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PROTEIN AND PEPTIDE DRUG DELIVERY SYSTEM

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ABSTRACT

Protein and Peptide drug delivery system are the Novel drug Delivery System. Proteins and peptides are the most abundant components of biological cells. They exist functioning such as enzymes, hormones, structural element and immunoglobulin. The twenty different naturally occurring amino acids join with each other by peptide bonds and build polymers referred to peptides and proteins. Although the distinction between peptides and proteins are peptide contains less than 20 amino acids, having a molecular weight less than 5000, while a protein possesses 50 or more amino acids and its molecular weight lies above this value. The most of pharmaceutical proteins and peptides are absorbed IM, IV and Subcutaneous route of Absorption, but the oral route is more convenient for absorption of protein as compared to other. Various problems associated with administration of protein and peptide drugs are needed to overcome by different pharmaceutical approaches. Several approaches available for maximizing pharmacokinetic and pharmacodynamics properties are chemical modification, formulation vehicles, mucoadhesive polymeric system, use of enzyme inhibitors, absorption enhancers, penetration enhancers etc. The Present Review is described Structure, classification of Protein, Need, Advantages, Function of protein and peptide drug delivery system. Route of Absorption, Pharmaceutical approaches, Incorporation of DDS, Stability aspect, Applications, Recent Advances and Marketed formulation of Protein and Peptide drug delivery system.

KEYWORDS: Protein, Peptide, Parenteral, Non-Parenteral, Pharmaceutical approaches, Novel drug Delivery System, immunoglobulin.

INTRODUCTION

The Protein and Peptide is a Novel Drug Delivery System and it is a Novel approach of drug delivery system.[1] Protein and Peptides are the Most Abundant Material of Living system and Biological cell.[2,4] Its act has Hormones, Enzymes, Structural Elements and Immunoglobulin's.[5] It is also important take part in Several Metabolic Process, Immunogenic Defense as well as its take part in several Biological activities.[6] Proteins are the one of the most abundant Organic molecule in Biological System, the term Protein first used has Berzelius.[7, 8, 9] The term Protein is derived from a Greek word Proteios Means Holding the first Place.[10] Proteins are the high molecular weight mixed polymer of Alpha amino acids joined together the Peptide Linkages.[11] In Protein mainly contain Carbon, Nitrogen, Oxygen and Sulphur Molecule.[12] Protein are the compounds having linear chain amino acids are held Together by the Covenant Linkages is called has Peptide Bonds.[13] Peptides are the Condensation Product of Alpha Amino acids.[14, 15, 16] The alpha amino group of one molecule of amino acid are condensed alpha carboxyl group of another amino acids.[17] Protein are occurs in every part of all living cells for giving nutritional activity for providing a body building ability.[18] It is Important Molecule for the Plant and Animal cells. In Protein is mainly act has Enzyme for catalysis of Biochemical reactions, It is applicable for the Transportation of Metabolites and Gene.[19] It is applicable for giving a definite shape, strength to the cell and tissues.[20] It is having a One of the Most Important Applicability to control the Metabolic Pathways, PH, Osmotic Pressure and Temperature.[21] The Protein Insulin Regulates the Blood sugar level.[22] It is Important for the muscle formation and Mechanical work.[23, 24] In case of Peptide the two amino acids are condensed to from dipeptides, three forms Tripeptides, Four to from Tetra peptide and Peptide for the 2-20 amino acids are Polypeptides.[25, 26, 27, 28] The Polymers of 100 and more than 100 Amino acid called has Proteins.[30] The Proteins are classified into two types first is depending on the solubility of proteins and another is complexity in structure of proteins. In fist case on the basis of solubility they are classified into two types Globular Protein and Fibrous Proteins, The proteins are soluble in water or common salts known has Globular proteins and the the proteins are insoluble in Water and common solvents are called has Fibrous Proteins.[31] In second case on the basis complexity Proteins are classified in three types Fist is simple protein it can contains only one amino acids, second is conjugated proteins it can contains amino acids and non protein parts, and third Derived Proteins it is hydrolysis product formed by the action of the physiological agents like heat, chemical agent, and enzymatic actions on the Protein molecules.[32] The structure of Protein is mainly classified into four types First is

Primary Structure of Protein the Primary structure of protein is referred as the number, nature and sequence of amino acids along with polypeptide chain, In this structure the N terminal of amino acids always shown in left end of Polypeptide and C terminal of amino acid shown in right side, The best example of Pri. Structure is an Insulin Molecule.[33] The Secondary structure of protein in which the Long Polypeptide chain are folded or collided in a different Geometric arrangements. The two types of arrangements of secondary structure of Protein Alpha helical Structure and Beta Pleated sheet.[34] In tertiary structure of proteins are the three dimensional coiling and folding of the chain, stabilized by the interaction between the sequences of amino acids, this folding results the (R-) group is side chai amino acids, these interaction are mainly (H-) bonded Interactions.[35] The final shape of the tertiary structure of protein is an elapsed, globe and any other irregular shape. In Quaternary structure of Proteins are the two or more polypeptide chain hold together by non covalent bond to give the quaternary structure of the proteins, Hemoglobin has Example of Quaternary structure of Proteins. Proteins and Peptide are applicable Endogenous functioning to maintain the Biological Environments.[36] The discovery of Numerous Hormones and Peptides are Applicable for the Pharmaceutical and Biopharmaceuticals, It is applicable in Pathophysiology of the Human diseases, The important application in Protein and Peptide in Medical Practices, Drug discovery Processes and Research activities.[37]

NEED OF PROTEIN AND PEPTIDE DRUG DELIVERY SYSTEM

1. The protein and peptides are very important in biological cells and Organic Molecules.[38]
2. In the Absence of proteins and peptides causes diseases like Diabetes mellitus. (Caused due to the lack of protein called INSULIN)[39]
3. Now a days R-DNA technology and hybridoma techniques also used in protein and peptide based pharmaceuticals.[40]

ADVANTAGES OF PROTEIN AND PEPTIDE DRUG DELIVERY SYSTEM

1. Erythropoietin is mainly used for production of RBC.[41]
2. The protein Tissue plasminogen activator is used for Heart attack, Stroke.[42]
3. Oxytocin is used in management of labor pain.[43]
4. Bradykinin increases the peripheral circulation.[44]
5. Somatostatin decrease bleeding in gastric ulcer.[45]
6. Gonadotropin induce ovulation.[46]
7. Insulin maintain blood sugar level.[47]

FUNCTIONS OF PROTEIN AND PEPTIDE DRUG DELIVERY SYSTEM

1. Transport and storage of small molecules and biological molecules.[48]
2. Coordinated motion via muscle contraction.[49]
3. The Mechanical support from fibrous protein.[50]
4. Generation and transmission of nerve impulses.[51]
5. Enzymatic catalysis in biochemical reactions.[52]
6. The Immune protection through antibodies.[53]
7. The Control of growth and differentiation via hormones.[54]

ROUTES OF ABSORPTION

The Proteins and Peptide drug delivery system in which Most of the Pharmaceutical Proteins and Peptides Formulations are the Formulated as a Solution, suspension, Emulsions and they are delivered in Invasive or Parenteral Route such as Intra muscular route (IM), Intravenous route (IV) and Subcutaneous route (SC) Injections. But, These all routes are arises its own Difficulties such as, Poor Patient Compliance, The pain and discomfort associated in this route (to inject injection in same site again and again it can arises Pain) and it is a Inconvenience to treat the Paediatric Patients. The oral route of administration in protein and peptide is suitable as compared to parenteral route, The Oral route having a One of the most convenient route of drug administration, in this type of route no pain and discomfort was arises and Maintained the Higher Patient Compliances or Acceptance. But, The Development oral Protein and Peptide Drug delivery arises several Problems for their Oral Administration of Drugs. This Problem is arises There Unfavourable and Undesirable Physicochemical Properties are such as The Large molecular size of the drug molecules, drug undergoes susceptibility to Biological and Enzymatic degradations, The oral drug having a short Plasma Half Life as compared to other drugs, it can having high Immunogenicity, The tendency of Protein undergoes Aggregations, Adsorption and it can undergoes Denaturation's, The Major Problem Orally Administered Proteins and Peptides are having a Lesser Bioavailability or Less Bioavailability is having a less than 1%. The other route of administration of protein and peptide is arises success for the administration of Proteins and Peptide drugs, the routes are Oral, Buccal administration, Intranasal administration, Pulmonary administrations, Transdermal, Rectal and Ocular administrations of Proteins and Peptide.[55, 56]

PROPERTIES OF PROTEINS AND PEPTIDES

The Protein are the most abundant biological and organic molecule they are soluble in water and it can formed a Colloidal solution with water. Protein and Peptides are the

physicochemically and Metabolically Stable System. In case oral administration of Protein and Peptide Drug delivery system Several Properties can affect the rate of absorption of Protein and Peptide in oral drug delivery system, the properties are such as, Absorption Properties, In case of Absorption Properties Molecular weight and size of the particle, Conformational studies and Steriospecification of Three Dimensional Arrangements in Space, Immunogenicity of drug molecules. Are affected the rate of Absorption of Protein and Peptide in Oral drug delivery systems. Another one is Physicochemical Properties such as, solubility and Lipophilicity of drug is major Criteria of absorption of drug, The aggregations and Hydrogen bonding of drug in oral administrations, The Physicochemical Properties are the major Criteria for the drug absorption in oral drug delivery systems, The drug absorption oral drug delivery system it an mainly arises two main Problems are the Metabolic degradation of Various forms of Protein and Peptides by interaction with the various Proteolytic Enzymes, and it is having Less Membrane Penetration Abilities. This all Criteria associated in Properties of Protein and Peptide drug delivery system is Applicable for determination of various Problem associated in oral drug delivery system and it is important to give idea on the basis Properties to prevent the problems in drug administration in oral Protein and Peptide in Oral drug delivery Systems.[57, 58, 59]

PHARMACEUTICAL APPROACHES

The protein and Peptides are having Four Approaches they has Follows

1. **CHEMICAL MODIFICATION**
2. **ENZYME INHIBITORS**
3. **PENETRATION ENHANCERS**
4. **FORMULATION VEHICLE**
5. **MUCOADHESIVE POLYMERIC SYSTEM**

1. CHEMICAL MODIFICATION (PRODRUG APPROACH) [60, 61, 62]

The Chemical Modification of Protein and Peptide Drug Delivery System of Drugs is Important to Improve the Enzymatic Stability as well as Membrane Permeations. It is Applicable for the reducing the Immunogenicity.

The Chemical Modification is Includes in Two Types of Modifications as Follows:

1. **Amino acid Modification**
2. **Hydrophobization**

1. Amino acid Modifications: The Modification of amino acid is one of the important approach in which the Substitution of the D- amino acid and L- amino acid is important to alter the Physiological Properties of Protein and Peptide Drug Delivery Systems.

Example: Desmopressin and Deaminovasopressin are the two important analogs of vasopressin, former involves deamination of first amino acid and replacement of last L-arginine D-arginine to give Deaminovasopressin.

Application: The Amino acid modification is important to enhance the Membrane Permeability and Maintain the Enzymatic Stability.

2. Hydrophobization: It is having an important approach for the Lipophilic Moieties.

Example: NOBEX INSULIN by the Palmitoylatios.

Description of Example: Conjugation of the Insulin Molecule to the 1, 3-dipalmitoylglycerol containing a free amino acid groups of glycine, Phenylalanine and Lysine molecule to from mono and insulin is important to facillated the transfer the insulin across the mucosal membrane of the large intestines. It is important to improve the Stability against the enzymatic degradations.

ENZYME INHIBITORS (PROTEASE)[63]

The enzyme (protease) inhibitors are the enzymatic approach of the Protein and Peptide drug delivery systems. GIT and Liver is play important role in Metabolization of the Protein and Peptides into smaller fragments of the two to ten amino acids with the help of the variety of Proteolytic Enzymes. This Protease inhibitors are CO- administered with Protein and Peptide to alter the Environment for the Enzyme stability to supress the Proteolytic activity. The enzyme proteases inhibiters are divided into four types they are Aspartic Proteases (Pepsin, Rennin), Cystinyl Proteases (Papain, Endopeptidase), Serinyl Proteases (Thrombin, Trypsin), and Metallo Proteases (Carboxypeptidase).

PENETRATION ENHANCERS[64]

Penetration enhancers are the one of the most important Component of Protein and Peptides formulation is responsible for the Disruption of the Mucosal Barriers and applicable to improve the Membrane Permeations of Large Macromolecular substances lie Proteins and Peptides. The Several classes of compounds are mainly used has a permeation enhancers are

such as Surfactant (Polysorbate, SLS, Pluronic F-68), Chelating agent (EDTA), Fatty acids (Sodium Carprate), Mucoadhesive Polymeric systems (Thiomers, Cellulose derivatives), Phospholipids (PC). The basic Mechanism of Penetration enhancers are the, detergent and surfactant molecules are the increases the transcellular transport of the drug material is responsible to disrupting the structure of the lipid bilayer of lipid membrane are having more permeability. Another mechanism is the calcium chelates are the responsible for the Exert the action of complex formation of the calcium ions and they are passing through the tight junctions and they are facillated the Paracellular transport of the hydrophilic drugs materials. Fatty acids are the important for the improving the paracellular absorption by phospholipases C activations and upregulation of intracellular Calcium ions, is leading to the contraction of actine myosin filaments.

FORMULATION VEHICLES[65, 66, 67, 68]

The Protein and Peptide Drug Delivery system is important for the Oral Delivery of Protein and Peptides can be successfully achieved by using various carrier systems are like

1. **Dry Emulsion**
2. **Microspheres**
3. **Liposomes**
4. **Nanoparticles**

1. Dry Emulsion: It is important application in drug delivery system s to prevent the instabilities of the long term storage of multiple emulsions. The novel approach at which multiple emulsion is replaced by dry emulsions. Dry Emulsion is prepared by the Spray drying, Lyophollization and evaporation Techniques. In dry emulsion preparation application of the PH responsive polymers like HPMCP, is important for the emulsions are the enteric coated and site specific achieved.

2. Microspheres: The uniform distribution of drug in oral drug delivery in Protein peptides drug are known as Microspheres. The PH responsive microspheres are the mainly used in oral delivery for the protection of the stomach from proteolytic degradations and Protection upper portion of small intestine from proteolytic degradations.

3. Liposomes: Liposomes are the small microscopic vesicles in which aqueous volume is entirely enclosed by the membrane composed lipid molecules. Liposomes in drug delivery system, the encapsulation of the insulin with sugar chain portion of mucin and PEG

completely suppressed the degradation of the insulin molecules in intestinal fluid. The uncoated from of liposomes are suppressed it on partially surface coating of the liposomes molecules in PEG or mucin gained resistances against dagestion by salts and increased the stability of GI tract.

4. Nanoparticles: Nanoparticles are Nano sized colloidal structure having size is 10-1000nm. The particles in nanometric sized range of the particles are absorbed intact by the intestinal epithelium and they are the less prone towards the enzymatic degradations. The particle size surface charges are the influencing the uptake of nanoparticle system in GI tract.

MUCOADHESIVE POLYMERIC SYSTEMS [69]

The mucoadhesive polymeric system is important to prevent the problem associated in Presystemic Metabolism or first pass metabolism and maintain its therapeutic efficacy. The residence time of this drug delivery systems at the site of action and the increasing or decreasing the drug clearance rate.

Examples: Thiomers, polyacrylic acid derivatives and cellulose derivatives. The stronger mucoadhesive properties of thiomers are believed to be based on covalent bonds between thiol groups of the thiomer and cystein- rich domains of mucus glycoproteins. (Higher amount of thiol groups is responsible for the stronger mucoadhesive properties).

INCORPORATION INTO DRUG DELIVERY MATRIX [70, 71, 72, 73, 74, 75]

The drug incorporate in the Protein and Peptide drug delivery system undergoes Three methods they as follows

1. **EMULSIFICATION**
2. **EXTRUSION AND SPRAY DRYING**
3. **POLYMERIZATION**

1. EMULSIFICATION

In this Process water soluble drugs is first dissolved in the aqueous (water solution) and it is soluble in Organic solvent. The two solutions are mixed with the appropriate Proportion to produce w/o emulsion. This prepared Primary emulsion is emulsified into aqueous solution containing emulsifier to produced w/o/w emulsion. Finally the organic solvent is mainly removed from emulsion by evaporation of solvent under reduced pressure by the filtration and increasing the Temperature.

2. EXTRUSION AND SPRAY DRYING

The extrusion and Spraying is employed to from microspheres and the core material or matrix containing drug, incorporated as Solution and the Particulate is mainly ejected from the orifice of fine tubes, syringe or nozzles to from micro droplets. The size of droplet is mainly depends upon the Properties of Liquid (melt, solution and suspension) and Orifice diameter to jet velocity.

3. POLYMERIZATION

Polymeriasation in hydrogels having a polymeric drug delivery system preparation by the mixing of monomer with the drug an initiator and a cross linking agents. The Intravascular delivery of the protein via hydro system that is photo polymerized in situ on the inner surface of blood vessel. The ♈-radiation are producing deleterious effect on integrity of protein molecules one of the drawback of Protein and Peptide drug delivery systems.

STABILITY ASPECTS [76, 77, 78]

In stability of protein and peptide is determined by the Protein degradations Pathways

In This drug delivery system under two Pathways of degradation of Protein and Peptide Molecules They has Follows

1. **Physical Degradation Pathways (Instability Aspects)**
2. **Chemical Degradation Pathways (Instability Aspects)**

The chemical degradation Pathways the Native or original structure of protein is changes by the modification of their Primary Structure of Protein Molecules.

The Physical Degradation Pathways the Native or original structure of Protein is Changes or Modified to from Higher order Structure of Proteins (secondary, tertiary or quaternary structure).

1. Physical Instabilities: In case of Physical Degradation the Primary Sign of Physical instability of the Protein Molecules. In case of globular Protein, the hydrophobic residue are buried in the interior and the hydrophilic residues. It is having interaction with the aq. Solvents. The denaturation of Protein Molecule refers to the loss or damage of the globular structure of protein molecule leads to protein unfolding. The physical denaturation is may be caused the changes in the environment of protein molecules such as temperature, pH, introduction of hydrophobic surfaces or by introduction of interfaces by the addition of organic solvents.

2. Chemical Instabilities: The chemical instability of the protein and Peptide can causes following four types of reactions.

1. **Oxidation**
2. **Deamination**
3. **Peptide bond hydrolysis**
4. **Disulphide exchange**

1. Oxidation

Oxidation is one of the most important chemical instability of Protein and peptide molecules. The Amino acid side chain of the protein and peptide are susceptible to oxidation, the oxidation is arises by the atmospheric oxygen molecule, various types of the metal ions like copper or iron, several reagents such as Hydrogen Peroxides.

Example: The Methionine residues under acidic conditions are especially prone to oxidation by reagents like hydrogen peroxide, producing methionine sulfoxide. (Hydrogen peroxide is used to sterilize formulation vessels or the formulation area).

2. Deamination: In this type of Instability is arises in hydrolysis of amide side chain of certain amino acid residue are mainly includes Glutamine and Aspargine, is known has Deamination. Some conditions are like changes in Temperature and PH are mainly shown to Facillated the Process of Deamination's of Biological Therapeutic Protein and Peptides.

3. Peptide bond Hydrolysis: In this Peptide bond Hydrolysis Process the aspartic acid residues are heated at 90-100^0 C, in PH 4 (acetate), the hydrolysis of the Asp-X bonds are leads to loss of the Biological activity.

4. Disulphide exchange: The Therapeutics Protein contain cystein residues that from disulfide bonds. These formed bonds are important components of the structural integrity of the Proteins. The incorrect linkage of peptide bonds lead to changes in the three dimensional structure of Protein Molecules and their biological activity.

APPLICATION

1. **CVS acing drugs Protein and Peptides** (Angiotensin 2 antagonist, Bradykinin, Captopril) is important for the Lowering blood pressure and improving peripheral circulation for Heart failure management.[79]

2. **CNS active Protein and Peptides** (Cholecystokinin, B-endorphin) is important for the Suppressing appetite and Relieving pain.[80]

3. **GI-active Protein and Peptides** (Gastrin antagonist, pancreatic enzymes) is important for the Reducing secretion of gastric acid and it is important for Digestive supplement.[81]

4. **Immunomodulation of the Protein and Peptides** (Bursin, Cyclosporin, and Interferon) is important for Selective B-cell differentiatingharmone Inhibits functions of T-lymphocyte Enhancing activity of killer cells.[82]

5. **Metabolism modulating Protein and Peptides** (Insulin, Vasopressin) is important for treating diabetes mellitus and treating diabetes insipidus.[83]

RECENT ADVANCES [84, 85, 86, 87, 88]

PEGylation

PEGylation is a Recent Advancement of Protein and Peptide Drug Delivery systems, PEGylation is a process of attaching the strands of the polymer PEG to most typical peptides fragments that can help to meet the protein and challenges of improving the safety and efficiency of many therapeutic macromolecules such as Protein and Peptides. It is widely used for the modification of proteins and peptides, antibody fragments and oligonucleotides. PEG are the Non-toxic. And non –immunogenic, it is having a specified Hydrophilicity and it is having high Flexibility. PEGylation is important to increases the Bioavailability, it is applicable for the optimized Pharmacokinetics, it is important for Decreasing Immunogenicity, It is important to Decreases the Frequency of administration. The PEGylation is important Mechanism for increasing the molecular weight of the molecules, it can increases the drug solubility and it is applicable for the protection against Proteolytic degradations, it is having an important mechanism to reducing the dosing frequency and maintain therapeutic activity.

MATKETED PREPARATIONS AND APPLICATIONS OF PEGylation

PEGylated interferon alpha -2a:	**In Hepatitis B Treatment.**
PEGylated interferon alpha-2b:	**In Hepatitis C Treatment.**
PEGylated liposome containing doxorubicin:	**In Cancer Treatment.**

Depo-Foam TECHNOLOGY [89, 90]

Therapeutic Proteins and Peptides are administered in IV or SC are often too rapid fro of the Circulation and it is need to inject to the frequent order of administration for maintaining their therapeutic level of the blood. Various types of liposomal formulations have been utilized as drug delivery vehicles for sustained release of proteins and peptides like unilamellar or Multilamellar vesicle systems but few deals with the mulltivesicular liposomes are called as "DepoFoam particles." The DepoFoam technology is capable of accommodating high drug loading and high recovery of drug material, it is having a high Encapsulation efficiency, it is important type of technique is applicable for the sustained delivery of macromolecular drugs. A unique feature of DepoFoam system is that inside each DepoFoam particle, discontinuous internal aqueous chambers ,bounded by a continuous network of lipid membranes render a higher aqueous volume to lipid ratio and much larger particle diameter as compared to SUV's or MLV's.

MARKETED FORMULATIONS [91, 92, 93, 94, 95, 96]

Product	Formulation	Route	Indication
Metrodin	FSH 75 IU	i.m.	Induction of ovulation
Pergonal	FSH and LH	i.m.	infertility
Profasi	HCG	i.m.	Infertility
Elspar	Asparginase	i.m. i.v.	Leukemia
Glucagon	Glucagon	i.m. i.v. s.c.	Hypoglycemia
Acthar	Corticotropin	i.m. i.v. s.c.	Hormone Deficiency

CONCLUSION

Protein and peptide based pharmaceuticals are rapidly becoming a very important class of therapeutic agents and are likely to replace many existing organic based pharmaceuticals in the very near future. Peptide and protein drugs will be produced on a large scale by biotechnology processes and will become commercially available for therapeutic use. This poses an urgent challenge to the pharmaceutical industry to develop viable delivery systems for the efficient delivery of these complex therapeutic in biologically active form. Their need in the clinical & therapeutic regions has intensified the investigation for their convenient & effective delivery through noninvasive system.

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REFERENCE

1. Nelson DL, Cox MM., Lehninger Principles of Biochemistry, 4th Ed., W.H. Freeman and Company, New York, 2005; 85-86.
2. Satyanarayan U, Chakrapani U, Biochemistry, 3rd Ed., Books and allied (p) Ltd., Kolkata, 2008; 43-44.
3. Smith EL, Hill RL, Lehman IR, Lefkowitz RJ, Handler P, White A, Principles of biochemistry: General aspects, 7th Ed., McGraw-Hill, New York, 1983.
4. Bummer PM, Koppenol S, Chemical and physical considerations in protein and peptide stability; In: Protein Formulation and Delivery, Drugs and the Pharmaceutical Sciences, McNally EJ, Marcel Dekker, New York, 2000; 15-18.
5. Langer R, Folkman J, Sustained release of macromolecules from polymers, Poly. Del. Systems, Midland Macro. Monograph, 1978; 5: 175-196.
6. Bergh VD, Gregoriadis G, Water-in-sorbitan monostearate organogels (water-in-oil gels), J Pharm Sci., 1999; 88: 615-619.
7. Murdan S, Gregoriadis G, Florence AT, Sorbitan monostearate/polysorbate20 organogels containing neosomes: a delivery vehicle for antigens, Euro J of Pharm Sci, 1999; 8: 177-186.
8. Sawhney AS, Pathak CP, Hubell JA, Bioerodible hydrogels based on photopolymeerized poly(ethyleneglycol)-copoly(alphahydroxy acid) diacrylate macromers, Macromolecules, 1993; 26(4): 581-587.
9. West JL, Hubell JA, Localized intravascular protein delivery from photopolymerized hydrogels, Proc Int Symp Control Rel Bioact Mater, 1995; 22: 17-18.
10. Vyas S.P. and Khar K.R., Targeted and controlled drug delivery Novel carrier system, CBS publishers and distributors, New Delhi. 505,507,511,537.
11. Banga A.K. and Chein Y.W, Systemic delivery of therapeutic peptides and proteins, Int. J. Pharmaceutics, 1988; 48: 15-50
12. Banerjee P. S. and Ritschel W. A., Int. J. Pharm. 1989; 49: 189-197.
13. Chein Y. W., Lelawongs P., Siddiqui O., Sun. Y. and W. M. Shi. W. M; Faciliated trandermal delivery of therapeutic peptides/proteins by iontophoretic delivery devices. J. Control. Rel., 1990; 13: 263-278.

14. Siddiqui O., Sun Y., Liu J. C. and Chein Y. W., Faciliated transdermal transport of insulin. J. Pharm. Sci., 1987; 76: 341- 345.
15. Sibalis D., Transdermal drug applicator. U. S. Patent, 1987; 4: 708-716.
16. Meyer B. R., Electro-osmotic transdermal drug delivery, in: 1987 Conference Proceedings on the Latest Developments in Drug Delivery Systems, Aster Publishing, Eugene, Oregon, (1987), 40.
17. Meyer et al. Transdermal delivery of human insulin to albino rabbits using electrical current. Am. J. Med. Sci., 1989; 297: 321-325.
18. Okabe K., Yamaguchi H. and Kawai Y., New iontophoretic transdermal administration of the beta blocker metaprolol. J. Control. Rel., 1986; 4: 79-85.
19. Chein Y. W., Siddiqui O. and Liu J. C., Transdermal iontophoretic delivery of therapeutic peptides/proteins. I. Insulin. Ann. N. Y. Acad. Sci., 1988; 507: 32-51.
20. Tahami. Alkhaled and Singh J., Recent patent on drug delivery and formulation, 2007; 1: 65-71.
21. Vyas S.P. and Khar K.R., Targeted and controlled drug delivery, Novel carrier system, CBS publishers and distributors, New Delhi.561.
22. Chein Y.W., Novel drug delivery systems, volume 50, second edition, 715.
23. Pekar A. H. and Frank B. H., Conformation of proinsulin. A comparison of insulin and proinsulin self-association at neutral pH. Biochemistry, 1972; 11: 4013-4016.
24. Banga AK etal; Hydrogel-based iontotherapeutic delivery devices for transdermal delivery of peptides-protein drugs. Pharm Res 1993; 10: 697-702.
25. Lee Ycetal;. Effect of formulation on the systemic absorption of Insulin from enhancer free ocular devices. Int J Pharm 1999; 185: 199-204.
26. Burgess DJ etal; editors. Biotechnology and Pharmacy. New York: Chapman and Hall; 1993; 116-51.
27. Aurora Jetal; delivery of protein and peptide –challenges and opportunities. Business Briefing: Future dry discovery, 2006; 38-40.
28. John M.etal; Shanafelt.Enhancing exposure of protein therapeutics. Drug Discovery today: Technologies 2006; 3: 87-94.
29. Yanagi H et al. Effect of inclusion complexation of decanoic acidwith _-cyclodextrin on rectal absorption of cefmetazole sodium suppository in rabbits. Yakugaku Zasshi. 1991; 111: 65-69.

30. Lin SY and Yang JC, Effect of _-cyclodextrin on the in vitro permeation rate an d in vivo rectal absorption of acetaminophen hydrogel preparations. Pharm. Acta Helv., 1990; 65: 262-268.
31. Arima H et al. Use of water soluble _-cyclodextrin derivatives as carriers of anti inflammatory drug bi phenylyl acetic acid in rectal delivery. Yakugaku Zasshi. 1992; 112: 65-72.
32. Brouard A et al. Rectal administration of carbamazepine gel. Clin. Pharm. 1990; 9: 13–14.
33. Levy R et al. Metabolism of Antiepileptic Drugs. Raven Press, New York. 1984; 61–71.
34. Graves NM et al. Relative bioavailability of rectally administered carbamazepine suspension in humans. Epilepsia., 1985; 26: 429–433.
35. Lambroso CT. Intermittent home treatment of status and clusters of seizures. Epilepsia., 1989; 30: S11–S14.
36. Moolenaar F et al. Biopharmaceutics of rectal administration of drugs in man. IX Comparative biopharmaceutics of diazepam after single rectal, oral, intramuscular and intravenous administration in man. Int. J. Pharm. 1980; 5: 127–137.
37. Gail D et al. Current oral and non-oral routes of antiepileptic drug delivery. Advanced Drug Delivery Reviews. 2012; 64: 911-918.
38. Maloney CM et al. The rectal administration of MS contin: clinical implications of use in end stage therapy cancer. Am. J. Hosp Care. 1989; 6(4): 34-35.
39. Batul N et al. Pharmacokinetics of two novel rectal controlled release morphine formulations. J. Pain Symptom Manage. 1992; 7(7): 400-405.
40. Warren DE. Practical use of rectal medications in palliative care. J. Pain Symptom Manage. 1996; 11(6): 378-387.
41. Sarwar, G. The protein digestibility-corrected amino acid score method overestimates quality of proteins containing antinutritional factors and of poorly digestible proteins supplemented with limiting amino acids in rats. Journal of Nutrition 1997; 127: 758-764.
42. Schaafsma, G. The protein digestibility-corrected amino acid score. Journal of Nutrition, 2000; 130: 1865S-1867S.
43. Sellmeyer, D.E., Stone, K.L., Sebastian, A. and Cummings, S.R. A high ratio of dietary animal to vegetable protein increases the rate of bone loss and risk of fracture in postmenopausal women. American Journal of Clinical Nutrition 2001; 73: 118-122.
44. St. Jeor, S.T., Howard, B.V., Prewitt, E., Bovee, V., Bazzarre, T. and Eckel, R.H. A statement for healthcare professionals from the nutrition committee of the council on

nutrition, physical activity, and metabolism of the American Heart Association. Circulation 2001; 104: 1869-1874.

45. Tarnopolsky, M.A., Atkinson, S.A., MacDougall, J.D., Chesley, A., Phillips, S.M. and Schwarcz, H. Evaluation of protein requirements for trained strength athletes. Journal of Applied Physiology 1992; 73: 1986-1995.
46. Tarnolpolsky, M.A., MacDougall, J.D. and Atkinson, S.A. Influence of protein intake and training status on nitrogen balance and lean body mass. Journal of Applied Physiology 1988; 64: 187-193.
47. Tikkanen, M.J., Wahala, K., Ojala, S., Vihma, V., and Adlecrerutz, H. Effect of soybean phytoestrogen intake on low density lipoprotein oxidation resistance. Proclamations of the National Academy of Science 1998; 95: P3106-P3110.
48. United States Dairy Export Council (1999) Reference Manual for U.S. Whey Products 2nd Edition.
49. Walberg, J.L., Leidy, M.K., Sturgill, D.J., Hinkle, D.E., Ritchey, S.J. and Sebolt, D.R. Macronutrient content of hypoenergy diet affects nitrogen retention and muscle function in weight lifters. International Journal of Sports Medicine 1988; 9: 261-266.
50. Zieve, D. (2009, May 2). In Protein in diet: MedlinePlus Medical Encyclopedia. Retrieved June 1, 2010, from http://www.nlm.nih.gov/ medlineplus/ency/article/002467.htm
51. Centers for Disease Control and Prevention, (2009, Nov. 9). In Nutrition for Everyone: Basics: Protein. Retrieved June 1, 2010, from http://www.cdc.gov/nutrition/everyone/basics/protein.html
52. Osterweil, N. (2004). In The Benefits of Protein. Retrieved June 1, 2010, from http://www.webmd.com/fitness-exercise/guide/benefitsprotein
53. Narashimhan B, Mallapragada SK and Peppas NA. Release kinetics, data interpretation. In: Encyclopedia of Controlled Drug Delivery. John Wiley and Sons, Inc, 1999; 921–935.
54. Higuchi T. Mechanism of sustained-action medication. J Pharm Sci 1963; 52: 1145–1149.
55. Paul DR and McSpadden SK. Diffusional release of a solute from a polymer matrix. J Membrane Sci 1976; 1: 33–48.
56. Peppas NA and Franson NM. The swelling interface number as a criterion for prediction of diffusional solute release mechanisms in swellable polymers. J Polymer Sci 1983; 21: 983–997.

57. Kwok WY, Kiparisider C, Yuet P, Harris TJ and Goosen MFA. Mathematical modelling of protein diffusion in microcapsules: a comparison with experimental results. Can J Chem Eng 1990; 68.
58. Carslaw HS and Jaeger JC. Conduction of Heat in Solids. Oxford: Clarendon Press, 1959.
59. Okhamafe AO and Goosen MFA. Modulation of membrane permeability. In: Kuhtrelber WM, Lanza RP and Chick WL(Eds.). Cell Encapsulation Technology and Therapeutics. Birkhäuser Boston Inc, 1999; 53–62.
60. Okhamafe AO, Amsden B, Chu W and Goosen MFA. Madulation of protein release from chitosan-alginate microcapsules using the pH-sensitive polymer hydroxypropyl methylcellulose acetate succinate. J Microencapsul 1996; 13: 497–508.
61. C. O. Tacket, M. B. Sztein, S. S. Wasserman, G. Loson-sky and K. L. Kotloff, “Phase 2 Clinical Trial of Attenu-ated Salmonella Enterica Serovar Typhi Oral Live Vector Vaccine CVD 908-htrA in U.S. Volunteers,” Infection and Immunity, 2000; 68(3): 1196-1201. doi:10.1128/IAI.68.3.1196-1201.2000
62. G. P. Li, Z. G. Liu, B. Liao and N. S. Zhong, “Induction of Th1-Type Immune Response by Chitosan Nanoparti-cles Containing Plasmid DNA Encoding House Dust Mite Allergen Der p 2 for Oral Vaccination in Mice,” Cellular & Molecular Immunology, 2009; 6(1): 45-50. doi:10.1038/cmi.2009.6
63. I. S. Kim, S. K. Lee, Y. M. Park, Y. B. Lee and S. C. Shin, “Physicochemical Characterization of Poly(L-lactic acid) and Poly(D,L-lactide-co-glycolide) Nanoparticles with Polyethylenimine as Gene Delivery Carrier,” Interna-tional Journal of Pharmaceutics, 2005; 298(1): 255-262. doi:10.1016/j.ijpharm.2005.04.017
64. ANGELINI, “EPAXAL®—Vaccine for Active Immuni- sation against Hepatitis A,” 2011. http://www.angelini.it/public/schedepharma/epaxal.htm
65. PEVION, “Virosomes Are the Only VLP Assembled in Vitro, Not by Host Cell,” http://www.pevion.com/index.php?page=723
66. M. R. Kumar, U. Bakowsky and C. M. Lehr, “Preparation and Characterization of Cationic PLGA Nanospheres as DNA Carriers,” Biomaterials, 2004; 25(10): 1771-1777. doi:10.1016/j.biomaterials.2003.08.069
67. Y. Yue, F. Jin, R. Deng, J. Cai and Z. Dai, “Revisit Com-plexation between DNA and Polyethylenimine—Effect of Length of Free Polycationic Chains on Gene Transfec-tion,” Journal of Controlled Release, 2011; 152(1): 143-151. doi:10.1016/j.jconrel.2011.03.020.

68. J. L. Italia, A. Sharp, K. C. Carter, P. Warn and M. N. V. R. Kumar, "Peroral Amphotericin B Polymer Nanoparti-cles Lead to Comparable or Superior in Vivo Antifungal Activity to That of Intravenous Ambisome® or Fungizone™," PLoS One, 2011; 6(10): 8. doi:10.1371/journal.pone.0025744
69. R. Rupp, S. L. Rosenthal and L. R. Stanberry, "VivaGel (SPL7013 Gel): A Candidate Dendrimer—Microbicide for the Prevention of HIV and HSV Infection," Interna-tional Journal of Nanomedicine, 2007; 2(4): 561-566.
70. C. S. Maia, W. Mehnert and M. Schäfer-Korting, "Solid lipid Nanoparticles as Drug Carriers for Topical Gluco-corticoids," International Journal of Pharmaceutics, Vol. 196, No. 2, 2000, pp. 165-167. doi:10.1016/S0378-5173(99)00413-5
71. H. Chen, X. Chang, D. Du, W. Liu and J. Liu, "Podo-phyllotoxin-Loaded Solid Lipid Nanoparticles for Epi-dermal Targeting," Journal of Controlled Release, Vol. 110, No. 2, 2006, pp. 296-306. doi:10.1016/j.jconrel.2005.09.052
72. M. Rother, E. J. Seidel, P. M. Clarkson, S. Mazgareanu and U. Vierl, "Efficacy of Epicutaneous Diractin (keto-profen in Transfersome gel) for the Treatment of Pain Related to Eccentric Muscle Contractions," Journal of Drug Design, Development and Therapy, 2009; 3: 143-149.
73. A. Rolland, N. Wagner, A. Chatelus, B. Shroot and H. Schaefer, "Site-Specific Drug Delivery to Pilosebaceous Structures Using Polymeric Microspheres," Pharmaceu-tical Research, 1993; 10(12): 1738-1744. doi:10.1023/A:1018922114398
74. B. Mahe, A. Vogt, C. Liard, D. Duffy and V. Abadie, "Nanoparticle-Based Targeting of Vaccine Compounds to Skin Antigen-Presenting Cells by Hair Follicles and Their Transport in Mice," Journal of Investigative Dermatology, 2009; 129(5): 1156-1164. doi:10.1038/jid.2008.356
75. A. Vogt, B. Combadiere, S. Hadam, K. M. Stieler and J. Lademann, "40 nm, but not 750 or 1500 nm, Nanoparti-cles Enter Epidermal CD1a+ Cells after Transcutaneous Application on Human Skin," Journal of Investigative Dermatology, 2006; 126(6): 1316-1322. doi:10.1038/sj.jid.5700226
76. F. F. Larese, F. D'Agostin, M. Crosera, G. Adami and N. Renzi, "Human Skin Penetration of Silver Nanoparticles through Intact and Damaged Skin," Toxicology, 2009; 255(1-2): 33-37. doi:10.1016/j.tox.2008.09.025
77. H. Maeda, J. Wu, T. Sawa, Y. Matsumura and K. Hori, "Tumor Vascular Permeability and the EPR Effect in Macromolecular Therapeutics: A Review," Journal of Controlled Release, 2000; 65(1-2): 271-284. doi:10.1016/S0168-3659(99)00248-5

78. H. Sarin, "Recent Progress towards Development of Ef-fective Systemic Chemotherapy for the Treatment of Ma-lignant Brain Tumors," Journal of Translational Medi-cine, 2009; 7: 77. doi:10.1186/1479-5876-7-77
79. National Diabetes Data Group, Diabetes in America: Diabetes Data Compiled 1984. Bethesda Md. NIH. 85, 1985; 146X.
80. Calceti, P. et al. Development and in vivo evaluation of an oral insulin-PEG delivery system. Eur. J. Pharm. Sci., 2004; 22: 315–323
81. Basu, A. et al. Structure-function engineering of interferon-beta-1b for improving stability, solubility, potency, immunogenicity, and pharmacokinetic properties by site-selective mono-PEGylation. Bioconjugate Chem. 2006; 17: 618–630
82. Wang, J. et al. Reversible lipidization for the oral delivery of salmon calcitonin. J. Control. Release, 2003; 26: 369–380
83. Kipnes, M. et al. Control of postprandial plasma glucose by an oral insulin product (HIM2) in patients with type 2 diabetes. Diabetes Care, 2003; 26: 421–426
84. Engerman R, Bloodworth JM, Nelson S. Diabetes 1977; 26: 760-769.
85. Engerman R, Kern TS. Diabetes 1987; 36: 8088812.
86. Wildmg R, Coupe AJ, Davis SS. Adv Drug Deliv Rev 1991; 7: 87-117.
87. Atchison JA, Grizzle WE, Pillion DJ. J Pharmacol Exp Ther 1989; 248: 567-572.
88. Manosroi KH Batter. Drug Dev Ind Pharm 1990; I6: 1521-1538.
89. Fukunaga M, Miller MM, Hostetler KY, Deftos LJ. Endocrinology I IS 1984; 757-61.
90. Jennifer's. Toxicology letter as 2001; 120: 59-66.
91. Celies WE et al. Drug discovery today 2007; 12: 674-81.
92. BilselL P et al. J Immunology 2005; 174: 3187-96.
93. Cindy H Dubin. Drug delivery technology march 2009; 8(3).
94. Marchant RE, Miller KM, Anderson JM. J Biomed Mater Res 1984; 18: 1169–1174.
95. Sewell WR, Wiland J, Craver BN. Surg Gynecol Obstet 1955; 100: 483–494.
96. Gourlay SJ, Rice RM, Hegyeli AF, Wade CWR, Dillon JG, Jaffe H, Kulkarni RK. J Biomed Mater Res 1978; 12: 219–232.