat action of the body increases the medication absorption, ultrasonic waves, which cause brief changes in the skin's physical makeup. Example: Erythropoietin and insulin

PENETRATION IMPROVEMENT: -The ability of penetration enhancers to reversibly lower the Layer's barrier resistance results in an increase in the quantity of medication that reaches the live tissue. For instance, azone, dimethylsulphoxide, surfactants, and oleic acid.

PRODRUGS: -Using prodrugs or their equivalents is another tactic that guarantees some promising outcomes, particularly with tiny peptides. The skin's enzymes renew the active medication in a targeted manner. Prodrugs with simulated physicochemical properties penetrated the skin better than drugs.

TRANS FEROSOMES: - Trans ferosomes are supramolecular aggregates made of phosphatidylcholine that are sufficiently flexible to pass through the intact epidermal barrier. The resulting vesicle membranes are particularly

flexible in their disposition because these carriers contain at least one polar amphiphilic component (such as cholate).

BUCCAL ROUTE OF ADMINISTRATION

Oral mucosa, such as the gingiva, buccal mucosa, and floor of the mouth, provide great accessibility and prevent first-pass hepatic metabolism.

Advantages: -

Less susceptible to permanent discomfort, even with prolonged therapy, very well received by the patients, and administration of dose types with ease

Disadvantages:

Obstacles to medication dispersion across the buccal membrane such as cellular barriers.

The dermis contains a large number of elastic fibres, peptidases in the mucus layer and saliva, and microbial flora.

VARIOUS STRATGIES EMPLOYED FOR BUCCAL ROUTE OF ADMINISTRATION

Adhesive Tablets: Adhesive tablets were created as eroding hydrocolloid/filler tablets for buccal administration. On the basis of hydroxypropyl cellulose, adhesive nitro-glycerine tablets were created.

Adhesive Gels: Using polyacrylic acid and polymethacrylate as the gel-forming polymers, viscous adhesive gels have been developed for local treatment. Gels are said to significantly lengthen their stay in contact with the mouth mucosa.

Adhesive Patches: In adhesive patches, the adhesive polymer may serve as both a drug carrier and a bonding agent between a layer that has been drug-loaded and the mucosa. The drug activity and the drug impact of additives are restricted to the site of application in this method, which also reduces drug loss to the saliva.

Absorption promoters: A few absorption promoters have been tested to improve the effectiveness of buccal peptide delivery. These include citric acid, sodium myristate, sodium glycocholate, sodium 5-methoxysalicylate, bile acids, sodium lauryl sulphate, and bile salts.

RECTAL ROUTE OF ADMINISTRATION

The rectum is a highly vascularized, villi-free bodily cavity. Suppositories, gels, and dry powders are all common drug delivery methods. EX: calcitonin, insulin

Advantages:

decreased breakdown caused by proteases, Steer clear of first pass metabolism, Systemic bioavailability is increased when absorption enhancers are also administered such as surfactants and large doses can be given.

Disadvantages

Delivered medication removal during bowel movement, Patient non-compliance and accessible surface area is little

NASAL ROUTE OF ADMINISTRATION

The nasal route has been used to produce local effect on the mucosa, which is more permeable highly vascularized mucosa, porous endothelium membrane, and has a smaller surface area than oral mucosa.

Advantages:

1. Quick start of action

2. It is possible to prevent first pass metabolism

3. Since the blood-brain barrier is bypassed, direct medication administration to the brain is possible.

Disadvantages:

Low systemic bioavailability and variable absorption, Long-term use irritates nasal mucosa and results in pathological alterations in them

Nasal membrane-associated peptidases and proteases act as an enzymatic barrier to prevent the absorption of proteins and peptides.

VARIOUS APPROCHES FOR NASAL DELIVERY OF PROTEIN AND PEPTIDE DRUGS: -

Viscosity modification: By utilising solutions with a greater viscosity, the nasal cavity's clearing time can be prolonged. For instance, 0.6% of hydroxypropyl methylcellulose might dramatically lengthen the half-time of clearance.

. pH Modification: Aggregate dissociation: In solution, proteins are prone to form higher-order aggregates. For instance, insulin mostly occurs in solution as hexameric aggregates at pH 7.0. Insulin is unable to pass through the nasal mucosa. In addition to preventing the synthesis of insulin hexamers, sodium deoxycholate also prevents the dissociation of insulin hexamers into dimers or monomers.

- Types of dosage form: -Nasal spray, Nasal drops, Aerosol

OCCULAR ROUTE OF ADMINISTRATION

Potential candidates:

Antibiotics made of polypeptides: cyclosporin, bacitracin, and polymyxin

Cyclosporine and interferons have immunomodulating properties. Enkephalins and substance P both reduce inflammation. fibronectin and epidermal growth factor have an impact on wound healing.

Disadvantages:

- Ocular tissues are sensitive to the presence of foreign chemicals;
- systemic bioavailability is poor;

Approaches to ocular delivery

Pro-drug strategy, transporters of mucoadhesive particulates and enhancers of penetration and nanoparticles

PARENTRAL ROUTE OF ADMINISTRATION

Due to their poor absorption and metabolic instability when administered via other alternative routes, parenteral mode of drug administration has been the most popular method for protein/peptide delivery. The parenteral medication delivery method comprises intrathecal, intramuscular, subcutaneous, and intraperitoneal administration. Particulates, soluble carriers, and other drug delivery methods are used for defined and controlled drug distribution through this channel.

PARTICULATES: -

MICROSPHERE: These are solid, spherical particles with a particle size between a few tenths of a micrometre and several hundred micrometres, and they can contain either a solution of drugs or a microcrystalline form of drugs that have been distributed.

Advantages: Because they may be given intravenously, subcutaneously, or intraperitoneally, the delivery device does not have to be implanted. They may be prepared inexpensively by using the right method and subsequent optimization. Drug release may not be well defined, which is a drawback. The blood constituents and they may engage in interactions or create complexes.

Microsphere-like nanoparticles with particle sizes in the nanometres range are known as nanoparticles (10-100nm). They can be used to deliver peptides and proteins precisely where they are needed. They can even fit through the sinusoidal gaps in the spleen and bone marrow due to their tiny size.

Liposomes: These act as a "depot," gradually releasing the medication after enzymatic breakdown.

Advantages:

flexibility in form, size, and colour, disposition with a low hazardous potential, and the capacity to encase both lipophilic and hydrophilic peptides/proteins.

Disadvantages:

The component phospholipids' innate propensity to bind with peptides and proteins is a drawback. This may have a negative impact on the liposomal preparation's release kinetics and shelf life. Liposome manufacture is not economically viable on a large scale.

Emulsions: Peptides can be delivered parenterally in the form of droplets that are colloidal in size. In addition to keeping hydrophilic or lipophilic medications from coming into touch with bodily fluids directly, this delivery mechanism can also be particularly useful for dispensing the medication over an extended period of time. The release of a medicine can be further delayed by using several emulsions. Pharmaceutical peptide/protein molecules can be enclosed in erythrocytes for delayed release or targeted delivery.

Advantages:

Biodegradability, profile that is not immunogenic, extended circulation life (up to 4 months), simple accessibility, provides the medicine that is entrapped with enzymatic and immunological protection.

Limitations: They are susceptible to a variety of medications, Long-term storage presents difficulties,

Conjugates, pharmaceuticals with chemical modifications, and hybrid proteins are examples of soluble carrier (macromolecular) systems. A polymer or macromolecule can be conjugated with the peptide or protein medication. This aids in achieving selective or targeted medication administration and improving stability and non-immunogenicity.

Macromolecule peptide/protein drug molecule derivative

1. Reduce immunological characteristics with bovine serum with PEG

2. Plasma half-life is prolonged by asparaginase with PEG.

3. Improved protease stability of asparaginase with DL-alanine-N-carboxy anhydride

A number of sophisticated methods have been developed for the regulated and targeted administration of peptides and proteins. MISCELLANEOUS: It contains an on-demand mechanism. Systems for externally increased demand delivery are very useful for delivering polypeptides like insulin. The drug release was boosted up to 30 times by the presence of an external oscillating magnetic field. When the magnetic field was switched off, the drug release rates resumed their previous levels.

Self-governing systems: - The self-regulated systems have a unique ability to provide diabetics with insulin in response to blood glucose levels.

Temperature-sensitive system: Some polymers, such as variants of polyacrylamide, naturally exhibit a swelling characteristic that is sensitive to temperature. This results in a release pattern for peptides and proteins that is temperature dependent.

PUMPS: - • There are several different kinds of pumps, including: 1) Implantable infusion pumps, which are used to provide medication subcutaneously.

• Pumps continuously supply medications to the central vein for 7–14 days.

2) Mechanical pumps, which are simple to operate, reliable, and adaptable enough to distribute peptidyl medicines in a variety of wave forms. An example is the effective delivery of insulin using a handheld syringe.

3) Subcutaneous implants can be used to place osmotic pumps. The following hormones have been administered via osmotic pumps: ACTH, calcitonin, LHRH, growth hormone, and vasopressin.

VIII. EVALUATION OF PROETIN AND PEPTIDE DDS

Stability evaluations

The capacity of a certain formulation to maintain its physical, chemical, microbiological, toxicological, and protective requirements in a specific container or closure system. Evaluates the impact of environmental elements on a pharmacological substance's or a formulated product's quality in order to forecast its shelf life and determine the best conditions for storage.

Bioassay

Although proteins are complicated, bioassays are necessary to evaluate the formulation's efficacy. There are two types of bioassays: in vitro and in vivo. In vitro bioassays track the way that cells react to hormones and growth factors. The pharmacological reaction of animals to proteins is seen in in vivo bioassays. During instance, post-injection BSL in rabbits is assessed for an insulin bioassay. Proteins with aromatic amino acid residues, including phenylalanine, tyrosine, and tryptophan, can be found using UV spectroscopy. The quality control of processes may be performed using ultraviolet spectroscopy. UV light is scattered by protein clumps, increasing absorption. Consequently, protein aggregation may be seen using UV spectroscopy.

BIURET TEST: Proteins and biuret have a similar structural makeup. In alkaline solutions, biuret converts copper to cuprous ions in the presence of proteins or peptides, resulting in the development of colour complexes.

The BRADFORD ASSAY is based on the idea that the Coomassie brilliant blue G-250 dye's maximal absorption varies when proteins are present in an acidic solution. The solution remains brown if there is no protein to bind to. By using Van der Waals forces, the dye forms a compound with the carboxyl terminus of proteins to create a blue-coloured solution. A spectrophotometer may be used to measure the colour of the coloured solution and calculate the amount of protein present in the sample.

Thermodynamic Analysis: -As a method for examining confirmation transitions as a function of temperature and, more significantly, the impact of putative stabilising excipients in a protein solution, differential scanning calorimetry (DSC) is becoming increasingly popular. The transition temperature is the peak of the endothermic curve between native and partially unfold confirmations.

ELECTROPHORESIS

Electrophoresis on sodium dodecyl sulphate-polyacrylamide gel is the method most frequently employed for protein products (SDS-PAGE). Boiling in the SDS solution causes proteins to become denatured. The negative charge of dodecyl sulphate masks all charges on proteins. As a result, protein only moves on polyacrylamide gel based on the size of the protein molecule. This method may be used to calculate the molecular weight of proteins. Silver nitrate and Coomassie brilliant blue dye are employed as reagents to see proteins on the gel.

LIQUID COLOR GRAPHICS

HPLC is a good tool to evaluate the stability of proteins and peptides. There are several modalities, including Normal Phase HPLC, Reverse Phase HPLC, and Ion Exchange and Chromatofocusing

IX. DEGRADATION PATHWAYS OF PROTEIN AND PEPTIDE FORMULATION

The stability issues that lead to protein breakdown are the fundamental barrier to the development of protein and peptide drug delivery systems.

The two processes by which protein and peptide molecules degrade are as follows: -

Pathways of Physical Degradation

Pathways of Chemical Degradation

Chemical decomposition Routes: By altering their primary structure of protein molecules, proteins lose some of their native or original structure.

Pathways of Physical DEGRADATION:

Higher order protein structures are altered or substituted for the native or original structure of proteins.

Recent advancement in protein peptide drug delivery system

1. Macro flux transdermal technology:-With the use of efficient macro flux transdermal patch technology, medications, proteins, and peptides are delivered to the patient while also providing comfort. A polymeric adhesive back is integrated, and titanium micro projection is fixed to it. This medication is absorbed through microcapillaries for systemic delivery.

2. Xenoport's transcytosis:-The technique that results in receptor-mediated transcytosis is remarkable. Such transcytosis combines protein therapeutics with the targeted ligand. When combined with peptide medicines, these ligands increase drug incorporation and intestinal permeability.

3. PEGylation:- Recent developments indicate that PEGylation is significant in peptide DDS. It increases the effectiveness of various medicinal macromolecules by attaching polymer PEG to certain peptide snippets. It alters oligonucleotides, antibody fragments, proteins, and peptides.

4. Depo-Foam technology :-For the controlled release of proteins and peptides, several kinds of liposomal formulations have been produced. With the use of this technique, large amounts of medicine may be loaded with improved recovery and encapsulation effectiveness. For smaller or bigger molecules, injectable medicines are used instead of sustained drug release formulations.

5. Polymeric micelles:- For medications that are amphipathic and less soluble, polymeric micelles serve as colloidal carriers. Compared to micelles, they are more stable and may dissolve amphipathic substances. They are also surfactants. Due to its tiny size and hydrophobic shell, this organism takes a long time to distribute throughout the body.

6. Rod-type covered technique for peptides:- Using silicon that has been transformed into a rod-like structure, a unique approach is needed to release protein medicines. Such a formulation is made in a gentle environment without the use of heat or organic solvents. It makes protein medications easier to administer accurately, increases the drug's effectiveness, and reduces the frequency of dosing and its adverse effects.

7. Microneedles:-Drug administration into the skin using microneedles is a simple approach that increases bioavailability and effectiveness. It might not cause discomfort or illness comparable to old needle stick wounds, allowing the patient to be cured. These microneedles, which are currently used for biosensors, fluid samplers, and micro-analysis, have the capacity to transport proteins and peptides in addition to vaccines, insulin, growth hormones, etc.

X. Marketed formulation of protein and peptide drug delivery system

TABLE I:- MARKETED PREPARATION OF PROTEIN AND PEPTIDE DRUG DELIVERY SYSTEM

XI. CONCLUSION

In the very near future, many currently used organic-based medicines are anticipated to be replaced by protein- and peptide-based medications, which are quickly emerging as a highly important class of therapeutic agents. Biotechnology technologies will be used to make peptide and protein therapeutics on a big scale, and they will then be made commercially available for therapeutic use. The pharmaceutical industry is now faced with the pressing task of creating effective delivery methods for the effective distribution of these complex therapeutics in biologically active form. The research into their practical & efficient distribution through non-invasive method has increased due to their requirement in the clinical & therapeutic fields.

Brand name	Type of preparation	Route of administration	Category
Lupron	Sterilized lyophilized microsphere contains Leuprolide acetate	IM	Prostatic Cancer
Pitressin tannate	Vasopressin tannate in peanut oil	IM	Antidiuretics
H.P. Acther gel	Adrenocorticotropic hormone in gelatine	IM, SC	Endocrine Cancer
Sandostatin LAR Depot	Octreotide	IM	Diarrhoea associated with metastatic carcinoid tumour
Neurotropin	Growth hormone	IM	Drowsiness
Metrodin	FSH 75 IU	IM	Induction of ovulation
Pergonal	FSH AND LH	IM	Infertility
Glucagon	Glucagon	IM, IV, SC	Hypoglycaemia
Elspar	Asparaginase	IM, IV	Leukaemia
Profasi	HCG	IM	infertility

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