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REVIEW ARTICLE

Nanoencapsulation system for delivery of protein and peptide-A review

Omdip R. Sohani*, Umesh B. Gaikwad, Pravin D. Chaudhari.

Progressive Education Society's, Modern College of Pharmacy, Nigdi, Pune-411 044, Maharashtra, India.

ABSTRACT

Various nanopaticulate systems are used for the nanoencapsulation of proteins and peptides for improving peptide drug accumulation inside the target site. Due to less stability of proteins; they get readily degraded there fore encapsulation of protein drugs provide sustained release and protect the non-released protein from degradation. Various natural as well as synthetic nanocarriers are used for encapsulation of proteins and peptides. Also there are different methods used for preparation of nanoencapsulant's but the choice of method for peptide encapsulation is entirely based on the physicochemical activity of protein and its application.

KEYWORDS: Nanoencapsulation, Nanocarriers, proteins and peptides.

INTRODUCTION[1,2]:

Proteins and other biomolecules such as antibodies, antigens, growth factors, and bioactive peptides are well known for their therapeutic application in various diseases. These therapeutic proteins and peptides are potential target for development of therapeutic nanoprotein and nanopeptides because of their nanoscale dimension, highly specific therapeutic activity, and numerous other specific enzymatic activities. A variety of nanoparticulate systems like liposomes and solid lipid nanoparticles, polymeric microspheres/nanoparticles, etc are being used for the nanoencapsulation of protein and peptides to improve protein drug accumulation inside target cells due to easy and efficient cellular internalization.

The protein and peptide nanoparticles are developed by adsorption on the nanocarrier surfaces, encapsulation in nanoparticles, bioconjugation on nanoparticles and by molecular self-assembly of small peptides into nanoparticles size. This encapsulated protein on nanoparticles has great potential to enhance therapeutic activity of peptides by sustained and targeted delivery to the active site, improve stability, better bioavailability.

As compare to conventional therapeutic small molecules, most of the therapeutic proteins and peptides are quite unstable, prone to denaturation and loss of activity. The therapeutic activities of peptides are lost due to aggregation, degradation and unfolding. Therapeutic peptides have short half-life due to proteolysis, rapid clearance from the blood stream and hence required repeated administration. Encapsulation of protein drugs on nanoparticles provides sustained release and protects the non-released protein from degradation. The retention of activity, structural identity, and stability of protein and peptides after encapsulation on suitable nanodevices are basic concern in development of protein and peptides nanomedicine. Nano carriers are one of the useful tools for achieving the main objective of protein therapeutics and its targeted delivery. Nanoencapsulation of protein to develop nano medicines require the complete information about the changes in cell receptors that occur with progression of disease, mechanism and site of action, retention of drugs, multiple administration, molecular mechanisms, stability and therapeutic activity of protein *in vivo* and pathophysiology of the disease.

NANOENCAPSULATION: IMPROVES THE THERAPEUTIC IMPORTANCE OF PEPTIDE DRUG:

The aim of the protein and peptide drug delivery systems is the interaction of protein and peptide drug into target cells. The desired feature of these efficient drug delivery systems is to deliver therapeutic peptides to active site at the right time in a therapeutically effective concentration and at highest patient compliance, with minimal side effects and low production cost.

Various proteases enzymes present at potential site of administration may inactivate or degrade these proteins and peptides. The elimination of these peptide drugs by body defense system is another major reason for less utilization of protein and peptides. These problems create the decrease in bioavailability and increases potential immune response against the protein and peptide drug which are administered. The alternative route of administration of the peptides therapeutics like nasal, transdermal, and pulmonary may be beneficial than conventional route, towards the reduction of above

*Corresponding author: Omdip R Sohani

mentioned problems and increase in their bioavailability at site of absorption [3].

Various methods have been widely proposed to efficient protein delivery. The most efficient non nanotechnological methods for efficient cellular delivery are transcriptional activator of transcription. These methods are used to improve the internalization of peptide drugs due to their unique potential to enter the cells in culture when added exogenously. Though these carriers systems have potential to deliver protein into the target cells but, many of them have shown inefficient delivery for the protein and peptide drug to their actual target site. In these strategies, a number of other problems, such as complex manipulation, cellular toxicity and immunogenicity are reported [2]. The retention of peptide structure and activity on nanoscale support are critical for their therapeutic applications. Another factor to be considered is the effect of size and surface chemistry of nanomaterial on structure, activity, and stability of nanoparticles conjugated proteins.

The study of lysozyme and human carbonic anhydrase adsorbed on silica nanoparticles reveals the change in structure, activity and loss of a helical contents depending upon the size of nanoparticles. This finding reveals that the smaller nanoparticles are favorable for protein stability due to higher surface curvature for the protein nanoencapsulation. The retention of protein structure on nanoparticles is also protein dependent. It is also reported that single walled carbon nanotubes stabilize the protein under harsh conditions like organic solvents, high temperature etc. [4, 8].

NANOCARRIERS FOR PROTEIN AND PEPTIDE ENCAPSULANT [1, 8]:

Nanocarriers are one of the useful tools for achieving the main objective of protein and peptides targeted delivery. Encapsulation of protein drugs on nanoparticles provides sustained release and protects the encapsulant protein from degradation and loss of its activity. The therapeutic protein drug has short half-life due to proteolysis, rapid clearance from the blood stream and hence required repeated administration. The therapeutic protein molecules interact with nanocarrier material by forming a coat or adsorption on the surface, or by bioconjugation (direct or using cross linkers). The choices of encapsulation methods and nanocarriers system entirely depends on the application and nature of protein to be encapsulated. Some proteins, enzymes, and DNA plasmids are encapsulated in biodegradable polymeric nanofibers by electro-spinning technique will help to retain their bioactivity.

ADVANTAGES OF NANOCARRIER FOR PEPTIDE DRUG:

- Selective targeting of peptide drug is achieved.
- Provide controlled release of encapsulant peptide drug.
- Increase residence of proteins molecule in the body.
- Protects the encapsulant protein from degradation and loss of its activity.

Following are the types of nanocarriers used for encapsulation of protein and peptide:

1. METALLIC NANOCARRIERS:

Protein and peptides encapsulated in semiconductor and metallic NPs having unique electronic, optical and catalytic properties. Proteins and peptides encapsulated on a tiny semiconductor device like quantum dot having composite material with new functionality. Horse spleen apoferritin was attached on gold nanoparticles by using technique of di-thiol bridge formation between peptide and gold nanoelectrode. These enzymes form a covering layer that acts as stabilizer for gold nanoparticles in solution. Hemoglobin and myoglobin are also bioconjugated on gold and silver nanoparticles by using the same technique. Similarly, interleukins conjugated gold nanoparticles show selective binding to macrophage cells. Silver nanoparticles interact with the HIV-1 virus via preferential binding to the gp120 glycoprotein knobs. Due to this interaction, silver nanoparticles inhibit the virus from binding to host cells. But in case of some enzymatic proteins molecules it may possible to lose their proper function and structural organization during encapsulation on nanoparticles [9, 10].

2. POLYMERIC NANOCARRIER [1, 3, 4, 11]:

The major benefit of polymeric system in protein and peptide delivery system are the controlled drug targeting, modified body distribution and enhancement of cellular uptake. The polymeric nanocarrier is biodegradable, non-antigenic, relatively easy to prepare and full control on size distribution. Polymeric materials used for the formulation of nanoparticles include-

A. NATURAL: (albumin, gelatin, alginate, collagen or chitosan).

B. SYNTHETIC:

- Poly (lactic acids) (PLA)
- Poly (lactic-coglycolic acids)
- Poly (L-caprolactone)
- Poly (methyl methacrylates)
- Poly (alkyl cyanoacrylates).

A. NATURAL POLYMERIC NANOCARRIERS:

Chitosan is the second most abundant polysaccharide in nature, and has attracted particular interest as a biodegradable material for mucosal delivery systems. Chitosan is a modified natural polymer prepared by the partial N-deacetylation of natural biopolymer chitin. There are at least four methods reported for the preparation of chitosan nanoparticles as ionotropic gelation, microemulsion, emulsification solvent diffusion and polyelectrolyte complex formation. More importantly, chitosan micro/nanoparticles can be spontaneously formed through ionic gelation using tripolyphosphate as the precipitating agent. This reduces the use of harmful organic solvents during preparation and loading of protein therapeutics.

Benefits of chitosan as material for nanocarrier:

- Low toxicity and highly susceptible to biodegradation.
- Mucoadhesive properties.
- Capacity to enhance protein drug permeability/absorption at mucosal sites.

Chitosan solubility is poor above pH 6.0 which is a major drawback of this system. At physiological pH, chitosan is known to lose its capacity to enhance drug permeability and absorption, which can only be achieved in its protonated form in acidic environments. In contrast, chemically modified form of chitosan e.g. quaternized chitosan derivative, N-trimethyl chitosan chloride shows solubility in water over a wide range of pH. In addition, these modified chitosan derivative nanoparticles shows bio-adhesive properties. Thus, it can be used for enhancement of permeability and absorption of variety of peptide drugs in neutral and basic-pH condition [12].

Insulin was observed to be directly internalized by enterocytes up on contact with intestine and retention of drugs at their absorptive sites by mucoadhesive carriers. Insulin loaded chitosan nanoparticles markedly enhanced intestinal absorption of insulin following oral administration. The hypoglycemia effect and insulinemia levels were significantly higher than that obtained from insulin solution and physical mixture of oral insulin and empty nanoparticles. The mechanism of insulin absorption seems to be a combination of both insulin internalization, probably through vesicular structures in enterocytes and insulin loaded nanoparticles uptake by Payers patches cells. It has been shown that ovalbumin loaded chitosan microparticles are taken up by the Peyer's patches of the gut associated lymphoid tissue (GALT).

Gelatin is one of the natural polymer which is extensively used in food and medicinal formulations. It is a natural origin nanomaterial used in controlled release of peptide therapeutics due to its nontoxic, and biodegradable nature. Gelatin nanoparticles can be prepared by desolvation/coacervation or emulsion method. This polymer consisting of both cationic and anionic groups along with hydrophilic functionality. Due to this unique nature, gelatin molecules are frequently used for encapsulation of both acidic and basic peptides [13].

B. SYNTHETIC POLYMERIC NANOCARRIERS:

Synthetic polymers can extend the drug release over periods from days to several weeks. Some of the biodegradable and biocompatible synthetic polymers like Poly (d,l-glycolide-colactide) (PLGA), poly (d,l-lactide) (PLA) and polycaprolactone are used to encapsulate proteins drug. PLGA (poly-d,l-lactide-co-glycolide) and PLA nanoparticles are one of the most successfully used nanosystem for the development of protein nanomedicines and shown to be biodegradable in nature. They undergo hydrolysis in the body to produce the biodegradable metabolite monomers, lactic acid and glycolic acid. Since the body effectively deals with these two monomers, it has minimal systemic toxicity [1, 14].

Insulin was encapsulated in a blend of poly (fumaric anhydride) poly (FA) and poly(lactideco- glycolide) (PLGA) at a 50:50 ratio (poly(FA:PLGA)) using the inversion phase method. Animals feeding the poly (FA: PLGA) – encapsulated insulin preparation showed a better ability to regulate glucose load than the controls. This gave an indication that the insulin is released from the microspheres in a biologically active form and crossed the intestinal barrier. Protection of insulin from the proteolytic enzymes can be done by encapsulation into poly (isobutylcyanoacrylate) (PIBCA) nanoparticles by interfacial polymerization methods and promote absorption of insulin by the intestinal mucosa [15].

Another example of a synthetic polymeric nanocarrier application is calcitonin; a peptide secreted by the parathyroid gland has been encapsulated into poly (isobutylcyanoacrylate) nanocapsules, polyacrylamide nanospheres, and chitosan nanoparticles. This nano encapsulated calcitonin shows decrease level of ionized calcium in blood than simple calcitonin up on oral administration [16].

3. PROTEIN AND PEPTIDE IT SELF ACTS AS NANOPARTICLES:

Peptides are excellent building blocks owing to the ease of their synthesis, small size, relative high stability and chemical/biological modification possibility. Proteins and Peptides can be design and synthesis of nanostructures because they show a great diversity of chemical and physical properties. They can be synthesized in large

amounts, can be modified and decorated with functional elements for application in the field of nanomedicine.

Native folded structures of proteins and peptides have capability to self assembles as protein fibers e.g. coil–coil or amylogenic peptides. Three classes of protein components as planar crystalline arrays, engineered proteins pores and molecular motors are mostly used for development of protein nanodevices. A good example of planar protein crystalline arrays is surface-layer proteins having square or hexagonal symmetry of 3–30nm unit cells with 5–10nm thickness. Various molecular motors used as nanodevices for various cystis fibrosis diseases like linear kinesin, myosin, and ATP synthase. Normal proteins are engineered to self-assemble into nanodevice and thus self-assembly at nanoscale is important for the fabrication of novel supramolecular structure, having applications in the field of nanotechnology and nanomedicines. Structural element as short as dipeptides can form well-ordered assemblies at the nanoscale and can be used in therapeutics [17, 18].

4. COPOLYMERIZED PEPTIDE NANOPARTICLES (CPP):

A modification of a polymer-based system is a novel approach utilized for delivery of therapeutic peptides as drug–polymer conjugates in which the drug moiety is covalently bound to the carrier instead of being physically entrapped. This system needs to be further explored for effective delivery of sensitive molecules such as peptides and proteins. [18]

METHODS OF PROTEIN AND PEPTIDE DRUG NANOENCAPSULATION [1, 18]:

The encapsulation of the proteins and peptides at nanoscale is the first step towards the development of peptides nanomedicines therapeutically. The suitable and specific nanoencapsulation of therapeutic protein and peptide may be achieved by utilizing the suitable method for nanoencapsulation.

Various methods are available for the adsorption or encapsulation of protein and peptides on nanocarrier are as follows:

1. Emulsion polymerization.
2. Interfacial polymerization.
3. Solvent evaporation.
4. Salting out.
5. Coacervation.
6. Combination of sonication and layer by layer technology.
7. Solvent displacement/solvent diffusion.

Each methods of protein encapsulation have their own advantage and disadvantage. Since each protein and peptides requires specific set of condition for its stability, solubilization, control releases. The choice of method for peptide encapsulation is entirely based on the physicochemical activity of protein and its application [19]. A short discussion on the each reported protein encapsulated methods along with detail characteristics are given below.

1. EMULSIFICATION–POLYMERIZATION:

The emulsification–polymerization method is classified in two categories, based on the use of organic or aqueous continuous phase. In the continuous aqueous phase, polymers and protein drugs are dissolved in aqueous solvent without surfactants or emulsifier by using anionic polymerization mechanism with high energy radiation. In the continuous organic phase, polymers are dissolved in organic non-solvent by dispersion via surfactants in to solvent. e. g. various enzymes like calcitonin are encapsulated with polyacrylamide nano/microparticles of <1000nm size nanoparticles[20, 21].

2. SOLVENT EVAPORATION:

In solvent evaporation method, the polymers along with proteins are dissolved in volatile organic solvent (DCM, acetone, $CHCl_3$, etylacetate, etc.) and poured into continuously stirring aqueous phase with or without emulsifier/stabilizer and sonicated. Most of the proteins are likely to denature after the sonication. Thus, a slow and intermittent sonication at low temperature is effective to retain the secondary and tertiary structure of protein drugs. E.g. Albumin and tetanus toxoid are successfully encapsulated on polylactic acid (PLA) nanoparticles of size 100–120nm and 150nm by these methods. Solvent displacement method is similar to solvent evaporation that is based on spontaneous emulsification of the organic internal phase containing partially dissolved polymer along with protein into the aqueous external phase. Insulin was nanoencapsulated on PLA nanoparticles are of size 105–170nm by using solvent displacement method [22].

3. SALTING OUT:

The protein and peptides are sensitive to unfolding or inactivation. These problems are minimized by the salting out methods for protein and peptide encapsulation. This method is based on the separation of a water miscible solvent from aqueous solution by adding magnesium chloride, calcium chloride, etc to the solvent mixture. The main advantage of salting out procedure is that it minimizes unfolding stress on to the protein to be encapsulated.

4. INTERFACIAL POLYMERIZATION:

In interfacial polymerization, the cyanoacrylate monomer and peptide drug are dissolved in a mixture of an oil and absolute ethanol. This mixture is then slowly extruded through a needle into a well stirred aqueous solution, with or without some ethanol containing surfactant. Oils have positive influence to reduce the unfolding of protein by ethanol. An advantage of interfacial polymerization technique is high efficiency drug encapsulation. E.g. Insulin was encapsulated on poly(ethylcyanoacrylate) and poly(isobutylcyanoacrylate) nanoparticles of particle size 151nm and 150–300nm respectively by using this method [23].

5. COACERVATION:

Coacervation is a process during which a homogeneous solution of charged macromolecules undergoes liquid–liquid phase separation, giving rise to a polymer rich dense phase. This method has been classified into simple and complex processes depending on the number of participating macromolecules. In simple polyelectrolyte coacervation, addition of salt or alcohol normally initiates coacervation. In complex coacervation, two oppositely charged macromolecules (or a polyelectrolyte and an oppositely charged colloid) can undergo coacervation process through associative interactions. The charges on the polyelectrolytes must be sufficiently large to allow significant electrostatic interactions but not so large to cause precipitation. The dilute liquid phase, (usually supernatant), remains in equilibrium with the coacervate phase. These two liquid phases are incompatible and immiscible. E.g. BSA has been successfully encapsulated on PLA and PLGA by using Coacervation [24].

6. EMULSIFICATION/SOLVENT DIFFUSION:

This is very efficient technique for encapsulation of protein on polymeric nanoparticles. This technique provides many advantages like maximum encapsulation efficiency without homogenization, high batch to batch reproducibility, ease of scale up, simplicity and narrow size distribution of protein nanoencapsulate. In these methods, the polymers with protein drug are dissolved in a partially water soluble solvent and saturated with water. Subsequently, the polymer–water saturated solvent phase is emulsified in an aqueous solution containing stabilizer, results in solvent diffusion to the external phase and the formation of the nanoparticles. E.g. Bovine serum albumin (BSA) and immuno-globulin (IgG) encapsulated on (PEO–PLGA) by using this method has high encapsulation efficiency (58.9%) and slow in vitro release rate. The problem of protein inactivation or irreversible aggregation inside PLGA or PLA nanoparticles can be prevented by incorporation of stabilizers such as polyethylene oxide (PEO) and its derivatives [25, 26].

7. SURFACE FUNCTIONALIZATION OF GOLD NPS:

Bayraktar et al. [27] demonstrated the ability to disrupt protein–protein interactions using surface-functionalized gold nanoparticles. They have designed nanoparticles that bound selectively to cytochrome c (Cyt c) or cytochrome c peroxidase (CCP), thereby inhibiting enzyme turnover. Surface-functionalized nanoparticles with gold cores (2 nm) are prepared using thiolates with oligo (ethylene glycol) groups terminated in carboxylate (Au- TCOOH) and trimethylamine (Au-TTMA) functionalities. Au-TTMA nanoparticles bind selectively to a negative patch on CCP surface, whereas Au-TCOOH nanoparticles bind selectively to basic amino acid rich surface of Cytochrome C. Tseng et al. [28] fabricated amino-acid-functionalized gold nanoparticles that modulate the catalytic activity of R-chymotrypsin. They proposed that the amino acid monolayer on the nanoparticles controls both the capture of substrate by the active site and the release of product through electrostatic interactions.

8. TAYLOR CONE JET METHODS:

Taylor Cone Jet is a new technique for protein encapsulation on nanoparticles to protect their structure and function. In this technique, protein solution is dispersed in organic phase (with DCM) dissolved PLGA by controlled sonication processes. Further, electrospray with well-defined potential difference is created between the nozzle and the ring by applying high voltage on the nozzle and a lower high voltage in the ring. The emulsion solution was pumped through nozzle to form liquid cone. This creates a thin jet from the apex to break up into monodispersed droplets [29].

Various methods used for preparation of nanoencapsulation are shown figure no.1 and various nanocarriers used in different methods given in table no.1.

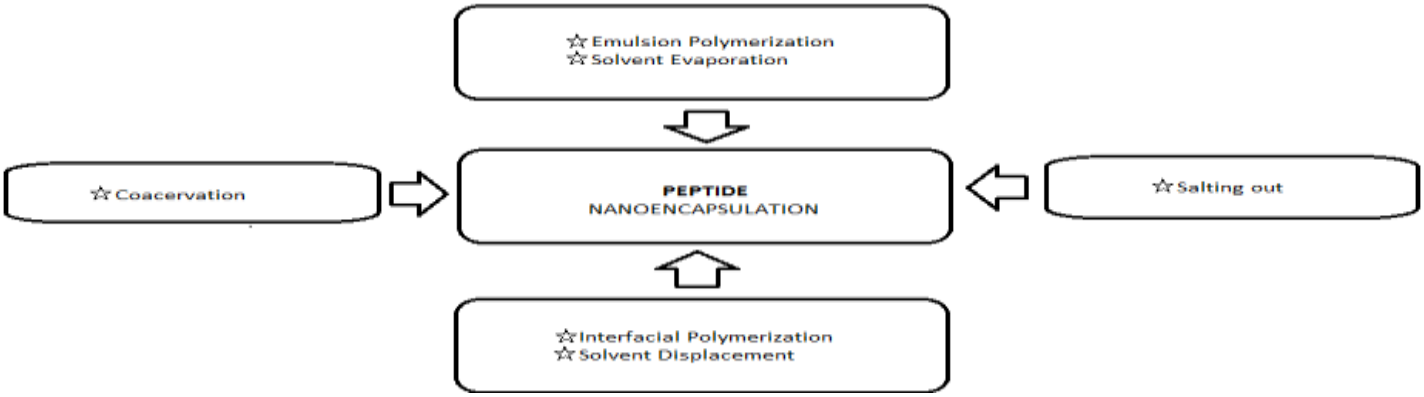

Figure No. 1: Methods of peptide Nanoencapsulation

Table No. 1: Protein and peptide Nanoencapsulation methods [1, 30]

Protein and peptide	Nanocarrier Material	Encapsulation Method	Advantage
Bioconjugation Type			
BSA	ter-butyl acrylate	Maleimide coupling	Biological activity retained
Tumor lysate	CNTs	EDC–NHS chemistry	Activity enhanced
Chymo-trypsin	Iron		Improved thermal stability
Bombesin peptide	Iron	Click chemistry	Targeted delivery
Encapsulation Type			
BSA	Gelatin	Emulsion	Activity Retained
Insulin	PLGA	Multiemulsion	Activity Retained
Lysozyme	Polymethylmethacrylate	Emulsion	shelf life increased
hGF2	PLA	Double emulsion	Activity retained
BSA	Chitosan	Taylor cone Jet	>80% of the BSA Bioactivity retained
Ganciclovir	Albumin	Coacervation	Increased release

CONCLUSION:

As the proteins and peptides are very prone to degradation therefore there is necessity to maintain their stability to retain their therapeutic activity. So that the nanoencapsulation system is very effective not only to maintain their therapeutic activity but also delivery the protein and peptide to the desired site of action and also maintain the concentration for longer period of time and give sustain effect. Based on the physicochemical activity and application of proteins various nanocarriers such as metallic, polymeric as well as some proteins and peptide are used along with suitable method for peptide encapsulation.

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